Indian J. Hort. 67(Special Issue), November 2010: 29-33

In-vitro multiplication of cherry rootstocks

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ABSTRACT

Two cherry root stocks mazzard (*Prunus avium* L.) and mahaleb (*Prunus mahaleb*) were used for in vitro multiplication studies. Different media combinations were treid to optimize the best combination fast and efficient proliferation and multiplications of root stocks. Meristem culture was done to produce virus free clonal root stocks. The best combination for meristem establishment and proliferation in culture was MS media with 1 mg/l Benzyl Adenine (BA) and for multiplication it was MS with 0.5 mg/l IBA plus 1 mg/l BA. The shoot elongation and multiplication was found better in mazzard rootstock than in mahaleb in all the treatments used. The rooting medium was half strength of Murashige and Skoog medium containing 2.0 mg/l Indole Butyric Acid (IBA). Rooted shoots showed very low survival rate.

Key words: Cherry, micro-propagation, mazzard, mahaleb, clonal root stocks.

INTRODUCTION

Micropropagation of many woody species is well extended. This holds true for the *Prunus* species (Snir, 19, Yang, 23, Preil, 17) as well. Many cherry rootstocks (Gisela 5, Weiroot, Maxma) are even micropropagated commercially, although several reports consider the micropropagation to be a technique not useful for producing rootstock commercially (Grant and Hammatt, 5, Osterc and Spethmann, 15). However, the micropropagation practice points to some steps that are not successful enough in *Prunus*, so they should to be clarified. Yang (23) reported difficulties in the rooting process in *Prunus* in his experiment, so the propagation process should have been optimised. He could achieve the best rooting with the use of NAA (á–naphthaleneacetic acid). The use of NAA was already suggested by Snir (19) who forced better rooting with the basal shoot wounding. Very good rooting results were achieved with the use of IBA (indole-3- butyric acid) in the experiment on the micropropagation of wild cherry *Prunus avium* L. (Meier-Dinkel, 10).

Mazzard and mahaleb rootstocks are the predominate rootstocks used. These are the main rootstock recommended even with their short comings such as: vigorous growth, large tree size, lack of precocity and slightly poorer yields. However they can be used in the majority of our soils and are well adapted to different cultivars with no reports of incompatibility problems with our scion cultivars. Colt has also been used in most parts of cherry grown countries. It is as vigorous as mazzard and produces a tree about the same size as mazzard. The major concern with Colt is the possibility of winter injury problems in some years and in the colder areas. Root stocks like Gisela7 5 (G5), Gisela7 6, Gisela7 12, and Weiroot 158 are new and more vigorous the mazzard, mahaleb and colt but use of virus free scion cultivars is extremely important as is fumigation of planting sites, especially if planting in an old cherry site while using these root stocks. Also, trees with these rootstocks need to be more intensively managed to maintain good vigour and optimum fruit quality. This includes adequate nutrition and water, weed control and careful pruning and training. Selffertile, precocious cultivars such as Sweetheart may not be a good combination with these rootstocks. Soil type also needs to be considered when choosing which rootstock to evaluate. Precocious rootstocks combined with precocious and very productive cultivars may lead to overcropping and eventual reduction in fruit quality and stunting of the tree. Mazzard and mahaleb root stocks on one side are vigorous but on other side these root stocks are tolerant to viruses like NRSV and PRV. Mazzard root stock is also tolerant to *Phytopthora* and mahaleb to crown gall (Perry, 16, Callasen, 4).

In order to overcome difficulties in vegetative propagation of cherry root stocks, methods of micropropagation were developed in past years. These methods are based on stimulation of axillary branching or adventitious shoot formation by addition of different phytohormones to the culture medium. Micropropagation systems for *Prunus avium* were mostly developed and tested for genotypes aimed for production of rootstocks (Muna *et al.,* 11). Nevertheless, it was proved that different

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genotypes do not respond in the same way during establishment and proliferation *in vitro* (Snir 19). Rapid shoot multiplication procedure for in vitro propagation of different cherry root stocks like dwarfing P-HL rootstocks ('P-HL A', 'P-HL B', 'P-HL C') for sweet cherries (*Prunus avium* L.), Maxma-14, CAB 6P, SL 64 and Gisela 5 using shoot tips were developed (Sedlak *et al.* 18, Xilogiannis *et al.* 22, Nacheva, *et al.* 13, Muna *et al.* 11).

Despite increasing numbers of reports of successful micropropagation, more research is still needed in this area. It is important to produce virus free planting material through tissue culture. The objective of this study was to standardize the tissue culture techniwue for production of virus free planting material using meristem as explant.

MATERIALS AND METHODS

The nutrient media contained inorganic and organic constituents according to Murashige and Skoog (12). Phytohormones were also added to the Murashige and Skoog medium in different concentrations and combinations. The pH of the medium was adjusted to 5.7 with either 1 N KOH or 1 N HCL. The culture media were autoclaved at 15 1b/in^2 at 121^{o}C for 15 minutes, then maintained at 27±2^{o}C in the culture room.

Plant materials required for all the meristem culture and tissue culture experiments were obtained from green-house grown plants at the Central Institute of Temperate Horticulture, Rangreth, Srinagar, J & K. The donor shoots were taken in the month of February and kept for sprouting in water treated with 0.1% Ziram at 25^{o}C in BOD. After 3-4 weeks sprouted buds were used for isolation of meristem. Actively growing shoot tips (5 to 10 mm in length) were cut from shoots. Excised shoot tips were used for isolation of meristem (under microscope).

Shoot tips were washed with Tween 80 and then rinsed with distilled water 3-4 times. Meristems were isolated from these shoot tips and surface disinfested with a 0.15% solution of mercuric chloride for 1 min. Under a laminar flow hood, they were rinsed with sterile distilled water. All the tools required for dissection were previously sterilized. Disinfected meristems were placed separately in culture tubes (25 mm) containing 15 ml of MS medium with various growth regulators. The culture tubes containing the explants were maintained in the laboratory at 25±2^{o}C. They were exposed to artificial illumination of 2000-3000 lux by placing them at 25-30 cm below fluorescent light for sixteen hours every day.

The experiment treatments included the auxins, kinetin and gibberellic acid in different concentrations and combinations in establishment and shoot multiplication as shown in Table 1 and 2. Rooting media was MS with different strengths plus different concentrations of IBA (Table 3). Proliferation rate was

Table 1. BA in different concentrations for culture establishment.

Treatment	BA(mg/l)
T1	0.1
T2	0.5
T3	1.0
T4	2.0

Table 2. Hormone concentrations/combinations for shoot multiplication.

Treatment	BA (mg/l)	IBA	Readymade SMM
T1	1.0	0.1	0.0
T2	1.0	0.2	0.0
T3	1.0	0.5	0.0
T4	0.0	0.0	Full

Table 3. Hormones concentrations and MS strength used in rooting media.

Treatment	IBA (mg/l)	MS
T1	1.0	Full
T2	1.0	Half
T3	2.0	Full
T4	2.0	Half
T5	3.0	Full
T6	3.0	Half
T7	4.0	Full
T8	4.0	Half

defined as the number of newly formed shoots (> 10 mm) per initial shoot tip/meristem after six weeks of culture. The shoot formation was recorded between the tenth and fifteenth subculture. In all experiments, 25 meristems were used. Each experiment was repeated four times. Data from four replications were pooled and expressed as the mean. Treatment means were compared with the standard error (SE) of the mean.

RESULTS AND DISCUSSION

Micropropagation of woody and semi-woody trees is reported as problematic (Babaolu *et al.,* 2) and to obtain a material without viruses is a long-term, complex process. To obtain stocks without viruses, it is necessary to use seeds without viruses as rootstocks. For this purpose, many *in vitro* experiments have been conducted for these economically important fruit trees (Kester and Asay, 8, Wang and Hu, 21, Hisajima, 7, Barghchi and Alderson, 3, Maynard *et al.* 9, Onay *et al.* 14, Adýyaman *et al.* 1, Tilkat *et al.,* 20). This study used two root stocks of cherry (mazzard and mahaleb) for standardization of

media conditions for *in-vitro* multiplication of virus free plants.

The effect of Benzyl Adenine (BA) on the culture initiation of the meristems isolated from mazzard and mahaled shoot tips was investigated. The number of shoots formed were 1.2 ± 0.15 in the control group (without BA), and 9.0 ± 1.13 and 12.7 ± 2.0 in 0.5 and 1.0 mgl^{-1} BA treatments, respectively. However, a decline in terms of new shoot formation in high BA concentrations (2.0 mg/l) was also observed. Consequently, the results suggest that different BA concentrations have an effect on new shoot formation. The rootstock mazzard showed better response than mahaleb with respect to shoot length, number of shoots developed and leaf number per explants to the treatment combinations used (Table 4). It was observed that with the increase in concentration of BA shoot multiplication rate increased, that shoot production decreased as BA concentrations decreases. The best multiple shoot initiation was obtained on the MS medium supplemented with BA at 1 or 2 mgl-1, with a average shoot number of 5.7 ± 1.02 and 5.2 ± 1.00 in mazzard and 5.4 ± 0.62 and 5.0 ± 0.60 per explant, in mahaleb respectively, on the 30th day of culture (Table 4). Readymade shoot multiplication media showed good response to shoot multiplication with 4.8±0.98 shoots per explants in mazzard and 4.6±0.86 in mahaleb root stock. At the same time, it was observed that there was better shoot multiplication in mazzard than in mahaleb root stocks. After shoot proliferation, the surviving explants were subcultured regularly every four weeks. To optimize an efficient proliferation method, one concentration of BA (1.0 mgl^{-1}) was used in combination with different combinations of IBA concentrations (0.1, 0.2 and 0.5 mgl-1). As can be seen from Table 5, the best response for shoot proliferation was obtained from the explants cultured on the MS medium supplemented with 0.5 mgl-1 IBA. Similar results were obtained by Jones and Hopgood (24) in cherry root stock F12/1. The results showed that using BA in the shoot multiplication was an absolutely necessity. But use of auxin induces callusing followed by shoot multiplication (Fig. 1).

Shoots obtained from *in vitro* conditions were used as material for rooting studies. For *in vitro* rooting of shoots, different concentrations of IBA (1.0, 2.0, 3.0 and 4.0 mg/l) were used. At the end of 40 days of culture, half of all explants (50%) cultured on half strength MS medium supplemented with 2.0 mgl^{-1} IBA were observed to have roots. The maximum number of roots and root length obtained was 1.20 ± 0.02 and 3.0 ± 0.22 cm, respectively. In our study, although the effects of light and darkness on rooting of shoots were analyzed, no positive results were obtained. Besides, half strength of MS medium supplemented with different concentration of IBA was also used for rooting. Consequently, the results indicated that shoots cultured on a half strength MS medium supplemented with 2.0 mgl^{-1} IBA showed root formation at the rate of 50%. In mazzard days for development of a100% roots on average were 60 and in mahaled it was 66 days. The survival rate of rooted plantlets was found very low (20%) in both the root stocks.

Table 4. Effects of different BA concentrations on shoot proliferation.

Treatment	Mean shoot length (cm)	Mean shoot number	Mean leaf number
Mazzard			
0.1 mgl-1 BA	2.8 ± 0.05d	2.0 ± 0.04d	4.7± 0.25d
0.5 mgl-1 BA	3.0 ± 0.10c	3.0 ± 0.04c	8.0± 0.20c
1.0 mgl-1 BA	4.0 ± 0.05a	5.7 ± 0.02a	10.0± 0.25a
2.0 mgl-1 BA	3.7 ± 0.04b	5.2 ± 0.03b	8.7b±0.15
Readymade SMM	2.9±0.04	4.8±0.05	4.0± 0.25d
LSD	0.201	0.144	0.122
Mahaleb			
0.1 mgl-1 BA	2.6 ± 0.05d	1.8 ± 0.04d	2.2± 0.19d
0.5 mgl-1 BA	3.1 ± 0.06c	2.9 ± 0.03c	6.7± 0.15c
1.0 mgl-1 BA	3.7 ± 0.03a	5.4 ± 0.03a	8.0± 0.20a
2.0 mgl-1 BA	3.3 ± 0.03b	5.0 ± 0.04b	7.7± 0.25b
Readymade SMM	2.8±0.03	4.6±0.06	2.6± 0.21d
LSD	0.129	0.66	0.61

Data was recorded on the 30th day and representing an average of 10 replicates per treatment with three repetitions of the experiment.

Table 5. Effects of BA (1.0 mgl^{-1}) used in combination with different concentration of IBA on the multiplication of shoots from mazzard and mahaleb explants.

Treatment	Mean shoot length (cm)	Mean shoot number
Mazzard		
0.1 mgl-1IBA + 1.0 mgl^{-1}BA	1.4 ± 0.02a	3.8 ± 0.04b
0.2 mgl-1IBA + 1.0 mgl^{-1}BA	1.3 ± 0.03a	3.4 ± 0.10c
0.5 mgl-1IBA + 1.0 mgl^{-1}BA	1.5 ± 0.07a	4.8 ± 0.10a
Readymade SMM (Himedia)	0.65 ±0.006b	3.0±0.10d
LSD	0.12	0.11
Mahaleb		
0.1 mgl-1IBA + 1.0 mgl^{-1}BA	0.8 ± 0.03b	2.7 ± 0.12b
0.2 mgl-1IBA + 1.0 mgl^{-1}BA	0.9 ± 0.03b	2.2 ± 0.09c
0.5 mgl-1IBA + 1.0 mgl^{-1}BA	1.0 ± 0.04a	3.0 ± 0.10a
Readymade SMM (Himedia)	0.85 ±0.03b	2.3±0.09c
LSD	0.27	0.30

Data was recorded on the 30th day and representing an average of 10 replicates per treatment with two repetitions of the experiment.

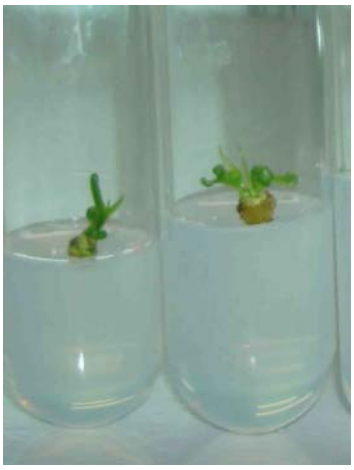

Fig. 1. Callus induction due simultaneous use of BA and IBA for shoot multiplication.

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Received: September, 2009; Revised: March, 2010
Accepted: August, 2010