

V. SUMMARY AND CONCLUSION

This study was carried out at the tissue culture laboratory, Research Desert Center, during 2006 and 2007 seasons. The main purpose of this study aimed to investigate the response of Hansen 536 peach rootstock and Mac9 apple rootstock to mass propagate for commercial production via tissue culture technique. Shoot tip (5- 10 mm) containing the apical meristem and stem one - node cutting (1- 2 cm length) explants were used as plant materials for this investigation .

Culture media:

Murashige and Skoog (1962) basal medium (Ms- medium) supplemented with 7.0 g/L agar, 30 g/L sucrose, was used as culture medium. Additive substances were also added to Ms medium according to the investigated stages of propagation as will be shown later.

The complete randomized design was used for arranging the treatments. Every treatment was replicated five times and each replicate, with 20 explants, was cultured individually in a culture tube.

Some experiments were conducted during each stage of propagation by using tissue culture technique, and subsequently the obtained data could be summarized as follows:

I. Surface sterilization

Three concentrations (1.0, 1.5 and 2.0%) of NaOCl (10 % clorox) combined with three immersion duration (10, 15 and 20 min) were investigated . Explant immersed in 2% NaOCl for 20

min were dipped first in 70% ethanol for 5 min. HgCl_2 at 0.1% for 10 min was used also as surface sterilized agent.

Data obtained from surface sterilization experiments for the two studied rootstocks, revealed the superiority of dipping the explants (shoot tip and stem node section) in 70% ethanol for 5 min followed by dipping in 2.0% NaOCl for 20 min, which proved to be the most effective method, where as it reduced both contamination and browning percentage, parameters, while it increased the other two investigated parameters (survival and growth percentages).

II. Establishment stage:

Shoot tips and stem node sections of both investigated rootstocks were cultured on MS based medium supplemented with either BAP or 2ip, each at three levels (0.5, 1.0 and 1.5 mg/L) as well as combination between BAP and 2ip each at three levels (1.5, 2.0 and 2.5 mg/L) besides cytokinin omitted medium to evaluate the best hormone and improve the investigated parameters browning, survival and growth percentages as well as shoot elongation.

The obtained results revealed that :

1. Shoot tip and stem one - node cutting of Hansen 536 peach and Mac9 apple rootstocks cultured in MS medium supplemented with 1.5 mg/L BAP or 2ip resulted in a significant reduction in browning while it maximized both survival and growth percentage as well as average shoot elongation.
2. The Maximum increment of both survival and growth percentages as well as the lower browning percentage of

Hansen 536 shoot tip culture were achieved with the addition of BAP and 2ip to establishment medium at 2.0 mg/L for each. However, 2.5 mg/L of each cytokinin was effective in improving shoot elongation.

3. All studied parameters of stem one - node cutting of Hansen 536 were enhanced with BA + 2ip each at 2.0 mg/L.
4. Parameters of both shoot tip and stem node section of Mac9, cultured in establishment media, were maximized with using the highest level of both cytokinins, BAP + 2ip (2.5 mg/L) except the stem one - node cutting browning was reduced significantly with 2.0 mg/L of each cytokinin.

III. Proliferation stage:

Proliferated shoots developed from shoot tip culture of both investigated rootstocks (Hansen 536 and Mac9) throughout establishment stage were cultured to investigate their multiplication response to cytokinin medium contents. Three combinations between BAP (1.0 and 1.5 mg/L) and 2ip (0.5, 1.0 and 1.5 mg/L) were added to MS basal medium as well as MS cytokinin omitted medium (control) were used.

Data obtained proved that :

1. Proliferation parameters (average number of proliferated shoot, proliferated shoot % and total length of proliferated shoots) of Hansen 536 shoot cultured, developed greatly when cytokinins (BAP and 2ip) were added to culture medium as compared with control. The highest values of the investigated parameters were achieved when BAP + 2ip were added to MS medium each at 1.0 mg/L. The proliferation rates were 3.2 and 5.6 fold for 1st and 2nd

seasons, respectively. As for average length of proliferated shoots parameter, it was maximized with 1.5 mg/L BAP + 1.5 mg/L 2ip.

2. Regarding the effect of different combinations between BAP and 2ip added to MS basal medium on some multiplication parameters of Mac 9 apple rootstocks, data reflected that adding BAP and 2ip each at the higher level (1.5 mg/L) was most effective in enhancing and maximizing the values of all investigated proliferaters during both seasons of study. The proliferation rates were 3.6 and 3.8 fold for 1st and 2nd seasons, respectively.

In order to study the effect of subculture number on shoot proliferation, shoots of both investigated rootstocks which were developed from shoot lip cultures throughout establishment stage were excised, transferred and cultured. The best culture medium for each rootstock which was able to improve proliferation parameters during estalbimsent stage was selected. Shoots of Hansen 536 and Mac9 rootstocks, were cultured in MS medium containing BA + 2ip each at 1.0 mg/L and 1.5 mg/L, respectively. The shoots were subculture on the same starting medium ever, 4 weeks. Data obtained including (average number, total and average length of proliferated shoots as well as proliferated shoot percentage) were recorded. The data revealed that :

1. The aforementioned parameters increased gradually by increasing the number of subculture on the sane initial medium for both rootstocks.

2. The fourth subculture was superior in this respect, where as it maximized the all studied parameters for both rootstocks.
3. The proliferation rate of Hansen 536 increased by increasing number of subculture. It was 5.2; 5.8 ; 8.0 fold for 1st, 2nd, 3rd and 4th subculture, respectively

Moreover, it was 5.8, 6.4 , 7.0 and 7.4 and 1st, 2nd , 3rd and 4th subcultures, of Mac9 rootstock, respectively

IV. Rooting stage:

Shoot of both rootstock which were raised throughout proliferation stage were used for rooting stage. Four combinations between IBA (0.5, 1.0 , 1.5 and 2.0 mg/L) and NAA (0.5, 1.0 , 1.5 and 2.0 mg/L) or PG at one level (100 mg/L) were added to ½ strength MS medium as well as auxin omitted medium were investigated regarding their effect on some rooting parameters (rooting percentage, average root number and average root length) for both rootstocks.

The obtained data revealed that:

1. The combinations between IBA + NAA each at 1.5 mg/L was the superior one, where as it enhanced significantly the rooting parameters of Hansen 536 peach rootstock.
2. Rooting percentage of Hansen 536 , shoot cultured in ½ MS + 1.5 mg/L , IBA + 1.5 mg/L NAA was 80% for both seasons of study .
3. ½ strength MS medium + 1 mg/L IBA + 100 mg/L PG gave the highest values of all investigated rooting parameters of Hansen 536 .

4. The aforementioned treatment achieved the highest Hansen 536 rooting percentage (80 and 100%) for 1st and 2nd seasons, respectively.
5. The most promoting response of both rooting percentage and average root number of Mac9 shoot culture, was achieved when ½ strength MS medium contained 1.5 mg/L of each IBA and NAA. The average root length character took the other was around. Such combination gave 60 and 80% rooting percentage for 1st and 2nd seasons, respectively
6. The highest level of IBA (2.0 mg/L) associated with 100 mg/L PG maximized the all investigated rooting parameters of Mac9 during both seasons of study. It maximized rooting percentage (100%) for both seasons of study

V. Acclimatization stage:

The healthy plantlets (rooted shoot) which were developed from rooting stage for both investigated rootstocks were rinsed with top water, immersed for 5 min. in 0.5% benelate fungicide , soaked in 2000 ppm IBA rooting solution and finally transferred individually (each in 15 x 15 cm dimation black plastic pot) filled with one of the following medial :

1. Sand + peat moss (at 1: 1 ratio v: v)
2. Sand + vermiculite (at 1: 1 ratio v: v)
3. peat moss + vermiculite (at 1: 1 ratio v: v)
4. Sand +peat moss + vermiculite (at 1:1:1 ratio v: v: v)

Survival percentage and average of stem length of both root stocks (Hansen 536 and Mac9), were recorded 8 weeks later:

The data revealed that :

1. Survival percentage and average of stem length of both investigated rootstock plantlets though the acclimatization stage was obviously affected by the different substrate mixture used as growing media.
2. Mixture of sand + peat moss + vermiculite at equal proportion by volume was statistically the superior growing medium for both rootstocks.
3. The growing medium (Sand + peat moss + vermiculite) 1:1:1 ratio, exhibited the maximum survival percentage (80%) for both rootstock plantlets as well as it maximized the average stem length of both studied rootstock plantlets.