INTRODUCTION.

1- INTRODUCTION

The almond (*Prunus dulcis* [Mill] D.A. Webb, Syn. *Prunus amygdalus*, Batch), has long been recognized in the front rank among edible nuts. It's kernel contains about 55.0% lipids, 21.0% proteins 17.3% carbohydrates. It is also a good source of vitamins specially niacin, riboflavin and thiamine **(Chandler, 1958).**

The almond tree belongs to the family *Rosaceae*. It apparently originated in south-eastern Asia, although both bitter and sweet almond have been cultivated in the Mediterranean region since before recorded history.

According to the latest statistics (1993) of the Ministry of Agriculture, Egypt, the total area planted with almond trees was 17105 Feddan. However, bearing trees cover only 13625 Fed., producing about 13774 tons of nuts per year. The largest areas of almond production are located in north Sinai (12768 Fed.) and Nubaria (3480 fed.) districts.

Almond trees, in Egypt, are mostly budded on bitter almond rootstock which requires deep and well-drained soils. Accordingly, almond production in Egypt is limited to areas where the soil is porous and the water table is deep. However, most of these soils are marginal because they lack fertility, and sometimes poor in quality. Furthermore, bitter almond roots are susceptible to nematode infection specially in well-drained soils.

In order to meet the high demand for almonds in Egyptian market, planting of almond trees need to be expanded to other areas of more fertile irrigated soils. Such development requires the selection of new rootstocks for almond that are adapted to different soil types and conditions.

Accordingly, this study was carried out to investigate the feasibility of using rootstocks other than bitter almond that are more adapted to the different soil types and conditions prevailing in lower Egypt. The research work included the following studies.

1- Clonal propagation trials of bitter almond, Okenawa peach and Marianna 2624 plum by cuttings to produce uniform rootstock nurslings to be budded by the almond scion.

2- A study on the performance of almond scion on the above mentioned three rootstocks.

3- A study on the application of tissue culture techniques for the micropropagation of the same rootstocks under study.

2- REVIEW OF LITERATURE

For the purpose of clarity, literature concerning the studies on propagation by cuttings, budding and micropropagation were grouped under the following topics:-

2- 1-Rooting of cuttings:

- 2- 1-1- Effect of species and cultivars.
- 2- 1-2- Effect of wounding.
- 2- 1-3- Effect of different concentrations of growth substances; IBA, NAA and pp_{333}.
- 2- 1-4- Anatomy of adventitious roots origin.

2- 2- Budding:

- 2- 2-1- Morphological studies:
  - 2- 2-1-1- Take and survival of scion.
  - 2- 2-1-2- Growth vigour.
  - 2- 2-1-3- Root distribution.
- 2- 2-2- Effect of different rootstocks on leaf elements content.
- 2- 2-3- Anatomy of budunion zone.

2- 3-Micro propagation studies:

- 2- 3-1- Type of explant.
- 2- 3-2- Time of collecting explants.
- 2- 3-3- Micropropagation stages:
  - 2- 3-3-1- Stage I : Establishment.
  - 2- 3-3-2- Stage II : Proliferation.
  - 2- 3-3-3- Stage III : Rooting.

2- 1- Rooting of Cuttings:

2- 1-1- Effect of Species and Cultivars:

Different genera vary in the rooting ability of their cuttings. Thus, while cuttings of grapes, figs and pomegranates are easy-to-root, those of other species as almond, pecan, mangoes, pears and peaches are difficult-to-root **(Chandler, 1958).**

Species belonging to the same genus *Prunus* such as plum, cherries and apricot differ greatly in the rooting ability of their cuttings. Moreover, the ability of cuttings to root vary greatly within different plum species. In this respect, Marianna plum cuttings are easy-to-root while those of European plum and Japanese plum are difficult-to-root **(Chandler, 1958).** Meanwhile, different varieties under the same specie may vary greatly in their rooting ability as reported by many authors **(Chauhan** and **Maheshwari, 1970, Nicotra** and **Demiano 1975; Hanson, 1978, Jawanda *et al.*, 1979, Robitaille** and **Yu, 1979, Erez** and **Yablowitz, 1981).**

2- 1-2- Effect of Wounding:

The usual procedure for preparing hardwood cuttings, is by making a horizontal cut at the base and oblique cut at the top. Nevertheless, some reports had pointed out that better rooting was obtained when cuttings were prepared with an oblique cut at the base **(Gemma *et al.*, 1982).** Furthermore, root production on stem cuttings in some different plant species my be promoted by

wounding the base of the cuttings. A vertical cut down on each side of the cutting for an inch or two penetrating through the bark and the wood my be enough. Wounded cells or adjacent ones near the base of the cutting stimulated cell division and initiation of root primordia. This is due, perhaps, to a natural accumulation of hormones and carbohydrates in the wounded area as well as to an increase in respiration. Moreover, stem tissues of some species, had a schlerenchymatic ring of tough fiber cells in the cortex external to the point of origin of adventitious roots which had difficulty to penetrate this band of cells. A shallow wound would cut through these cells and permit outward penetration of the developing roots more readily (**Hartmann** and **Kester, 1975).**

Wounding responses in hardwood cuttings of M-26 were studied to provide more information on tissues involved in rooting. Cuttings of different rooting ability were prepared by preparing them from stool shoots with already rooted portion removed or from a hedge in both cases either cut at a basal node or internode. Wounds of 2 cm. long were made at the base of cuttings. Two incisions were compared with a shallow or deep slice, the latter penetrating the xylem for most of the wounded area. Wounding increased rooting slightly and almost all cuttings had roots associated with wounds. Rooting after wounding was almost identical in both types of cuttings, with the deep wound being less effective than the other two methods **(Howard *et al.*, 1979).**

In other crops such as Myrobalan plum, splitting the base of cuttings longitudinally caused more callus formation and hence more rooting **(Howard *et al.*, 1983).**

Basal wounding of peach cuttings improved rooting (**Couvillon** and **Erez, 1980, Sen** and **Couvillon, 1983).**

On the other hand, best rooting of *Pyrus communis* cuttings were obtained when the cuttings were splitted at the base **(Ibrahim *et al.*, 1976).**

Ringing of peach cuttings improved the rooting ability and increased number of roots per cutting **(Yadava *et al.*, 1974).**

Besides, hardwood cuttings of Balady apple from mature shoots failed to initiate any adventitious roots even when treated with different root inducers. Cuttings prepared from juvenile shoots struck adventitious roots and survived successfully. Terminal juvenile cuttings treated with wounding and soaking in 300 ppm IBA, resulted in the highest percentage of success **(El-Tomi *et al.*, 1974).**

Moreover, M-27 stool shoots wounded at their base and treated with 2500 ppm IBA and 2 weeks at 21° C bottom heat gave 94% establishment against 38% without wounding treatment. Preliminary anatomical work suggested that wounding improved the lateral penetration of IBA and caused distraction of cortical tissue **Howard** and **Pontikis (1977)** and **Mackenzie (1978).**

Incision wounding of M-26 cuttings, which penetrated the sclerenchyma, enhanced rooting, particularly in non-basal (internode) cuttings where fiber was relatively continuous. Both terminal and basal cuttings prepared in December benefited from wounding, but in February the generally high rooting level in basal

2- 1-3-1- Effect of Indole Butyric Acid (IBA) on Rooting of Cuttings:

Hardwood cuttings with 4 - 6 buds of *Pyrus communis*, L. were prepared in January or February from root suckers and treated with IBA at 0 - 200 ppm. The highest rooting percentage was obtained for cuttings prepared in February and treated with 200 ppm IBA after splitting the base and planting in a glass house **(Ibrahim *et al.*, 1976).** Moreover, 200 ppm IBA caused disappearance of the endogenous root inhibiting activity in the basal segments of apple cuttings prepared from October to February. In this concern, cuttings prepared in February required 400 ppm IBA **(Shirzad** and **Miles, 1978).**

On the other hand, cuttings of sand pear (*Pyrus pyrifolia*) required 1000 - 2000 ppm IBA for good rooting **(Kahlon** and **Sukhdev, 1981, Pandey** and **Pathak, 1981).**

Hardwood cuttings of almond X peach hybrid gave the best rooting when treated with 1000 ppm IBA **(Kaundal** and **Bindra, 1984).**

Hardwood and semi-hardwood peach cuttings treated with 500 - 4000 ppm IBA gave best rooting only at 1000- 1500 ppm concentrations. **(Hartmann** and **Hansen, 1968; Daud** and **Carlson, 1972, Zyl** and **Carreiro, 1977; Erez** and **Yablowitz, 1981.** and **Erez, 1984).** Similarly, plum cuttings, responded well to the concentrations used for peaches of 1000 - 1500 ppm IBA **(Hartmann** and **Hansen, 1968;** and **Nicotra** and **Damiano, 1975).**

Softwood, hardwood and root cuttings of 6 clones of *Pyrus caucasiea* prepared in late October and stored at 5 - 8° C till early March were treated with 1500 - 3000 ppm NAA or 2500 - 5000 ppm IBA for 5 seconds. Hardwood cuttings of some clones only responded well to auxin treatments mainly 3000 ppm NAA **(Znajdek *et al.*, 1987).**

Hardwood cuttings of peach cv. Golden Queen were prepared during the winter at fortnightly intervals. Rooting development was measured after the cuttings had been dipped for 10 seconds in 1000 ppm IBA dissolved in 50% ethyl alcohol and treated for 6 weeks with bottom heat at 23° C. The percentage of satisfactorily rooted cuttings was highest in July (85%) **(Issel** and **Chalmers, 1979).**

Wounding and the application of IBA and basal heat to unrooted shoot from stools of M-27 apple rootstock greatly improved their establishment when planted back into the nursery for a second season **(Pontikis *et al.*, 1979).**

Clones of peach (*Prunus persica* L. Batsch) were propagated from sprouted nodal cuttings. Rooting was greatest under mist when the basal hardwood tissue was dipped for 10 seconds in 100 to 500 ppm of indolebutyric acid (IBA) before insertion into a mixture of equal parts of sphagnum peat moss and perlite over underlying gravel **(Robitaille** and **Yu, 1980).**

Furthermore, semi hardwood cuttings of 82 genotypes of peach (*Prunus persica* L. Batsch) and complex hybrids of peach with almond (*P. amygdalus* Batsch), *P. davidiana, P. kansuensis* and *P.*

persica vulgarise siberica were rooted under mist. Average rooting percentages ranged from 7% to 100%. Peach selections generally rooted well with the exception of hardy rootstock selection *P. davidiana* it rooted poorly but most of its hybrid progeny rooted well *P. kansuensis* and its hybrid progeny rooted well. Peach x almond F_1 hybrids generally rooted poorly, the rooting percentage was higher in backcrosses to peach **(Mehlenbacher, 1986).**

More than 400 genotypes of *Prunus* were evaluated in field for rooting and survival of fall-planted hardwood cuttings treated with 2000 ppm IBA. Cultivars of European and Japanese plums originating from species and inter specific hybrid of the section (sect. *Prunus*) had the highest survival. Cuttings from cultivars of sand cherry (sect. *Microcerasus*) and peach (sect. *Euamygdalus*) averaged 28% to 54% lower survival than European and Japanese plum. Few cultivars of almonds (sect. *Euamygdalus*) apricot (sect. *Armeniaca*) and American plums (sect. *Prunocerasus*) rooted from hardwood cuttings **(Reighard *et al.*, 1990).**

In trials with sour cherry, Zhagarskaya cv. softwood cuttings 10 - 12 cm. long with 2 - 3 fully developed leaves were prepared during 10 and 20 June. The basal part of each cutting was steeped for 5, 10 or 15 hours in a solution of TA - 12 (derivative of alpha NAA) at 10^{-3} M, IBA at 10^{-3}M or IBA at 2.5×10^{-3} M, and control cuttings in water. The cuttings were rooted in the green house in a 1 : 2 sand: peat substrate under mist. Duration of treatment had a marked effect on rooting, which was highest for all auxin treatments when applied for 15h (52.2 - 58.9 % rooting as compared with 8.9 % for the control) **(Prizhmontas, 1991).**

Dormant cuttings of 4 wild rootstocks (wild peach, wild apricot, Behmi [peach x almond] and bitter almond), 5 Clonal plum rootstocks (Myrobalan B, Brompton, pershore, Damas C and St. Julien A), plum Santa Rosa cv., apricot Newcastle cv., peach July Elberta and almond Nonpareil cv. and one [almond x peach] hybrid (GF-677) were prepared during the last week of February. Cuttings were dipped in IBA solution (50% ethanol) at 2000 or 6000 ppm for 10 seconds followed by dipping in 0.3% Captan fungicide solution, before being inserted in polyethylene bags filled with sand media and kept in partial shade. Rooting parameters were measured 8 months after planting. Highest percentage of rooting parameters was obtained with Myrobalan, closely followed by Santa Rosa rootstocks treated with 2000 ppm IBA. (mean rooting percentage was 31.11%). New castle Nonpareil, wild apricot, Behmi and bitter almond cuttings failed to root under any of the used treatments **(Rana** and **Chadha, 1992).**

Furthermore, peach hardwood (Jan.) and leafy (June and Sep.) cuttings 30 and 20 cm. long, respectively, were treated with 2000 ppm IBA. Hardwood cuttings were placed outside with bottom heat, while leafy cuttings were planted in greenhouse under mist. Rooting percentages, number of roots / cutting and survival percentages were studied. Only IBA-treated-cuttings rooted (with average of 40%). The average number of roots ranged from 4.2 in hardwood cuttings to 5.6 in leafy ones while the survival of rooted cuttings ranged from 1.8% in hardwood to 3.5% in leafy cuttings **(Fiorino** and **Mattii, 1992).**

In addition, hardwood cuttings were taken from trees of apricot cv. Amar. The basal end was dipped in 0 or 4000 ppm IBA for 10 seconds before inserting in a mixture of peat and sand under mist for rooting. Cuttings were sampled at weekly intervals to examine their anatomy in relation to rooting. IBA treatment promoted the initiation of adventitious roots from cambium, xylem and pith **(Salama, *et al.*, 1993).**

Stem cuttings of the current year's growth were taken from trees of apricot cv. Amar at monthly intervals from April to December. The base of each cutting was dipped in 0 or 4000 ppm IBA for 10 seconds before inserting in a mixture of peat and sand under mist for rooting. The highest percentage of rooting was obtained with cuttings taken in August and treated with IBA. Untreated cuttings failed to form any roots and/or callus as did cuttings taken from April to June **(Hassan *et al.*, 1993).**

Cuttings treated with IBA gave percentages of rooting ranging from 87 to 100%, respectively, for Red Beauty plum, cerasus tomentosa [*Prunus tomentosa*], pomegranate and *Furtunella*. On the other hand, percentage of rooting was 8% for peach, pear and apple **(Hu *et al.*, 1993).**

Furthermore, results of many investigations indicated that IBA increased rooting percentage, number of roots / cutting, length and fresh weight of roots. The highest results were recorded in October and November while the lowest were obtained in January and February. However, 4000 ppm IBA did not significantly affect rooting and inhibited root growth of Nemaguard peach rootstock

[(**Edriss *et al.*, 1993**); (**Abd Al-Hammed *et al.*, 1993**) and (**Edriss** and **Burger, 1993**)].

2-1-3-2- Effect of Naphthalene Acetic Acid (NAA) on Rooting of Cuttings:

NAA was noted to be less effective in inducing rooting of cuttings in apples, pears, peaches and plums than IBA (**Chauhan** and **Pundir, 1972**).

Softwood, hardwood and root cuttings of 6 clones of *Pyrus caucasiea* prepared in late October and stored at 5 - 8° C till early March were treated with 1500 - 3000 ppm NAA for 5 second. Hardwood cuttings of some clones only responded well to auxin treatments mainly 3000 ppm NAA (**Znajdek *et al.*, 1978**).

Eureka lemon, Baramasi and Kagzi-Kalan cuttings were treated with 2000 ppm, 3000 ppm and 4000 ppm NAA. The best rooting and survival were noticed for cuttings without leaves, and treated with NAA at 2000, 3000 or 4000 ppm for Baramasi, Kagzi-Kalan and Eureka, respectively (**Arora** and **Yamdagni, 1985**).

Softwood or semi-hard-wood guava cuttings with (a) 2 nodes + 2 leaves (b) 2 nodes + 4 leaves or (c) 3 nodes + 4 leaves, were taken from trees of 1 - 3 cultivar after fruit harvest. The cuttings, were treated with 2000 ppm NAA, then struck in vermiculite under partial shade. Softwood cuttings generally rooted better than semi-hardwood cuttings, particularly when treated with treatment (b), as it gave the highest rooting percentage (70) (**Pereira *et al.*, 1986**).

Sweet lime cuttings were taken in July and treated with NAA at 1500 - 6000 ppm and planted in pots filled with sand. Rooting

and subsequent sprouting and growth of plants was best with 1500 ppm NAA treatment **(Zora Singh** and **Sandhu, 1987).**

Semi-hardwood rough lemon cuttings were taken from 4-year-old trees at 15 - 20 cm. long in September and dipped in solutions of NAA at 50 - 200 ppm. Rooting percentage for 50 ppm NAA was 27.5 while rooting in the control cuttings was nil **(Zora Singh** and **Sandhu, 1987).**

Sprouted nodal peach softwood cuttings taken in May and dipped their basal cut ends in solution of 1500 ppm NAA gave the highest rooting percentage, but most roots (measured as fresh weight of roots per cutting) were obtained with 500 ppm NAA. Semi-hardwood cuttings treated with 1500 ppm NAA gave the highest rooting percentage **(Debnath *et al.*, 1988).**

In addition, treating cassanese cuttings taken in spring and autumn with 1000 and 2000 ppm NAA greatly improved rooting percentage **(Briccoli-Bati, 1991).**

2- 1-3-3- Effect of Paclobutrazol on Rooting of Cuttings:

Paclobutrazol at relatively low concentrations (3 to 6 mg/litre) increased the number of roots on hardwood cuttings of creeping Charlie (*Plectranthus australlis* R. Br.) and common bean (*Phaseolus vulgaris* L.) by about 100% and 85%, respectively. Paclobutrazol did not affect root length but reduced shoot length by about 20% as compared to the control **(Davis *et al.*, 1985).**

Furthermore, the difficult-to-root mature phase petiole cuttings of English ivy failed to root regardless of treatment. Pre-

treatment with paclobutrazol significantly promoted rooting in Juvenile stem cuttings However, the opposite was true concerning immature stem cuttings **(Geneve, 1990).**

Besides, paclobutrazol treatment enhanced the rooting of softwood peach cuttings (*Prunus persica* L. Batch). The survival rates of rooted cuttings obtained from paclobutrazol-treated plants were significantly higher than those obtained from untreated plants **(Wiesman *et al.*, 1989).**

In addition, paclobutrazol (PBZ) was supplied in nutrient solution culture to Nemaguard peach rootstock (*Prunus persica* x *P. davidiana*) at the concentrations of 0, 0.001, 0.1 mg-litre. PBZ increased root : shoot ratio and decreased root length by 5 fold over the range of PBZ concentrations tested. Root tip diameter, stele diameter and width of root cortex were not significantly affected by PBZ **(Rieger** and **Scalabrelli, 1990).**

2- 1-4- Anatomy of Adventitious Roots Origin:

Adventitious roots are widely distributed in all vascular plants and are originated from different plant tissues. The origin of roots in stem cuttings had been reported to be located in various tissues and varies from one specie to another.

In most plants, adventitious roots are originated in the vicinity of differentiating vascular tissue. The young roots were close to xylem and phloem which facilitates rapid establishment of a vascular connection.

Besides, the roots formed on cuttings of conifers were originated in one of four different ways from cambial and phloem regions of ray tissues, from leaf and branch traces, from irregularly arranged patches of parenchyma, and / or from callus tissues **(Satoo, 1956).**

In plants that are difficult-to root, rooting failure may partly be due to the presence of scleroids and sclerenchymatous cells in the cutting tissues. In addition, **Fahin, (1969)** mentioned that plants having narrow rays such as *Ceratonia, Pyrus* and *Carya* spp. rooted with difficulty.

Moreover, adventitious roots of some apple clones, red raspberries and *Hydrangea macrophylla;* were originated from phloem rays and the parenchyma cells in various locations **(Cummins, 1967), (Molnar** and **Laroix, 1972).**

In stem hardwood cuttings roots were originated at cut surface, either from the cambium or from the cells immediately outside **(Mittemprgher, 1964).**

Adventitious roots developed in almond cuttings were originated from an anomalous medullary rays **(Deidde, 1970).**

In addition, primary roots of guava cuttings resumed its activity by IBA + NAA treatments only whereas in untreated cuttings "control" the primary roots were not able to resume their activity and remained dormant and became compact **(Abou-Omera, 1976).**

Cambium activity was only observed in the treated guava cuttings. It was noticed that root initiation may take place in the cortex and / or in the pith **(Wally *et al.*, 1981).**

Moreover, roots developed on cuttings of woody perennial plants, often originated from young secondary phloem tissues, although such roots may arise from various tissues, such as vascular rays, parenchyma or pith **(Hartmann** and **Kester, 1992).**

In *Pyrus communis* pear hardwood cuttings adventitious roots were initiated from the cambial zone as well as from xylem rays **(Makarem, 1985).**

On the other hand, easy-to-root cuttings were associated with broad vascular rays such as in *Vitis vinifera* and *Tamarix* spp. **(Fahin, 1969).**

In addition, roots of both pear and peach cuttings were initiated from the combium zone. However, Bartlett pear cuttings failed to root due to the presence of schleroid cells which prevent root emergence **(El-Fakharani, 1986).**

However, microscopic inspection showed that in the walnut stem tissue structure, due to the presence of anomalous parenchyma rays, some meristematic cells develop from the rays to the cambial zone and under the cortex giving rise to root primordia **(Avanzato & Cappellini, 1988).**

In addition, the cambium appeared to be the only tissue that resumed its activity by cell division at the cambium zone and gave rise to different layers of tissues which formed the root initials and

root primordia of guava cuttings. Thereafter, the development of adventitious root primordia took place through the phloem tissue, cortex and periderm, and finally appears on the base of cuttings. Moreover, during the developmental stages of the adventitious roots, the vascular system of these roots is developed and made contact with the main vessels of the stem cutting **(Youssef *et al.*, 1991).**

Peach stems contained 3 leaf gaps (trilacunar type) immediately below the node area, where the median leaf trace was largest. The origin of the traces was the central core of the vascular tissue. Three orders of emergence for the leaf traces were observed which were affected by the distance of sectioning mode below the node. Moreover, the appearance of the traces led to the formation of gaps which were either associated with the traces in the area below the node, or disappeared where the traces were freed from the vascular tissue, leading to the formation of a large gap. The results also showed that no preformed roots were present in the stem tissue, thus adventitious roots in Flordabelle (FB) and Florda gold (FG) were either of nodal or internodal origin. In addition, parenchyma of all leaf gaps, such as parenchyma tissue outside the trace, parenchyma immediately interior to the bud scales and xylem parenchyma were the origin of adventitious roots. A sclerenchyma ring in the area of leaf and bud gaps was either not present or was disrupted in either cultivar and this was linked to the ease with which cuttings of these cultivars rooted **(Qrunfleh, *et al.*, 1992).**

Hardwood apricot cuttings cv. Amar were taken in November and the basal end of cutting was dipped in a solution of 0 or 4000

ppm IBA for 10 seconds. Cuttings were sampled at weekly intervals to study root ability in relation to cutting anatomy. IBA treatment promoted initiation of adventitious roots from cambium, xylem rays and pith **(Salama *et al.*, 1993).**

2- 2- Budding Studies:

Bitter almond is a suitable rootstock for commercial almond cultivars. However, the major defect of this rootstock is its susceptibility to nematodes and crown rot. Nematodes usually prevail in sandy soils while crown rot is associated with poorly aerated and highly wet soils **(Hartmann & Kester, 1975).**

Moreover, peach seedlings are resistant to root knot nematode. Young almond trees on Okenawa peach grow faster than on almond rootstock **(Hartmann** and **Kester, 1975).** In addition Marianna 2624 plum (*P. cerasifera* x *P. munsoniana*) is more tolerant to poor aeration and wet soil than bitter almond **(Monstra** and **Strada 1974** and **faynes, 1979).** Besides, Marianna 2624 is nematode-resistant **(Norlon *et al.*, 1963** and **Hartmann** and **Kester, 1975).**

2- 2-1- Morphological Studies:

Many studies have reported that rootstocks exert a considerable effect on compatibility, take and survival of scions. The following are a brief account of such reports.

2- 2-1-1- Effect of different rootstocks on take and survival of scion:

Compatibility between pear scions and quince rootstocks depends not only on scion variety and rootstock type but also on soil type and meteorological conditions, **(Indenko, 1965).**

Furthermore, three apricot selections (SH-6, SH-7 and curtis) grafted on six different rootstocks and grown on sandy loam and clay loam soils. Scion / rootstock incompatibility was more prevalent when peach seedlings were used as rootstocks some internal decay was found in graft union vicinity which apparently contributed to the decline of some trees **(Carlson, 1965).**

Two almond scion cvs. Ne Plus-Ultra and Nonpareil were grafted on the rootstocks: bitter almond, peach, apricot, *Prunus mume,* St. Julien, Damas, Marianna and Myrobalan plums. Both scion cvs. had poor graft union with St. Julien, apricot and Damas in comparison with other tested stocks **(Felipe, 1970).**

Seedlings of *Prunus* hybrid used as rootstock produced better take and survival rate for several almond scion cvs. than peach or almond control rootstocks **(Popok, 1981).**

Many apricot cvs were grafted on peach (*Prunus persica* L.) Dzanarika (*P. cerasifera*) and Belosljiva (*P. insititia*) rootstocks. The greatest survival rate was shown by the cv. Luized on Dzanarika **(Ninkovski, 1983).**

The highest take percentages for Bircher apple scions were on both Balady apple and quince (A) rootstocks in comparison with

quince (B) & (C). Thus, Balady apple seems to be the most compatible rootstock followed by quince (A) **(El-Fakharani, 1986).**

The bitter almond, apricot, common Marianna plum and Marianna 2624 plum rootstocks affected take and survival percentages. The highest values were noticed for common Marianna. Marianna 2624 came in the second rank descendingly followed by bitter almond while apricot yielded the lowest take and survival **(Marwad, 1989).**

On the other hand, budding and grafting of Nonpareil almond scions on four different rootstock of *Prunus* revealed that differences in the survival percentage were slight **(Sinha *et al.*, 1976).**

2- 2-1-2- Effect of Different Rootstocks on Growth Vigor:

Virginian crab apple have been used successfully as apple rootstock in many producing countries. It is quite well known that this rootstock react differently with various apple varieties. It acts as a dwarfing stock with some members of the triploid Winesap group while the opposite was true with other varieties. Moreover, such stock may be used as a dwarfing rootstock for very vigorous varieties which would be very advantageous for different cultural practices **(Maney, 1937).**

The vegetative growth of the tree depends on the weakest partner of the combination. The influence of this partner was greater, when it was used as the rootstock, the growth, even when a

a vigorous scion was used. The effect of the intermediate stock on the yield of the scion depended on rootstock **(Dehas, 1962).**

Some varieties of *Prunus cerasifera* and its inter- specific hybrids were tested as rootstocks for almond. Vigorous trees were obtained with Myrobalan P. 34-16, Marianna 2624 and GF 8-1. Almond varieties Ai, Ardechoise, Avola, IXL, Ferragnes, Jordonola, Ne Plus Ultra, Peerless and Texas were compatible with plum stocks, whereas cristomorto, Desmayo and Ferradual were incompatible **(Graselly, 1969).**

The effect of some almond rootstocks (almond, peach, Marianna 2624 and almond-peach hybrids) were described. Unions of almond / peach grafts often show a scion overgrowth but are quite acceptable. In irrigated soils, trees of such combination grow faster for the first several years and bear heavier crops during the first 15 - 20 years than those on almond roots. Almond trees on Marianna 2624 plum stocks are about one third smaller than those on other tested rootstocks. Not all almond cvs. are compatible with Marianna 2624. Those that can not be used include: Nonpareil, Davey, Milaw and Kapareil. A Clonal rootstock selection of *Prunus insitita* as Haven's 2B had been used as an interstock between Nonpareil and Marianna 2624 to overcome incompatibility. Almond trees on (almond-peach) hybrid stocks such as: (Titan Almond x Nemaguard peach) and (Nonpareil almond x Nemaguard peach) and the Clonal selections GF -677 & GF - 557 are noted for their vigour and excellent compatibility **(Hartmann and Kester, 1975).**

Ten *Prunus* hybrid seedlings were tested as rootstocks for some almond scion cvs. They gave tranplants of better conditions than on other peach or almond control rootstocks **(Popok, 1981).**

In addition to almond, a lot of information in this respect has been realised for apple trees.

As early as, it was known that some apple rootstocks may be used very effectively in reducing the vigor of varieties which naturally attain a great size **(Maney, 1937).**

Longley, (1963) concluded that Cortland, Delicious and Mc-Intoch apple trees on MM-106 were about one-half smaller than trees on other rootstocks. The growth of budded apple material depended on rootstock vigour **(Koval - skaya, 1973).** The rootstocks had an appreciable effect on trunk cross sectional area in the apple cv. Cox's Orange Pippin **(Kroop *et al.*, 1981).** Apple rootstocks exerted clear effect on trunk, girth measured 30 cm. above the ground, the thickest trunk was on Antonoka rootstock followed by those on A_2 and M-26. The number of shoots and their lengths followed a similar trend **(Ugolik *et al.*, 1981).**

Rootstocks exerted a marked effect on length of scion, main shoot and stem thickness (below, above and at the union zone). Scions on common Marianna stock were much longer than those on other tested stocks. Common Marianna also produced the thickest stems below and above union zone descendingly followed by Marianna 2624, bitter almond and apricot. All tested stocks produced an overgrowth at the union zone portion of stem,(such overgrowth is a sign of incompatibility). However, the overgrowth

was highest with apricot and common Marianna while was much lower with Marianna 2624 and bitter almond. Moreover, the rootstocks exerted an obvious effect on the whole nurseling, scion and stock fresh and dry weights. The upper-most values were exhibited by common Marianna followed by Marianna 2624, bitter almond and apricot in a descending order **(Marwad, 1989).**

Peach rootstock influenced tree survival, bacterial canker damages, root suckering, bloom date, degree of budbreak and fruit yield. However, rootstocks had little effect on bud density, fruit maturity and size, and time of autumn defoliation, and no influence on trunk circumference and bark gummosis. Cultivars differed in all characteristics except tree survival and canker damage. Tree survival was not negatively correlated with budbreak, bloom date, combial browning, *Pseudomonas* canker, suckering and defoliation. Lovell peach rootstock had the best overall PTSL-related performance, while Siberian C had the worst. Derby was the most desirable and Hamlet the least of the four cultivars evaluated by **Yadava** and **Daud (1989).**

Victoria plums grown on the French *Prunus* hybrid rootstock Ferlenain formed dwarf trees on smaller stature, than those on the dwarfing rootstock Pixy (*P. insititia*). cv. Czar initially grew more vigorously on Ferlenain than on pixy, but after 8 years in the orchard Czar trees on Ferlenain rootstock were the smallest. Ferciana (another French rootstock with *P. belsiana*, Myrobalan and peach in its parentage) formed larger trees similar in size to those on St. Julein A (*P. insititia*). Victoria grown on its own roots following micropropagation formed much larger trees than Victoria

on Ferciana. There was no evidence of increased shoot growth and/or delayed cropping due to scion rejuvenation following micropropagation **(Webster** and **Wertheim, 1993).**

2- 2-1-3- Root Distribution:

*** Effect of Rootstocks.**

The differences in root distribution of full bearing apple trees due to rootstocks (seedlings, M-II & M-IX) were studied. With seedlings and M-IX most of the main roots grow horizontally or sloped slightly downwards but with M-II they sloped downwards at a sharper angle. At 2 m. from the trunk the percentage distribution of absorbing roots at 0 - 50, 50 - 100, 100 - 150 and 150 - 200 cm. depth was 37, 33, 21 and 9 with the seedlings and 50, 48, 29 and 19 with M-II, respectively. With seedlings and Malling IX the top soil was well supplied with roots even at the periphery of the root area, but with M-II at some distance from the trunk a noteworthy distribution of roots was first observed below 40 - 50 cm depth **(Weller, 1965).**

Almond trees on common almond rootstock in irrigated soil had 43% horizontal and 57% vertical roots against 82% and 18%, respectively on peach rootstock **(Gretsinger & Gortanava, 1971).**

The root system of peach trees grafted on cherry plum, wild apricot, peach and almond rootstocks was studied. Wild apricot formed the strongest root system followed by peach, cherry plum and almond **(Skirtach, 1976).**

Grafted and own-rooted trees of sour cherries produced from softwood cuttings were found to have more skeletal and semi-skeletal roots spreading horizontally in the upper soil layer than trees on seedling rootstocks **(Revyakina, 1984).**

In the unbudded state, the tested stock plants revealed clear differences regarding characteristics of root distribution. The number of main roots (arising from the cutting or from the axial root) was greater with Marianna 2624, apricot and common Marianna in comparison with bitter almond. The main roots of Marianna 2624 were the thickest ones followed in descending order by common Marianna, apricot and bitter almond. The deepest penetration in soil was attained by apricot and common Marianna while Marianna 2624 and bitter almond exhibited a relatively shallow root penetration. Moreover, root distribution varied in response to the scion effect. The number of main roots of budded plants showed the same trend as with unbudded stock plants. However, differences between budded plants in thickness of main roots, depth and width of roots distribution were not as clear as for unbudded plants **(Marwad, 1989).**

2- 2-2- Effect of Different Rootstocks on Leaf Elements Content.

Nitrogen.

(a) Unbudded Stock Plants:

Determination of foliar N content of some unbudded apple rootstocks revealed that MM-104 exhibited the highest foliar N

level while quince showed the lowest level, MM-106, *M. communis* and Balady crab apple came in-between **(Kenawy, 1979).**

Studies on some unbudded apple rootstocks clarified that quince (A) exhibited the highest level while the lowest level was shown by quince (C). Foliar N content of Baladi apple and quince (B) came in between **(El-Fakharani, 1986).**

Meanwhile, unbudded stock plants revealed that leaf N content of common Marianna and Marianna 2624 leaves was higher than that of bitter almond and apricot **(Marwad, 1989).**

(b) Budded Plants:

Many workers reported that rootstocks affect scion leaf nitrogen content. Many of these reports related such effect with the compatibility between scion and rootstock.

It was noticed that dwarfing apple stocks promoted foliar N content of the scions **(Perfil, 1962).**

Apple rootstocks were found to exert obvious effect on foliar N content of the scions **(Tukey *et al.*, 1962).**

In this sphere, apple rootstock MM-III increased N content of apple scions, while MM-104 depressed it **(Carlson, 1965).**

The incompatibility of some central Russian apple varieties with Kitajka and Sibirka rootstocks was accompanied with losses in leaf total and protein N **(Leoncenko, 1967).**

The greater compatibility of peach scion grafted on peach stocks was associated with higher leaf and root contents of amide N. in comparison with components with lower compatibility [Peach on apricot] **(Zauarzin, 1967).**

The compatible (peach on peach & plum on plum) components were compared with incompatible (plum on peach, and peach on plum) ones. Concentrations of foliar nutrients were relatively low in trees with severe incompatibility symptoms **(Breen & Muraoka, 1975).**

Golden jubilee peach scions were grafted on almond No. 206, peach No. 22092, or cherry plum (*Prunus cerasifera*) rootstocks, Lesser fluctuations in mineral elements were observed in trees on the highly compatible rootstocks **(Syrbu & Stoyanov, 1984).**

Foliar N content of Bircher apple scion was stimulated by using Balady rootstock while it was relatively depressed when the dwarfing quince (C) rootstock was used **(El-Fakharani, 1986).**

Nitrogen content of scion leaves was greatly affected by the tested rootstocks. Ne Plus Ultra almond scion leaves on Marianna 2624 showed the upper most N level, while those on common Marianna recorded the lower most value. Scion leaves on both bitter almond and apricot exhibited intermediate N level **(Marwad, 1989).**

On the other hand, results of other investigators revealed measurable effect of rootstocks on scion foliar N content. This was clear in reports on plum trees grown from suckers or budded on

Myrobalan stock **(Dzomic *et al.*, 1966)**, and Bircher apple scion budded on five different rootstocks **(Kenawy, 1979)**, also Stanley plum trees grown on ten different seedling rootstocks **(Vitanova, 1982)** and Van and Kozarska cherry cvs. on seedling stock of the cv. Droganova Zhulta along with ten selected Mahaleb forms **(Koleva, 1986)**.

Phosphorus:

(a) Unbudded Stock Plants:

Studies on the foliar P content of unbudded five apple rootstock (MM-104, MM-106, *M. communis*, Balady crab apple and quince) revealed that quince and *M. communis* were superior in their foliar P content in all sampling dates **(Kenawy, 1979)**.

Also the foliar P content of some apple rootstocks in the unbudded state showed that Balady apple had the lowest level of phosphorus while quince (C) contained the highest level. Leaves of quince (A) and (B) exhibited intermediate P levels **(El-Fakharani, 1986)**.

The foliar P content of some almond rootstocks in the unbudded state showed that apricot had the highest level of phosphorus, while bitter almond contained the lowest level. Moreover, leaves of common Marianna and Marianna 2624 gave intermediate P. levels **(Marwad, 1989)**.

(b) Budded Plants:

Many workers reported that rootstocks affect foliar phosphorous content of the scion. Many of the available reports in this respect related such effect to the compatibility between scion and rootstock.

The transport rate of P_{32} to root and scion of apple trees grown in solution culture was paralleled to vigour of similar trees under field conditions **(Bukovac *et al.*, 1958).**

Leaf phosphorus content of bearing apple trees differed in the various combinations of rootstock, body stock, interstock and scion variety tested. The scion appeared to have the greatest effect which varied with planting locations, year and fertilizers **(Tukey *et al.*, 1962).**

The foliar P content was significantly different between plum plants grown from suckers and those grown on Myrobalan stock **(Dzamic et al., 1966).**

In compatible apple grafts P_{32} was translocated more freely within tree components in comparison with incompatible components **(Sygel, 1966).**

In addition, studies on distribution of P_{32} in the leaves, bark, and wood of Hungarian apricot trees on *Prunus institia*, apricot and Myrobalan plum rootstocks showed that the proportion of P_{32} in the leaves relative to the rootstock was lowest in trees of *P. institia*, and there was little difference between the proportion in the leaves and the rootstock with trees on apricot or Myrobalan plum. The

proportion of P_{32} in the bark and wood, relative to that in the rootstock was much lower when the rootstock was Myrobalan than with the other combinations studied, suggesting incompatibility between, Myrobalan and the apricot variety **(Modic, 1968).**

The uptake of P_{32} in two almond cvs. grafted on hybrid and commercial rootstocks was studied. The leaves of compatible rootstock / scion combination showed 3 - 4 times higher P_{32} concentrations than those of ungrafted rootstock plants used as control. The leaves of compatible combination showed lower P_{32} concentration than the controls **(Mitasov *et al*, 1973).**

In a study on plum trees it was found that the rootstock Zhulta Dzhanka (*Prunus cerasifera*) reduced foliar P content of Stanley scions in comparison with other tested rootstocks **(Vitanova, 1982).**

The concentration of foliar nutrients was relatively low in plum and peach trees with severe incompatibility systems **(Breen** and **Muraoka, 1975).** The leaf phosphorus content of Bircher apple scion was highest when quince (A) was used as rootstock, while it was lowest with Balady apple rootstock. Scions on quince (B) and (C) were of intermediate P content **(El-Fakharani, 1986).**

Phosphorus content of scion leaves was markedly affected by the tested rootstocks. Scion leaves on common Marianna showed the uppermost P level, while those on apricot recorded the lowermost values. Bitter almond and Marianna 2624 recorded intermediate values **(Marwad, 1989).**

On the other hand, results of other investigations revealed no apparent effect of rootstocks on scion foliar P content. This was clear in studies on apple where only slight differences were observed in translocation of P_{32} to scion (cv. Starking Delicious) grafted on a wide range of dwarfing rootstocks **(Cline, 1960).** Also, Bircher apple scion when budded on five different rootstocks (MM-104, MM-106, *M. communis*, Balady crab apple and quince) no significant differences were detected in foliar P content **(Kenawy, 1979).** In addition, when Van and Kozarska cherry cvs. were budded on seedling rootstocks of the cv. Droganova Zhulta and ten selected Mahaleb forms, the foliar P content was within the optional range with all tested rootstocks **(Koleva, 1986).**

Potassium:

(a) Unbudded Stock Plants:

The differences among apple rootstocks in K content were found to have no relation to the amount of root development or differences in selective uptake of the antagonistic cations Ca and Mg, but to varietal efficiency in K absorption **(Avent, 1957).**

Studies carried out on apple seedling rootstocks (MM-104 - 106, 109, 111 & Delicious) showed that they varied in leaf K content **(Titus & Ghosheh, 1963).**

The leaf K content of five unbudded apple rootstocks MM-104, MM-106, *M. communis*, Balady crab apple and quince was determined. Leaves of Balady crab apple showed the highest K

level, whereas leaves of quince contained the lowest level **(Kenawy, 1979).**

Moreover, the highest foliar K content belonged to Balady apple while the lowest content belonged to quince (C). Both quince (A), (B) recorded intermediate values in this respect **(El-Fakharani, 1986).**

Furthermore leaves of unbudded apricot plants exhibited the highest K content whereas Marianna 2624 leaves contained the lowest amounts. Bitter almond and common Marianna exhibited intermediate level **(Marwad, 1989).**

(b) Budded Plants:

Rootstocks markedly affect potassium content of scion leaves. Studies carried out on cherry trees clarified that rootstocks obviously affected foliar K content of scion **(Katzfuss, 1957).**

Also, foliar K content was significantly different between plum plants grown from suckers and those grown on Myrobalan stocks **(Dzomic *et al.*, 1966).**

Bircher apple scions were budded on five different rootstocks. Foliar K content of Bircher / MM-104 and Bircher / MM-106 were superior than those on quince, *M. communis* and Balady apple rootstocks **(Kenawy, 1979).**

Furthermore, it was found that the rootstock Zhulta Dzhanka plum (*Prunus cerasifera*) increased foliar K content of stanely cv. scions in comparison with other tested rootstocks **(Vitanova, 1982).**

Potassium content was determined in leaves of Bircher apple scions budded on different rootstocks. Scions on quince (A) rootstock are the highest in K content, while are lowest with Balady apple rootstock. Scions on quince (B) & (C) were of intermediate K content **(El-Fakharani, 1986).**

Potassium content in scion leaves was clearly promoted by apricot stock while reduced by Marianna 2624 stock. Scion leaves on common Marianna and bitter almond exhibited intermediate K levels **(Marwad, 1989).**

Calcium:

(a) Unbudded Stock Plants:

Calcium content was determined in leaves of five unbudded apple rootstocks (MM-104, MM-106, M. communis, Balady crab apple and quince). Samples taken in December showed that MM-104 accumulated the highest in this respect **(Kenawy, 1979).**

On the other hand, Balady apple rootstock exhibited the highest foliar Ca content while quince (C) gave the lowest content. Quince (A) & (B) had intermediate values **(El-Fakharani, 1986).**

Furthermore, leaves of unbudded apricot plants gave the highest Ca content, while Marianna 2624 recorded the lowest level. Bitter almond and common Marianna had intermediate levels **(Marwad, 1989).**

(b) Budded Plants:

Studies on cherry trees revealed a clear influence of different rootstocks on Ca content of scion leaves **(Katzfuss, 1957).**

The foliar Ca content of three apple scion cvs. was affected by their rootstocks as compared with trees grown on their own roots **(Molanov, 1968).**

As Bircher apple scion were budded on five rootstocks (MM-104, MM-106, M. communis, Balady crab apple and quince) the foliar Ca content of the scion was highest on MM-104, while it was lowest on the quince **(Kenawy, 1979).**

Concerning plum trees it was found that the lowest Ca content in stanely plum leaves was obtained when M, tna Byaka Bakinits (*P. domestica*) was used as a rootstock in comparison with other tested rootstocks **(Vitanova, 1982).**

Besides, studies on Bircher apple scions budded on different rootstocks showed that the highest foliar Ca content was noticed when Balady apple was used as rootstock, while the lowest level was with quince (A) rootstock. Scions on quince (B) & (C) were of intermediate foliar Ca content **(El-Fakharani, 1986).**

In addition, calcium content of Ne Plus Ultra scion leaves on apricot stock showed the uppermost values, while those on Marianna 2624 recorded the lowermost values. Scion on bitter almond and common Marianna recorded intermediate values **(Marwad, 1989).**

Magnesium:

Leaf magnesium was determined for bearing apple trees of various combinations of rootstocks, body stock, interstock and scion varieties. It was found that the influence, varied within years, and due to interactions between the plants and their parts **(Tukey *et al.*, 1962).**

Leaf magnesium content of apple cultivars was either decreased or increased by rootstocks as compared with trees grown on their own roots. The rootstock used for different scion varieties also caused changes in leaf magnesium content, thus a rootstock / scion interaction was observed in respect of nutrient uptake **(Molanov, 1968).**

Balady apple of unbudded rootstocks had the highest amount of Mg. while the lowest level of Mg existed in leaves of quince. Meanwhile, leaf nutrient content of Bircher apple budded on different rootstocks indicated that Balady apple rootstock, among the other used rootstocks, stimulated Mg content. On the contrary, the dwarfing quince C rootstock, induced the lowest content of Mg. **(El-Fakharani, 1986).**

Anatomy of Bud-union Zone:

Studies on stock / scion combinations of apple, clarified that cambial zone was initiated at the union and progressed into scion tissue. Phloem ray cells and sieve tubes of the scion became meristematic as the cambial zone formed a specific area. An interaction between stock and scion tissues changed the phloem ray

cells and sieve tubes of the scion, secondary phloem changed into thin walled parenchyma cells as a result of a wood stimulus for cambial initiation. Sieve tubes were necrotic in the immediate vicinity of the union. An irregular black line was formed on the stock side of the union, partially or completely encircling the tree trunk **(Simons, 1968).**

The sequence of events in the healing of graft union in woody plants was summarised as follows:

(a) The outer exposed layers of cells in the cambial region of both scion and stock produced parenchyma cell tissues.

(b) Certain cells of this newly formed callus which are in line with cambium layer of the intact scion and stock differentiate into new cambium cells.

(c) These new cambium cells produce new vascular tissues, xylem toward the inside and phloem toward the outside **(Hartmann & Kester, 1975).**

The peach cvs. Early and Diri Red formed normal graft unions with Brompton rootstock, but 126 A.D. united imperfectly. Patches of parenchyma were found interrupting the continuity of xylem of stock and scion of the incompatible combination, xylem necrosis was more intense in the stock than in the scion, the differences being most marked with incompatible combinations **(Cambra, 1967).**

The graft compatibility of some apricot cultivars on various *Prunus* rootstocks, was studied. The best graft union after five

years was between apricot scions and *P. domestica* rather than *P. cerasifera* or Marianna rootstocks. Incompatibility symptoms developed in certain combinations after one year and parenchymatous inclusions were visible in the xylem **(Duquesene, 1970).**

The formation of vascular connection was studied in compatible *Prunus* combinations, such as apricot cv. palonais 1331 on Myrobalan, a functional vascular connection was established 8 - 10 months after budding, meanwhile in combinations such as apricot cv. Canino 1343 on Myrobalan showed an imperfect vascular connection, apparently as a result of the abnormal functioning of the newly-formed cambium in the union zone **(Deliore & Hebant, 1983).**

Scion of *Prunus avium* cv. Sam were grafted on *P. avium* clone F 12 / 1 and *P. ceracus* clones (Cer. W 10, Cer. W 11 and Cer. W 13). In all combinations, the grafting process caused a shock phase of 3-4 weeks. The re-establishment of functioning phloem cells began in the 4th and 5th weeks, except on the rootstock Cer W 11, in which the process was blocked **(Feucht *et al.*, 1983).**

The Texas (mission) and Peerless almond varieties were found to be compatible with Marianna 2624 rootstock, Nonpareil and Davey were incompatible. Differences between compatible and incompatible combinations show both quantitative and qualitative aspects. Incompatibility of almond on Marianna 2624 involves the development of branch breakdown in the area of graft union whereas no obvious abnormality occurs in xylem tissue. The localisation of graft union failure could be readily observed micro-

scopically when the unions were examined at the end of the growing season. The incompatible combinations showed dead bark extending almost to the xylem, while the portion of the union in the wood stays mechanically strong and free of abnormalities. Microscopic examination in both mid-summer and during the dormant season of the living bark tissue in the area of graft union of incompatible combinations showed a range of symptoms. In some, a greenish-brown water-soaked area was observed, in other, a distinct dark line was present at the union, and in most cases, the bark tissue degenerated resulting in a separation of the living parts of the two components of the union by a plug of dead bark. Bark on incompatible combinations was thick at the union, as contrasted to that produced by compatible combination. Microscopic sections comparing Davey / Marianna 2624 at the end of the third growing season confirmed the continuity of xylem tissue between stock and scion in both combinations. There was disintegration of bark in Davey / Marianna 2624 but the details of phloem abnormalities could not be ascertained **(Kester *et al.*, 1986).**

The transverse section in union zone reveal better healing and callus felling as well as less necrotic tissues and air gaps with bitter almond stock in comparison with all other tested rootstocks. The most obvious defect appeared with almond/apricot nurselings. Nurselings on Marianna 2624 and common Marianna came in between **(Marwad, 1989).**

3- Micropropagation Studies:

Micropropagation is used specifically to refer to the application of tissue culture techniques to the propagation of plants starting with very small plants grown aseptically in a test tube or other container where the environment and nutrition can be rigidly controlled. Tissue culture procedures utilise an in vitro system of production that requires a laboratory-type-facility and aseptic techniques similar to those used in culturing fungi, bacteria and other micro-organisms. **(Hartmann** and **Kester, 1992).**

The following advantages can be cited for such wide interest in commercial nursery operations:

1- Mass propagation of specific clones; commercial micropropagation is particularly useful under the following conditions:

   (a) Plants whose natural rate of increase is relatively slow such as fruit trees and palm species.

   (b) New cultivars where commercial demand requires getting a cultivar on the market in as short a time as possible.

   (c) Cultivars that cannot readily or economically be clonally propagated by standard methods (e.g., peach-almond hybrid rootstocks, walnut rootstocks).

2- Production of pathogen-free plants, to maintain germplasm and source material in a pathogen-free condition, to allow movement of germplasm materials across quarantine barriers and to facilitate the distribution of commercial material in

international trade, which would be feasible with conventional propagation.

3- Clonal propagation of parental stocks for hybrid seed production.

4- Provide year round nursery production scheduled according to market demands. **(Hartmann** and **Kester, 1992).**

Propagation through tissue culture is affected by some major factors such as types of explant, time of collecting explants and kind of culture media. All these factors will be reviewed.

Type of Explant:

Different types of explants have been used for establishment of almond, peach and plum rootstocks and cultivars:

Dormant shoot buds, shoot tips (part of shoot of about 0.4 - 0.7 mm long) and cotyledons of almond were used by **(Tabachnik** and **Kester, 1977), (Rugini** and **Verma, 1983)** and **(Antonelli, 1992).**

Shoot tips, dormant meristem-tips and ovule explants of peach had been taken by **(Hammerschlag, 1981), (Ochatt** and **Caso, 1983),(Scorza** and **Cordts, 1989), (Allam** and **El-Rayes, 1991), (Pinto *et al.*, 1993)** and **(Jiang *et al.*, 1993).**

Shoot tips of plum were used by **(Rosati *et al.*, 1980), (Hammerschlag, 1982), (Pietropaolo** and **Reisch, 1984)** and **(Turk *et al.*, 1992).**

Time of Explants Collection:

Dormant shoot buds of almond and almond peach hybrid were collected in October, December and January. Buds collected in late December and January grew readily without chilling (70% or more good shoots) and scions could be stored for as long as nine months to provide a source of viable materials for culturing. Rate of shoot development appeared to be greater in material collected in January than that collected earlier **(Tabachnik** and **Kester, 1977).**

Shoot-tips of Mq, M-26 and M-106 were taken on May 28, June 20, July 14 and September 3, 1981. Better callus was formed when shoot-tips were taken in May, June and September **(Kim *et al.*, 1982).**

Explants (terminal buds) of almond cultivars were collected in April. The buds had already flushed at this time **(Rugini** and **Verma, 1983).**

Optimum time for shoot collection of MM-106 was mid-May **(Joung** and **Ko, 1983).**

Dormant buds of Red-leave-peach were collected during autumn and winter **(Ochatt** and **Caso, 1983).**

Cultures of Stanley plum were initiated from shoots collected in October and June. Shoots in culture was more difficult in October than in June. **(Pietropaolo** and **Reisch, 1984).**

However, aseptic cultures of Northern spy could be established at any time during the year although mid-spring and

mid-summer were the best **(James, 1984).** Shoot-tips, 5 mm long, of compact Redhaven peach were collected in the spring **(Scorza** and **Cordts, 1989).**

Actively growing shoots of Nemaguard peach were taken in late April **(Allam** and **El-Rayes, 1991).**

Culture Media:

Ingredients of the culture medium vary with kind of plant and the propagation stage at which one is working. These ingredients include (a) inorganic salts, (b) organic compounds, (c) complex natural ingredients and (d) inert supports **(Hartmann** and **Kester, 1992).**

The different media for establishment stage, shoot proliferation and root initiation of some stone fruit rootstocks and cultivars will be reviewed.

Establishment Media:

There are different establishment media for various explants of different rootstocks.

Modified knob's macro-element mineral solution, 2% sucrose, FeEDTA, micro-elements and organic supplementing of Murashige and Skoog medium were found to be suitable media for shoot tip culture of almond and almond-peach hybrid **(Tabachnik** and **Kester, 1977).**

Optimum shoot tip growth occurred on a liquid Murashige and Skoog salts medium supplemented with 0.01 mg/liter indole-3-butyric acid and 0.2 mg/liter and 0.2 mg/liter N-6 benzyladenine **(Hammerschlag, 1981).**

Shoot tips (5 mm long) of Myrobalan were cultured on micropropagation medium consisting of liquid modified Murashige and Skoog salt (MS) medium supplemented with 2% sucrose and in mg/liter: 0.4 thiamine HCl, 100 myo-inositol, 0.5 pyridoxine Hcl, 0.5 nicotinic acid, 0.1 P-aminobenzoic acid, 0.01 indolebutyric acid (IBA) and 0.26 benzylamino purine (BA) **(Hammerschlag, 1982).**

Shoot tips were excised to 1.0 to 1.5 mm long and placed onto a Murashige and Skoog basal medium (BM) supplemented with vitamins, glycine, myo-insitol, sucrose, agar and 0.1 mg./L indole-3-butyric acid (IBA) and 1.0 mg 6-benzyl aminopurine **(Ochatt** and **Caso, 1983).**

Murashige and Skoog medium supplemented with BA at 1 mg/L. and IBA at 1 mg/L with or without GA_3 at 0.1 mg/L. was found to be suitable medium for shoot tip culture of M-7, M-16 and MM-106 apple rootstocks **(Joung** and **Ko, 1983).**

Furthermore, modified Jones medium for apical meristem culture of M-9, M-26 and M-27 apple rootstocks were used by **(Stakanova** and **Abramenko, 1984).** Benzyladenine (BA) was added & both media of Murashige & Sokoog and Quoirion & Lepoivre for the meristem cultures of M-26, M-27 and M-106 apple rootstocks **(Golosin & Redojevic, 1985).**

Shoot tips were cultured on modified Ms medium supplemented with 1.0 ppm 6-BAP [benzyladenine] at 22°C and 16 h light/8 h dark **(Rodriguez** and **Muzas, 1992).**

Meristem culture of the early-ripening peach cv. Beinong Zaoyan was more successful on liquid medium than on solid medium, and 0.5 mm-long tips were more viable than 0.2 mm-tips **(Jiang *et al.*, 1993).**

Proliferation Media:

The media used in the proliferation stage vary mainly in their content of growth regulators.

Light and 6-benzyladenine (BA) at 1 mg / L. produced shoot elongation, at 1 mg/liter lateral shoot proliferation has been obtained from shoot tips of almond and almond-peach hybrid when cultured in 0.7 to 0.8 % agar, modified Knobs macro element mineral solution, 2% sucrose, FeEDTA, micro-elements and organic supplements of Murashige and Skoog **(Tabachnik** and **Kester, (1977).**

Shoot tips of Japanese plum proliferated at a rate of 10 : 1 to 20 : 1 per month when grown on a modified Murashige and Skoog medium with 3% sucrose, 0.75% agar and (in mg/L.) 0.4 thiamine Hcl-100 myo-inositol. 1.0 6-benzylamino purine (BA). 0.1 gibberellic acid (GA_3) and 0.1 indole-butyric acid (IBA). **(Rosati *et al.*, 1980).**

Rapid propagation of the apple rootstocks Malling Morton (MM) 104, MM-106 and MM-109 (Malus sp.) was achieved by shoot

tip proliferation using Murashige and Skoog basal medium with 1 mg / liter 6-benzylamino purine (BA) and 1 mg /liter indolebutyric acid (IBA). Improved proliferation was achieved by using a liquid media **(Snir** and **Erez, 1980)**. Shoot regeneration was induced on a half-strength MS medium supplemented with 0.5 mg / liter benzylamino purine (BA).

Moreover, proliferation of shoot tips of pear (*Pyrus communis* L. cv. Seckel) was obtained on Murashige and Skoog (MS) medium containing benzyladenine gibberellic acid (GA_3) and naphthalene acetic acid (NAA). Subculturing shoots on MS medium supplemented with 2 mg/liter (BA) resulted in the highest rate of shoot multiplication **(Singha, 1980).**

A. 10-fold multiplication rate of Myrobalan plum was achieved every 4 - 6 weeks when cultured shoots were transferred to multiplication medium consisting of modified Murashige and Skoog salts (MS) medium supplemented with 2% sucrose and in mg/liter: 0.4 thiamine Hcl, 100 myoinositol, 0.5 pyridoxine Hcl, 0.5 nicotinic acid, 0.1 p- aminobenzoic acid, 0.01 indolebutyric acid (IBA), 1.0 (BA) and 0.6% agar **(Hammershlag, 1982).**

Besides, an evaluation of 6-benzylamino purine (BA), isopentenyladenine (Zip), indolebutryic acid (IBA), and indoleactic acid (IBA) for shoot proliferation of pecan on a defined medium found that combination of 4 mg / liter BA and 1 mg / liter IBA was the most effective **(Wood, 1982).**

In addition, 25 - 30 fold multiplication was obtained of EMLA25 apple rootstock shoots by subculturing the shoots on

Murashige and Skoog medium supplemented with BA at 1 mg/L. and 0.2 mg/L. IBA **(Cheema** and **Sharma, 1982).** The best BA concentration for shoot proliferation was 5.0 µM for Macspur and M-26 but slightly higher for M-27 and M-9 **(Lane** and **McDougold, 1982).**

Shoot multiplication rates of six almond cultivars were obtained with 20-day periods of subculture continued for at least 24 months, on Murashige and Skoog (MS) medium with 0.9% agar, 0.7 mg/L 6-benzylamino-purine (BAP) and 0.01 mg/L naphthalene-acetic acid (NAA) **(Rugini** and **Verma, 1983).**

Moreover, a 500-fold increase in shoot tip of *Prunus* x Hally 'Cherry' was achieved after 25 weeks in culture, on a Murashige and Skoog medium containing 0.1 mg/liter naphthaleneacetic acid (NAA) and 1.0 mg/L. benzylamino purine (BA) **(Lineberger, 1983).**

Proliferated shoot cultures were established from shoot tips and nodal bud segments of *Pistacia vera* L. excised from seedlings germinated aseptically and cultured on Murashige and Skoog medium supplemented with BAP plus NAA **(Barghchi** and **Alderson, 1983).**

Shoot tips of 'Stanley' plum were multiplied 10 times per month on MS inorganic salt with (in mg/liter) thiamine-Hcl (1.0), nicotinic acid (1.0), pyridoxine Hcl (1.0), myo-inositol (1.0), sucrose (30, 000) agar (7, 000), and BA (1.1) **(Pietropaolo and Reisch, 1984).**

A walnut specific medium, DKW, had been defined as supporting optimum multiple shoot development under 4.5 µ M

benzyladenine (BA) and 5 µM indole butyric acid treatment **(Driver** and **Kuniyuki, 1984).**

Single-node cuttings obtained from 2-month-old seedlings of pecan were induced to break buds and form multiple shoots in liquid, woody plant medium (WPM) and 2% glucose supplemented with 6-benzylamino purine (BA) at 3 mg/liter **(Hansen, 1984).**

Micropropagation of carob is possible from seedlings and mature trees using Murashige and Skoog medium with 5 µM Zeatin for shoot multiplication **(Sabastian** and **McComb, 1986).**

Proliferation of Japanese cherry was achieved on solid medium [agar at 8 g/liter] containing Gamborg inorganic salts, myo-inositol [100 mg/liter], thiamine-Hcl [1 mg/liter], BA [1 mg/liter] and sucrose [30 g/liter] **(Katano, 1987).**

Proliferation of Nemaguard peach rootstock was obtained on MS media supplemented with different combinations of Benzyl amino purine (BAP) and indole butyric acid (IBA), and containing 3.0 mg/L charcoal. A combination of 5 mg/L BAP + 3 mg/L IBA was found to enhance the proliferation in five days **(Allam** and **El-Rayes, 1991).**

Shoot differentiation of sweet cherry occurred from shoot tips on half-strength MS medium with 0.5 or 1 mg BA and 0.1 or 0.5 mg IBA/liter **(Oh *et al.*, 1991).**

Proliferation in the bistrica ecotype was achieved on MS medium containing 0.25 mg benzyl adenine and 0.1 mg IBA/liter. The addition of 0.1 and 0.05 mg gibberellic acid / liter to the

medium did not enhance shoot proliferation or shoot elongation. On medium containing zip elongation of the transferred explants was good, but there was a lack of multiplication (**Turk *et al.*, 1992**).

Shoot proliferation of Prunus rootstock was generally better when the de Fossard components were added to culture medium, with most NAA/BA combinations, and this treatment also resulted in the formation of good quality shoots in all cases (**Arena** and **Caso, 1992**).

Rooting Media:

The rooting media must be free of cytokinin and contain mainly some growth regulators which induce rooting. Shoot tip of almond and almond-peach hybrid can be cultured in 0.7 to 0.8 % agar, modified Knob's macro elements mineral solution, 2% sucrose, FeEDTA, micro elements and organic supplements of Murashige and Skoog medium. Application of auxins as low as 0.01 mg/liter shifted development from shoot growth to callus production. Indole acetic acid caused less callus formation and allowed more shoot growth. Several rooting experiments were conducted with limited success (**Tabachnik** and **Kester, 1977**).

Successful rooting was related to exposure of shoot cultures to hormone-free media. This procedure prevented callus formation and led to a three-fold increase in root number per rooted culture compared to continuous contact with hormone, when shoot culture of apple rootstock Mq grown on a Linsmaier-Skoog medium (**James** and **Thurbon, 1979**).

However, the rooting of cherry shoots was better on basal nutrient medium used by **Jones** ***et al.,*** **(1979)** containing 0.2 mg/ L x - NAA **(Ivanicka** and **Pretova, 1980).**

Best rooting results of Japanese plum were obtained on modified Murashige and Skoog medium with 2 or 4 mg/liter indolebutyric acid (IBA) at 21° C. Raising the temperature to 26° C or 30° C slowed and decreased rooting unless 0.1 mg/liter GA_3 was included in the medium. Activated charcoal in the medium drastically reduced rooting **(Rosati,** ***et al.,*** **1980).**

Two media were selected for rooting of apple rootstocks Malling Merton (MM) 104, MM-106 and MM-109 (*Malus* sp.), the first using a Murashige and Skoog basal medium with IBA for rooting initiation and the second was MS without IBA but plus 0.25% activated charcoal for improving root development **(Snir** and **Erez, 1980).**

Rooting of Malling M7 apple rootstock was induced on a third strength MS medium containing 0.27% agar and supplemented with either 1.0, 2.0 or 3.0 mg/liter indolebutyric acid (IBA). Shoot subcultured on medium containing 2.0 mg/liter IBA rooted within 28 days **(Werner** and **Boe, 1980).**

Rooting of pear was achieved by transferring individual shoots to MS medium containing NAA.

Up to 80% of apple cv. Granny Smith cuttings formed roots when grown in continuously agitated liquid culture (half-strength MS) with continuous illumination and constant temperatures 26° C

± 2°C). Exogenous auxin was essential for rooting; IBA at 10 µM and NoA at 10 µM both promoted root formation but 2, 4 -D was inhibitory. The optimum sucrose concentration was 1 % [W / v] **(Srick *et al.*, 1981).**

Complete rooting (100%) of Myrobalan plum occurred at four weeks when in vitro-proliferated shoots were transferred to MS medium supplemented with 2.5 - 5.0 mg/liter indole acetic acid (TAA) and incubated in the dark for two weeks at either 21° C or 26°C. Poor rooting occurred in the light (1.0 KLX, 16-hr photoperiod). Shoots incubated at 26° C rooted more quickly than at 21°C. Shoots cultured on MS medium with IAA and incubated in the dark for two weeks rooted significantly better than shoots on medium with IBA and incubated under dark or light conditions. Addition of chlorogenic acid to the rooting medium significantly increased rooting in the light **(Hammerschlag, 1982).**

When shoots of Red-leaf peach 2.0 cm. long or more from stage II were transferred to Murashige and Skoog's basal medium (BM) supplemented with vitamins, glycine, myo-insitol, sucrose, agar and 1.0 mg/L. IBA but lacking cytokinins and GA_3. Only limited rooting has been obtained, they were very short (5 mm or less). If these rooted shoots were transferred to soil they always failed to survive on account of desiccation **(Ochatt and Caso, 1983).**

Higher IBA concentration (2.0 and 5.0 mg/L), the addition of either 0.25% activated charcoal or 0.162 g./L phloroglucinol, the omission of agar from the MS medium (using instead filter paper support for the explants), and the reduction of salt concentration by half failed to improve rooting **(Ochatt and Caso, 1983).**

Cultured shoot tips of 'Hally Jolivette' cherry exposed to different levels of NAA and BA (0, 0.1, 1.0, 2.5 and 5.0 mg/liter of each in all combination) formed roots, shoots, or basal callus depending on the concentrations of added growth regulators **(Lineberger, 1983).**

Cultured shoots of *Pistacia vera* L. were rooted in *vitro* using MS medium (half strength macro nutrients) containing IBA for rooting initiation followed by subculture onto hormone-free medium for root development. **(Barghchi** 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), myo-inositol (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).**

In vitro-derived shoots of pecan soaked in 1 - 3 mg/liter indolebutyric acid (IBA) produced adventitious roots in liquid, woody plant medium [WPM] **(Hansen, 1984).**

Shoots of St. Julien A. (*Prunus institia* L.) were placed in modified half-strength MS medium with indolebutyric acid (IBA) or indole -3- acetic acid (IAA) with or without a 16/8 light-dark incubation. Light (16-h photoperiod) inhibited rooting. IAA (4 mg/L) was ineffective in promoting rooting. Rooting was best when shoots were incubated in the dark with IBA (4 mg/L). **(Reeves *et al.*, 1985).**

Rooting was induced by subculturing plantlets MS medium containing 1 mg/L BAP and 5 mg/L IBA without either GA_3 or riboflavin **(Allam** and **El-Rayes, 1991).**

Shoots of the cultivar Santa Rosa plum, raised in vitro, were placed for rooting in MS medium with one-third the normal concentration of mineral salts and with IBA at 0.2, 0.5 or 0.8 mg/liter. The explants were exposed to 1000, 2000 or 4000 Lax emitted by fluorescent lamps (Gro-lux) or lamps providing cool white light or mixture of both. Light treatments had little effect on rooting. However, rooting was improved as the IBA concentration decreased, and amounted to 91.11, 83.88 and 80.55 % with IBA at 0.2, 0.5 and 0.8 mg/liter, respectively. **(Junior** and **Peter, 1991).**

Root formation of sweet cherry took place on transfer to rooting medium with 0.5 mg IBA and 0.1 or 0.5 mg BA/liter. Callus formation was effective with 2.4; D at 0.1, 0.5 or 1.0 mg/liter with or without BA at 0.1 or 1.0 mg/liter. Root initiation from callus was best with 0.1 or 1.0 mg/L IBA combine with 0.1 mg BA/liter, but IBA alone had little effect **(Oh *et al.*, 1991).**

The shoots of apple rootstock (M-26) were transferred to MS medium coloured with a red pigment or non-coloured, supplemented with 0.3 or 0.5 ppm IBA or 0.3 or 0.7 ppm NAA. Root number was evaluated after thirty five days under two lights intensities (1000 or 2000 lux). The best results were obtained with shoots cultured on coloured MS medium supplemented with IBA and held under 1000 lux (80% of shoots with 3 - 5 roots). **(Rodriguez** and **Muzas, 1992).**

Moreover, micro cuttings of two almond (*Prunus dulcis*) genotypes (cv. Supernova and rootstock Sel M-51) placed 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 + light, whilst supernova rooted only with IAA or IBA + dark (3.7 and 58%, respectively). **(Caboni and Damiano, 1994).**