1-INTRODUCTION

Stone fruit (***Prunus*** **spp.**) trees have an extremely economical and nutritional importance. These plants may be grown either for their edible fruits and nuts or for their value as rootstocks and ornamental trees.

Economically the most important stone fruits are; apricots (***Prunus armeniaca***), peaches and nectarines (***Prunus persica***), European and Japanese plums (***Prunus domestica*** and ***Prunus salicina***, respectively), sweet and sour cherry (***Prunus avium*** and ***Prunus cerasus***, respectively) and almonds (***Prunus amygdalus***). Stone fruit trees can grow in different temperate and subtropical regions of the world, depending on the availability of the chilling requirements of the trees, as well as, the suitability of other factors of the environment.

According to the latest Agricultural Year Book*, the total areas under stone fruits production are; 7363 feddans for apricots, 83746 fed. for peaches, 7610 fed. for plums and 18272 fed. for almonds. Meanwhile, the total number of stone fruit transplants produced by different Egyptian nurseries during the 1993/1994 season reached 713616 transplants.

Commercial stone fruits production in Egypt is limited to areas where soils are porous and water table is deep. However, soils in these areas may suffer from lack of fertility, water stress, salinity or dominating nematodes. Therefore, finding out a suitable solution for such problems is a crucial step to reduce costs of cultural treatments, as well as, to increase both horizontal and vertical expansion of stone fruits production. One good example in this respect is the introduction of Nemaguard peach rootstock **(Brooks and Olmo, 1961).**

*Agricultural Economic Reports (1993), Ministry of Agriculture, Dokki, Giza.

Stone fruits, in Egypt, are grown either on their own roots or budded on rootstocks that are mostly propagated by seeds. But seed propagation , generally, increases genetic variability that may lead to a great diversity in vegetative growth, yield and fruit quality parameters.

Since 1980, the Ministry of Agriculture has encouraged research projects aimed to improve stone fruits production to meet the requirements of the international market standards. This trend has been recently emphasized after the GAT agreement. Several trials and experiments have been applied to introduce new rootstocks and cultivars as well as to select superior trees from local seedling trees that are free of known diseases. The success achieved, so far, in selecting superior trees of apricot as well as other stone fruits has stressed the need for the application of recent developments in clonal propagation to propagate such superior trees in large numbers, in a short time, with the maintenance of cultivar stability. Micropropagation, or *in vitro* propagation, can be utilized to achieve this goal.

Therefore, the aim of the present study is to develop the suitable *in vitro* propagation techniques for apricot, peach and almond plants. In this sphere, experiments were conducted to select the best nutritive medium, as well as, the suitable explant, additives and growth regulator types and concentrations to achieve the highest plantlets regeneration, proliferation and rooting of these stone fruit plants.

2-REVIEW OF LITERATURE

The review of literature includes the following topics:

2-1. Establishment stage :-

2-1-1. Effect of explant type and nutrient medium.

2-1-2. Effect of antioxidant treatments.

2-1-3. Effect of medium strength.

2-1-4. Effect of additives.

2-2. Proliferation stage :-

2-2-1. Effect of Cytokinin type and Cytokinin like compounds.

2-2-2. Effect of Cytokinin concentration.

2-2-3. Proliferation curve.

2-3. Rooting stage :-

2-3-1. Effect of Gibberellic acid concentration.

2-3-2. Effect of medium strength.

2-3-3. Effect of auxin type and concentration.

2-3-4. Effect of different photoperiods.

2-3-5. Effect of etiolation treatments.

2-1. Establishment stage :

2-1-1. Effect of explant type and nutrient medium.

Tabachnik and Kester (1977), Rugini and Verna (1983) and Antonelli and Chiariotti (1988) stated that, dormant buds, cotyledons and 0.4 - 0.7 mm shoot tips of almond are preferable as explants.

Also, shoot tips of plum were recommended by **Rosati *et al.*, (1980), Hammerschlage (1982b), Pietropaolo and Reisch (1984) and Turk *et al.*, (1992).**

In addition, **Hammerschlage (1981), Allam *et al.*, (1991b), Jiang *et al.*, (1993) and Pinto *et al.*, (1993)** found that, shoot tips, dormant meristem tips and ovule explants were preferred for peach culture.

Hammerschlage (1982-b), stated that, the best length of peach shoot tips used *in vitro* propagation was 0.5 - 1.0 cm.

Different explant types have been used for the establishment stage of apricot, peach and almond rootstocks and cultivars. In this respect, nodal explants were superior than shoot tips of northern Spy apple rootstock **(James, 1984).**

Jaiswal and Amin (1987) mentioned that, shoot tips and nodal explants were successfully used for the micropropagation of "Banaves local" guava cv. While, **Fitchet and Purnell (1990) and Khattak *et al.*, (1990)** have made good use of 10 - 25 mm length shoot tips.

El-Wakeel (1991) stated that, the survival percentage of either shoot tips or one node cuttings of MM-106 apple rootstock were similar.

Optimum shoot tip growth occurred on liquid Murashige and Skoog salts medium supplemented with 0.1 mg/L. Indole-3-butyric acid and 0.2 mg/L. N^{-6} benzyladenine (**Hammerschlage, 1982-a**).

Moreover, Murashige and Skoog medium was suitable for culturing shoot tips of M-16 and M-106 apple rootstocks **(Joung and Ko, 1983).**

Ochatt and Caso (1983) recommended 1.0 to 1.5 mm long shoot tips cultured on Murashige and Skoog medium.

2-1-2. Effect of antioxidant treatments :

Meyer and **Anderson (1970)** explained the effect of ascorbic acid in the medium as it may operate as a hydrogen carrier and it can be oxidized indirectly in plant tissues through a reaction with a quinone resulted from the action of polyphenol oxidase.

Murashige (1974) and El-Bahr *et al.*, (1993) reported that, ascorbic acid combined with citric acid were mainly used to retard browning of freshly excised tissues.

Anagnostakis (1974) and Bajaj and Nitsch (1975) pointed out that addition of activated charcoal at optimal concentrations i.e. 1% or 2% in the culture medium prevented the accumulation of inhibitory substances in the culture medium.

Moreover, **Preip and Englehart (1977)** found that, activated charcoal inhibited several shoots formation in azalea plants when added at the establishment stage.

Meanwhile, **Sondahi (1977)** clarified that, activated charcoal has no detectable effect on the growth or development of cultured coffee leaf explants.

Vieitez and Vieitez (1980) partially overcame the phenolic oxidation by soaking *Castanea sativa* explants in sterile distilled water for 2 - 3 hours as pretreatment.

Pierik (1987) reported that, the addition of activated charcoal to the medium at the rates of 0.2 - 3.0% w/v was effective in reducing the toxic phenolic compounds. He added that, the effect of activated charcoal resulted from the adsorption of toxic material, auxins, cytokinins, ethylene, vitamins, Fe and Zn chelates.

Miller *et al.*, (1982) suggested that, ascorbic acid was very effective in preventing the accumulation of the toxic oxidation products in peach medium.

Ziv and Halevy (1983) and Hildebrandt and Horney (1988) found that, pretreatment of sterilitizia explants with reducing agents (ascorbic acid and citric acid) minimized the potential of the explant. However, the addition of 2.5 gm/liter activated charcoal to the initiation medium favoured establishment of "Jinhue" explants and 100 mg/liter ascorbic acid + 150 mg/liter citric acid were effective with Fuji **(Wang *et al.*, 1994).**

2-1-3. Effect of medium strength :

Modified Murashige and Skoog medium was found to be the most suitable medium for shoot tip culture of almond and almond peach hybrid **(Tabachnik and Kester, 1977).**

Meanwhile, shoot regeneration was induced on a half strength MS medium **(Snir and Erez, 1980).**

Also, **Miller *et al.*, (1982)** reported that, Nemaguard peach rootstock was successfully propagated on a modified Murashige and Skoog (MS) medium.

On the other hand, **Stakanova and Abramenko (1980)** recommended modified Jones medium for apical meristem culture of M-9, M-26 and M-27 apple rootstocks.

Chiariotti and Antonelli (1988) carried out a study on nectarine, cv. Mayfair and 2 semidwarf peach selections. Shoot tips were subcultured on MS medium supplemented with BA (1.4 - 5.0 mg/liter).

Kuhne *et al.*, (1988) claimed that, one-half or one-third MS strengths were very effective with cultured shoot tips of stone fruits.

Furthermore, **Bajaj (1986)** indicated that, media with high levels of mineral salts have been used for peach shoot tips.

Shoot differentiation of sweet cherry occurred when shoot tips were cultured on half-strength MS medium with 0.5 or 1.0 mg/liter BA and 1.0 or 0.5 mg/liter IBA **(Oh, *et al.*, 1991).**

Rodriguez and Muzas (1992) suggested that, shoot tips were successfully propagated on a modified Murashige and Skoog (MS) medium.

2-1-4. Effect of additives :

The promotive effect of adenine sulphate on shoot initiation was firstly indicated by **Skoog and Tsui (1948).**

Miller and Skoog (1953) showed that, adenine sulphate reversed the anticaulogenr effect of auxin. They added that, a competition between adenine sulphate and IAA on shoot formation of tobacco stem sections were noticed.

Besides, **Dodds and Roberts (1982)** stated that, some morphogenic responses can be initiated with the addition of adenine sulphate.

Reisch (1986) reported that, the combination of adenine sulphate with BA decreased proliferation in grape as it is governed by the interaction between cytokinin and genotypes. However, the addition of adenine sulphate alone had no effect.

Bhagyalakehmi and Narendra (1988) reported that, meristems of ginger were induced to form shoots on three-quarter

strength MS medium containing 6% sucrose, 20% coconut milk, 0.5 mg/L. BAP and 0.4 mg/L. IBA.

Starrantino and Caruso (1988) found that, the highest rate of shoot multiplication for citranges and citrumelo was achieved on MS medium supplemented with 1 mg/L. BAP, IBA at 0.5 mg/L. and adenine sulphate at 40 mg/liter. However, the best multiplication for trifoliate orange occurred on MS medium supplemented with BA at 0.5 mg/liter, IBA at 0.25 mg/liter and adenine sulphate at 40 mg/liter.

2-2. Proliferation stage :

2-2-1. Effect of Cytokinin type and Cytokinin like compounds:

Barlass and Skene (1982) indicated that, supplementation of 10 μM BAP of the modified medium consisted of Knops organics and Murashige and Skoog micro-elements stimulated multiple shoot production in sour orange, Carrizo citrange and Cleopatra mandarin as compared with the same concentration of Kinetin and 2ip (N^{-6}-iso-pentyle).

On the other hand, maximum shoots proliferation of Chenin blanc (grape cv.) occurred when either BA or zeatin riboside was supplemented to the medium **(Goussard, 1982).**

Reisch (1986) reported that, 4 *Vitis* genotypes grew best *in vitro* on a medium containing 6-benzylaminopurine (BA) with a variable response concentration. However, Kinetin had no effect upon 2 interspecific hybrid cultivars but evoked a slight response with 2 genotypes.

Gray and Benton (1990) indicated that, BA at the rates of 5 - 20 μM and thidiazuron (TDZ) at the rates of 1 to 5 μM produced equal number of Carlos, Dixic and Fry grape cvs. Shoots produced on TDZ containing medium were stunted and distorted as compared to those from medium with BA. Kinetin was ineffective, being comparable to medium with no cytokinin. Therefore, BA was superior to both Kinetin and TDZ in this respect. The best response of thidiazuron (TDZ) alone or combined with BA or Kinetin proved to be cultivar dependent. TDZ alone was adequate for inducing shoot proliferation. However, the addition of BA or Kinetin into TDZ containing medium resulted in an increased

number of nodes. In addition, wide differences were observed. Time of exposure on media containing TDZ was critical for subsequent shoot proliferation **(Sudarsono and Goldy, 1991).**

Gray and Benton (1991) found that, the highest average number of shoots/cultured apex (3.4 - 3.8) occurred when 5, 10 and 20 μM BA (Benzyladenine) or 5 μM TDZ (thidiazuron) were added to the nutrient medium.

Huetteman and Preece (1993) revealed that, thidiazuron (TDZ) was the most active cytokinin for *in vitro* manipulation of many woody species. Low concentrations (< 1μM) induced greater axillary proliferation than many other cytokinins.

2-2-2. Effect of Cytokinin concentration :

In a study on micropropagation of almond and almond peach hybrid, **Tabachnik and Kester (1977)** pointed out that, 0.1 mg/liter BA induced shoot elongation, while, 1.0 mg/liter enhanced lateral shoot proliferation.

Hammerschlage (1980) reported that, optimum growth of peach rootstock, with minimum nicroses and callus formation occurred on MS medium supplemented with 0.2 mg/liter benzyladenine (BA) and 0.01 to 1.0 mg/liter (IBA).

Japanese plum proliferated at the rate of 10 : 1 to 20 : 1 per month when grown on a modified Murashige and Skoog medium supplemented with 1.0 mg/liter 6-benzylaminopurine (BAP), 0.1 mg/liter gibberellic acid (GA_3) and 0.1 mg/liter indolebutyric acid (IBA) **(Rosati *et al.*, 1980).**

Singha (1980) reported that, 2 mg/liter BA induced the highest proliferation of pear (*Pyrus communis* L. cv. Sekel).

Wood (1982) indicated that, the combination of 4 mg/liter BA and 1 mg/liter IBA was the most effective as compared to the other combinations of isopentenyl adenine and either of IBA or NAA for pecan.

Lineberger (1983) found that, the addition of 1.0 mg/liter BAP to the Murashige and Skoog medium supplemented with 0.1 mg/liter NAA resulted in 10 fold increase in Prunes X Hally cherry plantlets.

Reeves *et al.*, (1983) showed that, overall growth of Nemaguard peach rootstock was better on MS medium supplemented with 1.0 mg/liter (BA) and 0.01 mg/liter (IBA).

Driver and Kunijuki (1984) found that, the combination of 4.5 μM BA and 5 μM IBA to DKW medium resulted in development of optimum multiple shoots of walnut plants.

Moreover, "Stanley" plum multiplied 10 times per month when cultured on Murashige and Skoog medium supplemented with 1.1 mg/liter BA. **(Pietropaolo and Reisch, 1984).**

Furthermore, Murashige and Skoog medium supplemented with 5 μM zeatin enhanced shoot multiplication of carob **(Sabastian and McComb, 1986).**

Besides, **Hammerschlage *et al.*, (1987)** reported that, using 8.8 μM BAP induced the highest proliferation of "Nemaguard" peach rootstock and 8 scion peach cultivars.

Proliferation of Japanese cherry was achieved on solid Gamborg medium supplemented with 1 mg/liter BA **(Katano, 1987).**

Chiariotti and Antonelli (1988) carried out a study on nectarine, cv. Mayfair and 2 semidwarf peach rootstocks. They found that, 5.0 mg/liter BAP induced vitrification with repeated subcultures for obtaining a higher proliferation rate. Thus, they recommended the use of lower concentrations of BA (1.4 - 2.8 mg/liter).

Gunidy (1990) reported that, the best shoot multiplication of Nemaguard and *Prunus davidiana* was achieved on Murashige and Skoog (MS) medium supplemented with 1 mg/liter BA and 0.02 - 0.2 mg/liter (IBA).

In addition, **Allam and *et al.*, (1991b)** mentioned that, the highest proliferation of "Nemaguard" peach rootstock occurred on MS medium supplemented with a combination of 5 mg/liter BAP + 3 mg/liter IBA as well as 3.0 mg/liter charcoal.

Huetteman and Preece (1993) reported that, lower concentrations of thidiazuron (< 1 μM) induced greater number of

axillary proliferation. However, higher concentrations (> 1 μM) stimulated callus formation.

2-2-3. Proliferation curve :

Skirvin and Chu (1977) suggested that, shoot tips of peach cv. Redhaven cultured on modified Murashige and Skoog medium, resulted in the development of new axillary shoots within 6 weeks, which produced more shoots when transplanted on a fresh medium.

In addition, 10 fold multiplication rate of Myrobalan plum was achieved every 4 - 6 weeks when shoots were transferred to multiplication medium consisting of modified Murashige and Skoog Salts (MS) medium supplemented with 0.01 mg/liter (IBA), 1.0 mg/liter BA and 0.6% agar. **(Hammerschlage, 1982a).**

Shoot multiplication rates of six almond cultivars were obtained with twenty days period interval of subculture that continued for at least twenty-four months, on Murashige and Skoog (MS) medium with 0.9% agar, 0.7 mg/liter benzylaminopurine (BAP) and 0.01 mg/liter Naphthalene acetic acid (NAA). **(Rugini and Verna, 1983).**

The rate of shoot proliferation was slow during culture establishment for the first eight weeks for all levels, but increased rapidly thereafter. Relatively rapid increase of shoots were obtained on 5, 10, 20 or 40 μM BA treatments after 12 weeks, the cultures with two higher BA concentrations had dense, unexpanded shoots with high mortality. After sixteen weeks, the greatest number of total shoots was obtained with 10 μM BA. **(Lee and Wetzstein, 1990).**

Rama and Pontikis (1990) reported that, shoot proliferation of olive was obtained on MS modified medium supplemented with 10 mg/liter zeatin and at 7.5 mg/liter BA in 12 and 6 weeks, respectively.

2-3. Rooting stage :

2-3-1. Effect of medium strength :

Verner and Boe (1980) recommended one-third strength of Murashige and Skoog (MS) medium for rooting of M-7 apple rootstock.

However, **Barghehi and Alderson (1983)** stated that, one-half macro-nutrient strength of Murashige and Skoog (MS) medium was suitable for rooting of *Pistacia vera* plants.

In addition, **Reeves *et al.*, (1983)** indicated that, modified half-strength MS medium was optimum for rooting of *Prunus institia* L.

Besides, the best rooting occurred in plum when one-half strength of Murashige and Skoog (MS) medium was used **(Vysnotskii and Olesko, 1988).**

Furthermore, **Manta *et al.*, (1989)** pointed out that, rooting of peach and plum were superior when semi-solid half-strength Murashige and Skoog (MS) medium supplemented with 2.5 - 5.0 p.p.m. IBA was used.

Meanwhile, **Ognjonov and Vujanic (1989)** reported that, one-half strength Murashige and Skoog (MS) medium enhanced the highest rooting of peach.

In contrast, **Junior and Peters (1991)** mentioned that, using one-third concentration of mineral salts of Murashige and Skoog medium encouraged rooting of Santa Rosa plum.

2-3-2. Effect of Gibberellic acid concentration :

Reeves *et al.*, (1985) found that, culturing of peach and plum rootstocks was better on Murashige and Skoog (MS) medium supplemented with 12.5 mg/liter GA_3 in producing elongated shoots suitable for rooting.

However, **Turk *et al.*, (1992)** reported that, the addition of 0.1 and 0.5 mg/liter gibberellic acid to the medium have no effect on shoot proliferation or elongation of plum ectotype (*Prunus domestica* L.).

2-3-3. Effect of auxin type and concentration :

Bonner and Galston (1959) stated that, NAA is more suitable than IAA since it is not attacked by IAA oxidase in the plant, and it is readily and cheaply synthesized.

It has been suggested that, IBA has a higher resistance to oxidation because of the side chain length and therefore, persists at the site of induction longer than IAA. **(Fawcett *et al.*, 1969)**

A study carried out by **Hackett (1960)** involved the comparison between NAA, IAA and IBA growth regulators and their effect on root development. He found that, NAA is more active than IAA in promoting root development, while, IBA was in between for its intermediate susceptibility to various kinds of oxidative destruction.

James and Thurboon (1979) clarified that, rooting was greatly affected by pre-rooting culturing of M-9 apple rootstock on Linsmaier and Skoog (LS) medium containing hormones.

Conjugate formation after the application of IBA is possibly also important in rooting because conjugation can serve as a mechanism to protect auxins from oxidation and allow for the slow release of free auxin **(Cohen and Bandurskirs, 1982).**

The formation of adventitious roots depends upon numerous endogenous factors, among which growth substances such as auxin and polyamines that are believed to play an important role **(Altman, 1989 and Batten and Goodwin, 1978).** Auxins appear to be the primary phytohormones involved in this process since the application of synthetic auxins alone can stimulate root initiation.

In some cases, an early increase in IAA levels was followed by a decline that was observed during the rooting process **(Blakesley *et al.*, 1991).** In other cases, no increase in IAA concentration was found, but instead only a gradual decline was measured. **(Berthon *et al.*, 1989; Blakesley *et al.*, 1991)**

Culture of *Prunus cistena* did not root well when agar was present in the medium, while the reverse was true in liquid medium with filter paper as an explant support **(Lane, 1979).**

Best rooting results of Japanese plum were obtained on modified MS medium supplemented with 2 or 4 mg/liter IBA. **(Rosati *et al.*, 1980)**

In addition, Murashige and Skoog medium with or without IBA were selected for rooting of apple rootstocks. **(Snir and Erez, 1980).**

Moreover, **Hammerschlage (1982b)** reported that, culturing of Myrobalan plum shoots on MS medium supplemented with 2.5 - 5.0 mg/liter NAA for 4 weeks then incubated under dark conditions for 2 weeks resulted in a complete rooting (100%).

" Nemaguard" peach rootstock achieved 95% rooting when cultured on MS medium supplemented with 50 mg/liter ascorbic acid and 0.1 mg/liter NAA **(Miller *et al.*, 1982).**

Skirvin *et al.*, (1982) found that, within 3-5 weeks of transferring single shoots from a shoot proliferation medium onto a new rooting medium (MS-H) 71 out of 144 Harbrite peach shoots, 7 of 9 Stanley plum and 2 of 51 Montmorency cherry shoots developed multiple roots. The MS-H medium is composed of Murashige and Skoog high mineral salts with : (amounts in mg/L.) 200 myoinositol, 1000 casein hydrolysate, 2 glycine, 0.05 biotin, 2 thiamin-HCl, 1 chorine chloride, 2.5 nicotinic acid, 0.25 pyridoxine-HCl, 1 P-amino-benzoic acid, 0.25 folic acid, 0.5 pantothenic acid, 3 IBA, 1 NAA, 0.1 GA_3, 0.04 Kinetin, 0.01 BA and 20 g sucrose and 6.0 g agar.

Cultured shoots of *Pistacia vera* L. rooted well by using half-strength macro-nutrient Murashige and Skoog medium containing IBA for rooting initiation followed by subculture on hormone-free medium for root development. **(Barghehi and Alderson, 1983).**

Sufficient rooting response of "Stanley" plum occurred on MS inorganic salts with : (mg/liter) thiamine-HCl (1.0) nicotinic acid (1.0), pyridoxine-HCl (1.0), myoinositol (100), sucrose (15.000), agar (7.000) and 0.5 - 2.5 IBA for five weeks or 2.0 - 6.1 mg/liter IBA for three weeks **(Pietropaolo and Reisch, 1984).**

Cultured shoot tips of "Hally-Jolivette" cherry exposed to different levels of NAA and BA (0.0, 0.1, 2.5 and 5.0 mg/liter of each in all combinations) formed roots, shoots, or basal callus

depending on the concentration of added growth regulators **(Lineberger, 1983).**

Hammerschlage *et al.*, (1987) working with 8 peach Scion cultivars and one rootstock "Nemaguard" exposed all cultivars to half-strength MS medium supplemented with 28.5 M of either IAA, IBA or NAA. NAA was the best one in achieving rooting.

Wanas (1987) stated that, the addition of 10μM (NAA) to Murashige and Skoog medium enhanced 100% rooting as compared with 10 μM of IBA which encouraged only 82% rooting of pear plants.

On the other hand, rooting was induced when Murashige and Skoog (MS) medium supplemented with IBA at 1.0 to 1.5 mg/liter were used depending on the cultivar of peach **(Paoli and Depaoli, 1986-1988).**

Gunidy (1990) compared the effect of either NAA or IBA at the rates of 0.1 to 20 mg/liter on three peach rootstocks. He found that, NAA was better than IBA in encouraging rooting specially with Okinawa and Nemaguard rootstocks. Rooting was increased by increasing the concentration of any of the auxins used in his study. Moreover, rooting was induced by subculturing plantlets on Murashige medium containing 1 mg/liter BAP and 5 mg/liter IBA for "Nemaguard" peach rootstock. **(Allam and El-Rayes, 1991a).**

Junior and Peters (1991) revealed that, rooting of Santa Rosa plum was improved and reached 91.11, 83.88 and 80.55% when IBA was used at the rates of 0.2, 0.5 and 0.8 mg/liter, respectively.

Root formation of sweet cherry took place when rooting medium was supplemented by 0.5 mg/liter IBA and 0.1 or 0.5 mg/liter BA **(Oh *et al.*, 1991).**

Moreover, successful rooting of pear was achieved using 1 mg/liter IBA. **(Moretti *et al.*, 1992).**

Also, the best rooting for apple rootstock M-26 was obtained with the use of 0.2 mg/liter IBA under dark conditions during the

induction phase. However, higher concentrations of IBA up to 2 mg/liter encouraged callus production and inhibited root formation **(Welander, 1991).**

Also, adding 1 µM putrescince to the rooting medium increased rooting of apple and basal olive explants. However, harmful effect on rooting was noticed in Walnut **(Rugini and Verna, 1983).**

2-3-4. Effect of different photoperiods :

Hammerschlage (1982a) showed that, the combination of 2.5 to 5.0 mg/liter IAA and the dark conditions for 2 weeks increased rooting of Myrobalan plum up to 100% in comparison with that occurred under light conditions (1.0 Klux, 16 hours photoperiods) with addition of IAA.

Reeves *et al.*, (1985) mentioned that, the combination of 4 mg/liter Indole-3-butyric acid (IBA) and dark conditions improved rooting of peach and plum rootstocks as compared with the combination of 4 mg/liter IBA and 16 hours photoperiod which inhibited root formation.

Hammerschlage *et al.*, (1987) found that, prior to rooting shoots were transferred to one-half strength MS medium and incubated in the dark at 4°C for 35 - 40 days. Rooting medium consisted of one-half strength MS medium supplemented with either IAA, IBA or NAA with various concentrations. All shoots were incubated on rooting medium in the dark at 26°C for two weeks and then transferred to one-half strength MS medium and incubated for two weeks at 26°C with 16 hours photoperiod, about 98%, 78%, 90% of Nemaguard shoots were rooted in medium containing 28.5 µM of IAA, IBA and NAA. respectively.

In addition, a period of darkness promoted rooting of stone fruits. Further, root growth was promoted by 16 hours light/8 hours dark cycle. **(Kuhne *et al.*, 1988).**

Morini *et al.*, (1990) studied the effect of different photo-period cycles on rooting of peach. They found that, fresh weight, dry weight of shoot clusters, number of shoots, leaf area, and root length were considerably greater with a treatment of 4 cycles/24 hours of 4 hours of light and two hours of dark than with the regular photoperiod treatment of 16 hours of light and 8 hours dark.

Moreover, in a study involving the exposure to 1000, 2000 or 4000 Lux emitted by fluorescent lamps (Gro-Lux) or lamps providing cool white light or mixture of both. Showed that, light treatments had little effect on rooting **(Junior and Peters, 1991).**

Morini *et al.*, (1991) studied the effect of different photoperiod cycles on rooting of plum. They were cultured under 4 different light - dark regimes : 16 h light - 8 h dark (16L : 8D) : 8 h light - 4 h dark (8L : 4D) : 4 h light - 2 h dark (4L : 2D) and 2 h light - 1 h dark (2L : 1D). They found that, 16 hours light and 8 hours dark encouraged longer shoots with low number of plants, while *vice versa* occurred when 4 hours light and 2 hours dark photoperiod was used.

Prunus dulcis cv. Tuono embryos with shoot axis and root primordia showed higher response in light than those in dark condition **(Antonelli and Chiariotti, 1992).**

Morini *et al.*, (1993) studied the effect of different photoperiod cycles on rooting of plum and peach rootstocks. They found that, 4 hours of light followed by 2 hours of darkness increased shoot culture fresh weight and dry weight and the number of new formed shoots as compared with the exposure of 16 hours of light and 8 hours of darkness.

Moreover, micro cuttings of two almond (*Prunus dulcis*) genotypes (cv. Supernova and rootstock Sel-M-51) cultured on Bourgin and Nitsch medium supplemented with 10 µM and subjected to a dark treatment for twelve days, produced roots. Sel-M-51 rooted in all treatments and showed the highest rooting

percentage (95%) with IAA plus light, whilst, Supernova rooted only with IAA or IBA plus dark (37 and 56%, respectively) **(Caboni and Damiano, 1994).**

2-3-5. Effect of etiolation treatments :

Rosati *et al.*, (1980) studied the effect of active charcoal on rooting of apple rootstocks Malling Merton (MM) 104, MM-106 and 109 (***Malus* sp.).** They found that, activated charcoal at 0.25% improved root development.

Boxus (1981) found that, rooting occurs on the medium supplemented with 0.05 activated charcoal and the buds developed into rooted plantlets in 4 -5 weeks.

Hammerschlage (1982-b) pointed out that, complete rooting (100%) of Myrobalan plum shoots occurred after 4 weeks in Murashige and Skoog medium supplemented with 2.5 - 5.0 mg/liter NAA and incubated in the dark for two weeks.

In addition, activated charcoal at the rate of 1g/liter enhanced rooting percentage and plantlets growth of guava plants **(Amin and Jaiswal, 1987).**

On the other hand, **Antonelli and Chiariotti (1988)** found that, using activated charcoal with Indole-3-butyric acid at the rate of 0.5 to 2.5 in the rooting media, caused inhibition of rooting in all cases of two peach genotypes, nectarine cultivar Mayfair and Semidwarf peach.

In addition, a period of darkness promoted rooting of stone fruits. Darkness for about one week, for at least, the base of the shoot is recommended for rooting. **(Kuhne *et al.*, 1988).**