

**Histology of auto- and heterografts of tomato,
eggplant and pepper and influence of rootstock
on broomrape infestation**

by

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Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in
Plant Production

Faculty of Graduate Studies
University of Jordan

1998


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This thesis was successfully defended and approved on July 27, 1998.

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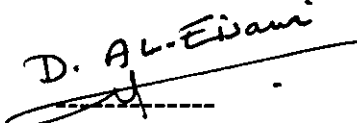
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DEDICATION
TO MY FATHER AND MOTHER

Acknowledgement

Sincere gratitude and appreciation are expressed to my advisor Dr. M. A. Suwwan for his patience, and efforts in guidance, supervision encouragement and invaluable advice throughout this study and preparation of the thesis.

I would like also to extend my sincere thanks to Dr. Walid I. Abu-Gharbieh, Dr. Dawud Al-Eisawi, Dr. Mahmoud A. Kasrawi and Dr. Esam B. Al-Emam for serving on my examination committee and reviewing the manuscript.

Finally, heartfelt gratitude is due to my father, mother, sister, brothers and friends for their encouragement and help.

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Abstract

**Histology of auto- and heterografts of tomato, eggplant and pepper
and influence of rootstock on broomrape infestation***by*

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Reciprocal auto- and hetero-cleft grafting of tomato (*Lycopersicon esculentum* Mill.) cv. 'GS12', eggplant (*Solanum melongena* L.) cv. 'Shantta' and pepper (*Capsicum annuum* L.) cv. 'Maram' were performed.

Autograft combinations of tomato, eggplant and pepper as well as compatible heterografts of tomato and eggplant showed high survival ratios. In these graft combinations, an isolation layer was formed at the graft interface 5 days after grafting. Callus proliferation was observed after 10 days from grafting and vascular cell differentiation at the graft union occurred 15 days after grafting. By the 20th day vascular connections were completed. Fresh and dry weights of the scion and rootstock and plant height were significantly greater in compatible combinations.

On the other hand, the incompatible graft combinations (tomato/pepper, pepper/tomato, eggplant/pepper and pepper/eggplant) showed no survival ratios and the isolation layer continued to separate the cells of the two partners of the graft union until 20 days after grafting; no vascular connections were formed and the scion died.

Whole plants of tomato and eggplant as well as their compatible auto- and heterografts were highly infested with *Orobanche ramosa*. In contrast whole plants of pepper and their autografts showed light infestation.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is considered the most important vegetable crop in Jordan (Statistical Yearbook, 1995). In Japan and USA the average productivity of tomato was about 54 and 43 ton/ha, respectively, whereas in Jordan the average productivity did not exceed 40 ton/ha (FAO, 1996). Nevertheless, many studies were undertaken to increase the productivity of tomato plants through clipping and multiple application of tomatotone (Al-Maslamani and Suwwan, 1987 and Suwwan, 1988), increasing plant population (Shibli and Suwwan, 1987 and Mahrakani and Suwwan, 1987), ethephon application (Suwwan, 1986 and Mahrakani and Suwwan, 1987), reducing plant spacing (Al-Maslamani and Suwwan, 1987) and using plastic mulches (Haddadin *et al.*, 1985).

Among the factors contributing to reduced tomato yields under local conditions, spread of diseases, nematodes and parasitic weeds (*Orobanche* spp.) are of prime importance. In the middle and northern parts of Jordan, the damage caused by *Orobanche ramosa* is severe, and heavy infestation could result in high yield losses or even in total failure of the tomato crop (Abu-Irmaileh, 1979). Several attempts had been carried out to control this parasite including, cultural practices (Abu-Irmaileh, 1982; Abu-Irmaileh, 1984; Sherif and Gelma, 1992; Abu-Irmaileh, 1994b and Labrada, 1994), chemical methods (Jacobsohn and Kelman, 1980, Kotoula-Syka and Eleftherohorinos, 1989 and Garcia-Torres *et al.* 1991), biological control with fungi (Labrada, 1994) and insects such as *Smicronyx cyaneus* GYLL. (Nadjia, 1997) and resistant varieties (Kasrawi and Abu-Irmaileh, 1989 and Qasem and Kasrawi, 1995). Control methods are not yet satisfactory and the search for practical control continues.

It appears that no previous attempts have been made to examine the effect of grafting tomato on rootstocks (pepper and eggplant) resistant or tolerant to such weed parasite (broomrape) in Jordan. This could be a good approach to avoid its infestation effect. Hence this research was initiated to:

1. follow the histological changes of auto- and heterografts of tomato, eggplant and pepper during growth and development.
2. study the compatibility relationships among 'GS12' tomato, 'Shantta' eggplant and 'Maram' pepper.
3. investigate the responses and resistance of grafted plants to the infection with branched broomrape (*Orobanche ramosa* L.).

2. Literature Review

2.1 Grafting Effects on Vegetables

As cited by Lee (1994), Ashita (1927) reported that growing grafted vegetables was first launched in Japan and Korea in the late 1920s by grafting watermelons to gourd rootstocks. Recently, grafting eggplants (Honma, 1977) tomato, (Yamakawa, 1982) cucumbers (Oda *et al.* 1993) and watermelons (Yamasaki *et al.* 1994) has been practiced.

The main objective of grafting technique has been applied to attempt growth promotion and yield increases in vegetables (Lee, 1994). Earlier flowering was induced when 'Pennorange' tomato scion was grafted on an early tomato cultivar 'Farthest North'; this was accompanied by a reduction in the number of nodes below the first inflorescence, suggesting the presence of a graft transmissible flowering stimulator in the early cultivar (Phatak and Wittwer, 1965). Among reciprocal cleft grafting of 29 tomato genotypes, 'Alcobacca' and 'Moneymaker', as rootstocks induced early flowering and a high truss weight, whereas 'KNVF' promoted vegetative growth (Zijlstra and Den Nijs, 1987).

Biesiada (1994) proved that the advantageous effect of long growing period of tomato is due to use of transplants grafted on resistant 'KNVF Tm' tomato rootstock. As cited by Lee (1994), Kato and Lou (1989) reported that highest cytokinin (trans-zeatin) concentrations were found in sap collected from eggplant grafted onto 'VF' rootstock, which was positively correlated with eggplant yield. Leaf area, early and total yield and total number of fruits of two eggplant cultivars were significantly increased when cleft-grafted on two tomato cultivars as compared with ungrafted plants (Vuruskan and Yanmaz, 1990). When 'BTS' common bean was grafted onto 'Nep-2' or 'C-70001' bean rootstocks

with greater root biomass than its own, a concomitant increase of yield, dry matter production and assimilate distribution efficiency was observed (Izquierdo and Hosfield, 1982).

Yamasaki *et al.* (1994) concluded that in watermelon/squash combination, the rate of mineral absorption and cytokinin synthesis by squash rootstock is greater than those in the watermelon/bottle gourd and non-grafted watermelon seedlings. Polson and Smith (1972) indicated that ion accumulation in a plant of soybean with two shoots, an original and grafted one with another soybean genotype, is largely independent, suggesting that scion control of ion accumulation is accomplished via self-contained mechanism within the shoots.

Plants of *Momordica dioica*, using the rootstocks of five different *Cucurbita* species, produced 2-3 times greater leaf area (Mian *et al.*, 1993) and dry matter (Mian *et al.*, 1992) than those of non-grafted plants at both vegetative and reproductive stages. Irrespective of root genotype, the plant combinations with shoot of the cultivated soybean type, gave a significantly greater leaf area and dry weight of nodules as compared with the shoot of the wild soybean type; this suggests the importance of the contribution of the shoot growth rate for whole plant growth determination (Fujita *et al.* 1991).

Grafting has also been shown to be important for the scope of prevention of occurrence and damage associated with nematodes (Trudgill, 1986) and soil-borne diseases such as brown root rot (Yamakawa, 1982) and *Verticillium* wilt (Honma, 1977 and Yamakawa, 1982) and *Fusarium* wilt (Yamakawa, 1982 and Kuniyasu and Yamakawa, 1983).

Several types of grafting has been used, but approach and cleft grafting are most common (Yamakawa, 1982). Cleft grafting was found to be the most efficient (Honma, 1977) in terms of survival ratio (Oda *et*

al. 1994) and growth and yield (Vuruskan and Yanmaz, 1990; Oda *et al.* 1994 and Oda *et al.* 1995).

2.2 The Graft Union

The developmental process of the graft union has been extensively studied. Several investigators have commented on structural events which could be responsible for the development of cohesion between the stock and scion during graft ontogeny for herbaceous plants including *Lycopersicon esculentum* (Lindsay *et al.*, 1974; Yeoman and Brown, 1976; Deloire and Hebant, 1982; Parkinson and Yeoman, 1982; Jeffree and Yeoman, 1983; Rachow-Brandt and Kollmann, 1992 and Yang *et al.*, 1992), *Capsicum annuum* (Deloire and Hebant, 1982), *Solanum pennellii* (Moore, 1984; Moore and Walker, 1981b,c and Moore and Walker, 1983), *Solanum tuberosum* (Rachow-Brandt and Kollmann, 1992), *Nicandra physaloides* (Yeoman and Brown, 1976 and Parkinson and Yeoman, 1982), *Datura stramonium* (Parkinson and Yeoman, 1982), *Helianthus annuus* and *Vicia faba* (Kollmann and Glockmann, 1985; Kollmann *et al.*, 1985 and Rachow-Brandt and Kollmann, 1992), *Sedum telephoides* (Moore and Walker, 1981a,b,c and Moore and Walker, 1983), pea roots (Stoddard and McCully, 1979) and *Amaranthus tricolor* (Yang *et al.*, 1992).

Generally, graft formation of herbaceous plants have been followed up through: (1) Tensile strength (Moore, 1984) and breaking weight (Lindsay *et al.*, 1974), (2) Using ^{14}C -labeled materials (Rachow-Brandt and Kollmann, 1992), (3) Fresh weight of scion (Moore, 1984), (4) Measuring electrical resistance (Yang *et al.*, 1992) and (5) Anatomical studies (Stoddard and McCully, 1980 and Moore, 1984).

The breaking weight in tomato autografts was observed to increase in two phases over a period of 7 days (Lindsay *et al.*, 1974 and Yeomen and Brown, 1976) and 14 days (Parkinson and Yeoman, 1982). According to Moore (1984), the tensile strength in *Solanum pennellii* horizontal autografts increased until day 15 after grafting. This increase was parallel to the increase in number of wound vessel members (Parkinson and Yeoman, 1982) or number of tracheidal elements (Lindsay *et al.*, 1974). Yeomen and Brown (1976), claimed that the increase in breaking weight of the first phase is due to the intensification of cohesion between parenchymatous cells while the continuing increase in the second phase is a consequence of lignification of cells at the junction. Using ^{14}C -labeled materials, assimilate transport across the graft interface, started in the compatible heterografts (*Lycopersicon/ Solanum*) and homografts (*Lycopersicon*, *Helianthus* and *Vicia*) combinations 5-7 days after grafting which was positively correlated with the number of sieve tubes (Rachow-Brandt and Kollmann, 1992).

As a response to successful graft union, Moore (1984) observed that the fresh weight of the scion in *Solanum pennellii* autografts increased by 5% d^{-1} during the first 2 days after grafting and 9.2% d^{-1} between 2 and 15 days after grafting.

Electrical resistance across tomato autografts rapidly increased for the first 2-3 days in step with formation and thickening of the isolation layer (Yang *et al.*, 1992); in the next 3-8 days this resistance decreased steadily, as the isolation layer ruptured and disappeared during callus proliferation and interdigitation. These observations on tomato autografts were conformable with autografts of *Sedum telephoides* (Moore and walker, 1981a), *Coleus blumi* (Stoddard and McCully, 1980), pea roots (Stoddard and McCully, 1979) and *Vicia faba/Helianthus annuus* heterografts (Kollmann and Glockmann, 1985).

In incompatible grafts of 'Yollowonder' pepper / 'St Pierre' tomato well-developed wound periderms are commonly differentiated by both partners at the graft line. These wound periderms may also differentiate in successful unions 'Doux des Landes' pepper / 'St Pierre' tomato, but they decidedly and normally appear outside the vascular zone (Deloire and Hebant, 1982). By five days after autografting of *Solanum pennellii*, the graft interface was comprised of callus cells, followed by vascular differentiation at 9-12 days, then completion of vascular connection at 21 days after grafting (Moore, 1984). According to Stoddard and McCully (1980), components of autografting process in *Coleus blumi* include: callus formation on the cut surfaces, necrotic layer removal, cohesion of the graft partners and bridging of the graft by xylem, phloem and cambium.

In contrast to compatible autografts, the curve of electrical resistance in incompatible heterografts (*Amaranthus tricolor* and *Lycopersicon esculentum*) rises successively as the isolation layer remains unruptured and prevents the cell contact and the unification of the graft union (Yang *et al.*, 1992). Development of wound periderms and lack of vascular connections between pepper and tomato were attributed to peroxidase activity that can be associated with the deposition of lignins and polyphenols at the contact layer (Deloire and Hebant, 1982). Cellular necrosis in the *Sedum* partner characterized the incompatibility response between *Sedum* and *Solanum* and resulted in formation of a necrotic layer of collapsed cells which was never ruptured by callus proliferation (Moore and walker, 1981b). This incompatibility has been claimed to involve the failure of *Sedum* to isolate hydrolytic enzymes from the cytosol, which subsequently lead to cellular necrosis (Moore and Walker, 1981c).

2.3 *Orobanche ramosa*

At least seven *Orobanche* species including *Orobanche ramosa* parasitize economically important crops in different areas of the world (Garcia-Torres, 1994). According to Parker (1994), tomato is probably the single most important crop being affected. Infection of tomato roots was detected after 12 days and occurred in the root hair zone and branching points of the roots (Hameed and Foy, 1991). Seed germination of *Orobanche* is initiated by a stimulant present in the root exudate of the tomato (Wegmann, 1994); after germination is induced, a germ tube emerges from the seed and, if it meets a host root, attaches itself to the root tissue by an appressorium and then penetrates the root tissue.

There is considerable interest in the control of this parasitic flowering plant, which causes a serious damage to the tomato crop (Abu-Irmaileh, 1994a). The control of such weeds is still difficult because economically effective means of control are not yet available (Abu-Irmaileh, 1982). Cultural practices such as manual removal (Abu-Irmaileh, 1994b) does not prevent the damage already inflicted upon the plant before emergence of the parasite (Garcia-Torres, 1994). Crop rotation with trap crops is recommended to reduce the infestations in affected fields (Labrada, 1994), but its negative side is manifested in loosing potentially economical crops for years (Garcia-Torres, 1994). Trap and catch crops were used by farmers (Abu-Irmaileh, 1984), but it is partially efficient, since many seeds do not germinate during the trap crop growing period and remain viable (Garcia-Torres, 1994). High levels of nitrogen (higher than 60g/pot) reduced *O. ramosa* infestation in tomatoes (Abu-Irmaileh, 1981), but it would not be practical to use such high levels because of high cost limitations and possible underground water contamination (Garcia-Torres, 1994). Several important herbicides have been used for broomrape control, including glyphosate (Jacobsohn and Kelman, 1980

and Kotoula-Syka and Eleftherohorinos, 1991), chlorsulfuron, imazaquin (Kotoula-Syka and Eleftherohorinos, 1991) and imazapyr (Garcia-Torres *et al.*, 1991) as pre-emergence herbicides and imazethapyr (Garcia-Torres *et al.*, 1991), metsulfuron-methyl, sulfometron-methyl and triasulfuron (Adam and Drennan, 1992) as post-emergence herbicides. However, herbicide treatments can be recommended in some crops but not in others (Jacobsohn and Kelman, 1980). According to Garcia-Torres *et al.* (1991) the rates of herbicides should be adjusted according to the environmental conditions and cropping system. However use of herbicides is expensive (Garcia-Torres, 1994).

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Resistance to *O. ramosa* in tomato germplasm was found at varying levels in accessions from *Lycopersicon peruvianum* (L.) Mill., *L. pimpinellifolium* Mill. and *L. esculentum* var. *cerasiforme* (Kasrawi and Abu-Irmaileh, 1989). From twenty-five tomato cultivars and one accession of wild tomato, 'Tiny Tim' showed the highest level of resistance (Qasem and Kasrawi, 1995). Moreover, the limited practical success of introducing genetic resistance against *Orobancha* in many crops, is attributed to existence of very diverse, complex *Orobancha* population, which easily select/shift to more aggressive biotypes/strains adapted to the newly introduced cultivars (Garcia-Torres, 1994).

3. Materials and Methods

3.1 Plant Material

Two experiments were conducted in 1997 in the glasshouse at University of Jordan. Seeds of 'GS12' tomato (*Lycopersicon esculentum* Mill.), 'Shantta' eggplant (*Solanum melongena* L.) and 'Maram' pepper (*Capsicum annuum* L.) were provided by local dealers⁽¹⁾. On basis of preliminary experiments, to obtain simultaneous germination, pepper seeds were sown in plastic trays filled with peatmoss mixed with perlite (1:1 v/v) 10 days earlier (March 18) than those of tomato and eggplant (March 28). The plants were grown in a glasshouse maintained at a day temperature of 22-25°C (measured at 9 AM, 12 midday and 3 PM with LCD Digital Thermometer model Mannix) and a night temperature of about 15°C (measured at 9 PM with the same device) up to the 7th leaf stage (Table 1).

3.2 Grafting Procedure

Cleft autografts and reciprocal heterografts were performed midway between the cotyledons and the first true leaf as described by Honma (1977). The grafting was made when tomato and eggplant seedlings (45 days old) had four to five leaves and pepper seedlings (55 days old) had six to seven leaves. Care was taken to assure that stems of stock and scion seedlings had nearly the same diameter within the range of 2.5-2.7mm at the first internode (Table 1). The rootstock portion of the first internode was split from the top downward for about 1cm with a sterilized razor blade (Plate 1A).

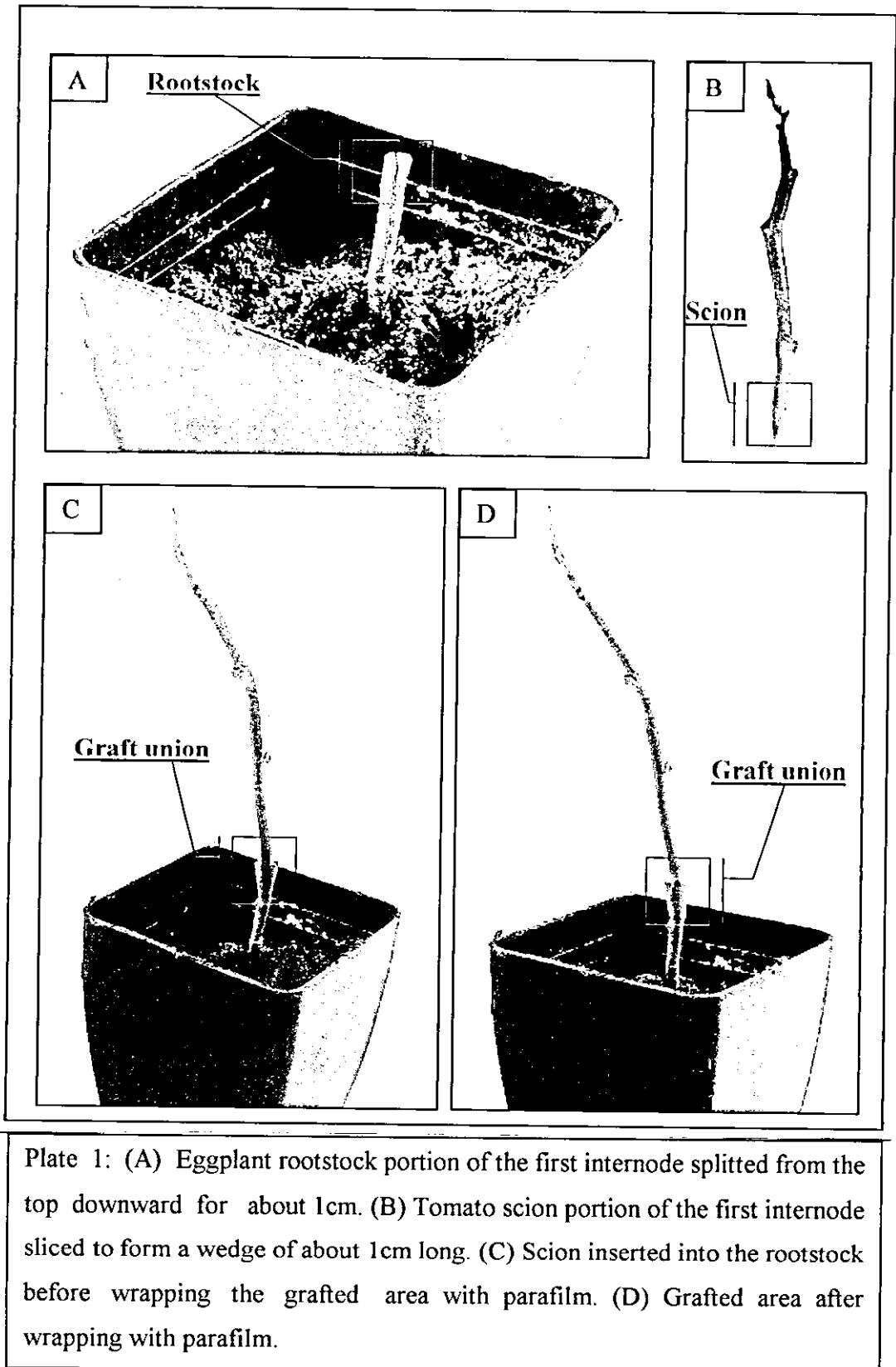
(1) 'Shantta' and 'Maram' purchased from Royal Sluis Company and 'GS 12' purchased from Agriculture Supply Company (ASCO).

Table 1: Diameter of the first internode (site of later grafting) of tomato, eggplant and pepper seedlings.

Leaves (No.) \ Plant	2	3	4	5	6	7
	Diameter (mm)					
Tomato	1.8	2.3	2.7	3.2	3.7	4.3
Eggplant	1.6	1.8	2.4	2.7	3.2	3.7
Pepper	1.4	1.6	1.9	2.0	2.6	2.7

Leaves on the scion were removed, except for those folded leaves at the top. The scion portion of the first internode was sliced to form a wedge of about 1cm long (Plate 1B) and inserted into the rootstock (Plate 1C). The grafted area was wrapped by parafilm (Beineke, 1978) to ensure adequate contact between tissues of the scion and rootstock (Plate 1D).

The grafted plants were transferred to a bench covered with a plastic tunnel equipped with a misting system (Plate 2). The system was operated for 10 seconds every 15 minutes for the first 2 days. Then from day 3 until day 7 gradual increase in the intervals of misting was performed (plus 15 minutes each day). Then the plants were transferred to another bench covered with a plastic tunnel for 3 days. Thereafter, plants were transferred and placed under greenhouse conditions. Thirty-six grafted plants of each of 9 treatment combinations (auto- and heterografts) (Plate 3 A, B and C), respectively were prepared and arranged on the bench in plastic trays using a completely randomized design (Plate 4).



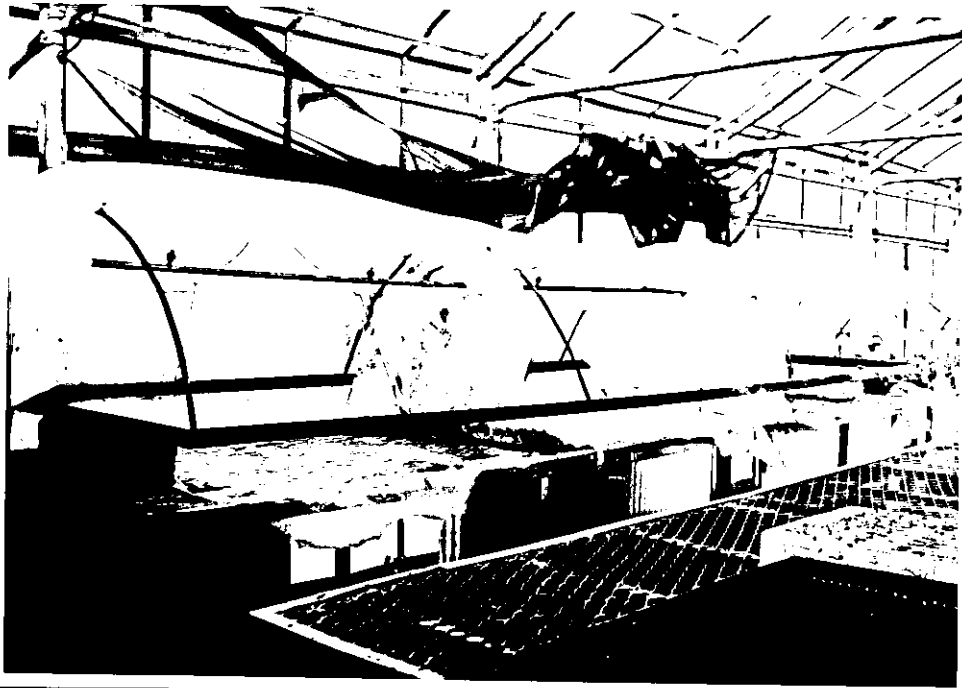


Plate 2: A bench covered with a plastic tunnel equipped with a misting system.

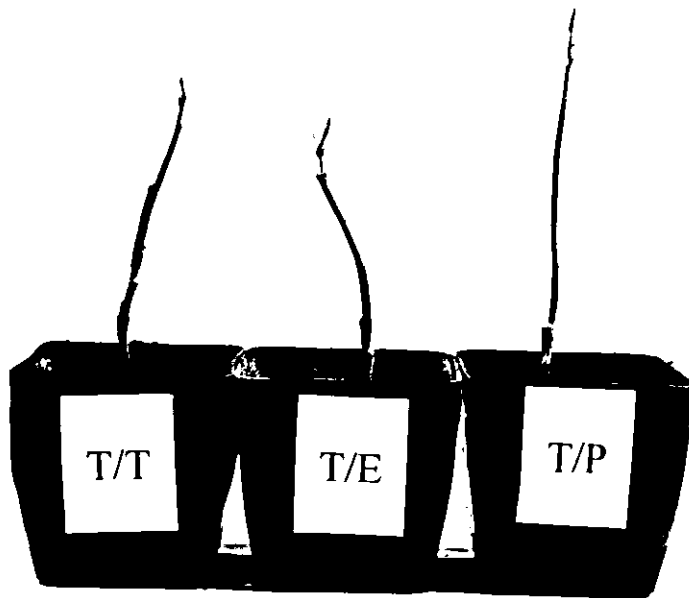


Plate 3: (A) Three grafting combinations: tomato/tomato (T/T), tomato/eggplant (T/E) and tomato/pepper (T/P).

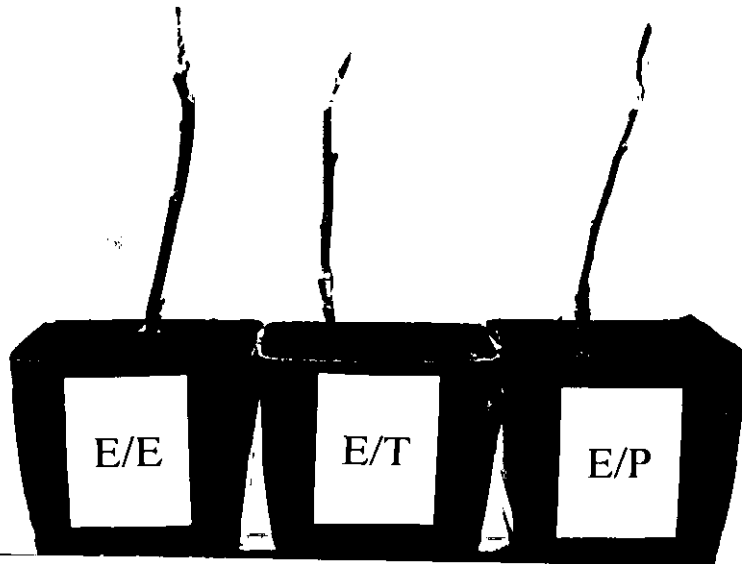


Plate 3: (B) Three grafting combinations: eggplant/eggplant (E/E), eggplant/tomato (E/T) and eggplant/pepper (E/P).

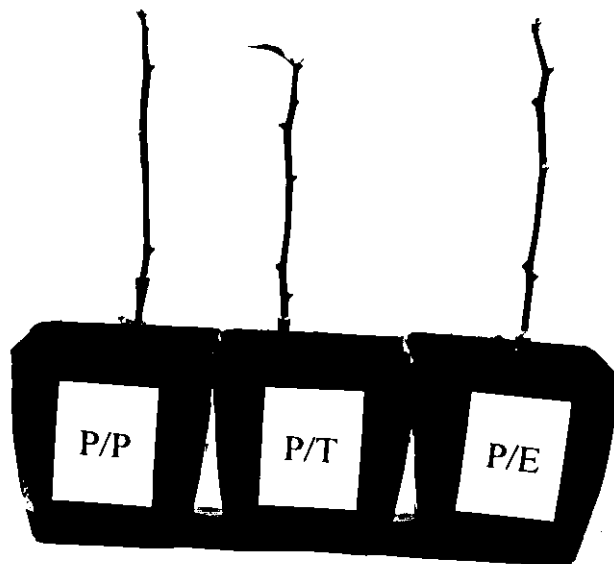


Plate 3: (C) Three grafting combinations: pepper/pepper (P/P), pepper/tomato (P/T) and pepper/eggplant (P/E).

Plate 4: Layout of the experimental seedlings in plastic trays in the greenhouse.

9		1		6		9	2			9
5	6	4	8	3		5		9		6
1		3	4	2	3	4	1	8	2	9
		5		1	5	7			8	3
3	6	3	8	6	4	2	7	9		7
6	1	4	2		6		4	8	1	4
		8		7	5	8	3		8	9
4	2	5		6	3	6	5		1	7
5	6	4	8	3	9	2	7	8	9	6
2	8	2	3	7		8		1	1	4
6	3	1		6	5		6	5	3	5
	4	5	2		2	3	7		2	7
5	8		8	3	7			9	6	
2	1	2	5	6	9	8	3	1		7
3		3		7	4	2	5	7		3
	4	7	1	8	5		6	4	5	
3	6	1	4	3	7	4	9	1	7	9
5		3	6	2	8		2	5	4	5
4	2	7	9		7	9		6		9

	7	4	1	2	6	9		4	6	4
3	8			7		1	8	5	1	9
9	3	5	8	4	9	6	5		2	
2	6	3		1	8		1	9		4
1	7	9	5		6	1		6	2	1
8			8	7	3	7	5	3	5	9
9	4	1		1	9		2	8	4	
	2	9	5	6	8	5	6		7	1
8	6	3	8	7	2	7	4	2	5	6
2	7			1	9	1		8	3	
5	3	5	2	6	3	7	9	1	9	3
	8		4		8	4	2	1		8
1		7	8	7	9	5		4	3	
	2	4		4	3		7	9	1	4
8	6	7	5	8		1	2		7	3
2	3	9	6		7	4		4	5	6
	1	4		5	1		7		1	8
2	9	6	8	9		5	2		3	
	2			2	4		9	7	9	6

- | | | |
|--------------------|-----------------------|---------------------|
| 1: Tomato/tomato. | 4: Eggplant/eggplant. | 7: Pepper/pepper. |
| 2: Tomato/eggplant | 5: Eggplant/tomato. | 8: Pepper/tomato. |
| 3: Tomato/pepper. | 6: Eggplant/pepper. | 9: Pepper/eggplant. |

3.3 Measurement of Growth Characteristics

Throughout the experiment, measurements were made on plant growth parameters including plant height, fresh and dry weights of scion and rootstock. These measurements were recorded at four developmental stages (5, 10, 15 and 20 days). A sample of four plants was taken at random from each group of graft combinations. Each plant was considered a replicate. Plant material was oven dried to a constant weight at 75°C for 48 hours, then the dry weight was recorded for each part.

3.4 Anatomical Study

Graft unions at various developmental stages (5, 10, 15 and 20 days after grafting) were surgically isolated and immediately fixed in formalin-acetic acid fixative solution (ethanol 50 ml, acetic-acid glacial 5ml, formalin 10ml and distilled water 35ml) for 72 hours at room temperature. Then, dehydrated and infiltrated (Kiswani, 1994) as shown below (Table 2).

Table 2: Tissue dehydration and infiltration series.

Dehydration			
Step	Ethyl alcohol (%)	Chloroform	Time (hr.)
1	50	-	3
2	60	-	3
3	70	-	3
4	80	-	3
5	90	-	3
6	100	-	over night
Infiltration			
7	3	1	3
8	1	1	3
9	1	3	3
10	-	Absolute	3
11	Melted paraffin at 60 °C		48

Some wax chips (60°C melting point) were added to the vials containing graft unions and absolute chloroform. Vials were placed on a hot plate at about 40°C and more wax chips were added every 6 hours. After 48 hours the wax was replaced with a new molten one. The vials were placed inside an oven at 60°C for another 3 days replacing the wax every day to insure that all the chloroform had completely evaporated (Kiswani, 1994).

Embedding was done using iron embedding molds. The contents of the vials were poured into the molds and the graft unions were oriented well using hot needle. Wax was allowed to solidify and harden at room

temperature for few minutes. The wax blocks were sectioned (15 μ m) using rotary microtom (American Optical Model). Five ribbon sections were laid on surface of 40°C hot water for 15 seconds in order to be stretched. Sections were then mounted on a clean slide smeared with a thin film of egg albumen (Kiswani, 1994). The egg albumen was prepared by filtrating 5gm albumen powder plus 100ml distilled water plus 0.5gm sodium chloride by Buchner Funnel and adding 50 ml glycerin to 50ml of the filtrate. Few crystals of thimol were added to prevent fungal development (Lutfe and Al-hajj, 1984). The slides were then left to dry at room temperature for overnight followed by wax removal. Staining was performed after the procedure of Kiswani (1994) (Table 3).

Table 3: Staining schedule of graft union sections.

Step	Time
Xylene	2-5 min
Xylene: Absolute alcohol ⁽¹⁾ (1:1)	5 min
Absolute alcohol	5 min
95% alcohol	5 min
90% alcohol	5 min
80% alcohol	5 min
70% alcohol	5 min
60% alcohol	5 min
50% alcohol	5 min
Safranin ⁽²⁾ (in 50% alcohol)	1-2 min
Alcian blue ⁽³⁾ (in 50% alcohol)	1-2 min
50% alcohol	3-5 sec
60% alcohol	3-5 sec
70% alcohol	3-5 sec
80% alcohol	3-5 sec
90% alcohol	3-5 sec
95% alcohol	3-5 sec
Absolute alcohol	3-5 sec
Clove oil	10-15 sec
Xylene	10-15 sec

(1) Ethanol. (2) 5gm. (3) 5gm.

Few drops of D. P. X. ⁽¹⁾ were added on the slides and covered with a coverslip. The slides were left on a hot plate at 40°C (Stuart Scientific Model) for 2 days until the D. P. X. dried up (Lutfé and Al-hajj, 1984 and Kiswani, 1994). The slides were then examined for vascular connections across the graft union using a compound light microscope (HM-Lux 3 Model).

3.5 *Orobanche ramosa* infestation study

A pot experiment was conducted in a glasshouse maintained at a day temperature of about 22-25 °C and a night temperature of about 15°C during 1997. Seeds of *Orobanche ramosa* collected from the Jordan Valley were obtained from Plant Protection Laboratory, Faculty of Agriculture, University of Jordan. 500mg of broomrape seeds were mixed with 300g of methylbromide fumigated soil sand mix [3 : 1(v/v)]. The mixture containing broomrape seeds was thoroughly homogenized with 2.2kg of fumigated sandy-soil and transferred to a two-liter plastic pot.

Twenty days after grafting, seedlings were transplanted on May 25, 1997. Ungrafted control plants of each cultivar were also included in the experiment. Each treatment consisted of one plant per pot. The twelve treatments were arranged according to a randomized complete block design with 4 replicates. Plants were irrigated with Haogland Solution (Table 4) at 250ml per liter once every 10 days. Protective pest sprays were done fortnightly using Afugan (12 cm³/20L) and Dursban (40 cm³/20L). Ten weeks after infestation, when some of the broomrape plants had emerged, the soil mix was washed carefully from the roots and the following parameters were recorded: mean number, length, fresh and

(1)D. P. X.: 'Distrene 80' 25g, Tri *p* tolyl phsphate 18.75ml and Xylene 100ml (Peacock and Bradbury, 1973).

dry weight of parasitic shoots emerging above and below soil. Mean fresh and dry weight of plants, shoots and roots were also recorded. Plant shoots, roots, *Orobanche ramosa* flowering shoots and haustoria were dried to a constant weight at 75°C for 48 hours.

Table 4: Haogland Solution (Hammer *et al.*, 1978).

<u>Concentrate A</u>		<u>Concentrate B</u>	
Salt	(g/liter)	Salt	(g/liter)
KNO ₃	50.55	Ca(NO ₃) ₂ .4H ₂ O	118.08
KH ₂ PO ₄	13.61		
MgSO ₄ .7H ₂ O	49.30		
NaCl	5.85		
<u>Concentrate C</u>		<u>Concentrate D</u>	
Salt	(g/liter)	Salt	(g/liter)
FeSO ₄ .7H ₂ O	2.49	H ₃ BO ₃	2.850
KOH (85%)	1.77	MnSO ₄ .H ₂ O	1.538
Fe-EDTA	2.61	ZnSO ₄ .7H ₂ O	0.219
		CuSO ₄ .5H ₂ O	0.078
		H ₂ MoO ₄ (85% MoO ₃)	0.020

3.6 Statistical Analysis

The experiment related to measurement of growth characteristics was arranged according to a completely randomized design, while the experiment related to *Orobanche ramosa* infestation study was arranged according to a randomized complete block design. The data was analyzed by computer using analysis of variance through the SAS program (SAS Institute, Inc., 1988). Mean separation was performed by using Duncan Multiple Range Test (DMRT) for the two experiments.

4. Results

4.1 Compatible Combinations

Graft unions at 5, 10, 15 and 20 days after grafting were surgically isolated in order to investigate the histological changes during graft growth and development. Five days after grafting, initial cohesion was observed between compatible graft combinations including tomato/tomato [Plate 5: (I a)], tomato/eggplant [Plate 5: (II a)] and eggplant/tomato [Plate 6: (I a)]. Cells adjacent to the cut surfaces of stock and scion were stimulated to divide, giving rise to callus. This callus was associated with the vascular cambium, endodermis, phloem parenchyma, xylem parenchyma and the outermost layers of the cortex. In both compatible auto- and heterografts, some adjacent cells, by contrast, were ruptured when making the graft incision. Such ruptured cells collapsed when scion and stock were placed together. These collapsed cells formed a distinct dark line; the isolation layer (Plate 7), which was initially continuous along the graft interface, delimiting the scion from both parts of the stock. At this stage of development absence of vascular connections or even primary cohesion in eggplant/eggplant [Plate 5: (IIIa)], eggplant/tomato and pepper/pepper [Plate 6: (Ia), (IIa), respectively] was observed. This disconnection was reflected by presence of the isolation layer and gaps between stock and scion.

Ten days after grafting in the compatible auto- and heterograft systems including; tomato/tomato, tomato/eggplant, eggplant/eggplant [Plate 5: (Ib), (IIb), (IIIb), respectively], eggplant/tomato and pepper/pepper [Plate 6: (Ib), (IIb), respectively], callus cells (Plate 8) proliferated and expanded filling the graft gap thus covering the severed xylem and phloem elements.

Plate 5

Cross sections of auto- and heterografts of tomato/tomato, tomato/eggplant and eggplant/eggplant (①: Scion, ②: Stock and ③: The isolation layer) showing:

- (I)
 - a. Five-day-old tomato/tomato. Callus cells division at the graft interface. permit initial cohesion between scion and stock. X100.
 - b. Ten-day-old tomato/tomato. Graft bridging cambium has been established across the graft interface. X100.
 - c. Fifteen-day-old tomato/tomato. Differentiation of vascular tissues across the graft interface is very evident. X100.
 - d. Twenty-day-old tomato/tomato. Completion of vascular connections with little patches of the isolation layer at pith region. X100.
- (II)
 - a. Five-day-old tomato/eggplant. Callus cells division at the graft interface, permit initial cohesion between scion and stock. X100.
 - b. Ten-day-old tomato/eggplant. Graft bridging cambium establishment across the graft interface. X100.
 - c. Fifteen-day-old tomato/eggplant. Establishment of vascular connections between stock and scion. Partial disconnection between epidermis and cortex is observed. X100.
 - d. Twenty-day-old tomato/eggplant. Complete graft union between scion and the two parts of the stock. X40.
- (III)
 - a. Five-day-old eggplant/eggplant. Connection layer has been not yet established. X40.
 - b. Ten-day-old eggplant/eggplant. Graft bridging cambium has been established across the graft interface. Pith region lack callus division. X100.
 - c. Fifteen-day-old eggplant/eggplant. Differentiation of vascular tissues across the graft interface is very evident, with callus proliferation and interdigitation at pith region. X40.
 - d. Twenty-day-old eggplant/eggplant. Completion of vascular connections with very limited patches of the isolation layer at pith region. X100.

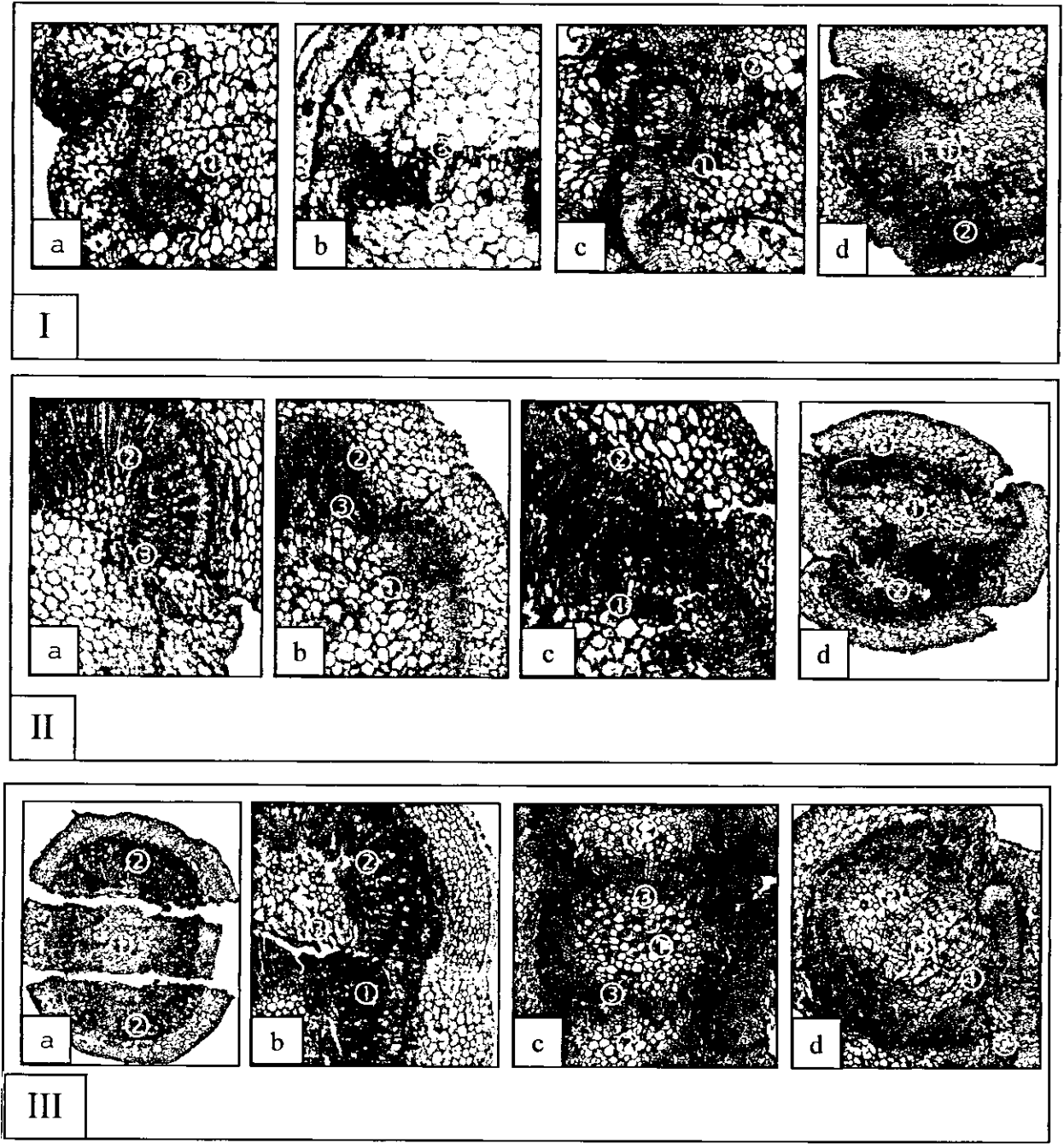


Plate 5

Plate 6

Cross sections of auto- and heterografts of eggplant/ tomato, pepper/pepper and tomato/pepper (①: Scion, ②: Stock and ③: The isolation layer) showing:

- (I) a. Five-day-old eggplant/tomato. Callus cells division at the graft interface, permit initial cohesion between scion and stock at one side. X40.
 b. Ten-day-old eggplant/tomato. Graft bridging cambium establishment across the graft interface, associated with cohesion at the epidermis and cortex regions. X100.
 c. Fifteen-day-old eggplant/tomato. Establishment of vascular connections between stock and scion. Callus proliferation permit rupture of the isolation layer. X100.
 d. Twenty-day-old eggplant/tomato. Complete graft union between scion and stock. X40.
- (II) a. Five-day-old pepper/pepper. Disconnection between scion and the two parts of stock due to existence of gap and the isolation layer. X40.
 b. Ten-day-old pepper/pepper. Graft bridging cambium has been established across the graft interface. Pith region lack callus division. X100.
 c. Fifteen-day-old pepper/pepper. Differentiation of vascular tissues across the graft interface is very evident, with callus proliferation and interdigitation at pith region. X100.
 d. Twenty-day-old pepper/pepper. Completion of vascular connections with very limited patches of the isolation layer at pith region. X40.
- (III) a. Five-day-old tomato/pepper. Disconnection between scion and the two parts of stock due to existence of gap and the isolation layer. X100.
 b. Ten-day-old tomato/pepper. Callus cell divisions were limited with very distinct isolation layer between stock and scion. X100
 c. Fifteen-day-old tomato/pepper. Partial disconnection as gap is observed. X100.
 d. Twenty-day-old tomato/pepper. Progressive thickening in the isolation layer. Vascular connections are absent. X100.

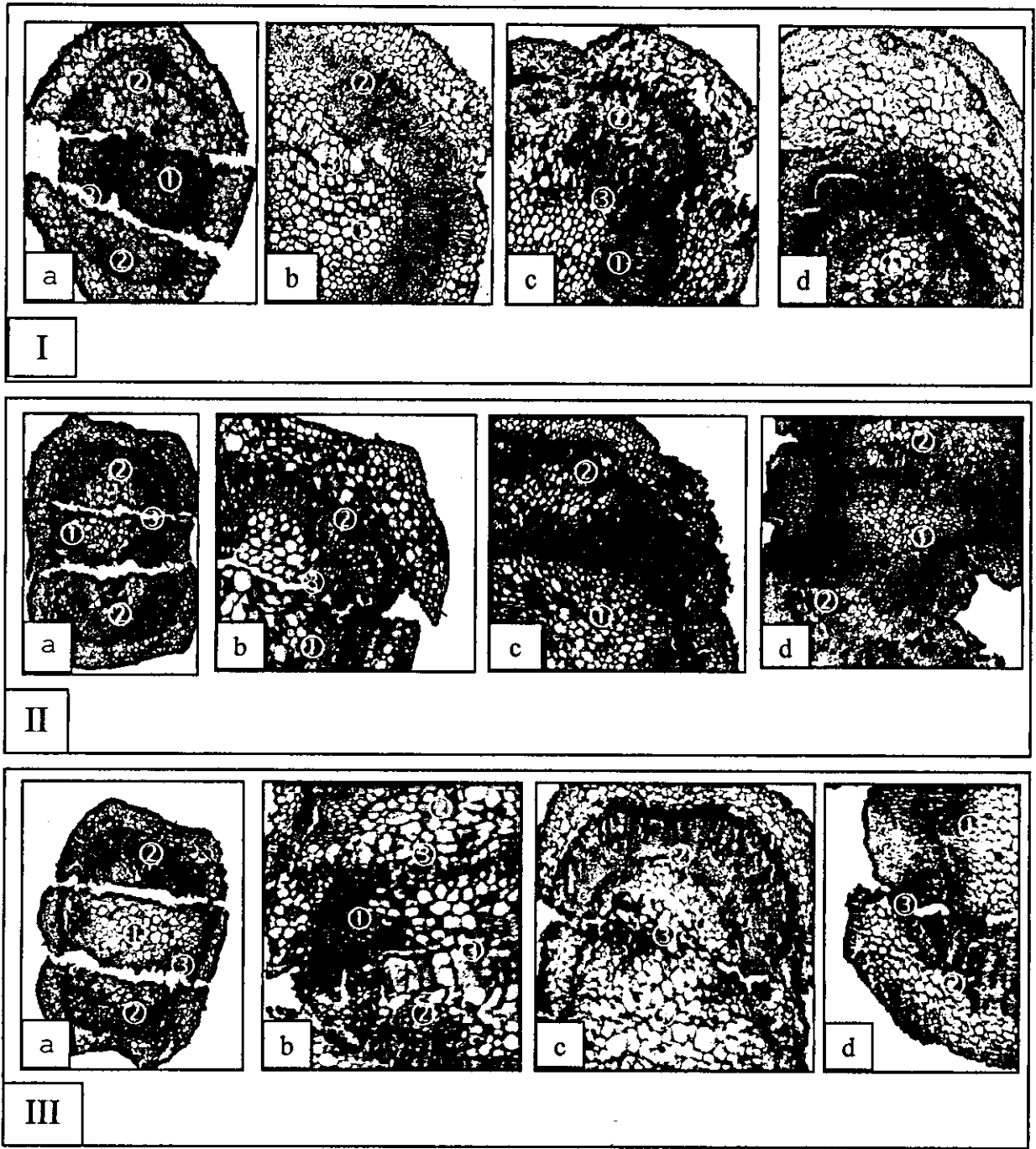


Plate 6

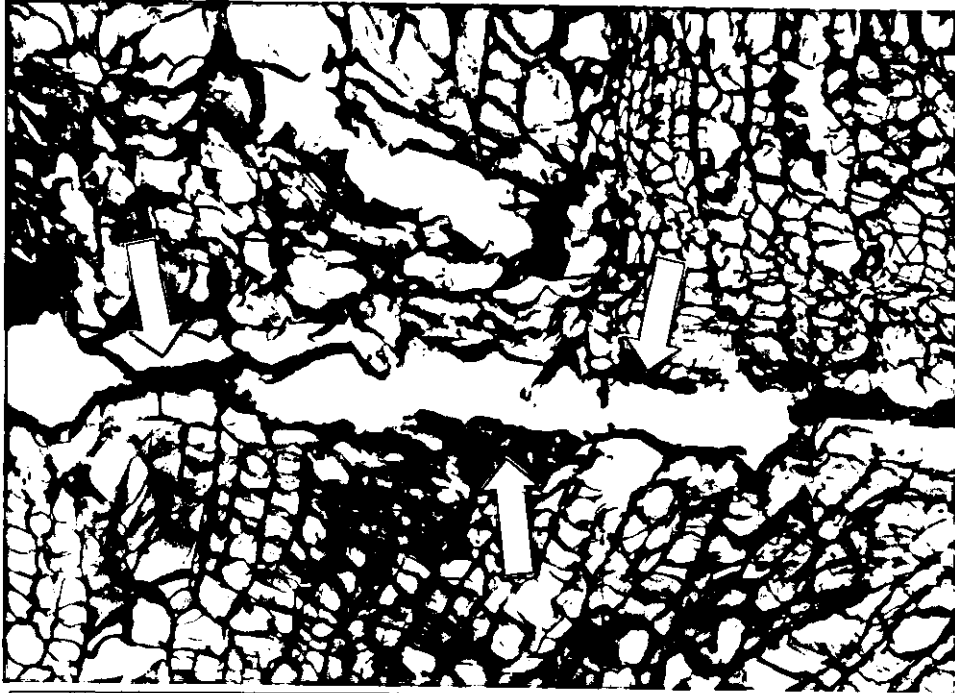


Plate 7: The isolation layer (white arrows) in a 5-day-old cleft graft of tomato/tomato. X400.

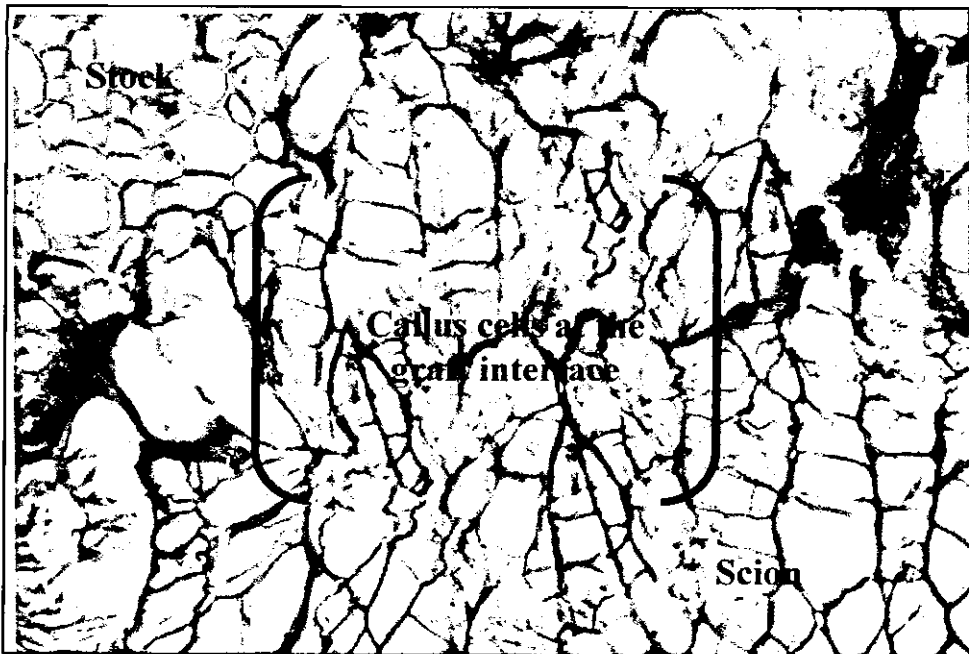


Plate 8: Callus cells at the graft interface of a 10-day-old cleft-graft of tomato/eggplant. X400.

The line of union becomes quite irregular as groups of callus cells push past each other and interdigitate. The orientation of these cells was perpendicular to the original graft interface. Thus, a substantial 'Callus Bridge' was formed between the two graft partners. This activity is more pronounced in the scion [Plate 5: (Ib)] than in the stock which may reflect the photosynthetic capacity of the scion. Proliferation and interdigitation of the callus cells resulted in the rupture and gradual fragmentation of the initially continuous isolation layer with the remaining fragments appearing as patches of collapsed cell walls. In eggplant/eggplant [Plate 5: (IIIb)], eggplant/tomato and pepper/pepper [Plate 6: (Ib), (IIb), respectively] these collapsed cell walls were interspersed between the large callus cells largely at pith region with some gaps that disconnect the two graft partners from each other.

Cambium differentiation across the graft interface occurred 15-days after grafting. These cambium cells appeared as continuous strand that differentiated through the homogenous 'callus bridge' forming a graft bridging cambium to connect the vascular tissues of stock and scion (Plate 9). This bridge has the shape of an arc that curved out-ward toward the cortex [Plate 5: (Ic)]. Establishment of graft bridging cambium was applicable to all compatible graft combinations at this stage of development. The xylem elements were differentiated from the inner face of bridging cambium, whereas the outer face of the cambial cells produced a narrow layer of parenchyma cells among which were occasional phloem elements (Plate 9). The isolation layer has in many places been surrounded by the invading callus and is much reduced in amount. Furthermore, complete union between the epidermal and outer cortical layers of the graft partners was observed in tomato/tomato, eggplant/eggplant [Plate 5: (Ic), (IIIc), respectively] and pepper/pepper [Plate 7: (IIc)].

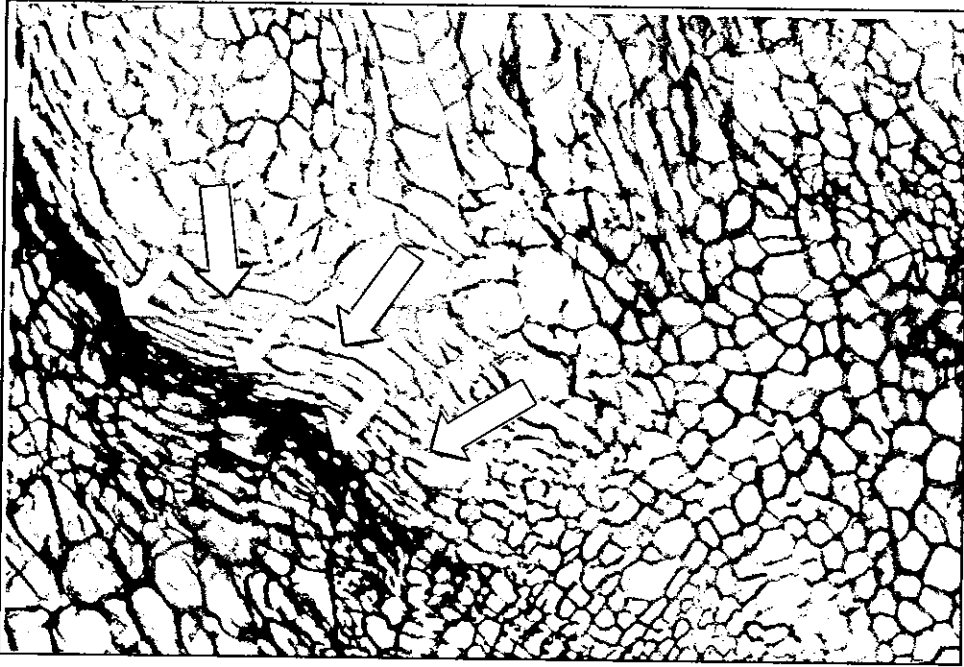


Plate 9: Tomato autograft at 15 days after cleft grafting: (A) bridging cambium (yellow arrows) and (B) Connections of vascular tissues (white arrows) of stock and scion. X400

The orientation of many cells filling the gap at the graft interface, indicate that the cells are derived from the scion part [Plate 5: (Ic)]. While the pith parenchyma cells in 15-day-old tomato/tomato, tomato/eggplant, eggplant/eggplant [Plate 5: (Ic), (IIc) and (IIIc), respectively] and eggplant/tomato [Plate 6: (Ic)] have not yet proliferated and the isolation layer overlaying it was not affected, the 15-day-old pepper/pepper graft combinations showed slight callus interdigitation across the graft interface at pith region [Plate 7: (IIc)]. Such cell division of parenchyma cells crossing the graft interface at pith region largely proceeded by the scion.

By twenty days after grafting xylem differentiation across the graft interface was complete [Plate 5: (IIIId)]. This tissue is made up of vessels and tracheid cells with thick lignified walls. The phloem elements are similarly differentiated across the graft interface. The divisions in the pith parenchyma also have been observed forming cells that were perpendicular to the cut surfaces. The isolation layer is largely eliminated and the graft line is eventually obliterated mostly between xylem and the epidermis. In tomato/eggplant, eggplant/eggplant [Plate 5: (IIId), (IIIId), respectively] and eggplant/tomato [Plate 6: (Id)] the exact location of the graft interface was difficult to be determined. In tomato/tomato [Plate 5: (I d)] and pepper/pepper [Plate 7: (IIId)] the isolation layer is largely invaded by interdigitated callus cells and have been seen only in small places at the pith region as thin wall.

Correspondingly, the structural development of tomato/tomato, tomato/eggplant, eggplant/eggplant [Plate 5: I, II and III, respectively], egg-plant/tomato and pepper/pepper [Plate 6: I, II, respectively] that showed compatible grafting, was supported by high survival ratios that were observed in autografts of the three cultivars as well as in the reciprocal heterograft combinations between tomato and eggplant (Table

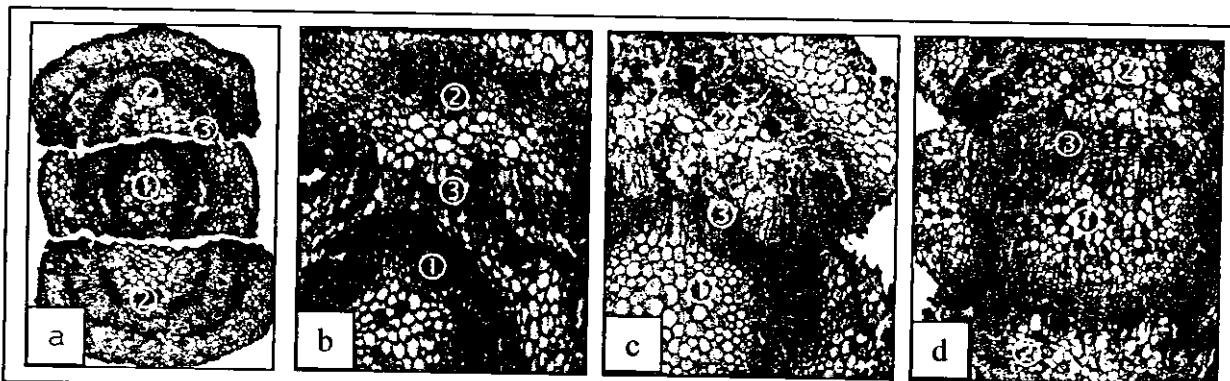
5). This indicates that successful graft formation has been established between previous combinations.

Table 5: Survival ratio (%) of auto- and heterografts of tomato, eggplant and pepper.

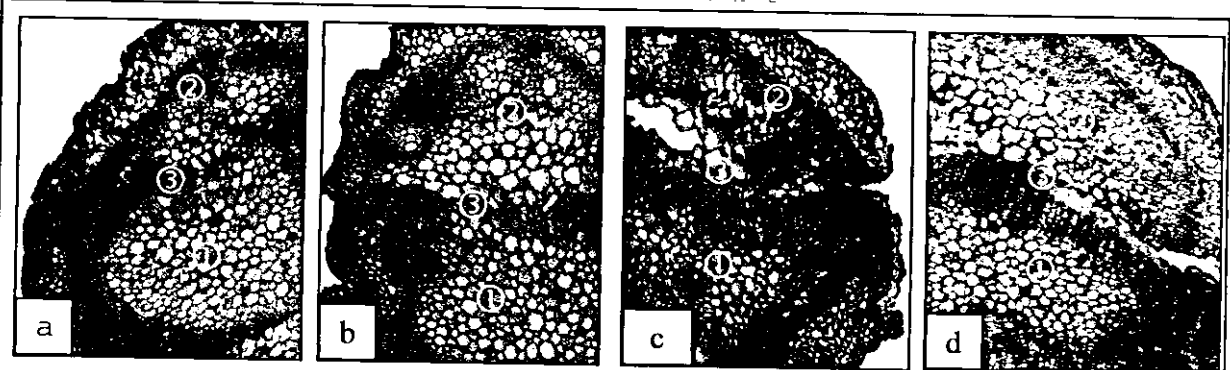
Stock	Scion		
	Tomato	Eggplant	Pepper
	Survival Ratio (%)		
Tomato 'GS 12'	99	98	0
Eggplant 'Shantta'	97	99	0
Pepper 'Maram'	0	0	98

4.2 Incompatible Combinations:

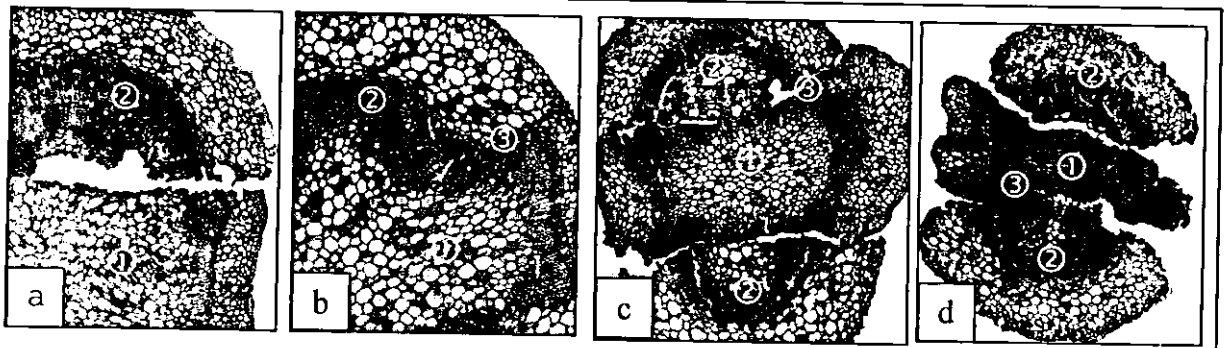
Five days old grafts including tomato/pepper [Plate 6: (IIIa)], eggplant/pepper and pepper/eggplant [Plate 10: (IIa) and (IIIa), respectively] revealed complete separation and disconnection imitated by existence of large gaps associated with the isolation layer between stock and scion along the graft interface. On the other hand, slight initial cohesion has been noticed at five days after grafting in pepper/tomato [Plate 10: (Ia)]. This primary cohesion was as a result of few callus divisions at the site of vascular tissues. Ten-day-old tomato/pepper [Plate 6: (IIIb)], eggplant/pepper and pepper/eggplant [Plate 10: (IIb) and (IIIb), respectively] grafts showed very low callusing along the graft union, with almost absent expansion of those new divided cells across the graft interface.



I



II



III

Plate 10

The isolation layer was very distinct, and delimited the scion from both parts of the stock. Accordingly, cohesion between the graft partners was very weak, since it occurs at cell walls from which the materials of the isolation layer has disappeared, or at walls of cells invading the gap that has never had such a layer. In pepper/tomato [Plate 10: (IIb)] a cohesion apparently occurred at the cortical and epidermal regions between stock and scion since callus cells division and interdigitation have been established at these regions. On the other hand, the isolation layer at the cut surface between pepper and tomato and along the pith and vascular tissues was still distinct. When the graft combinations were examined at fifteen days after grafting, it was found that the graft interface contained numerous new cells. In tomato/pepper [Plate 6: (IIIc)], pepper/tomato and pepper/eggplant [Plate 10: (IIc), (IIIc), respectively] there was neither cambium differentiation nor any xylem strands connections. In eggplant/pepper [Plate 10: (Ic)] callus cells division and proliferation along the graft union was obvious. The cells were oriented perpendicular to the graft interface, but xylem and phloem bridge formation was absent. Twenty-day-old grafts of tomato/pepper [Plate 6: (IIId)] and pepper/eggplant [Plate 10: (IIId)] revealed obvious disconnection of scion from both parts of stock. This was attributed to existence of gaps and progressive increase in the thickening of the isolation layer along the graft interface. Similarly, the isolation layer was also observed along the interface in pepper/tomato [Plate 10: (IIId)]. Cambial differentiation and vascular connections were absent. Although, callus cells division and proliferation was noticed along the graft union in eggplant/pepper after 20 days of grafting [Plate 10: (IIId)], the rupture and disappearance of the isolation layer was not observed. The existence of the isolation layer starting from five-day-old grafts until twenty days after grafting was

apparently distinct and comprised the graft interface of tomato/pepper (Plate 6: III), eggplant/pepper, pepper/ tomato and pepper/eggplant (Plate 10: I, II and III, respectively). In all previous incompatible systems the isolation layer was never ruptured and prevented cell contact between stock and scion. The absence of callus proliferation and interdigitation at the graft interface presumably prevent bridging cambium to be established, which cause lack of vascular junction and prevent the movement of water and nutrients across the grafts union leading to the death of scion. Support for this view comes from nil survival ratio for previous graft combinations (Table: 5). In other words, the compatibility between tomato and pepper and between eggplant and pepper was absent.

Unnaturally, one replicate of eggplant/pepper [Plate 11: (A)] and pepper/tomato [Plate 11: (B)] as incompatible heterografts remained stunted for three months after grafting then died. From anatomical view, eggplant/pepper [Plate 12: (A)] revealed that large callus cells have been differentiated from both partners along the graft interface. As a result, cohesion was established between the parenchyma cells of both partners. Nevertheless, the isolation layer resulted from collapsed cells at the graft interface was very distinct and preventing xylem bridging to be established across the graft line. In pepper/tomato graft [Plate 12: (B)] although callus cells exhibited high differentiation and proliferation along the graft interface from both graft partners with minimal xylem bridging establishment, the isolation layer was still distinct. Existence of the isolation layer largely at the vascular tissues for both parts of the graft prevents complete vascular tissues to be connected. Furthermore, many places at the graft interface revealed progressive increase in the thickening of the isolation layer which finally lead to complete disconnection between stock and scion.



P



P/P

Plate 16: Ten-week-old pepper (A) and pepper/pepper (B) parasitized with branched broomrape.

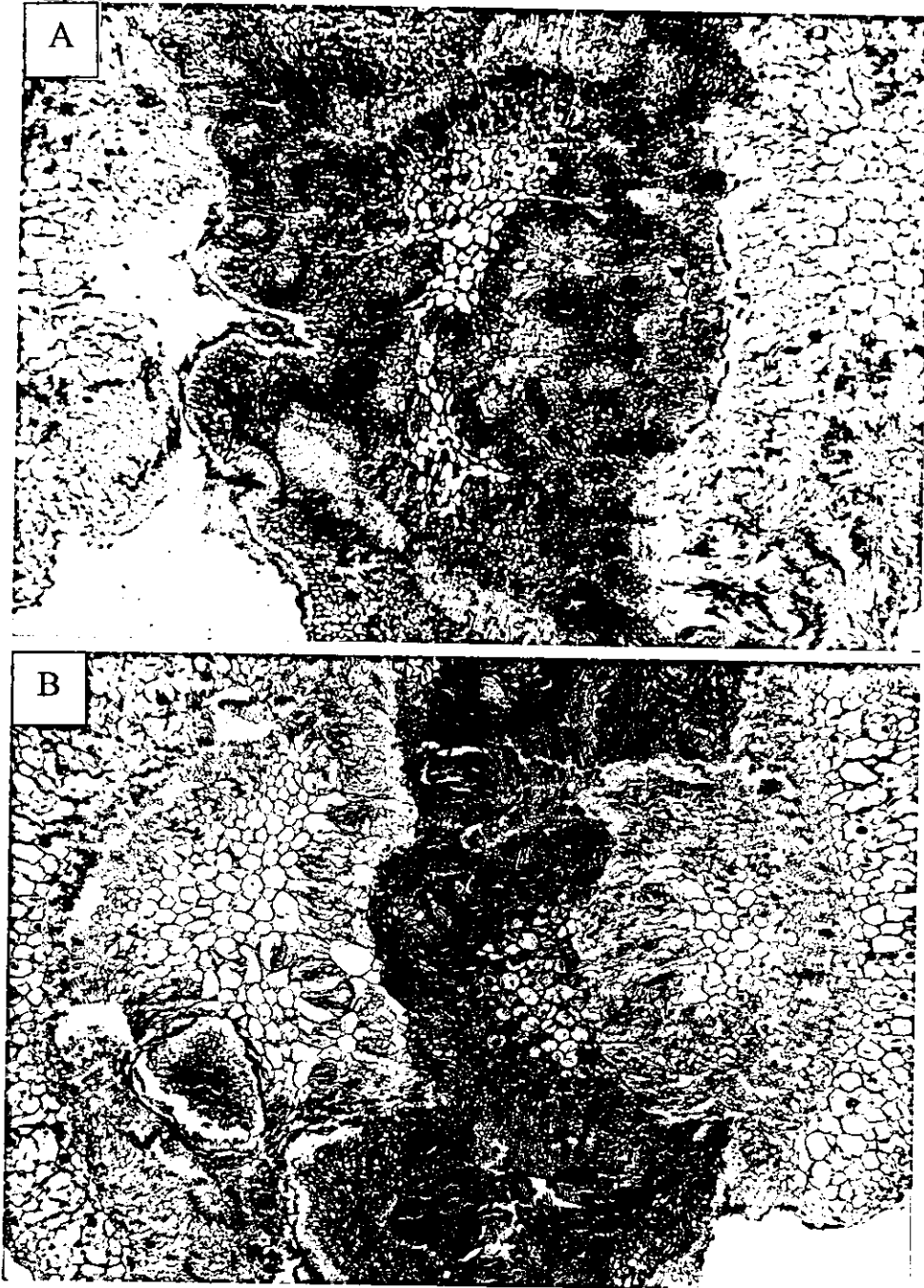


Plate 12: A cross-section in a three-month-old graft of eggplant /pepper (A) and pepper/tomato (B)

4.3 Resistance of grafted plants to *Orobanche ramosa*

Four parameters related to *Orobanche ramosa*, i.e., mean number, length, fresh and dry weight of parasitic shoots emerging above and below soil surface were recorded as a measurement for the resistance of compatible graft combinations to *Orobanche* after ten weeks of infestation. Although non-of the tested treatments were absolutely free from this parasite, differences in responses of graft combinations were observed for all measured parameters.

Expressing resistance by mean number of parasitic shoots that emerged above soil, indicated significant variation among treatments that ranged from 18.75 and 15.75 for tomato and tomato/eggplant, respectively, to 2.31 and 0.50 for pepper and pepper/pepper, respectively (Fig. 1). Furthermore, pepper and pepper/pepper produced significantly fewer mean number of parasitic shoots below soil surface (3 and 2.25, respectively) as compared with tomato, tomato/tomato and eggplant/tomato which exhibited the highest values of 27.25, 27.50 and 25.75, respectively (Fig. 1).

The average length of parasitic shoots above soil surface, was lowest for pepper and pepper/pepper (2.00 and 2.75cm, respectively) and highest for eggplant and tomato (13.92 and 11.26cm, respectively) (Fig. 2); furthermore, eggplant gave significantly greater length of parasitic shoots as compared to pepper and pepper/pepper. Average length of parasitic shoots below soil showed that the only significant variation observed was among eggplant, pepper and pepper/pepper (Fig. 2); pepper and pepper pepper have the lowest average length (0.25 and 0.50cm, respectively) while eggplant showed the highest average length (4.7cm).

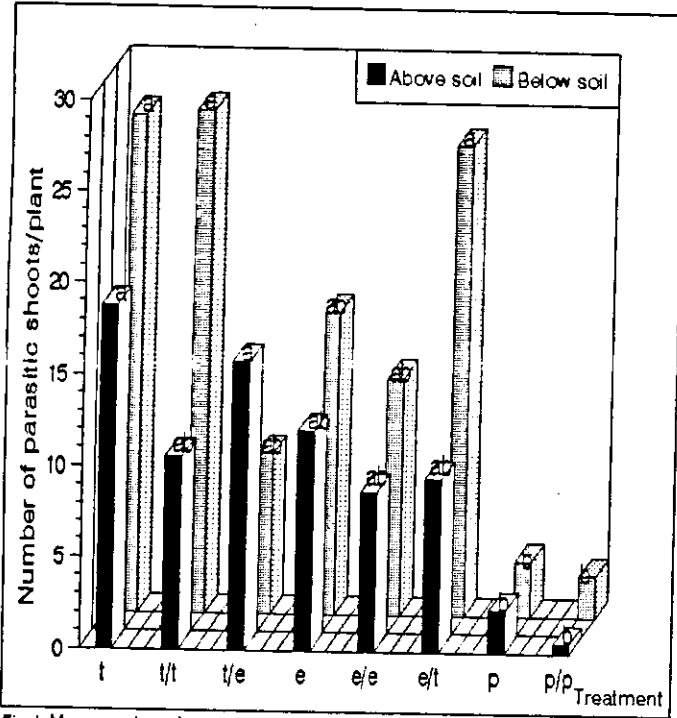


Fig.1: Mean number of parasitic shoots above and below soil surface per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

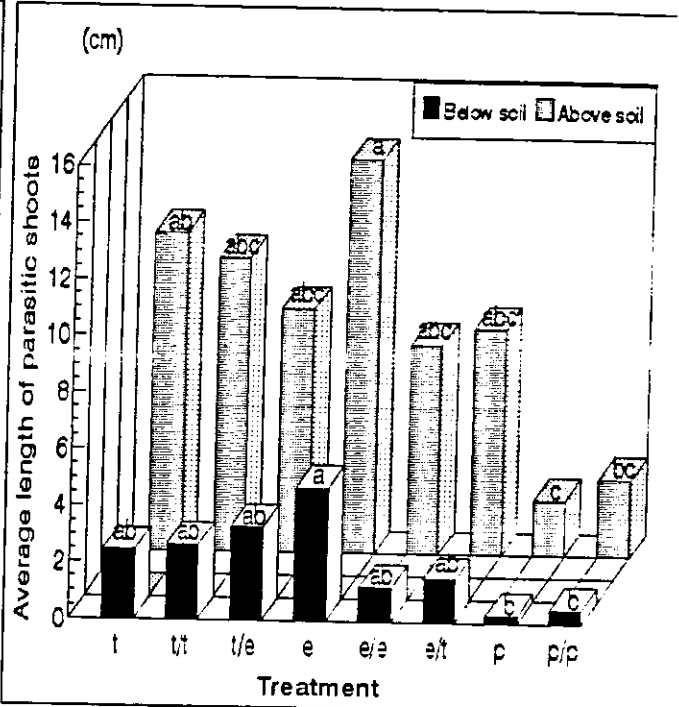


Fig.2: Average length of parasitic shoots above and below soil surface per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

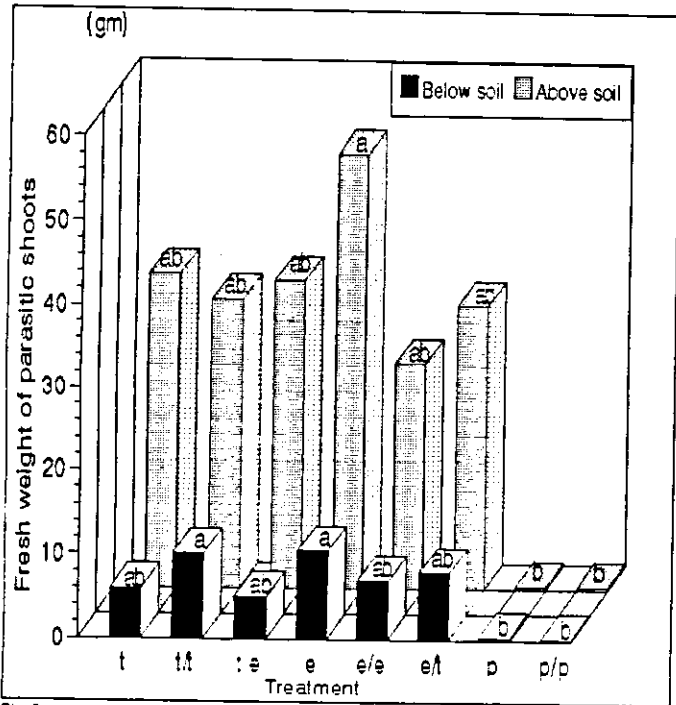


Fig.3: Mean fresh weights of parasitic shoots above and below soil surface per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

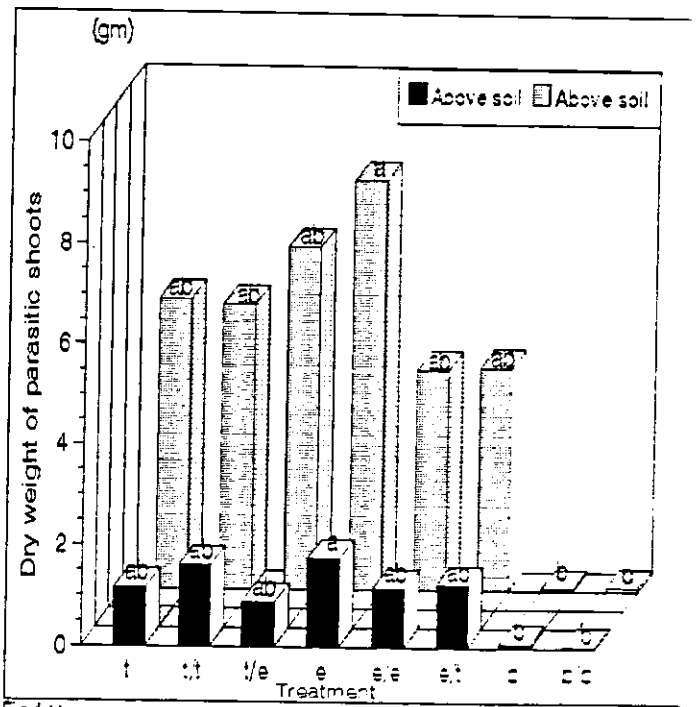


Fig.4: Mean dry weights of parasitic shoots above and below soil surface per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

Regarding mean fresh weight of parasitic shoots above soil surface, pepper and pepper/pepper have significantly lowest values (0.30 and 0.32gm, respectively) as compared to eggplant which gave the highest value (51.95gm) (Fig. 3); moreover, fresh weights of parasitic shoots below soil surface were significantly lowest in pepper, pepper/pepper (0.187 and 0.078gm, respectively) compared with those of tomato/tomato and eggplant (10.01 and 10.44gm, respectively). Pepper and pepper/pepper exhibited significantly lower mean dry weights of parasitic shoots above soil (0.032 and 0.062gm, respectively) compared with eggplant (8.15gm) (Fig. 4); the dry weight of parasitic shoots below soil surface were significantly greater for eggplant as compared with pepper and pepper/pepper; the remaining treatments had almost similar dry weights.

Mean fresh weight of plant and shoot exhibited no significant variation among treatments, except for mean fresh weight of root (Fig. 5), where pepper and pepper/pepper have the highest values (25.71 and 23.50gm, respectively) while eggplant gave the lowest value (7.15gm). Mean dry weight of shoot showed no significant variation among treatments (Fig. 6). In contrast mean dry weight of root was significantly different among pepper and pepper/pepper and eggplant treatments, where pepper and pepper/pepper exhibited high values (3.15 and 2.43gm, respectively) and eggplant has the lowest value (0.76gm).

According to the previous results, ungrafted control plants of tomato and eggplant as well as their compatible auto- and heterografts were highly infested with *Orobanche ramosa* [Plate (13a,b), (14a,b) and (15a,b)]. In contrast, ungrafted control pepper plants and their compatible autografts exhibited light infestation [Plate (16a,b)].

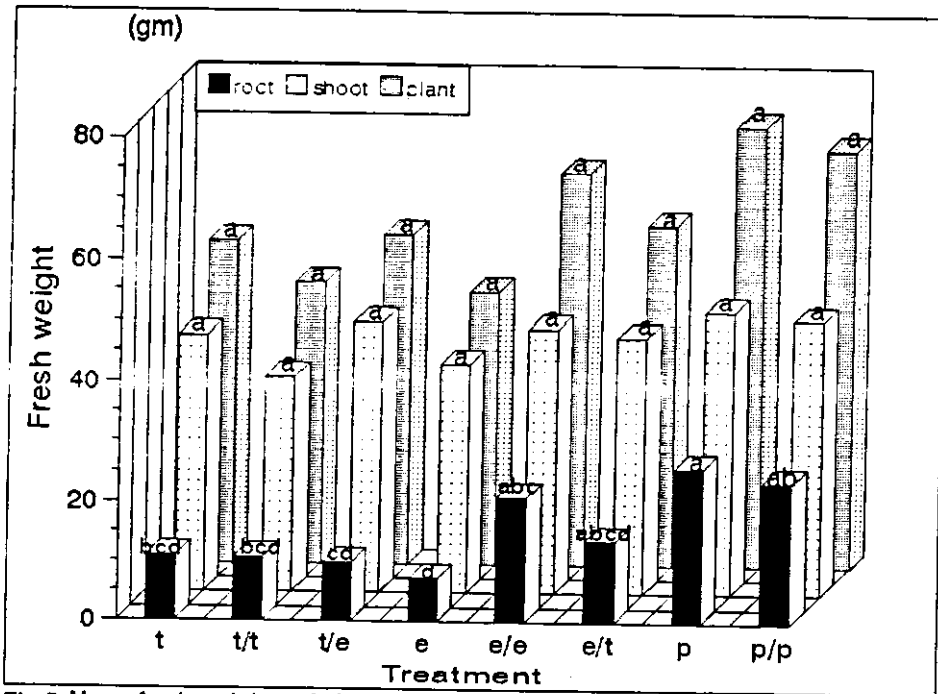


Fig.5: Mean fresh weights of plant, shoot and root per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

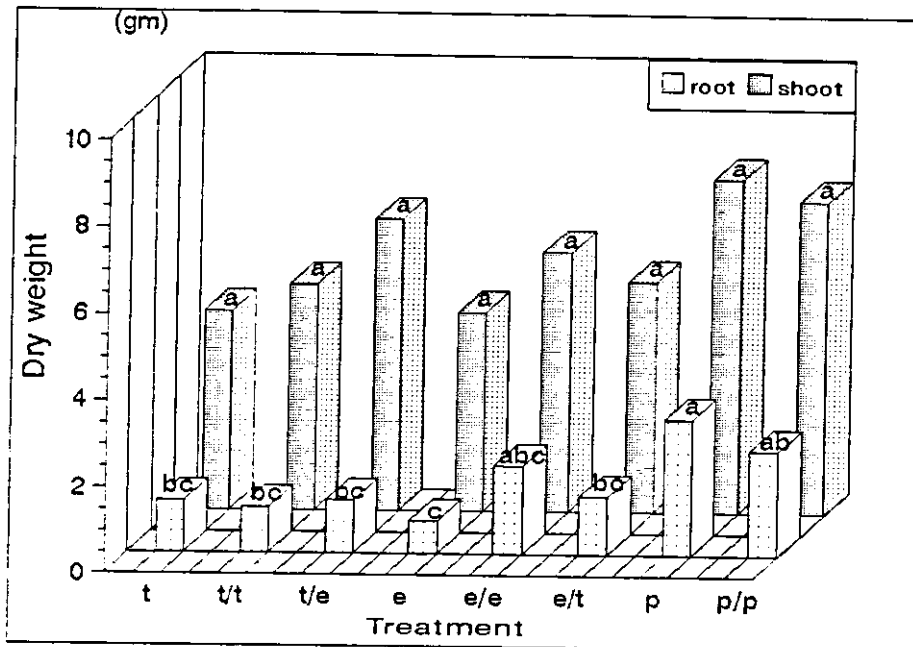


Fig.6: Mean dry weights of shoot and root per plant after 10 weeks of infestation with *Orobanche ramosa*. (Treatments followed by different letters are significantly different at 0.05 level according to DMRT).

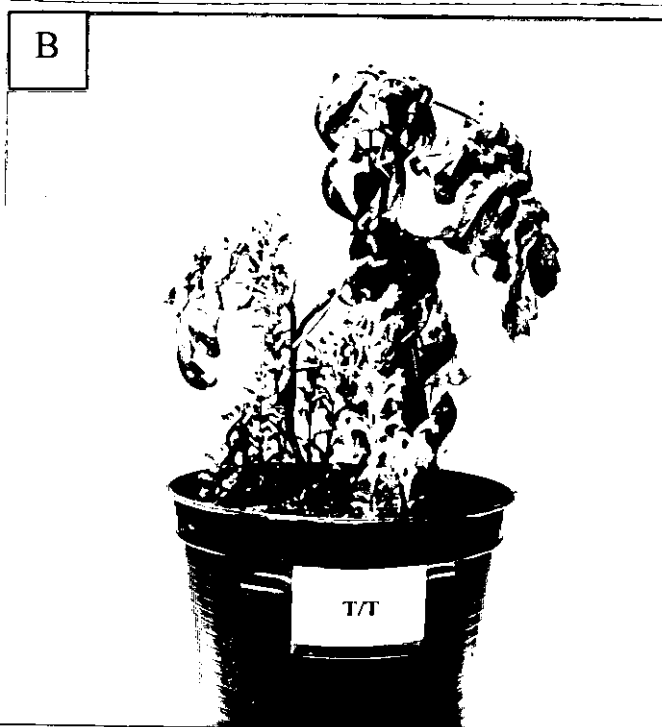
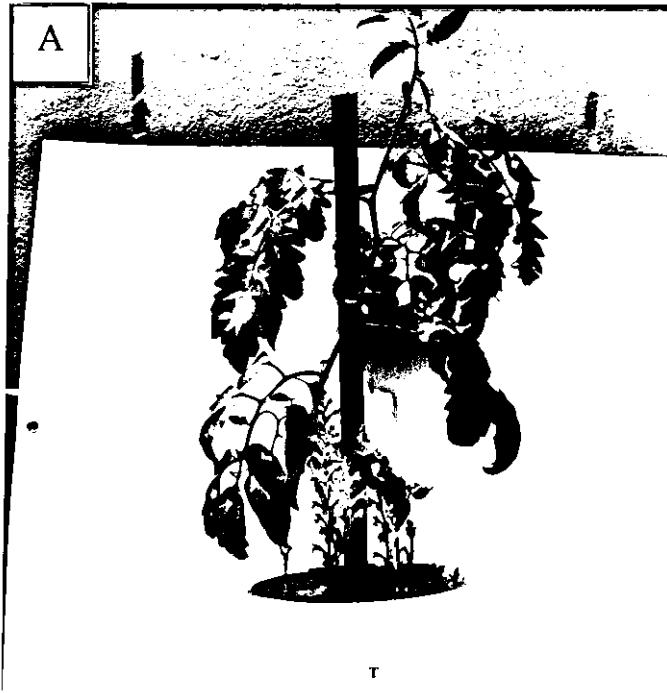


Plate 13: Ten-week-old tomato (A) and tomato/tomato (B) parasitized with branched broomrape.

A



T/E

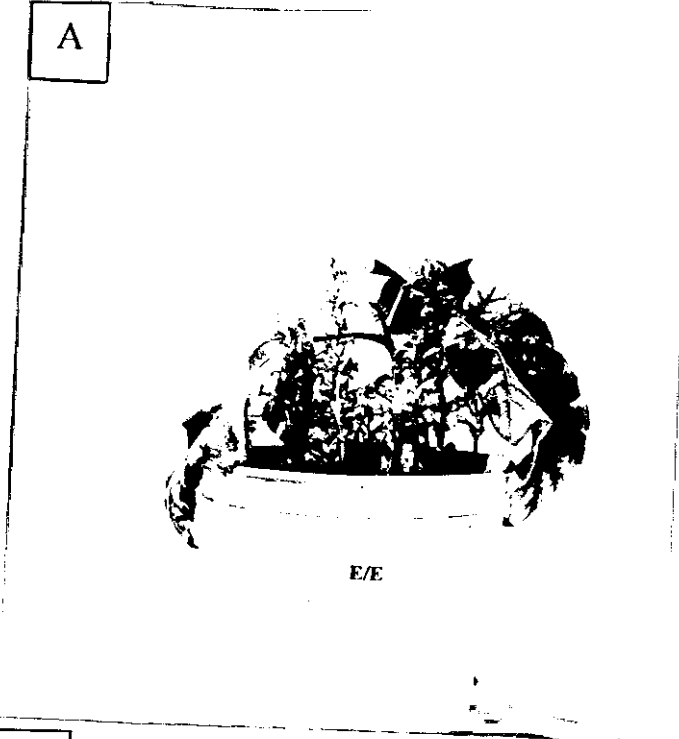
B



E

Plate 14: Ten-week-old tomato/eggplant (A) and eggplant (B) parasitized with branched broomrape.

A



B

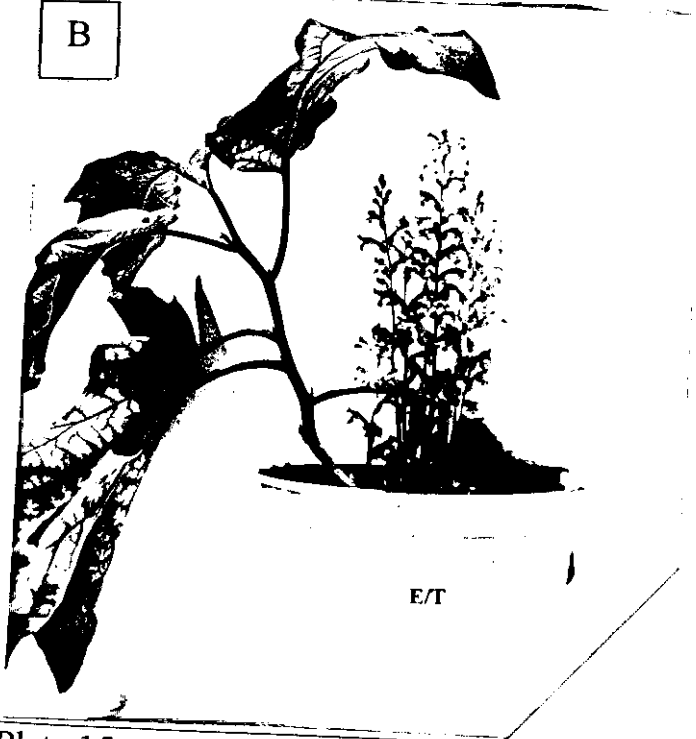


Plate 15: Ten-week-old eggplant (A) and eggplant parasitized with omrape.

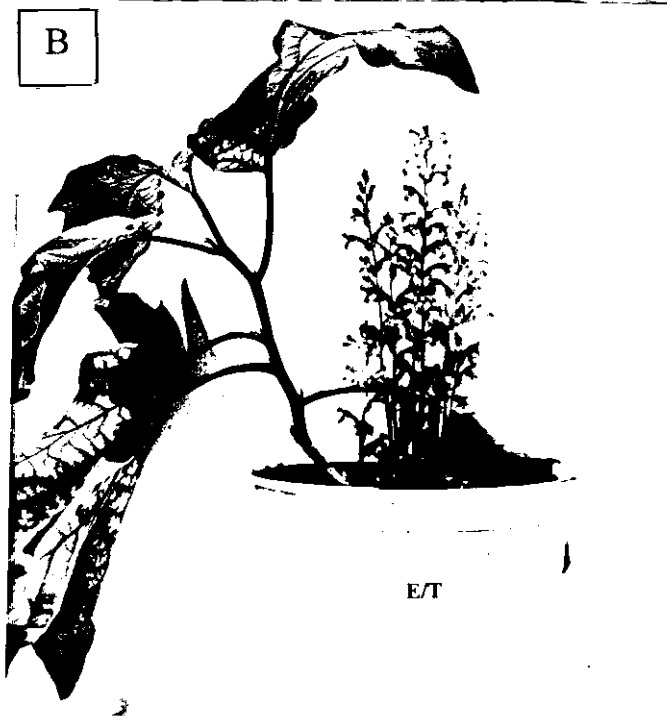
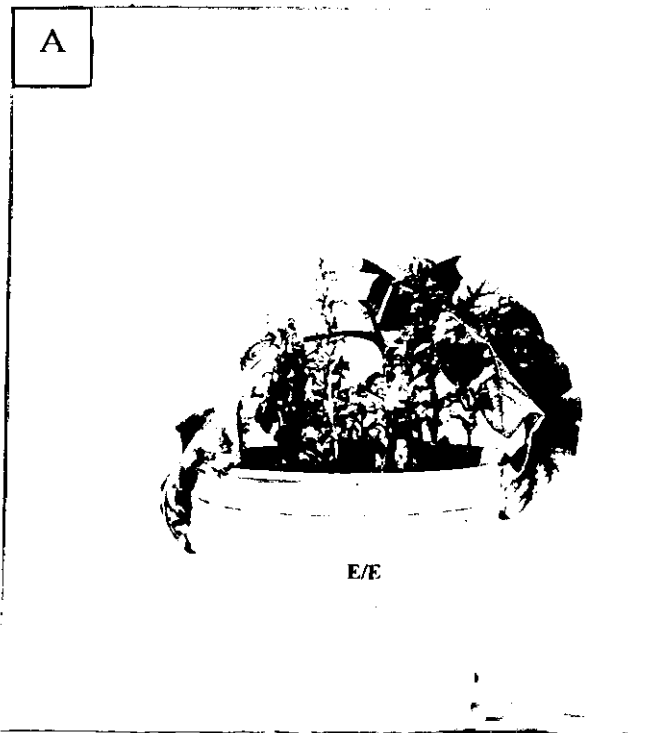


Plate 15: Ten-week-old eggplant/eggplant (A) and eggplant/tomato (B) parasitized with branched broomrape.

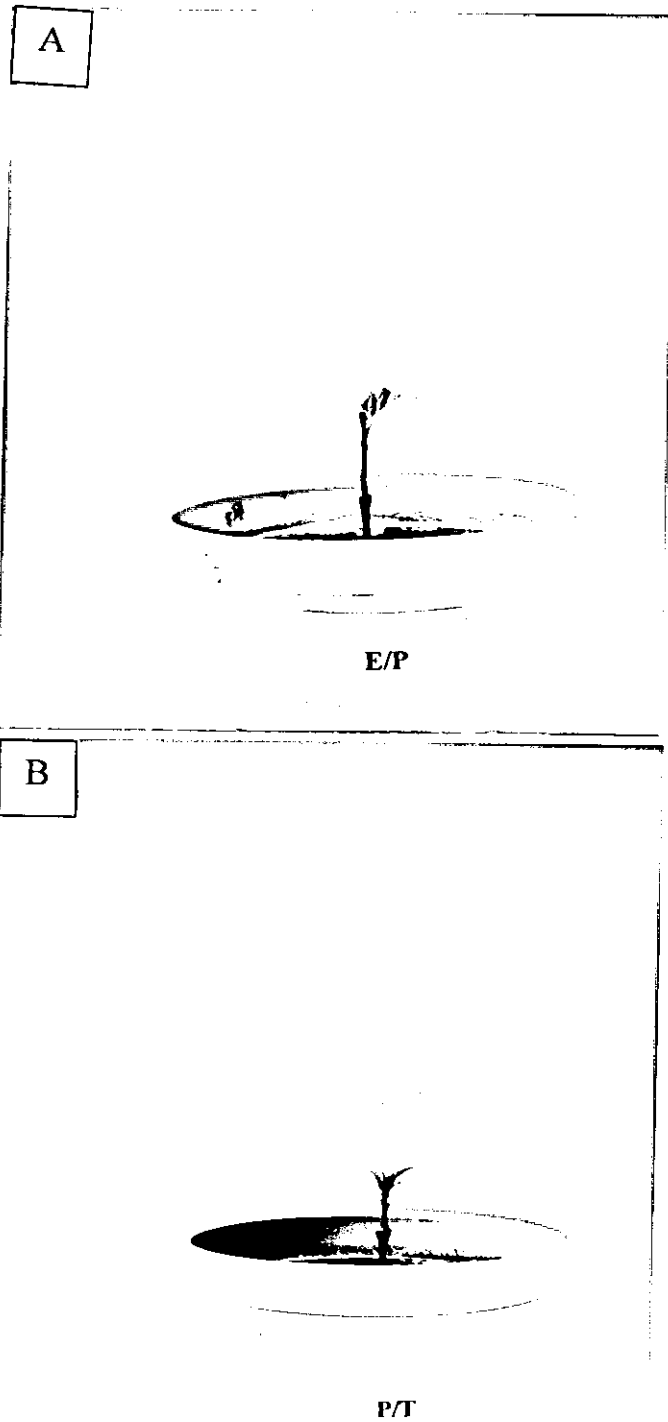


Plate 11: Three-month-old eggplant/pepper (A) and pepper/tomato (B) as incompatible heterograft combinations.

5. Discussion

The developmental process of graft union in compatible combinations including tomato/tomato, tomato/eggplant, eggplant/eggplant [Plate : I,II and III, respectively], eggplant/tomato and pepper/pepper [Plate 6: I and II, respectively] involves a sequence of stages each of which has distinctive features. According to the present anatomical investigation, compatible grafting included the formation of the isolation layer [Plate 7] and its subsequent reduction or elimination, growth of callus [Plate 8], cohesion of stock and scion [Plate 5: (Ib), (IIb) and (IIIb) and Plate 6: (Ib) and (IIb)] and the differentiation of graft bridging vascular tissue and cambium [Plate 5: (Ic,d) and (IIc, d), Plate: (Ic,d) and (IIc, d)]. Depending on microscopical observations and tensile strength measurements Moore (1984), found that the structural development of a compatible graft in *Solanum pennellii* consists of cohesion of stock and scion, proliferation and interdigitation of callus cells at the graft interface and differentiation of vascular tissues across the graft interface.

Due to cutting, an isolation layer has been established [Plate 7], delimiting the scion from both parts of the stock. In compatible tomato autografts Yang *et al.* (1992) observed a pronounced accumulation of dictyosomes and mitochondria in cells of the stock and scion. These dictyosomes typically possessed numerous associated vesicles that appeared to be in various stages of movement toward and fusion with the plasmalemma; the contents of these vesicles are released into the cell wall and thus contributing to the formation of the isolation layer (Moore and Walker, 1981 b and Yang *et al.*, 1992). In autografts of pea roots (Stoddard and McCully, 1979) and *Coleus blumei* (Stoddard and McCully, 1980) a necrotic layer formed after cutting from the walls and contents of

killed cells along the graft interface. Similar observations were noticed by Kollmann and Glockmann (1985), in 17-day-old *Vicia/Helianthus* grafts, in which a distinct dark line, the isolation layer or necrotic layer delimited the scion from both parts of the stock.

In tomato/tomato, tomato/eggplant [Plate 5: (Ia) and (IIa), respectively], eggplant/tomato [Plate 6: (Ia)], and pepper/tomato [Plate 10: (IIa)] the cells of stock and scion beside the isolation layer have divided since day 5 after grafting. Hence, an initial cohesion has been established between the two partners. Lindsay *et al.* (1974), suggested that when division is continuing in tomato autograft interface, each dividing cell releases a catalytic complex which promotes formation of polysaccharides that deposited between the opposing surfaces and secure the cohesion between them. In autografts of tomato (Jeffree and Yeoman, 1983), *Solanum pennellii* (Moore, 1984) and *Sedum telephoides* (Moore and Walker, 1981 a) an initial cohesion was suggested to be due to deposition and subsequent polymerization of cell wall materials in response to wounding.

Callus proliferation and interdigitation brought rupture of the isolation layer in 10-day-old compatible grafts [Plate 5: (Ib), (IIb) and (IIIb) and Plate 6: (Ib) and (IIb)] a feature that was rarely observed in incompatible grafts [Plate 10: (Ib), (IIb) and (IIIb)]. Rupturing of the isolation layer in a 6-day-old tomato autograft (Yang *et al.* 1992), 9-day-old *Solanum pennellii* (Moore, 1984) and 10-day-old *Coleus blumei* (Stoddard and McCully, 1980) was explained by callus proliferation and interdigitation at the graft interface (Yang *et al.* 1992). Stoddard and McCully (1979), noted a marked callus proliferation in successful autografts in pea roots but observed that little cell division occurred in the scion of grafts that were not successful. This absence of cell division was also correlated with the failure of vascular differentiation and degradation or displace-

ment of the initial necrotic layer in the pea root system (Stoddard and McCully, 1979). Thus, callus proliferation during grafting probably serves two functions (Moore and Walker, 1981 a) critical to a successful graft: 1) Providing undifferentiated cells at the graft interface through which vascular tissue may subsequently differentiate, and 2) Fragmentation of the necrotic layer to create direct contact of the living cells. When young internodes of *Capsicum annuum* L. and *Lycopersicon esculentum* Mill. were wounded by longitudinal splitting into halves, cells near the cut surface proliferated to form a callus within which vascular tissues differentiated and tended to restore a vascular cylinder in each half (Wilson and Grange, 1984).

In ten-day-old compatible grafts, callus cells adjacent to the pith region remained undifferentiated [Plate 5: (Ib) and (IIb)]. By determining the breaking weight for 7-day-old tomato autografts, Lindsay *et al.* (1974), found that the separation of the grafts occurred only in the central medullary region while the vascular tissues remained continuous. After fifteen days of grafting cambium differentiation associated with xylem and phloem bridges have been established [Plate 5: (Ic), (IIc) and (IIIc) and Plate 6: (Ic) and (IIc)]; whereas callus differentiation and interdigitation at pith region remained very limited. This confirmed previous reports of Kollmann and Glockmann (1985), who found that small regions of the pith started new cell divisions at 17-day-old *Vicia Helianthus* graft. As cited by Moore (1984), Sachs (1981) suggested that vascular elements are the preferred channels of transport of signals (e.g., auxin) which induce their own differentiation. As a result, in the absence of this signal, callus cells adjacent to the pith remain undifferentiated (Moore, 1984). The delayed cell division and vascular differentiation in the stock of *Coleus blumei* autograft was must be due to hormones originating in the scion and making their way across the graft union (Stoddard and McCully,

1980). From the investigation of Parkinson and Yeoman (1982) it was clear that successful graft formation in autografts of *Lycopersicon esculentum*, *Datura stramonium* and *Nicandra physaloides* was obtained by apical application of 0.2 to 2.0 mg L⁻¹ IAA. Correspondingly, in tomato autografts Yeoman and Brown (1976), supposed that auxin is generated in the scion and flows downward in the vascular elements and is released into the zone of tracheids. In view of previous fact, this may explain that the scion took the initiation of callus division and proliferation and establishment of graft bridging cambium across the graft interface [Plate 5: (IIIc) and Plate 6: (IIId)]. Furthermore, in decapitated bean plants, auxins were found to be involved in cambial cell activities that results in the formation of secondary xylem cells (Harrison and Klein, 1979).

Consistent with successful graft union formation, the fresh and dry weights of scion and rootstock and the plant height measured from day 5 until day 20 after grafting for tomato/tomato, tomato/eggplant, eggplant/eggplant, eggplant/tomato and pepper/pepper were significantly increased [Appendix B: Fig.1: (A), (B), (C), (D) and (E), respectively]. This increase in mean fresh and dry weights and mean plant height for previous graft combinations definitely attributed to the establishment of vascular connections between stock and scion that was demonstrated anatomically [Plate 5: I, II and III and Plate 6: I and II, respectively]. Parkinson and Yeoman (1982), reported that it is the xylem elements which contribute most to the mechanical strength after 14 days in tomato autograft union and enable the transport of essential nutrients. In agreement with Moore (1984), the fresh weight of the scion in *Solanum pennellii* autograft was increased by 5% per day during the first 2 days and by 15 day after grafting the fresh weight increased by 9.2% per day. Furthermore, ¹⁴C-transport and sieve tube number in *Lycopersicon Solanum* grafts were

increased progressively 5-12 days after grafting permitting assimilates translocation (Rachow-Brandt and Kollmann, 1992).

When stock and scion are incompatible, the graft partners may reject each other at any step of graft formation (Stoddard and McCully, 1980). In incompatible heterografts initial cohesion was observed as a result of callus cell division at the graft interface [Plate 10: (Ib, c) and (IIb)]. Yeoman and Brown (1976), found that the heterografts of *Lycoopersicon* and *Nicandra* are clearly incompatible, but nevertheless they do develop a residual cohesion at 4 days after grafting. Cell division did not bring about establishment of vascular connections between scion and stock. Instead, progressive increase in thickening of the isolation layer was observed until twenty day after grafting [Plate 6: III and Plate 10: III]. Cambium of stock and scion failed to unite, leading to interruption of vascular continuity and scion death. Support for this view comes from results of Moore and Walker (1981b), where the isolation layer did not rupture by callus proliferation and vascular redifferentiation did not occur in the callus masses at the graft interface 14 days after grafting. According to Stoddard and McCully (1979), the necrotic layer gave positive reaction for phenolics and pectin. In incompatible heterografts between pepper ('Yollowonder' and 'Floridae') and tomato ('Saint Pierre') wound periderms were differentiated by both partners at the graft line; establishment of wound periderms resulted from increased peroxidase activities that persisted for several weeks, leading to lack of vascular junction and the accumulation of lignin and polyphenols at the level of the contact layer (Deloire and Hebant, 1981). Furthermore, peroxidative enzymes were found to be responsible for IAA oxidation (Taiz and Zeiger, 1991).

Among other factors that may contribute to incompatibility between stock and scion, Moore and Walker (1981b), observed that starch

deposition persisted in some cells throughout lethal cellular senescence, which resulted in deposition of the necrotic layer which insulated *Sedum* from *Solanum*. In the same incompatible graft combination, high activity of acid phosphatase in the *Sedum* cytosol was correlated with cellular autolysis, death and eventual cell collapse to form the characteristic necrotic layer (Moore and Walker, 1981c).

The significant decrease in the fresh and dry weights of scion and rootstock and plant height that were observed in tomato/pepper, eggplant/pepper, pepper/tomato and pepper/eggplant after 20 days of grafting [Appendix B: Fig. 2: (A), (B), (C) and (D), respectively] support that establishment of vascular connections between scion and rootstock were absent. According to Deloire and Hebant (1982), in incompatible combinations ('Yollowonder' pepper on 'St Pierre' tomato) no vascular connection was established or only very weak one is differentiated; as a result, early degeneration of incompatible grafts occur; in some cases, this degeneration is delayed, although these remaining individuals will not normally survive the flowering stage. Data of Rachow-Brandt and Kolmann (1992), suggested that most of the phloem elements developed in the graft union of less compatible system of *Vicia Helianthus* were non-functional.

If the treatments were compared among each other with respect to fresh and dry weights of scion and rootstock and plant height and when tomato is the scion, no significant variations were observed for 5-day-old grafts [Appendix B: Fig. 3: (A)]. From 10-20 days after grafting, tomato/pepper revealed the lowest significant values compared to tomato/tomato and tomato/eggplant [Appendix B: Fig. 3: (B), (C) and (D)]. When eggplant is the scion, no significant variations were observed among 5-day-old grafts [Appendix B: Fig. 4: (A)]. At 20 days after grafting eggplant/pepper was significantly the lowest [Appendix B: Fig. 4: (D)]. At this

stage of development eggplant/tomato showed less significant values than eggplant/eggplant in relation to dry weights of scion and rootstock [Appendix B: Fig. 4: (D)]. When pepper is the scion, the variations among treatments were non-significant at 5 days [Appendix B: Fig. 5: (A)], whereas 10-day-old grafts showed low significant values for pepper/tomato and pepper/eggplant with respect to fresh and dry weight of scion, dry weight of rootstock and plant height [Appendix B: Fig. 5: (B)]. These of 15- and 20-day-old pepper/tomato and pepper/eggplant were significantly lower than those of pepper/pepper [Appendix B: Fig. 5: (C) and (D)]. In general, by comparing the nine treatment combinations among each other it was found that the reciprocal combinations between tomato and pepper and between eggplant and pepper tended to show lower significant values than other treatments starting from 10 days until 20 days after grafting [Appendix B: Fig. 6: (B), (C) and (D)].

In view of present results, the principle of establishment of xylem and phloem bridges between stock and scion is very important factor. Consequently, successful graft union formation is produced upon which movement of water and nutrients between the two partners is provoked through new differentiated xylem and phloem elements.

When the grafting area of three-month-old incompatible eggplant/pepper and pepper/tomato [Plate 11: (A, B, respectively)] have been sectioned and analyzed under light microscope, large callus cells divisions by both partners were observed along the graft interface [Plate 12: (A, B)] with very limited vascular differentiation. Deloire and Hebant (1981), reported that accumulation of lignin and polyphenols at the level of the contact layer restricted the movement of water and of organic and inorganic nutrients between pepper and tomato. This results in progressive depletion and final death of the scion, which may occur after a time lapse of between 2 weeks and 3 months. Callus cell division at the graft

interface may provide a symplastic route through plasmodesmata through which movement of water and nutrients occur. In the fused cell walls between *Vicia* and *Helianthus* cells, plasmodesmata interconnected the protoplasts of the incompatible unrelated cells (Kollmann and Glockmann, 1985).

Measurements related to *Orobanche ramosa* (Figs. 1, 2, 3 and 4) showed very limited significant variations among tomato, tomato/tomato, tomato/eggplant, eggplant, eggplant/eggplant and eggplant/tomato. The above treatments were highly susceptible to *Orobanche ramosa* infestation and exhibited a significant reduction in mean fresh and dry weights of roots only (Figs. 5 and 6). Qasem and Kasrawi (1995), observed that the reductions in the percentage of dry weights of tomato shoots and roots due to *Orobanche ramosa* infestation were relatively high. Also, Jain and Foy (1989), found that shoot and root fresh weights of tomato plants parasitized by branched broomrape were significantly reduced; parasitized plants were also smaller in size. Hameed and Foy (1991), found that the first sign of parasitism in the root hair zone of tomato and branching points of the roots by *Orobanche ramosa* occurred within 12 days of infestation. According to Zwanenburg *et al.* (1986), the germination of *Orobanche* seeds is initiated by a stimulant present in the root exudate of the host plant. As cited by Wegmann (1994), Mathews *et al.* (1991) isolated and partially identified germination stimulants from tomato that highly stimulated seed germination of *Orobanche aegyptiaca*.

On the other hand, pepper and pepper/pepper showed resistance to the broomrape as reflected by the very limited infestation with the parasite (Figs. 1, 2, 3 and 4) and the high fresh and dry weights of root (Figs. 5 and 6). According to Wegmann (1994), *Orobanche* does not parasitize resistant plants; at least they do not allow regular development of the *Orobanche* plant to maturity. Abu-Irmaileh (1984), found that pepper prod-

uced a significant reduction in broomrape parasitism on tomato as judged by decrease in mean dry weight of the parasite plant; this reduction was explained by the suicidal germination of some seeds of the parasite. On the other hand, eggplant and tomato did not produce a significant reduction of broomrape dry weight (Abu-Irmaileh, 1984). In addition, when pepper was the first crop in the rotation with tomato, certain percentage of broomrape seeds germinated then died; but when tomato was the first crop in the rotation, comparable percentage of broomrape seeds germinated and infested the tomato, producing broomrape shoots which were subsequently removed before they set seeds (Abu-Irmaileh, 1982). Of the other resistance manners that can be accomplished by plant to such weed parasite, Antonova (1978) as cited by Teryokhin (1994) demonstrated that the resistance reaction of sunflower to *Orobanche cernua* was accompanied by an accumulation of lignin-like compounds in the cell walls of xylem cells wherever the haustoria contacted them.

6. Conclusions

1. The success of grafting depend on attainment of differentiation and complete vascular connections between stock and scion after 20 days of grafting through which movement of water and nutrients occurred.
2. The reciprocal cleft-grafting between tomato cv. 'GS 12' and eggplant cv. 'Shantta' was successful, where as the reciprocal cleft-grafting between tomato cv. 'GS 12' and pepper cv. 'Maram' and between eggplant cv. 'Shantta' and pepper cv. 'Maram' was failed.
3. Tomato and eggplant whole plants as well as their compatible auto- and heterograft combinations were highly infested with *Orobanche ramosa*. In contrast, pepper whole plants and their compatible auto-graft combinations revealed the highest level of resistance to *Orobanche ramosa* infestation for all measured parameters.

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Abbreviations

T/T	: Tomato/Tomato
T/E	: Tomato/Eggplant
T/P	: Tomato/Pepper
E/E	: Eggplant/Eggplant
E/T	: Eggplant/Tomato
E/P	: Eggplant/Pepper
P/P	: Pepper/Pepper
P/T	: Pepper/Tomato
P/E	: Pepper/Tomato
s	: scion
rs	: rootstock
FWT	: Fresh weight
DWT	: Dry weight
PH	: Plant height

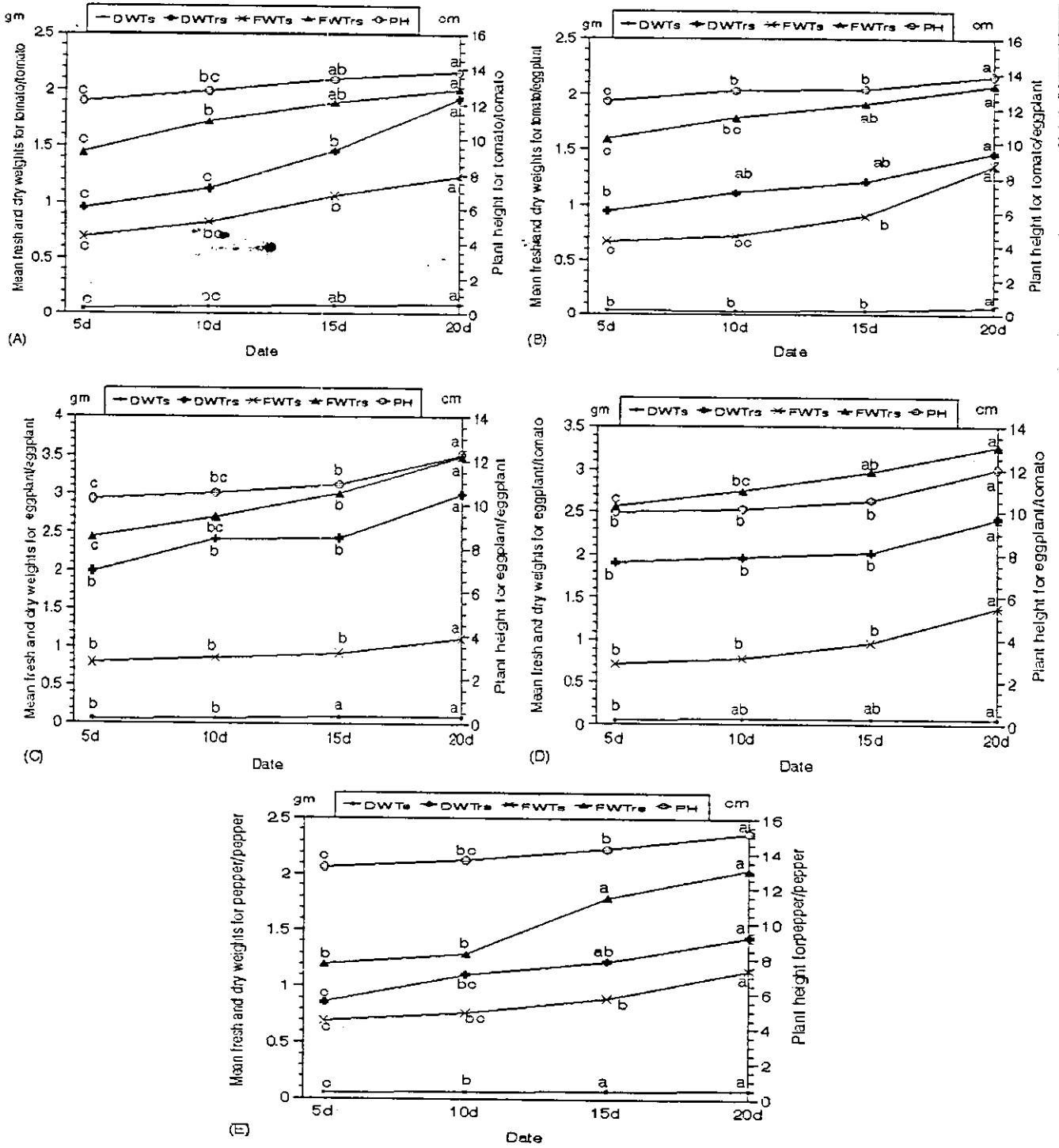


Fig.1: Mean fresh and dry weights of scion(FWTs,DWTs) and rootstock(FWTrs,DWTrs) and mean plant height(PH) for: (A)Tomato/tomato, (B)Tomato/eggplant, (C)Eggplant/eggplant, (D)Eggplant/tomato and (E)Pepper/pepper measured at 5,10,15 and 20 days after grafting. (For each parameter, dates followed by different letters are significantly different at 0.05 level according to DMRT).

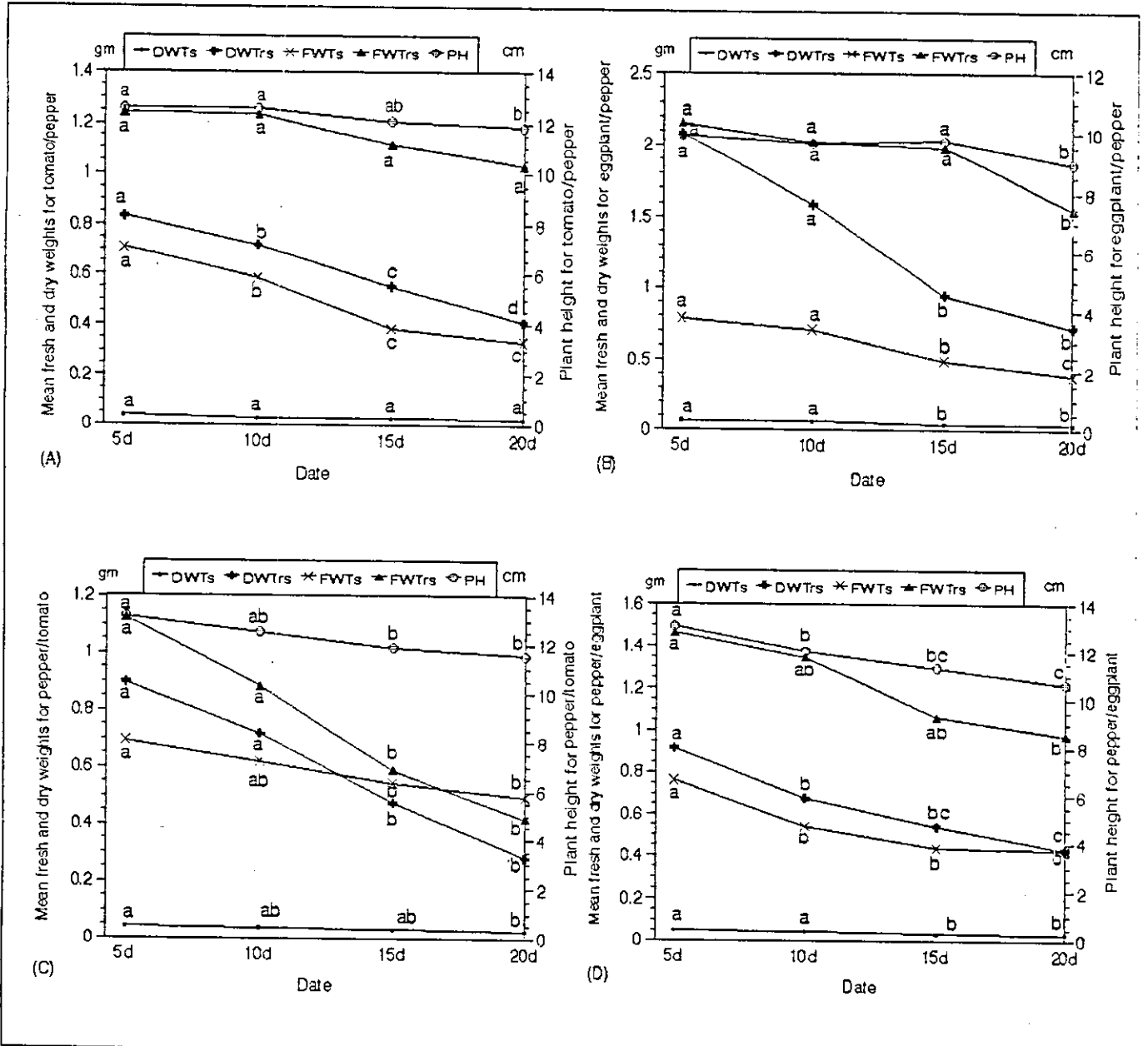


Fig.2: Mean fresh and dry weights of scion(FWTs,DWTs) and rootstock(FWTrs,DWTrs) and mean plant height(PH) for: (A)Tomato/pepper, (B)Eggplant/pepper, (C)Pepper/tomato and (D)Pepper/eggplant measured at 5,10,15 and 20 days after grafting. (For each parameter, dates followed by different letters are significantly different at 0.05 according to DMRT).

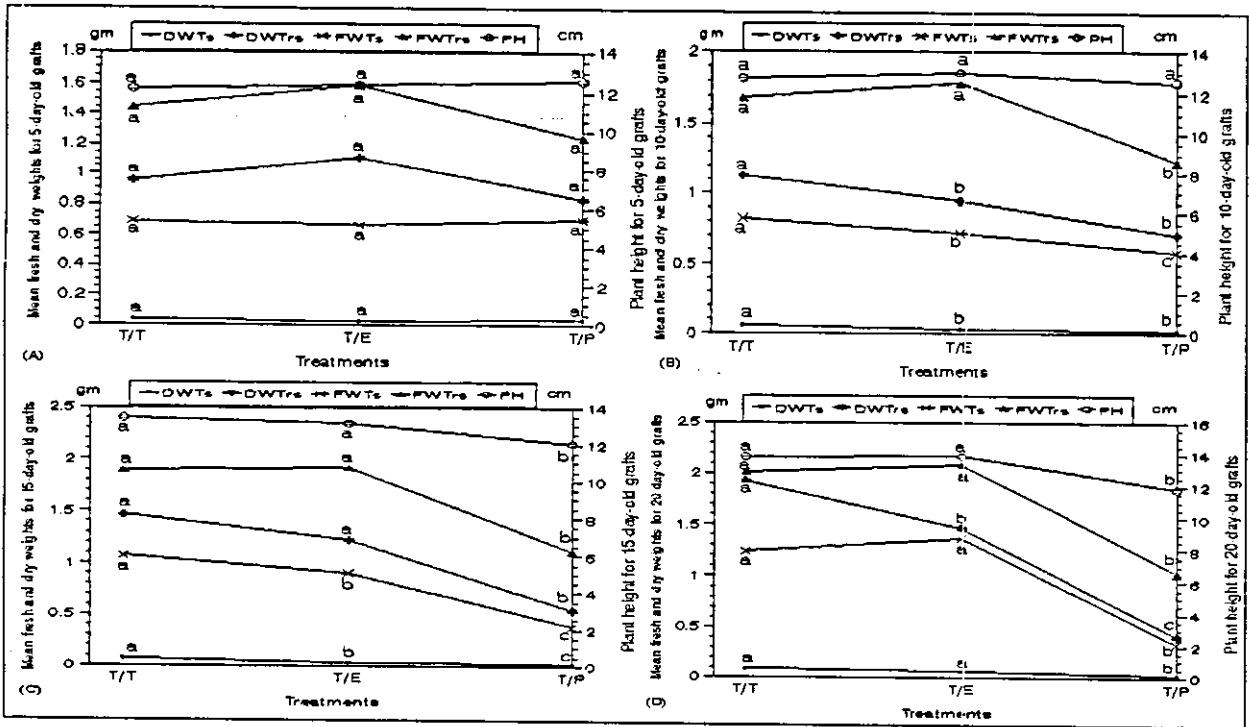


Fig.3: Mean fresh (FWT) and dry (DWT) weights and mean plant height (PH) for: Tomato/tomato, tomato/eggplant and tomato/pepper at 5 (A), 10 (B), 15 (C) and 20 (D) days after grafting. (For each parameter, treatments followed by different letters are significantly different at 0.05 level according to DMRT).

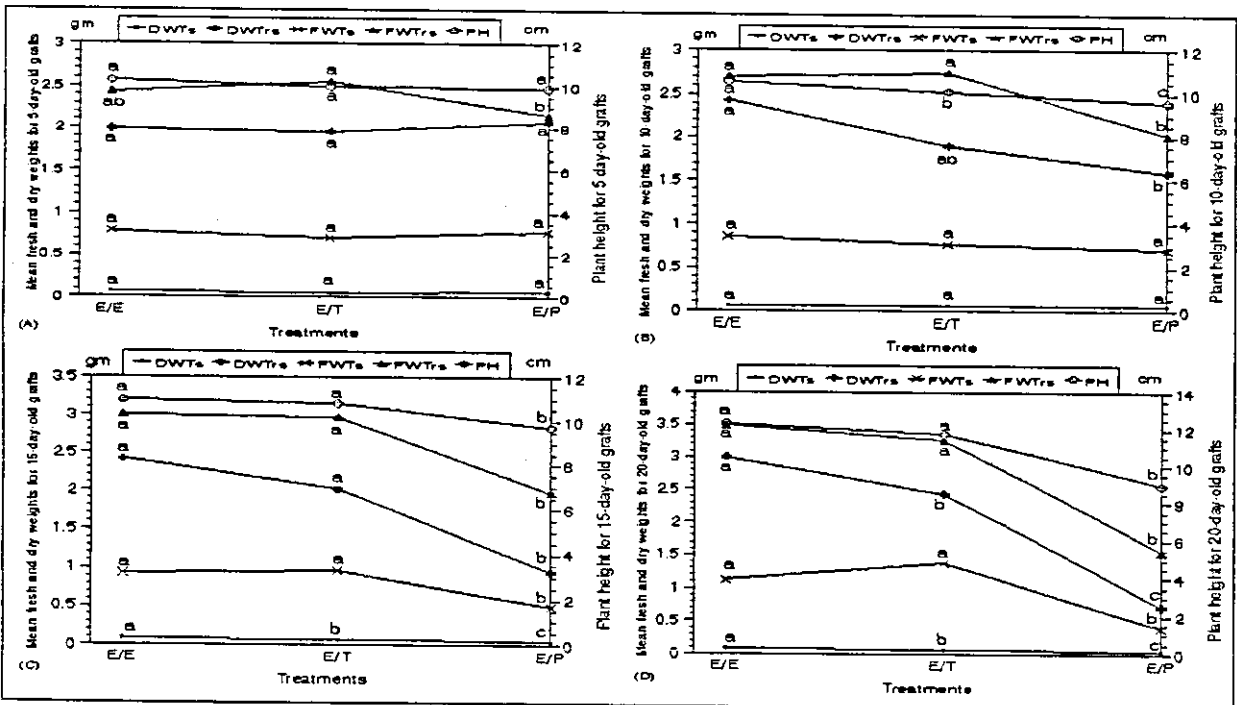


Fig.4: Mean fresh (FWT) and dry (DWT) weights and mean plant height (PH) for: Eggplant/eggplant, eggplant/tomato and eggplant/pepper at 5 (A), 10 (B), 15 (C) and 20 (D) days after grafting. (For each parameter, treatments followed by different letters are significantly different at 0.05 level according to DMRT).

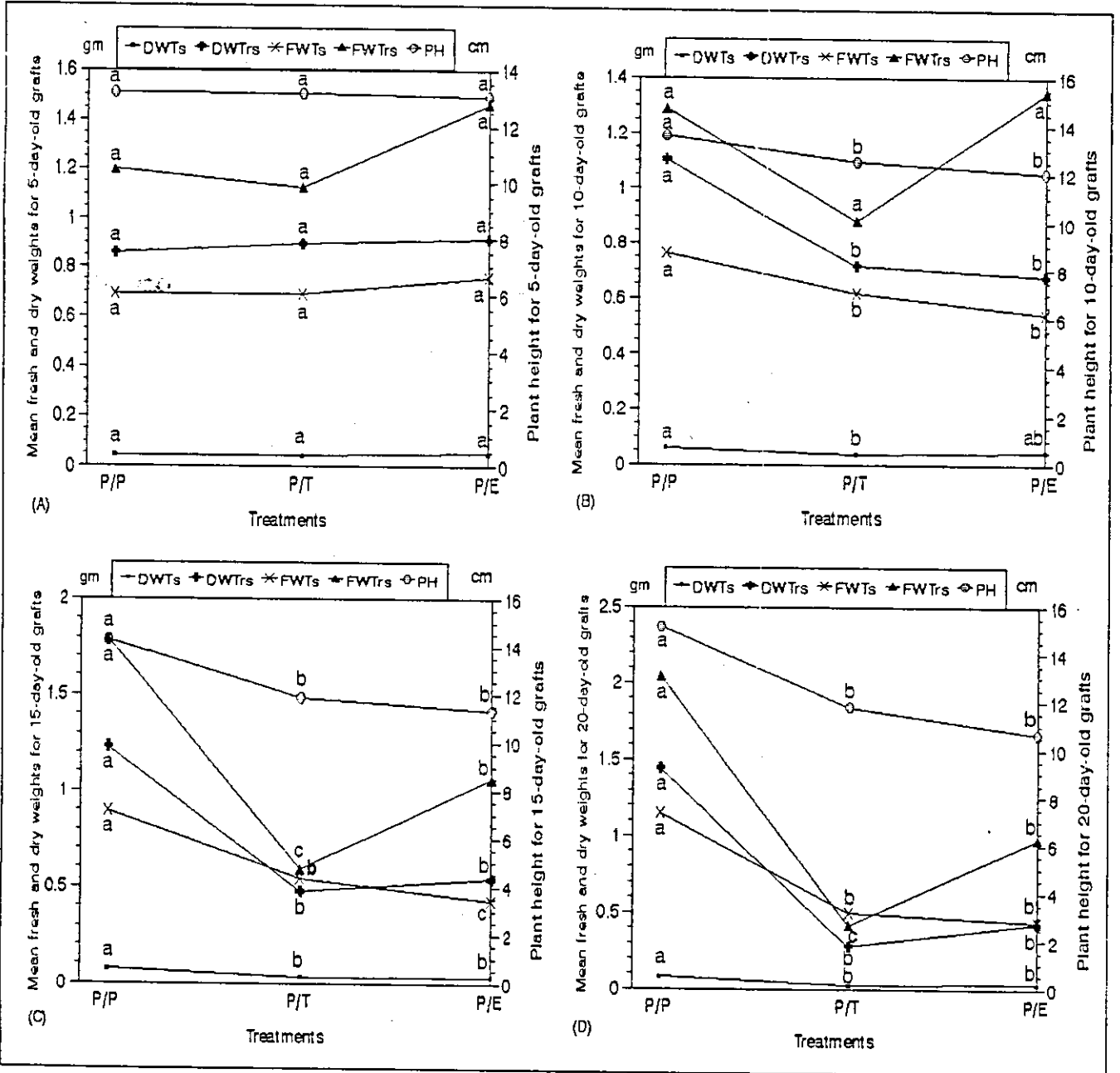


Fig.5: Mean fresh (FWT) and dry (DWT) weights and mean plant height (PH) for: Pepper/pepper, pepper/tomato and pepper/eggplant at 5 (A), 10 (B), 15 (C) and 20 (D) days after grafting. (For each parameter, treatments followed by different letters are significantly different at 0.05 level according to DMRT).

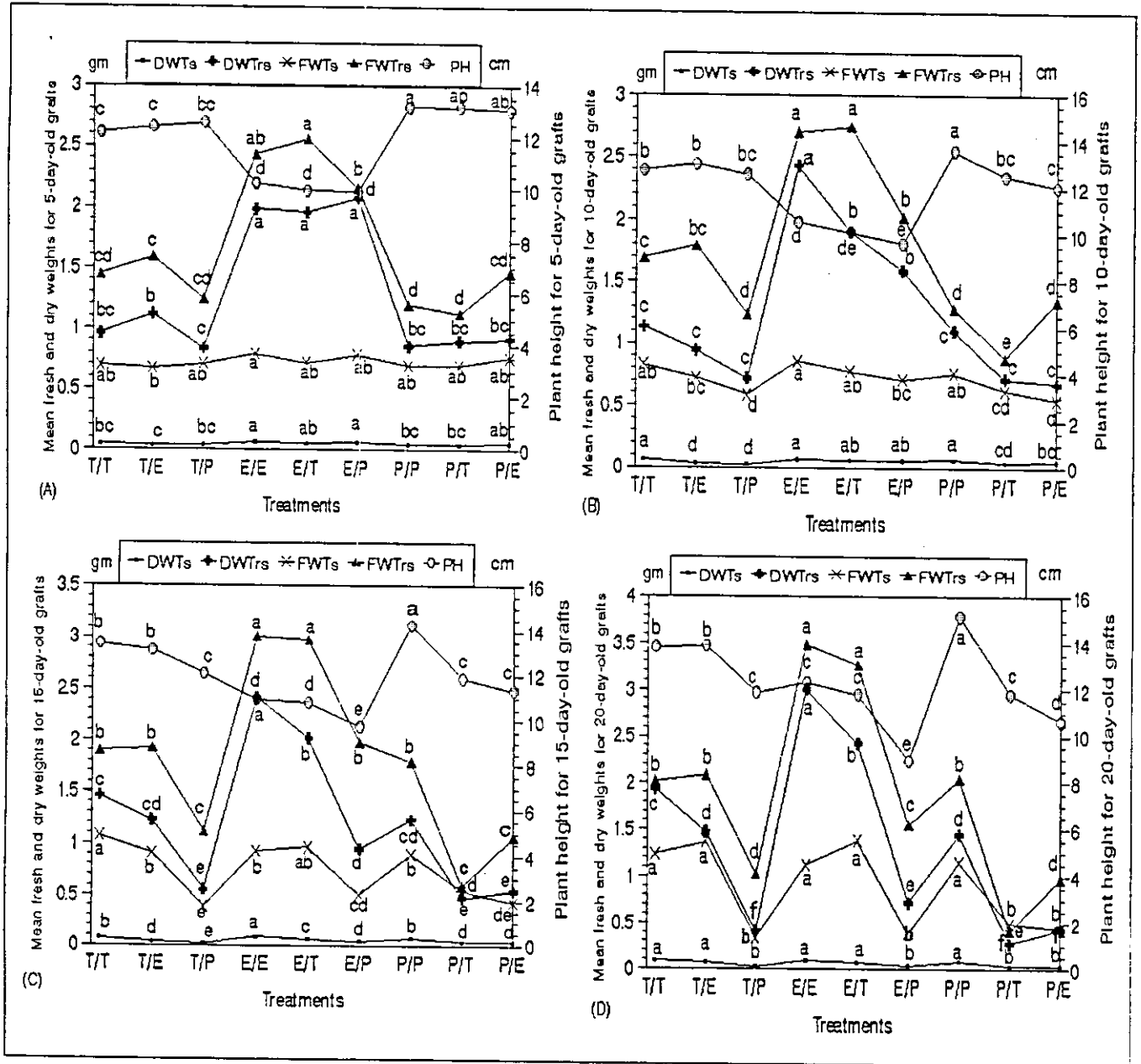


Fig.6: Mean fresh (FWT) and dry (DWT) weights and mean plant height (PH) for nine graft combinations at 5 (A), 10 (B), 15 (C) and 20 (D) days after grafting. (For each parameter, treatments followed by different letters are significantly different at 0.05 level according to DMRT).

Appendix table 1: ANOVA for plant growth parameters of tomato/tomato autografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.2399**	0.2455**	0.0013**	0.7364**	2.1280**
Error	12	0.0253	0.0139	0.0001	0.0245	0.1977

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 2: ANOVA for plant growth parameters of tomato/eggplant heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.4055**	0.1764**	0.0011*	0.1932 ^{ns}	1.4723**
Error	12	0.0215	0.0170	0.0002	0.0581	0.1102

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 3: ANOVA for plant growth parameters of tomato/pepper heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.1199**	0.0426 ^{ns}	0.0002 ^{ns}	0.1376**	0.5340*
Error	12	0.0034	0.0525	0.0001	0.0047	0.1144

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 4: ANOVA for plant growth parameters of eggplant/eggplant autografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.0826**	0.8184**	0.0008**	0.6917**	3.2758**
Error	12	0.0093	0.0740	0.0001	0.0930	0.1825

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 5: ANOVA for plant growth parameters of eggplant/tomato heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.3581**	0.3672**	0.0002**	0.2247**	3.5073**
Error	12	0.0498	0.0526	0.0001	0.0293	0.1677

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 6: ANOVA for plant growth parameters of eggplant/pepper heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.1330**	0.2722*	0.0006**	1.5257**	0.7323**
Error	12	0.0048	0.0648	0.0001	0.1346	0.0831

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 7: ANOVA for plant growth parameters of pepper/pepper autografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.1634**	0.6525**	0.0007**	0.2404**	2.9875**
Error	12	0.0097	0.0541	0.00003	0.0293	0.2896

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 8: ANOVA for plant growth parameters of pepper/tomato heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.0296*	0.3918**	0.0002*	0.2911**	2.0773*
Error	12	0.0066	0.0279	0.00006	0.0217	0.4560

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 9: ANOVA for plant growth parameters of pepper/eggplant heterografts at 5, 10, 15 and 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Date	3	0.0958**	0.2135 ^{ns}	0.0003**	0.1748**	4.2823**
Error	12	0.0063	0.0808	0.00005	0.0172	0.2431

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 10: ANOVA for plant growth parameters of tomato/tomato, tomato/eggplant and tomato/pepper 5 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0016 ^{ns}	0.1213 ^{ns}	0.00007 ^{ns}	0.0798 ^{ns}	0.1608 ^{ns}
Error	9	0.0024	0.0580	0.0001	0.0453	0.095

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 11: ANOVA for plant growth parameters of tomato/tomato, tomato/eggplant and tomato/pepper 10 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0595**	0.3477**	0.0016 ^{ns}	0.172**	0.2058 ^{ns}
Error	9	0.0031	0.006	0.00001	0.0063	0.1494

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 12: ANOVA for plant growth parameters of tomato/tomato, tomato/eggplant and tomato/pepper 15 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.5113**	0.8437**	0.0030**	0.8900**	2.01**
Error	9	0.0090	0.0127	0.00002	0.0296	0.1447

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 13: ANOVA for plant growth parameters of tomato/tomato, tomato/eggplant and tomato/pepper 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	1.2634**	1.3953**	0.0037*	2.4282**	5.3358**
Error	9	0.0525	0.0295	0.0005	0.0354	0.1739

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 14: ANOVA for plant growth parameters of eggplant/eggplant, eggplant/tomato and eggplant/pepper 5 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0072 ^{ns}	0.1712 ^{ns}	0.0002 ^{ns}	0.0158 ^{ns}	0.1225 ^{ns}
Error	9	0.0066	0.0486	0.0001	0.0250	0.0894

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 15: ANOVA for plant growth parameters of eggplant/eggplant, eggplant/tomato and eggplant/pepper 10 days after grafting.

Source	df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0219 ^{ns}	0.6588 ^{**}	0.00005 ^{ns}	0.7253 ^{ns}	0.8108 ^{**}
Error	9	0.0094	0.0305	0.0001	0.2098	0.0342

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 16: ANOVA for plant growth parameters of eggplant/eggplant, eggplant/tomato and eggplant/pepper 15 days after grafting.

Source	df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.2677 ^{**}	1.3711 ^{**}	0.0019 ^{**}	2.3072 ^{**}	1.7100 [*]
Error	9	0.0028	0.0335	0.00005	0.0930	0.3956

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 17: ANOVA for plant growth parameters of eggplant/eggplant, eggplant/tomato and eggplant/pepper 20 days after grafting.

Source	df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	1.0536 ^{**}	4.4819 ^{**}	0.0025 ^{**}	5.6064 ^{**}	12.986 ^{**}
Error	9	0.0665	0.1427	0.0000	0.0145	0.1975

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 18: ANOVA for plant growth parameters of pepper/pepper, pepper/tomato and pepper/eggplant 5 days after grafting.

Source	df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0062 ^{ns}	0.1277 ^{ns}	0.0001 ^{ns}	0.0033 ^{ns}	0.0175 ^{ns}
Error	9	0.006	0.0541	0.00003	0.0105	0.2883

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 19: ANOVA for plant growth parameters of pepper/pepper, pepper/tomato and pepper/eggplant 10 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0492**	0.2567 ^{ns}	0.0006*	0.2221*	2.5225**
Error	9	0.0039	0.1045	0.0001	0.0333	0.1797

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 20: ANOVA for plant growth parameters of pepper/pepper, pepper/tomato and pepper/eggplant 15 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.2372**	1.4661**	0.0020**	0.6964**	9.7275**
Error	9	0.0044	0.0133	0.00004	0.0128	0.3828

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 21: ANOVA for plant growth parameters of pepper/pepper, pepper/tomato and pepper/eggplant 20 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.6255**	2.7250**	0.0034**	1.6066**	22.1258**
Error	9	0.0158	0.0453	0.00003	0.0342	0.5397

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 22: ANOVA for plant growth parameters of 9 treatment combinations 5 days after grafting.

Source	Df	Mean square				
		FWT. of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0080 ^{ns}	1.2081**	0.0004**	1.1968**	7.8963**
Error	9	0.0050	0.0536	0.00001	0.0269	0.1576

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 23: ANOVA for plant growth parameters of 9 treatment combinations 10 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.0475**	1.6836**	0.0009**	1.4772**	7.8878**
Error	9	0.0055	0.04821	0.0001	0.0831	0.1211

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 24: ANOVA for plant growth parameters of 9 treatment combinations 15 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.2827**	2.6916**	0.0021**	1.8268**	8.2525**
Error	9	0.0054	0.0198	0.00004	0.0451	0.3077

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 25: ANOVA for plant growth parameters of 9 treatment combinations 20 days after grafting.

Source	Df	Mean square				
		FWT. Of scion	FWT. of rootstock.	DWT. of scion.	DWT. of rootstock.	Plant height.
Treatment	2	0.8122**	4.18501**	0.0026**	3.7522**	13.8586**
Error	9	0.0449	0.0725	0.0002	0.0281	0.3037

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 26: ANOVA for number of parasitic shoot of *Orobanche ramosa* emerged above soil.

Source	Df	Mean square
Treatments	7	151.1805 ^{ns}
Blocks	3	24.6478 ^{ns}
Error	21	61.6895

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 27: ANOVA for number of parasitic shoot of *Orobanche ramosa* emerged below soil.

Source	df	Mean square
Treatments	7	441.000*
Blocks	3	147.5833 ^{ns}
Error	21	158.5833

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 28: ANOVA for average length of parasitic shoot of *Orobanche ramosa* emerged above soil.

Source	df	Mean square
Treatments	7	66.07904 ^{ns}
Blocks	3	25.3988 ^{ns}
Error	21	28.4832

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 29: ANOVA for average length of parasitic shoot of *Orobanche ramosa* emerged below soil.

Source	df	Mean square
Treatments	7	9.0012 ^{ns}
Blocks	3	1.3752 ^{ns}
Error	21	5.9428

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 30: ANOVA for fresh weight of parasitic shoot of *Orobanche ramosa* emerged above soil.

Source	df	Mean square
Treatments	7	1349.8684 ^{ns}
Blocks	3	520.5675 ^{ns}
Error	21	697.2975

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 31: ANOVA for fresh weight of parasitic shoot of *Orobanche ramosa* emerged below soil.

Source	df	Mean square
Treatments	7	62.7108 ^{ns}
Blocks	3	62.4632 ^{ns}
Error	21	36.1308

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 32: ANOVA for dry weight of parasitic shoot of *Orobanche ramosa* emerged above soil.

Source	df	Mean square
Treatments	7	34.9796 ^{ns}
Blocks	3	11.6192 ^{ns}
Error	21	18.1273

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 33: ANOVA for dry weight of parasitic shoot of *Orobanche ramosa* emerged below soil.

Source	df	Mean square
Treatments	7	2.5818 ^{ns}
Blocks	3	3.1046 ^{ns}
Error	21	1.2612

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 34: ANOVA for fresh weight of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	404.3387 ^{ns}
Blocks	3	1099.6046 ^{ns}
Error	21	817.3937

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 35: ANOVA for fresh weight of shoot of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	55.8439 ^{ns}
Blocks	3	501.2115 ^{ns}
Error	21	477.0952

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 36: ANOVA for fresh weight of root of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	198.9535*
Blocks	3	148.3089 ^{ns}
Error	21	65.9275

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 37: ANOVA for fresh weight of fruits of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	5741.3775**
Blocks	3	262.9324 ^{ns}
Error	21	363.7730

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 38: ANOVA for dry weight of shoot of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	5.6944 ^{ns}
Blocks	3	9.5366 ^{ns}
Error	21	7.6598

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 39: ANOVA for dry weight of root of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	2.6284**
Blocks	3	0.7368 ^{ns}
Error	21	0.6829

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

Appendix table 40: ANOVA for dry weight of fruits of T, T/T, T/E, E, E/E, E/T, P and P/P.

Source	df	Mean square
Treatments	7	31.3330**
Blocks	3	1.3898 ^{ns}
Error	21	1.4080

*: significant at 0.05 level, **: significant at 0.01 level, ns: non-significant.

أبدت نباتات البندورة و الباذنجان (غير المطعمة) بالإضافة إلى مجموعات التراكيب الذاتية و المتغايرة الخاصة فيها إصابة مرتفعة جدا بالهالوك. و أظهرت نباتات الفلفل و مجموعات التراكيب الذاتية الخاصة فيها إصابة ضئيلة جدا.