# *V. SUMMARY AND CONCLUSION*

This study was carried out during 1994 and 1995 seasons, preceded with a preliminary work in 1993 year. The main purpose aimed to investigate possibility of using tissue culture techniques as a rapid mean for clonal propagation of three imported peach cultivars namely : Florida Sun, Florida Prince and Early Grand. Shoot tip (0.5-1.0 cm. containing the apical meristem) and single node cutting (stem segment with one node of 1-2 cm. length) explants were the plant materials used in this investigation. Experiment were conducted in the Plant Tissue Culture Experimental Lab. of Agric. Development System Project (ADS) belonging to Minst. of Agric.

## Preparing of Culture media :

Murashige and Skoog (MS-62) medium at full strength was used for different stages (except rooting stage, at 1/2 strength), besides rather additive substances were also involved to be investigated within different experiments conducted during various stages (steps) of propagation process as will be shown later.

## Experimental Layout and Culturing :

The complete randomized design was used for arranging treatments of every experiment. Every treatment was replicated three times and each replicate was represented by 10 explants cultured individually i.e, each in a single tube.

## Experiments Included in this Work:

For achieving the aimed purposes from this work, some experiments were conducted during each stage (step) of tissue culture technique and consequently the obtained data could be summarized as follows:

### Part one :-

### V.1. Experiments aimed to investigate possibility of overcoming some technical problems:

Two factors i.e, methods of surface sterilization and adding of PVP to culture were studied regarding their effect on the two main earlier technical problems (browning and contamination of cultured explants) and their refliction on survival potentiality.

## V.1.1. Experiments of surface sterilization :

Three methods of surface sterilization namely:

1- Immersion for 10 min. in 0.5 % NaOCl (10 % clorox), 2- Immersing for 10 min. in 0.5 % NaOCl + dipping for 2 min. in 70% ethanol and 3- [0.5 % NaOCl (10 min.) + 70% ethanol (2 min.) + 0.1 % $HgCl_2$ (1 min.)] were investigated in this respect. Taken into the consideration that tween 20 at 0.01 % was used as surfactant and all treated explants were rinsed 3 times after each treatment had been done.

Data obtained from the three experiments conduced for the three peach cvs. "each was devoted for two explants of every culivar " revealed obviously the superiority of the third treatment i.e, (immersion for 10 min. in 0.5 % NaOCl + successive dipping in 70% ethanol and 0.1 % $HgCl_2$ for 2 and 1 min., respectively) which proved to be the most effective surface sterilization method whereas it reduced both contamination and browning rates, while raised the survival % of both shoot tip and single node cutting explants of the Florida Sun, Florida Prince and Early Grand peach cvs.

## V.1.2. Experiments of adding PVP "Polyvinylpyrrolidone" to culturing media :

Four concentrations of PVP (10, 20, 40 and 80 mg/L) added to MS basal medium were investigated regarding their effect on browning and survival % of cultured explants (shoot tips and single node cutting) excised from the three peach cultivars during both 1994 and 1995 seasons.

Data obtained declared that adding PVP to culturing media at 40 mg/L was statistically the most effective for reducing the browning in cultured shoot tip and single node cutting explants not only as compared to control "PVP omitted medium" but also than the PVP supplied media at other concentrations. On the other hand, number of survived explants was significantly increased by the aforesaid superior treatment especially with both Florida Sun and Florida Prince peach cvs., while with Early Grang cv. adding PVP to culture at both 40/80 mg/L were statistically the same and resulted in the highest survival %.

## Part two:-

## V.2. The morphogenesis of Peach Shoot tip and nodle cutting:-

### V.2.1. Experiments conducted during establishment of explants (stage,1) :

Different combinations of (IBA + BA) added to culturing media and excising date of peach explants were the two investigated factors during this stage pertaining their effect on number of proliferated shoots per cultured explant of both shoot tip and single node cutting during two seasons of 1994 and 1995.

#### V.2.1.1. Experiments of investigating added (IBA + BA) combinations :

Four combinations between two concentrations of both IBA (0.01 and 0.02 mg) and BA (0.1 and 0.2 mg added to one liter of MS basal medium, beside neither IBA nor BA added (control) were the five investigated media within the three experiments conducted (each was devoted for both explant kinds of every peach cv.).

Data obtained during both seasons indicated obviously that culturing explants of both shoot tip and single node cutting on MS basal medium supplemented with activated charcoal at 3 g per liter and any of (IBA + BA) combinations increased significantly the number of

proliferated shoots per each over control. However, MS basal medium supplemented with 3 g activated charcoal per liter plus any of the two combinations between the lower IBA rate (0.01 mg) from one hand and 0.1/0.2 mg BA per liter from the other were the most favourable during establishment stage, regardless of either explant kind or peach cultivar.

### V.2.1.2. Experiments dealing with investigating effect of excising date :

Seven dates for excising both shoot tip and node cutting explants from the three peach cultivars were investigated. Explants were monthly collected during the growning season starting from March till September.

Data obtained disclosed the closed relationship between excising date of peach explants from one hand and their poteniality to be proliferated during establshment stage,. Hence, May excised explants (shoot tip and single node cutting) exhibited statistically the greatest number of proliferated shootlets per each followed by those excised either in April and August (for Florida Sun and Florida Prince) or June and July for Early Grand cv. On the contrary, all explants excised in March from any peach cultivar, as well as in September (in most cases) were the inferior in this concern.

## V.2.2. Experiments conducted during multiplication of shoots (stage,2) :

Proliferated-shoots induced from both explant kinds throughout the previous stage (establishment) were cultured to be multiplicated and investigating their response to supplying culturing media with BA either soleby or in combination with IBA. Therefore, two experiments were conducted for each peach cv. to study the following :

### V.2.2.1. Effect of various BA concentrations:-

Three levels of BA (0.1, 1.0 and 2.0 mg) added to one liter of auxin omitted MS basal medium were investigated regarding their effect on number of developed axillary shootlets (laterals) and average length per each cultured shoot. Three experiments were conducted, whereas an experiment was devoted for each peach cv.

Data obtained proved that both number of proliferated shootlets per each cultured shoot and average length of each were in a firm relationship with the added rate of BA. Since, the highest values of both number and length of developed shootlets were always concomitant with culturing in IBA omitted MS basal medium supplemented with (the intermediate level of BA " 1.0 mg" + 40 mg PVP) per liter during multiplication stage for the three peach cultivars irresepective of the origin explant from which the employed shoots were induced.

### V.2.2.2. Effect of different combinations between IBA and BA:-

Four combinations between two levels of both BA (1.0 & 2.0 mg) and IBA (0.02 & 0.2 mg) added to one liter of MS basal medium supplemented with 40.0 mg PVP were investigated. Three experments were conducted, each included the aforesaid four treatments to investigate their effect on average number of developed axillary shootlets (laterals) per individual cultured shoot of three peach cv. (Separately as an experiment was devoted for every peach cv.).

From the obtained results it could be clearly observed that proliferation during multiplicotion stage (expressed as an average of developed shootlets per each cultured shoot) was responded obviously to the different IBA + BA combinations . However, each peach cultivar followed its own trend, but it could be safely concluded to great extent that supplying MS basal medium with 40.0 mg PVP and the combination of 1.0 mg BA + 0.2 mg IBA was the most favourable culturing medium for both Florida Sun and Florida Prince peach cultivar. While, with the Early Grand cv. the combination between the higher rate of both BA + IBA (2.0 + 0.2 mg) was the superior in increasing the number of proliferated shootlets per each cultured shoot. Such trends were true with cultured shoots originated from any of two peach explant kinds.

Nevertheless, proliferated shoots from the shoot tip explant surpassed obviously those of the single node cutting regarding the number of developed shootlets per each as both were compared after culturing on the same medium, regardless of peach cultivar.

Conclusively, it was quite evident that number of proliferated shootlets was increased vigorously by supplying culturing media with various combinations of (BA + IBA) rather than BA alone.

### V.2.3. Experiments conducted during rooting stage :

During this stage three experiments were conducted for each peach cultivar to investigate effect of adding three concentrations of both IBA and NAA either each was added solely or both in combination to the MS basal medium (1/2 strength) with the 1st, 2nd and 3rd experiments, respectively. Shoots of 4-5 cm. in length regenerated from shoot tips or single node cuttings were used.

#### V.2.3.1. Effect of IBA level added to rooting media:-

Three levels of IBA (0.5, 1.0 and 2.0 mg/L) were added to charcoal supplemented MS basal medium (at 1/2 strength) to investigate the response of callusing, rooting and survival percentage of cultured shoots initiated from

both shoot tip and single nod cutting explants of three peach cvs.

Data obtained disclosed that callusing takes place only under culturing in IBA supplemented media either at 1.0/2.0 mg/L. Meanwhile, callus was completely absent under IBA omitted MS basal medium, and to great extent the 0.5 mg/L IBA supplemented medium with few excptions i.e, the cultured shoots proliferated from single node cutting of Florida Sun and Florida Prince peach cvs., whereas small quantity of callus was observed.

As for the potentiality of cultured shoots to root and remain survive in response to IBA rate, data obtained showed that both parameters were in positive relationship and followed the same trend from one hand. On the other hand the peach cultivar and origin of employed shoots each exhibited some influence. Hence, highest level of IBA (2.0 mg) was the most effective for proliferated shoots from single node cuttings of all three peach cultivars, while those induced from shoot tip the IBA at 1.0 mg was the superior for Florida Prince and Early Grand. No difference was occurred between 1.0 and 2.0 mg IBA for Florida Sun peach cv.

### V.2.3.2. Effect of NAA added to rooting media :-

Adding of NAA to rooting media was investigated at the same levels previously mentioned with IBA.

Data obtained showed that, however callusing of cultured shoots in response to NAA added followed nearly the same trend previously found with IBA, but NAA was relatively more active in this concern.

As for the survival percentage of cultured shoots and average number of developed rootlets per each as influenced by the level of NAA added to rooting media, it was quite evident that both characteristics were in closed relationship and followed the same trend. Each parameter reached its peak under culturing on the rooting medium consisting of MS basal medium (one half strength) supplemented with 3g activated charcoal + 2.0 mg NAA per one liter, regardless of either peach cultivar or explant kind from which cultured shoots were initiated.

### V.2.3.3. Effect of adding various combinations between IBA + NAA to rooting media:-

Callusing, rooting and survival % were investigated regarding their response to charcoal supplemented MS medium employed during rooting stage of cultured shoots proliferated from two explant kinds i.e, shoot tip and single node cutting of three peach cultivars.

Data obtained pointed out that callusing did not take place when shoots where cultured on auxins omitted MS

basal medium. However, callus was observed as any of (IBA + NAA) combinations was added to rooting media for shoots of both two peach explants, except those of Florida Sun cv. cultured on IBA + NAA (each at 0.5 mg/L) supplemented MS medium. Moreover, induced quantities of callus was gradually increased by applying the IBA + NAA combinations of higher added rates from each. Such increase was varied from one cultivar to another and in some cases from the explant type to another.

Referring the rooting and survival % in response to different combinations of (IBA + NAA) added to rooting media employed during rooting stage, it could be noticed obviously the great correlation between both parameters, since they showed statistically the same response. On the other hand, all rooting media supplemented with any of the IBA + NAA combination increased significantly the measurement values of both characteristics than control (charcoal supplemented MS medium-auxin free). Since, control was the inferior whereas it showed the least values of both parameters for Florida Sun and Florida Prince peach cvs. However, Early Grand cv. showed complete failure to root or survive under culturing on omitted auxin media.

Nevertheless, three studied peach cultivars varied greatly in their response to the various IBA+NAA combinations added to MS basal medium (1/2 strength and contained 3g activated charcoal). Shoots proliferated from both shoot tip and single node cutting explants collected from Florida Sun peach cv. exhibited the maximum values

of average number of developed rootlets and survival percentage by culturing on rooting medium consisting of 3 g/L charcoal supplemented MS medium plus combinations between 0.5 mg NAA and 1.0 or 2.0 mg IBA. However, both combinations were equally effective with proliferated shoots from shoot tip but the (0.5 mg NAA +1.0 mg IBA ) was more effective than that of 0.5 mg NAA + 2.0 mg IBA with shoots initiated from single node cutting. Meanwhile, the MS basal medium ( 1/2 strenghth ) supplied with charcoal + NAA + IBA at 3.0, 1.0 and 1.0 mg/L was the superior medium for rooting shoots of Florida Prince peach cv. In addition, with shoots of Early Grand peach cv., adding the NAA + IBA combination (each at the lowest level i.e, 0.5 mg/L) was statistically the most favourable treatment .

Any how, all other NAA + IBA combinations varied in their effeciency on rooting and surviving of cultured shoots from one peach cultivar to another, but all were generally less effective than the above mentioned superior oves.

Generally, it could be safely concluded from experiments conducted during rooting stage that callus was absent under culturing in auxin omitted rooting medium (MS basal medium + 3g charcoal). However, adding of IBA and NAA either solely or in combinations, especially at the higher rates (1.0 or 2.0 mg/L) stimulated callus formation with all peach cultivars with an obvious tendency to be

more pronounced with proliferated shoots from single node cuttings than those of shoot tips .

Neverthelss, the most favourable combinations of NAA + IBA required to be added to rooting media for obtaining the satisfactory results concerning survival % and rooting varied from one peach cv. to another. Since, combinations of (0.5 g NAA + 1.0/2.0 mg IBA/L), (NAA + IBA each at 1.0 mg/L) and (NAA + IBA each at 0.5 mg/L) were the superior treatments for rooting shoots of Florida Sun, Florida Prince and Early Grand peach cvs., respectively.

Florida Sun cv. surpassed the two other peach cultivars regarding the higher potentiality of its cultured shoots to root and survive, irrsespective of the origin from which they initiated. On the contrary, Early Grand showed the lowest ability, while Florida Prince cv. was in between in this respect .

## V.3. Changes in phenolic compounds contents as influenced by excising date from three peach cultivars :-

Both explant kinds ( shoot tip and single node cutting ) were periodically excised during growing season of 1993 year at one month interval ( March till September) from trees of the three peach cvs. to determine their contents of phenolic-compounds.