PHYSIOLOGICAL AND ANATOMICAL CONSIDERATIONS IN THE PROPAGATION OF TOMATOES (Lycopersioon esculentua Mill.) BY SEED, VEGETATIVE STEM-CUTTINGS AND TISSUE CULTURE

BY

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DEDICATION

I wish to dedicate this work to my late mother, Mrs. Lami Mary Bako who believed so much in my abilities but could not live to see the result of her efforts.

Thank you, Mom. This one is for you.

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First, I thank GOD for life, health and the energy to complete this work, despite all the difficulties encountered.

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S.P. Bako
December 1989

APPROVAL PAGE

This thesis titled "PHYSIOLOGICAL AND ANATOMICAL CONSIDERATIONS IN THE PROPAGATION OF TOMATOES (Lycopersicon esculentum Mill.) BY SEED, VEGETATIVE STEM-CUTTINGS AND TISSUE CULTURE" by S.P. Bako meets the requirements governing the award of the Degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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CERTIFICATION

I hereby certify that this thesis is completely a product of a research work initiated and carried out by me and that all contents are original (except where acknowledged) works of mine. I therefore bear complete responsibility for any errors and credits herein.

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ABSTRACT

Experiments were carried out to evaluate qualitatively and quantitatively the performance of seed-propagated and stem-cutting propagated tomato plants under varying levels of nitrogen, phosphorus and potassium, The effects of growth hormones on physological and anatomical aspects of growth of stem-cutting propagated tomato and shoot-tip culture of the plant were also investigated. Planting and other cultural practices for tomato, recommended by the agricultural extension and Research Liaison Services (A.E.R.L.S.), Ahmadu Bello University, Samaru, Zaria, were adopted in the experiments. Three rates of fertilizer (NPK 15.15.15) were applied as zero, 300 and 600kgNPK./ha.

Stem-cutting propagated plants had a shorter life-span (6-7 weeks) and came to bearing earlier than seed-propagated plants (7-9 weeks), The zero and high fertilizer levels (600kg NPK/ha) delayed flowering by 6 and 13 days respectively in seed-propagated plants. The zero fertilizer treatment delayed flowering in stem-cutting propagated plants by 8 days. The rate of plant height increase was higher in stem-cutting propagated than seed-propagated plants during the first 14 days after transplanting (DAT) particularly for fertilizer treated plants. Plant height at 42 DAT was significantly higher for seed-propagated and stem-cutting propagated plants for all fertilizer treatments, as compared to the unfertilized plants. Kegardless of mode of propagation, plant height at 42 DAT was in the order 600kg >300kg >OkgNPK/ha. Auxin treatments did not have a significant effect on plant height.

Dry matter increase was generally but not significantly (P = 0.05/ higher in seed-nropagated plants (between 28-42 DAT) than in stem-cutting propagated plants, for all fertilizer treatments. While fertilizer treatments significantly increased DM production, auxin levels did not.

Tissue auxin increased steadily with age in plants not treated with fertilizer but fluctuated in treated plants with peaks at 3 weeks after transplanting (WAT) and 5 WAT. Higher activity was in 300KgNPK/ha treatments at 5 WAT, At 6 WAT highest activity was in the no-fertilizer treatment.

Total soluble carbohydrate (TSC) was significantly higher in plants with no fertilizer than for either 300kg and 600kgNPK/ha throughout the study period. This increased steadily with age and was highest at 6 WAT for untreated and 300kgNPK/ha treated plants, although for the latter category and 600kgNPK/ha treatments, TSC fluctuated with age.

Plant tissue N level was lowest in plants given no fertilizer- It increased steadily and significantly up to 6 WAT for non-fertilizer and 600kgNPK/ha treated plants. In 300kgNPK/ha treated plants, it fluctuated with age, but had its highest level at 6 WAT.

Carbohydrate: Nitrogen ratio (CHO:N) was generally lowest for 600kgNPK/ha treatments, followed by 300kgNPK/ha and highest for untreated plants throughout the study period.

Generally, IAA gave the earliest and most uniform rooting response than other auxin treatments and untreated controls regardless of fertilizer levels. For all fertilizer levels, IAA at 0.05g/l gave the earliest rooting, highest number and length of roots followed by IAA at 0.1g/l. For IAA/KAA treatments, rooting response was earliest at 0.02£g/l. This concentration also generally gave the highest number and length of roots. For NAA treatments, rooting response and root number were earliest and highest in 300kgNPK/ha at 0.10g/l. Root lengths were highest in 300kgMPK/ha and 0.05g/l. Generally, 300kgKPK/ha treatments gave better rooting response regardless of auxin treatments. High rooting

percentages (80 - 100%) were recorded for all fertilizer and auxin levels.

Adventitious roots originated from pre-formed parenchymatous root initial cell clumps in the cortex.

Seed-propagated plants had higher mean number and weight of fruits than stem-cutting propagated plants for all fertilizer and auxin treatments. Auxin levels did not significantly affect fruit number or size. The pattern of response in terms of fruit number at first harvest was 600kg> 300kg>0kgNPK/ha for seed-propagated plants and stem-cutting propagated plants. Fruit weight was 300kg>600kg>0kgNPK/ha for seed-propagated plants and 600 kg> 300kg>0kgNPK/ha for stem-cutting propagated plants. Fruit weight decreased significantly with harvest date for all fertilizer and auxin treatments as well as modes of propagation. Fruits from seed-propagated plants took 7 ± *1* days to 50% full ripening, while those from stem-cutting propagated plants remained unripened (orange-yellow) up to 12 days.

Seeds extracted from fruits of seed-propagated plants were heavier and had higher percentage germination (86.7%) than seeds from stem-cutting propagated plants (23.33%).

Shoot-tips aseptically cultured on Murashige and Skoog (MS) medium, pH 5.8 at 4000 lux light intensity, 27°C and 16 hours photoperiod, gave the best root regeneration and shoot growth response at IBA/K (indole-butyric acid/Kinetin) 10.0/0.01uM. Callus formation was prominent at IBA/K, 1.00.1uM. Shoot growth was good at 0.1/1.0UM IBA/K, but root regeneration and callus formation were poor. 2,4-D was generally unsuitable for shoot-tip culture, causing injury and death probably due to its phytotoxicity.

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CHAPTER ONE

I N T R O D U C T I O N

1.1 History:

Literature has it that the genus Lycopersicon originated from South America; for instance, Zeven and Zhukovsky (1975) traced the origin of the genus to a narrow belt of the South American West Coast limited by the equator (0°) latitude and long. 30°S; the Andes and Galapagos Islands. These authors further suggested that the vera-cruz-puebla area of Mexico was most likely the source of cultivated tomatoes of the world.

The ancestor of the tomato is probably var. Cerasiforme (Dun.) Alef. (Zeven and Zhukovsky, 1975). Outside its primary gene centre, the tomato plant is self-compatible. In Peru and Ecuador, Cerasiforme spontaneously crosses with L. pimpinellifolium. Tomato flowers pollinated by the pollen of L. peruvianum results in the induction of parthenocarpic fruits. In some cases, F_1 seeds may be set, resulting in hybrids with varying degrees of fertility and viability.

1.2 Botany:

According to Cobley and Steele (1976) tomatoes are annuals, or short-lived perennials cultivated as annuals. They have weak, trailing, much-branched stems covered with glistening reddish-yellow glandular hairs as well as pointed non-glandular hairs. The stem bears numerous spirally arranged leaves which are unevenly pinnate with variously indented and lobed margins.

Branching at the base of the stem is often monopodial, but tends to be sympodial higher up, with small cymose inflorescences developed from the apical bud. The flowers (with superior ovaries) are borne on short pedicels, and are often shed 2-3 days after they have opened. The floral parts are usually in sixes, but some cultivars have pentamerous flowers. The persistent, united sepals (calyx) enlarge with the fruit and has six sepals. The six bright-yellow petals are hairy on the outside, and are fused to form a rotate corolla tube.

Some cultivars are self-pollinated, while others are cross-pollinated. The ovary contains numerous ovules in axile placentation on a large fleshy placenta. The fruit is a red or yellowish globose berry with a smooth skin although cultivars vary in size and shape of fruits, thickness of the fleshy mesocarp and in the development of the placenta. The colouration of the fruit is due to the pigments carotene and lycopersicin which occur in different concentrations in fruits of different colours.

Tomato seeds are flat, kidney shaped and hairy, light-brown in colour and contain a curved embryo. After tomatoes have been processed to make juice, paste or puree, the press cake is used as a fertilizer or live stock feed (Cobley and Steele, 1976).

The crop is propagated from seed, which is commonly sown in nursery beds or seed boxes. When the seedlings are transplanted, their taproots are commonly damaged, and in mature plants, these are replaced by an extensive copiously branched adventitious root system. The adventitious roots arise from the base of the stem and stem cuttings root easily.

1.3 Importance:

While positive changes in yield patterns of cereals in the tropics have been recorded, tomatoes and other vegetables have not had such significant improvements. For example, FAO (1975) estimated an annual average increase in production of 2.6% for cereals like rice, maize and wheat, while vegetables like cabbage, green beans, sweet potatoes and tomatoes had an average of only about 0.87% for the period 1961-1975.

The tomato fruit has potential as a major source of vitamins and mineral elements. For example in Hawaii tomato fruits ranked 8th highest in protein and vitamin C, 9th in iron and 13th in vitamin A. It is also a good source of calcium, phosphorus, potassium, thiamin, riboflavin, niacin and ascorbic acid. The levels of these vitamins can be improved by breeding (Villareal, 1980).

Tomatoes can be a major source of these vitamins and minerals, if consumed in large quantities. In developing countries the consumption of tomatoes can be increased, causing them to become a larger source of vitamins if supplies are greater and more [illegible] available to consumers all year and at relatively cheaper prices.

1.4 Problems Associated with seed-production tomatoes:

Although wild ancestors of the tomato plant are out-breeding plants, the tomato has become predominantly in-breeding in cultivation (Cobley and Steele, 1976). In recent times various cultivars have been bred and selected for fruit size and quality and also for their ability to grow in different environments.

For instance, different cultivars have been bred for different uses, some are good for vegetable salad, while others are better for making puree, paste, ketchup, and juice. Others are more suitable for canning, pickling, etc. Consequently, tomato crop is now quite diverse in fruit size, shape, texture, etc.

Tomatoes grow well in the tropics. In humid lowlands however, the single most serious factor limiting its production is disease. The crop is often seriously attacked by fungal, bacterial, viral diseases as well as insect pests. Everywhere, susceptible cultivars suffer from these diseases and often massive infections of root (soil) nematodes, sometimes leading to serious losses of the crop. For example, in a study by Mayol (1980) it was observed that the dry weight of the foliage of plants innoculated with root-knot nematode Meladogyne incognita decreased by as much as 39% in 7 weeks. This decrease was due to premature senescence as a result of root necrosis and destruction of feeder roots.

Similarly, Elkholei et al. (1982) found that the nematode M. javanica caused a significant decrease in N, P, K, contents and dry matter accumulation in tomato plants. In fact the effect of nematodes were even more pronounced than the effects of soil salinity to which the tomato plant is known to be sensitive (Salama et al., 1981; Elkholei et al., 1982; Csizinszky and Schuster, 1982).

Some authors have attributed tomato plant susceptibility to diseases to cultural practices. For example, Spiegel et al. (1982) suggested that increasing the NO_3-N nutrition of tomato

plants increases the plants tolerance to the root-knot nematode M. javanica. It is however not desirable to increase the NO_3-N nutrition of vegetables, particularly those that may be eaten raw, because NO_3- may accumulate in the edible portions of the plant. This NO_3- is subsequently converted to NO_2- and nitrosamines, which when present in high doses are toxic to man. When nitrites are absorbed in quantity, ferrous haemoglobin is oxidised to ferric haemoglobin, and methemoglobinemia results. When this occurs, oxygen is not transported, and tissues are subjected to anoxemia. Since nitrite is the toxic substance, nitrate concentration serves as a direct index of the potential for nitrite toxicity (Maynard and Barker, 1972).

Due to these reasons, breeders have developed several varieties of tomato which are resistant to diseases. Examples are the 'Rubyvee'-resistant to Fusarium oxysporum; 'Wondervee'-resistant to Verticillium wilt; "Ontario 7710" resistant to Pseudomonas syringae Pv. tomato and 'Veeking' tomato-resistant to Verticillium and fusarium wilt as well as being tolerant to metribuzin (herbicide) (Karr and Cook, 1983, 1984).

Locally, varieties like 'Roma VF', 'Cirio 56', 'Marzanino', 'Piacenza 0164', 'Harvester', 'LaBonita', 'Chico', 'Gamado', 'Gemed F', 'Ronita', 'Ife 1' and 'Enterpriser' are all resistant to fusarium wilt; none however is resistant to bacterial wilt and some are susceptible to leaf-spot (A. solani and Septoria lycopersici) and Leaf mould (Cladosporium fulvum) (A.E.R.L.S.).

However, even where and when these varieties have been developed, several problems still exist; sometimes a seed-propagated cultivar that is produced initially in one location

may undergo a 'genetic shift' if it is grown for several consecutive generations in a different location (Hartmann and Kester, 1975) Loss of heterosis (hybrid vigour) in succeeding (inbred) generations is a second problem (cultivated tomatoes are inbreeding). To maintain maximum hybrid vigour, it is necessary to repeat the original parental cross for each new crop. This the local farmer cannot do. However, if a plant can be propagated vegetatively, the hybrid vigour can be maintained indefinitely by cuttings, bulbs, corms, tubers, tissue culture, etc.

The tomato plant does not produce bulbs, corms or tubers. Its stem cuttings however, root very easily. It also has potentials for propagation by grafting, in fact several workers, for instance Reboul et al. (1981) and Pereguine and Ahmed (1982) have both tried to induce resistance on tomato plants by grafting tomato scions unto rootstocks from solanum species resistant to the bacterial wilt caused by Pseudomonas solanacearum. Similarly, Thorpe and Jarvis (1981) found that grafting the susceptible greenhouse tomato cv. MR13 to rootstocks of the resistant cultivar IRB-301-31 confered total escape from Fusarium foot and root rot caused by Fusarium oxysporum F. sp. radicislycopersici.

Grafting however, has several disadvantages. First, special techniques are involved, which might be difficult for the local farmer to employ. There also has to be compatibility between the scion and the rootstock or else the graft union would be rejected. This means, empirical

trials, which are time consuming. Poor graft union due to lack of expertise and lack of uniformity within grafted seedlings are other problems involved. In addition, certain undesirable metabolites from the rootstock might be translocated into and accumulate in the fruits of tomato plants reproduced from such graft unions. For example, tomato scions grafted unto rootstocks of Datura stramonium which was resistant to the root-knot nematode, produced fruits with as much as 0.03mg of alkaloids per gram of fresh fruit, whereas ungrafted plants had zero alkaloid content (Lowman and Kelley, In: Hartmann and Kester, 1975).

Two attractive techniques that present possibilities for vegetative propagation of tomatoes, are by rooting of stem cuttings (cuttage or macropropagation) and tissue culture (micropropagation). These have several advantages. These are that many new plants can be started in a limited space from a few stock plants. From each cutting that roots, and each plantlet that establishes and grows into a new plant, several other plants can be obtained. Cuttage is rapid, inexpensive and so simple that a farmer with the barest minimum of expertise can start new plants from cuttings. For instance the cultivated cassava (Manihot sp.) is commonly propagated by stem cuttings. Micropropagation though not so inexpensive or simple, provides a means by which plantlets can be regenerated in high frequency. In addition, disease-free plants can be obtained and maintained from almost any part of the plant.

Greater uniformity is obtained by the absence of variation which sometimes appears owing to the variable seedling rootstocks of grafted plants and more often than not, the parent plant is reproduced exactly; with no genetic change. Furthermore, the time taken from the planting of tomato seeds to harvest of the fruit may be greatly reduced, if the plants are started as cuttings or micropropagated plantlets. Moreover, when new, resistant and high yielding cultivars are developed, they can be propagated continuously as vegetatively reproducing clones, without fear of loss of heterosis or a genetic shift, since all individuals in the clone are genetically identical.

Where it becomes necessary to modify the genetic composition of the clone, induced mutation can be employed. For example, Moh and Alan (1973) have used induced mutation to improve the growth characteristics of cassava stem cuttings. Similarly, chemical mutagens have been employed to improve the genetic structure of banana cultivars in Jamaica (Menendez, 1973).

This thesis evaluates the efficiency of two methods of vegetative propagation, namely: macropropagation (by stem cuttings) and micropropagation (plant tissue culture) in the production of Lycopersicon esculentum (Mill) 'Roma VF', taking into consideration' nutritional (CHO:N ratio, and vitamins) hormonal (endogenous and exogenously applied auxin) and environmental (light, temperature and humidity) that influence these techniques.

Evidence as to the nature of the photoperiodic response of the tomato plant is conflicting. While workers like Cooper (1927, 1962) suggested that it is a weak long-day plant, others like Wittwer (1963) say it is a facultative short-day plant. Evans (1969) concluded that a true photoperiodic behaviour may reside in the cultivated tomato as a result of cross-pollination with short-day perrenial species in the course of evolution of modern varieties. He further stated that there is no evidence against the proposal that the tomato is a facaultative short-day plant. Eguchi et al. (1948) however proposed that the tomato plant is a 'nutritional type' rather than a photoperiodic plant in its flowering behaviour.

The cultivar 'Roma VF' is an early to medium paste type with determinate growth habit during the dry season, but in the rains growth is much taller and vigorous. Fruits are medium sized pear shaped with a uniform green colour when immature (Quinn, 1980).

Specific objectives include:

- To evaluate the effect of the CHO:N status of the CHO:N ratio of stock plant on rooting of stem cuttings taken from them.
- To trace the fluctuations in the level of endogenous auxin-like substances, with age of plant.
- To relate the NPK-nutrition of tomato plants to the level of auxin-like substances.
- To evaluate the effect of two exogenously applied auxins on the rooting, establishment and survival of rooted cuttings.
- To determine the origin of adventitious roots in tomato stem cuttings.
- To determine the optimum requirements, in terms of temperature, light intensity and duration, humidity, nutrient and hormones, for the micropropagation of tomato.
- To assess qualitative and quantitatively the influence of methods of propagation on seed viability, fruit quality, growth characteristics and fruit yield of tomato.
- To evaluate the effect of NPK fertilization on the growth of tomato plants propagated by seed and stem-cuttings.

CHAPTER TWO

.2 LITERATURE REVIEW

2.1 GENERAL ASPECTS OF VEGETATIVE PROPAGATION

Plant breeding and selection aim at choosing and multiplying crop plants that are genetically vigorous and most able to satisfy human needs (Karikari, 1988).

Vegetative propagation is the process of reproducing new plants from parts of parent plants, such as stems, leaves, or even cells of the plant concerned without the sexual processes involved in propagation by seeds. Such propagation entails mainly the mitotic process of cell division and multiplication. Plants propagated in this way are genetically similar to the parent plants, and such propagation mechanisms preserve the unique characteristics of any single plant which are perpetuated in the propagation of a clone.

A clone has been defined by Hartmann and Kester (1975) as a group of plants that are of uniform genetic make-up derived from a single individual and propagated exclusively by vegetative means, such as cuttings, grafts, layering, divisions or tissue culture, which all require manipulation by man. Other means of propagation which occur in nature include rhizomes, corms, stolons, runners tip layers, bulbs, etc. Some of these methods of propagation are fairly simple and straightforward. Others like the use of cuttings, tissue culture and sometimes layering require the use of several plant growth regulators particularly hormones like auxins, kinetins, as well as several other growth promoting substances.

Many clones can perpetuate themselves successfully in nature better than some seeds as long as the environment remains reasonably stable. On the other hand, rare and

drastic changes may occur in the environment in which case, clones are handicapped since they may not have the opportunity to evolve new forms better adapted to the new environment by themselves due to lack of cross-fertilization (Hartmann and Kester, 1975).

Karikari (1988) listed the main purposes for which vegetative propagation methods are used as:

(i) to establish clones from selected high yielding parent plants;

(ii) to propagate plants which do not produce viable of sufficient seed e.g. banana, pineapple and navel orange;

(iii) to select for sex in dioecious trees such as nutmeg;

(iv) To obtain earlier fruiting; plants propagated vegetatively generally come to bearing quicker than corresponding seedlings;

(v) to obtain various advantages which may result from a suitable combination of rootstock and scion, such as modification of vigour, resistance or immunity to pests, diseases or wind damage, suitability to different soils, climatic conditions or cultural systems.

Cuttings for macropropagation can be obtained from plant parts like the hardwood, semi-hardwood, softwood (greenwood), leaves, leaf buds and roots. Similarly, material for micropropagation can be obtained from vascular cambia, storage parenchyma, pericycle of roots, endosperm, cotyledons, leaf mesophyll and provascular tissue (Street, 1977).

2.2 PROPAGATION BY STEM CUTTINGS

Plant propagation by stem cuttings is based on the ability of plant stem sections to produce adventitious roots at the base of stem cuttings. This is due to the ability of endogenously produced hormones (auxins) to induce rooting. These auxins are synthesized in the meristematic regions and translocated basipetally to initiate new roots at the target area. After the formation of roots, the plant usually is established and new growth proceeds. Auxins have also been applied exogenously to cuttings of plants that either do not produce enough to favour rooting or for some other reason fail to root readily.

The most common auxins employed are indole-3-acetic acid (IAA) which is a native auxin usually extracted from plant tissues; indole-3-butyric acid (IBA); Naphthalene acetic (NAA); and sometimes 2,4-Dichlorophenoxy acetic acid (2,4-D). These later three auxins are synthetic compounds commonly employed in root initiation. Other rooting promoters, such as phenols and their derivatives also occur both naturally in plants and as synthetics e.g. Pyrogallol, cathecol, salicyclic and tannic acid. Several auxin types have been used to induce rooting of cuttings in various plant species. These chemicals are available in commercial preparations; dispersed in talc or liquid formulations which can be diluted with water to the proper strength. The purpose of applying these chemicals, particularly in difficult-to-root species, is to increase the percentage of cuttings which form roots, to hasten root

initiation, to increase uniformity of rooting and to increase the number and quality of roots produced per cutting.

2.2.0 FACTORS AFFECTING PROPAGATION BY STEM CUTTINGS

(i) Chemical Applications:

Auxin applications in various forms, combinations and concentrations are known to influence the success of rooting greatly. Torre et al. (1980) found in red-raspberry, that root numbers were strongly increased by IAA at $2x10^{-4}M$, Potassium-indole-3-butyrate at $2x10^{-5}M$ and NAA at about $2x10^{-5}M$. Active range of IAA was narrow, with shoot damage occuring at $4x10^{-4}M$ or greater and much reduced rooting at $1x10^{-4}M$ or less. Prolonged exposure of cuttings to $2x10^{-4}M$ IAA increased the number of roots per cutting. In apple (cv. red delicious) cuttings treated with 5, 10, 20 and 40ppm IBA and IAA, highest number and maximum length of roots were recorded in cuttings treated with IAA at 20ppm (. Khan, 1982). Similar results were obtained in Dalbergia latifolia cuttings treated with 1000ppm, IAA, IBA or NAA for 48 hours, where there was an increased percentage rooting over controls dipped in water. IAA was found to be the best treatment (Negi, 1982).

On the other hand, Li and Eaton (1984) observed that in two grape cultivars, IBA pre-treatment at $2.46x10^{-3}M$ and $3.94x10^{-3}M$ gave better results in rooting than BA (Benzyladenine) and NAA. Geneve and Heuser (1982) found in Mung bean (Vigna radiata) cuttings that NAA and IBA were most effective in stimulating rooting at 10^{-7} to $10^{-3}M$ with IAA intermediate at 10^{-4}-10^{-3} and 2,4-D least at $10^{-3}M$ only.

In peppers, Pillay et al. (1982) found that dipping stem cuttings in 1000ppm IBA for 5secs gave the highest number of rooted cuttings at 20 and 90 days. Similarly root initiation and development of black pepper roots was significantly increased by NAA. A 0.1ppm concentration of NAA increased root weight per plant by 182% as compared to no application of NAA (Khoon and Talib, 1985). Dipping Barberry cuttings, in 3000ppm IBA was found to be the best treatment for rooting (Satomi and Preece, 1987) and IBA has also been found to induce profuse root formation and sprouting in yam tubers. IBA at 500mg/l^{-1} gave 100% sprouting within 3 weeks (Okezie, 1985). Rooting percentages of up to 95% have been reported for Arizona rosewood stem cuttings with 4000 -8000ppm dips of IBA (Smith and Lee, 1983).

In three species of Chickpea, namely Cicer arietinum, C. cuneatum and C. reticulatum, IBA and IAA enhanced rooting at concentrations 0, 0.2, 0.4, 0.6, 0.8 and 1ppm. Results showed that although IBA was a better rooting compound than IAA, the different species, required different optimum concentrations of either of the two (Bassiri et al., 1985). Similarly, other workers like Khan (1982) found no significant differences between auxins IBA and IAA or within treatment levels in the rooting of Ficus sp. grown in solid medium in the glasshouse.

Sometimes a combination of two or more of the auxins produce better results. For example, mixtures of equal parts of indole-butyric acid and naphthalene-acetic acid at

a low concentration have given more roots and higher percentage of rooted cuttings in arbovitae (Thuja sp.) and bittersweet (Celastrus sp.) (Malstede and Haber, In: Hartmann and Kester, 1975). Similarly, IBA up to 4000ppm and IBA + NAA up to 2000ppm have been found to give similar results in the rooting of Popolus tremula (Al-Kinamy, 1981).

There are also reports that low concentrations of Kinetin stimulate root initiation. For instance a concentration of 0.1ppm Kinetin, more than doubled the rooting of terminal cuttings of one clone of the difficult-to-root Feijoa sellowiana (Meredith et al., In: Hartmann and Kester, 1975). Sometimes, chemicals applied to control diseases may adversely affect the rooting response of cuttings. For example fungicides applied as dips to polyurethane propagation cubes prior to propagation of poinsettia (Euphorbia pulcherimma Willd.) cuttings, inhibitted rooting. Least inhibition of rooting was from either 37mg metalaxyl, 25WP plus 300mg ferban, 76WP, 500mg, ferban 76WP or 599mg benomyl 50WP (Lee et al., 1983).

2.2 (ii) Physical Environmental Factors:

A wide variety of rooting media are used and these include soil, sand, peat moss, shredded sphagnum moss, vermiculite, perlite or even water. Sometimes the quantity and quality of roots depends on the type of medium used. For example, Pokorny and Austin (1982) in a study with blackberry (Vaccinium ashei) softwood cuttings, found that

rooting was best in media containing milled pine bark alone or mixed with equal parts of perlite compared to sphagnum peat moss alone or mixed with equal parts perlite.

The environment in which cuttings are rooted, particularly, light, moisture and temperature have pronounced effects on the rooting response of propagated plants. Hardwood stem cuttings started out of doors in the nursery require only the usual care given to other crop plants, such as adequate supply of moisture, freedom from weed competition, insect and disease infestation. Leafy softwood or semi-hardwood stem cuttings and leaf bud or leaf cuttings are rooted under high humidity and these require close attention throughout the rooting period. Temperatures should be closely regulated and cuttings must not be allowed to show wilting for any length of time. Adequate drainage must be provided (Hartmann and Kester, 1975).

The necessity for environmental control is probably better illustrated by a few examples; Kelly and Moser (1983) found that in tulips (Liriodendron tulipfera L.) root regeneration and shoot growth of seedlings increased as temperatures of either the soil or aerial growing environment increased from 10° to 21°C. IBA applied at 1000mg lit^{-1} increased root regeneration and shoot growth when both air and soil temperatures were held at either 15.5° or 21°C. When air temperature was maintained at 21°C and soil temperature was varied from 10°C to 21°C, root regeneration and shoot growth increased as soil temperatures increased. Similarly, cuttings of three avocado cultivars were found to root best under mist with IBA treatment and a soil temperature of about 25°C (Ernst and Holtzhausen, 1982).

Although varying light intensity made no difference to the percentage of Euonymus japonicus softwood cuttings that rooted, the number and weight of roots per cutting were lower at lower than at higher light intensities (Ooishi et al., 1981).

To effectively control the environment of cuttings during rooting, several types of propagating frames have been designed and are currently in use at commercial installations. These include plastic and glass covered green houses, hot beds, cold frames, lath-houses, flouresecent light boxes, propagating cases,

2.2 (iii) Juvenility (Age):

Apart from purely environmental factors, certain properties of the stockplant as well as the cutting itself influence the success of rooting. One of such is the age, otherwise referred to as the juvenility factor. For example, juvenile cuttings of pecan (Carya illinoensis) produced more roots and a larger percentage of rooted cuttings than adult cuttings when treated with 1 or 2% solution of IBA (Smith and Chiu, 1980). Similarly in both apple (Malus sylvestris L.) and Peach (Prunus persica L.), juvenile cuttings rooted better than adult cuttings. For both types of apple cuttings, 0.8% IBA (in talcum) gave the highest % rooting and the greatest number and length of roots; and for both types of peach cuttings, best results were obtained with 0.1% IBA (in talcum) or 200ppm IBA in solution (Nyomora and Mnzawa, 1982). Related to juvenility, the position of the cutting on the plant at the time of cutting is also important. For example, semi-hardwood

cuttings of peach taken from terminal portions rooted better than from basal portions and this was related to stem diameter (Marini, 1983). However, in sand pear (Pyrus pyrifolia) rooting was not significantly different between basal, sub-apical and apical parts of 1-year old shoots (Sandhu et al., 1982).

Particularly for species that exhibit bud dormancy, the time of the year during which cuttings are taken influences rooting and this is related to the general metabolic state of the buds. This has been demonstrated in species like Malus sylvestris (apple) and Prunus persica (Peach)(Nyomora and Mnzawe, 1982), where cuttings taken in July at the beginning of dormancy, rooted better than those taken in September at the end of dormancy. Similarly, rooting percentages of juvenile cuttings of pecan (Carya illinoensis) was greater with February, June and August cuttings than April, October and December cuttings (Smith and Chiu, 1980). Rooting percentages were similarly higher in May than in April, June or July cuttings of Arizona rosewood (Vanquelinia californica) (Smith and Lee, 1983).

2.2 (iv) Anatomical basis for rooting initiation in stem cuttings:

The formation of adventitious roots in most plants species, takes place after the cutting is made. These roots usually originate from a certain group of cells, which become meristematic probably due to stimulation by auxins, just like callus formation takes place after wounding. The type of tissues involved at the site of origin depends on the kind of plant. For example, Priestly and Swingle (1929) observed that in herbaceous plants, adventitious roots usually originate just outside and between vascular bundles.

In woody perennials, adventitious roots in stem cuttings usually originate in the young, secondary phloem although they may also arise from other tissues such as vascular rays, cambium or pith (Bhella and Roberts, 1975; El-Toni and Galal, 1980).

Generally however, the origin and development of adventitious roots take place next to and outward from the central core of the vascular tissue. Upon emergence from the stem, the adventitious roots have developed a root-cap and the usual root tissues, as well as a complete vascular connection with the orginating stem (Esau, 1965).

2.2 (v) Physiological basis for root formation in stem cuttings:

Adventitious root formation in stem cuttings depends on the interaction among several compounds which include auxins, enzymes, phenols, carbohydrates and nitrogenous compounds. Bouillene and Bouillenewalrand in: Hartmann and Kester (1975) proposed that a rooting factor, the 'rhizocaline' is the determinant. These authors proposed that, the rhizocaline is made up of three components: a specific factor translocated from the leaves with the chemical properties of an orthodihydroxy-phenol, a non-specific factor (auxin) which is also translocated and exists in low concentrations and a specific enzyme located in cells of certain tissues, which is probably of the polyphenol oxidase type. In their contention, the orthodihydroxyphenol reacts with the auxin in the presence of the enzyme to form the complex 'rhizocaline' as the initial step to root formation.

Recent work has thrown more light on the process of root initiation in stem cuttings and generally agree with the proposed 'rhizocaline' system. For example, Tognoni and Lorenzi (1972) found that, acidic extracts of difficult-to-root cultivars of Picea had a high concentrations of substances which inhibited the growth of Avena first internodes, while easy-to-root-cultivars of Chamaecyparis had a low concentration of these substances. The substances stimulated root formation in mung bean cuttings and showed a dose response effect over a concentration range of 0-1.106g fr. wt. An additive effect was also noticed, between extract and IAA and the possibility

of rooting being determined by a hormone balance was suggested. Chromatograms derived from alcoholic extract of cuttings of plum, yielded presumptive IAA upon re-chromatography and KOH and peptidase treatment of alcohol insoluble residues yielded a radioactive substance with chromatographic properties of IAA (Kay Ryugo and Breen, 1974). Earlier, Haissig (1970) found that IAA always influences the rooting process both by cell division and cell extension. The role of IAA is however, limited by the presence of the enzyme IAA-oxidase, which has been found to be the same enzyme that acts as guaiacol peroxidase (Hoyle, 1972). In pea seedlings, Bryant and Lane (1979) found that peroxidative and non-peroxidative forms of the enzyme exist. From their study, the plumule hook , which is a tissue that has high IAA oxidase activity, contained no detectable peroxidases. They concluded that non-peroxidative IAA oxidases might play a major role in the regulation of IAA content of pea seedlings.

In Zea mays the oxidation of exogenously applied IAA to ox-IAA is the major catabolic pathway for IAA in endosperm tissue. Here ox-IAA was found to be naturally occuring and the amount of ox-IAA in both shoot and endosperm tissue approximate the amount of free IAA. Ox-IAA has been reported to be inactive in growth promotion and thus the rate of oxidation of IAA to ox-IAA could play a role in the regulation of IAA mediated growth (Reinecke and Bandurski, 1983). From their work with peach seeds, Ritzert et al. (1972) agreed with this and further found that, the enzyme had a pH optimum of 4.5-5.0 and required Manganese ion (Mn^{2+}) and 2,4-D for optimum activity. The reaction product

however appeared to be 3-methylene oxindole. Catechin a naturally occuring polyphenol was found to inhibit the IAA oxidase system and had a synergistic effect on IAA activity in the Avena coleoptile straight growth test. Kinetic studies demonstrated that catechin inhibition was non-competitive with substrate. On the other hand, there are indications that exogenously applied growth regulators such as GA promote IAA oxidation and thus rooting (Ockerse and Waber, 1970). Basu et al. (1970) also reported that ABA promotes the rooting of cuttings.

IAA oxidase activity seems to increase with maturity of plant material and IBA applications have caused an initial decrease in activity soon after application, followed by a dramatic increase prior to root formation (Al Barazi and Schwabe, 1984).

Generally, orthodihydroxyphenols act as inhibitors of the IAA oxidase system (Heuser and Hess, 1972) and twenty one phenolics including catechol, pyrogallol, salicyclic acid and tannic acid have been shown to stimulate rooting. Catechol stimulated rooting in softwood cuttings of Acer saccharinum. Likewise softwood cuttings of Acer griseum rooted 100% when treated with a combination of catechol (4.5×10^{-3}M) and IAA (1.1×10^{-3}M) for 24 hrs. (Kling and Meyer Jr., 1983).

Similar findings have been reported by Bose et al. (1972) and Roy et al. (1972) in 10 sub-tropical shrubs and vines; and mungbean cuttings respectively.

Earlier, Hess (1962) had proposed that the synergistic effect of catechol with IAA is due to adjacent hydroxyl groups and a free para position on the aromatic ring. Phenolic

compounds without adjacent hydroxyl groups and a free para position would not stimulate rooting with IAA, in Hedera helix and Hibiscus rosasinensis.

Evidence presented here, suggests that stem sections low in phenols and high in the IAA-oxidase enzyme, may not root readily. Bansal and Nanda (1981) have however, shown a positive correlation between IAA-oxidase activity and rooting response in stem cuttings of some forest tree species.

Another enzyme involved in rooting of cuttings is polyphenol oxidase (PPO). This enzyme is situated around the root initiation area around the secondary xylem and phloem. In Pistachia vera it has been found to decrease with maturity of plant tissue (Al Barazi and Schwabe, 1984). In apple, PPO-activity and phloridzin content in the xylem increased with endogenous rooting co-factor activity and the rooting of cuttings. When tested, many oxidised phenolic substances improved rooting. Furthermore, the reaction product of PPO and phloridzin showed certain similarities with extracted endogenous co-factor whose activity during a period of poor rooting was increased by adding PPO and phloridzin. Rooting of IBA treated cuttings was also increased by pre-treatment with the products of a PPO and phloridzin reaction (Bassuk et al., 1981). However, no significant rooting responses were found in pepper cuttings treated with the phenols Phloroglucinol and Phloridzin (Khoon and Talib, 1985).

The presence of endogenous and sometimes exogenously applied rooting inhibitors is another key factor in the rooting of stem cuttings. For instance, Paton et al. (1970), and Crow et al. (1971) found that in the difficult-to-root adult tissues of Eucalyptus grandis, there is normally a high concentration of compounds that blocked adventitious root formation. Three of such inhibitors were found to be naturally occurring derivatives of the 2,3-dioxobicycle (4,4,0) decane system. These inhibitors were not present in the easily rooted juvenile tissue. The action of these chemicals is probably as a result of blockage of RNA synthesis. For instance, Kefford (1973) found that the application of actinomycin-D inhibits RNA synthesis. Similarly, Jain and Nanda (1972) demonstrated that applications of cycloheximide and actinomycin-D inhibited the rooting of Salix tetrasperma. These chemicals are known to inhibit protein and RNA syntheses respectively.

Mineral elements in various combinations also play a physiological role in rooting. For example, Gorst et al. (1982) found in Eucalyptus ficifolia that there was a positive interaction between five major and minor nutrient combinations and the efficiency of IBA as a rooting agent.

As soon as adventitious roots are initiated in cuttings, a lot of metabolic activities take place as the new root tissues develop and roots grow through and out of the surrounding stem tissue to become external functional roots. For example Molnar and Lacroix (1972a & b) observed in Hydrangea that, root

initials originated from the phloem ray parenchyma, with roots emerging 10-12 days after the cuttings were made. The total protein content of root initials were found to increase over 100 percent in the first four days after the cuttings were made, but there was no significant increase in the DNA content of the cells until the sixth day. The same authors in 1972(a) found significant increases in enzyme activity during adventitious root formation; peroxidase, cytochrome oxidase, succinic dehydrogenase and starch hydrolyzing enzymes were observed in the phloem and xylem ray cells of the vascular bundles. During root development, enzyme activity shifted from the vascular tissues to the periphery of the bundles. Breen and Muroaka (1973) reported that developing callus and roots act as a 'sink' for the movement of soluble carbohydrates from the top of the cutting.

According to Hartmann and Kester (1975) it is likely that some proteins play specific roles in the activation of the root primordia. For example, peroxidase may destroy some inhibitors; which may be blocking metabolic processes leading to root formation. Increases in the activity of other enzymes may only be associated with resumption of rapid general cellular activity. Succinic dehydrogenase and cytochrome oxidase are involved in cellular respiration, while amylase releases sugars from starch as a general substrate for various continuous synthetic processes.

2.2 (vi) Role of Organic Nutritional Status of stockplant and Cuttage:

The carbohydrate and nitrogen nutrition of the stockplant is recognised as one of the very important factors that affect the success of propagating plants from stem cuttings. Plants which are in good nutritional state and are growing under good environmental condition of temperature, moisture and light, will have a relatively high carbohydrate-nitrogen ratio in their tissues. Cuttings collected from such plants, will produce a greater number of roots than those taken from plants having a low carbohydrate-nitrogen ratio. In a study by Stolt and Hess (1968) in Chrysanthemum cuttings, there was a positive correlation between the carbohydrate storage in stems and the rooting response of plants. Similarly, the hop (Humulus sp.) plant cuttings responded to sugar pre-treatments by large increases in root production (Howard & Sykes, 1966). Also, Gautheret (1969) in Helianthus tuberosus and Olieman et al. (1971) in Phaseolus and Rhododendron found that glucose was essential in nutrient medium for maximum rooting response. Related to this Greenwood and Berlyn (1973) found that sucrose was also essential for good rooting of Pinus lambertiana embryo cuttings.

In his study, Winkler, in: Hartmann and Kester (1975) found that 63% of the high-starch cuttings of grapes rooted, 35% of the medium starch cuttings rooted and 17% of the low-starch cutting rooted. Reuveni and Adato (1974) found in Phoenix dactylifera that carbohydrate content was higher in

easy-to-root than in difficult-to-root offshoots although the latter contained more reducing sugars, which might indicate a higher rate of hydrolysis of carbohydrate as a result of higher vegetative growth. In Mung bean hypocotyl cuttings, 50% of the decrease in root initiation could be regained by the application of glucose and fructose thus pointing out the importance of CHO supply in root initiation, however the other 50% loss could not be recovered, probably due to loss of some other factors like phenols (Hauser and Hess, 1972).

Reuveni and Raviv (1981) also found that in Avocado cuttings, starch accumulation at the cutting bases occurred while the cuttings were under mist; this was correlated with the rooting capability. Similarly, in Pisum sativum, Davis and Potter (1981) found that treatments which caused a reduction in the rate of photosynthesis lowered sucrose and glucose levels in the basal portion of the cuttings. These findings indicated that the supply of current photosynthates to the base of cuttings is important to rooting. In apple cuttings Cheffins and Howard (1982) found that root production was associated with a marked redistribution of carbohydrates, an increase in the rooting zone being at the expense of adjacent tissues. Conditions which led to more rooting caused greater losses of CHO and establishment appeared to be controlled partly by the level of carbohydrate remaining after the root-stimulating phase.

Active buds on rooting cuttings of apple appear to be a preferential sink for carbohydrates and bud activity during the rooting period appears to compete with the important basal sink for carbohydrate, lessening the ability of cuttings to establish when transplanted (Cheffins and Howard, 1982, b). Related to this, it was found that in cocoa hybrids at IITA (Nigeria), the presence of only one leaf per cutting significantly reduced rooting. Two leaves per cutting were adequate. Reduction in the size of leaves by half was found to have no adverse effects on rooting and this even enhanced the survival of cuttings under polythene sheets, probably due to reduced transpiration (Odegbaro and Adedipe, 1986).

The importance of nitrogen in rooting activities cannot be over-emphasised. For example, Pearse, in: Hartmann and Kester (1975) found that grape cuttings from stock plants grown under reduced nitrogen, had high rooting response. This was substantiated by tests within geranium where low and medium nitrogen content of stock plants gave better rooted cuttings than the high nitrogen treatments (Hann and Cornell, in: Hartmann and Kester, 1975).

Extreme nitrogen deficiency however, decreased rather than increased rooting (Pearse, in: Hartmann and Kester, 1975). In Blue berry cuttings, Smagula and Hepler (1980) found that cuttings grown under field conditions for 6 months, responded to the N-treatments by producing larger root systems,

more aerial growth and more rhizomes. Scott et al. (1982) found that the number and length of roots per explant of Rose shoots increased as the concentration of total N in the medium was reduced from 60-7.5mM. There was no effect on rooting with as much as a 16-fold reduction in the concentration of the remaining salts in the medium when total N was kept at a constant 7.5mM. It appears then that there is a minimum and maximum level of nitrogen requirement. Outside this range it seems rooting is impaired.

In tomatoes, the dry weight of vegetatively propagated shoots was closely related to the crude protein content of the stock plants. The dry weights of roots from terminal cuttings was also positively correlated with the crude protein content of the stock plants. The crude protein accumulated by the new shoot was proportional to the size of the shoot (Lowe and Ries, 1972). It is not clear why high levels of nitrogen are detrimental to good rooting, however for root iniation to take place, it is necessary to supply nitrogen for nucleic acid and protein synthesis.

2.3 PROPAGATION BY TISSUE CULTURE

The term 'plant tissue culture' generally refers to all types of aseptic micropropagation techniques including cultures of seedlings or large plants (Plant cultures); of isolated mature or immature embryos (embryo culture); of isolated plant organs (organ culture), including cultures of root tips, stem tips,

leaf primordia, primordia or immature parts of flowers, immature tissues arising by proliferation from segments (explants) of plant organs (tissue or callus cultures); of isolated cells or very small cell aggregates remaining dispersed as they grow in liquid media (suspension cultures). Almost any tissue of the plant can be cultured in the appropriate medium. These include vascular cambia, storage parenchyma, pericycle of roots, endosperm, cotyledons, leaf mesophyll and provascular tissue (Street, 1977).

The fragment of a plant or tissue used to initiate a culture clone is referred to as an 'explant'. Since plants are inevitably contaminated with micro-organisms, there is a vital need for sterilization of both plant material and the instruments used. A wide variety of sterilizing agents are used for sterilization. Antibiotics are used to check bacterial growth in the cultures.

To take explants, fairly large pieces of tissue are generally favoured, however such explants should be taken so as to obtain the maximum number of explants from a single source (Street, 1977).

After the explant has been obtained and sterilized, it is necessary to provide the right nutrient medium for regeneration. All nutrient media are basically mixtures of the essential macro- and micro-elements together with a source of carbon usually sucrose. For many plant tissues, a number of additives are essential. These include vitamins, amino acids, sugar

alcohols, auxins and related growth regulators, gibberellins, kinetin or other cytokinins. A full review of the principles and practice of plant tissue culture is available in Street (1977); Hartmann and Kester (1975).

Plant tissue culture is used as a technique for the rapid clonal propagation of plant material, with the minimum loss of genetic attributes. Various workers have employed various modifications of this method, with varying results. For example, Murashige et al. (1972) propagated in vitro, Asparagus officinalis (L.) from surface sterilized (0.5% sodium hypochlorite) single shoot explants. Cultures were maintained under 16 hr daily low intensity illumination at 27°C. After 6 weeks it was found that initiation of adventitious roots at the base of the explant and elongation of the apex had been completed. NAA concentrations of 0.3mg/l enabled good rooting without inhibiting spear formation. IAA was effective and could not replace NAA. Kinetin enhanced spear and root initiation but reduced rate of survival of cultures. 0.1mg/l Kinetin was about optimum. Yang and Clore (1974) obtained similar results with the same techniques and the same species.

In Garlic (Allium sativum L.) meristems were cultured on the Murashige and Skoog (MS) or Nitsch (N) media at 970lux with 16 hr photoperiod at 25°C. The best medium for tissue increase was MS medium supplemented with 1mg/l IAA, 1mg/l 2,4-D and 25ml of deprotenized coconut milk. MS medium with 1mg/l IAA and kinetin did not permit extensive callus growth but shifted growth towards differentiation (Kehr and Schaeffer, 1976). Using standard MS medium with 30g/l sucrose and pH 5.8,

Litz and Conover (1978) found that sweet potato axillary buds and shoot tips initiated callus at cut surfaces of explants within 2 weeks. Shoot induction from quiescent appical meristems occurred at 5 weeks and small rooted transferable plantlets were formed within 8 weeks. In Ficus lyrata, surface sterilized leaf fragments were cultured on a modified Nitsch and Nitsch medium, containing thiamine 15uM and supplemented with 24uM BA (Benzyladenine) + 24uM 2-iP) (N-(3-methyl-2-butenyl) 14-purin-6-amine) + 24uM kinetin + 12.5uM IBA. After 3 months, the explants started to proliferate on the edge of the cuttings producing many adventitious buds that developed into shootlets. During a further growth period of 1 month, full elongation was attained. The plants were then transferred to a rooting medium containing Knopps macro-elements, modified Nitsch and 5uM of NAA. After 1 week, the shootlets were deprived of NAA and thus induced to root within 1 week (Jona and Gribaudo 1987).

Similarly, Omura et al. (1987) have used callus tissue from leaf explants of pomegranate (Punica granatum L.) clones to produce plantlets. Here a modified MS based salt medium supplemented with a range of 0.5-10.0uM BA and 0.05-5uM NAA were used. Shoot elongation was stimulated most efficiently when the initial calli were transferred from the shoot induction medium to a half strength MS supplemented with 2uM BA. Elongated shoots rooted easily on a medium of half MS and 0.1uM NAA. With tomato leaves, best sprout formation was obtained with a medium containing 4mg/l IAA and 4mg/l kinetin (Santana, 1985).

Similarly, a combination of IAA (5.7uM/l) and kinetin (4.7uM/l) was found to be equal to zeatin in shoot regeneration efficiency and reduced the time required for shoot production. Rooting was achieved by transplanting the in vitro produced shoots to 'pro-mix' under intermittent mist in a greenhouse (Uddin et al., 1988). In lettuce (Lactuca sativa L.) Blocksberg and Saltveit (1986) using the MS basal medium, developed a procedure to regenerate plants from excised axillary buds of harvested and stored heads. This procedure permitted the rescue of lettuce germplasm that showed resistance to post-harvest physiological disorders and diseases. At IITA, Nigeria, a meristem (shoot tip) culture technique to obtain clonal plant material free of viruses occuring in sweet potato has been developed. Over the period 1981 - 1986, 60 countries received tissue culture materials from IITA. At present this organisation has developed 33 improved sweet potato varieties available for distribution (Ng, 1987).
In tomatoes, shoot apical meristems isolated from 7 day old seedlings have been aseptically cultured on MS medium supplemented with varying concentrations of auxin and cytokinins under defined environmental conditions. Benzyladenine (BA) or Zeatin (Z) at various concentrations from 0.1 to 10uM induced shoot differentiation in high frequency. All levels of BA or Z in conjunction with IAA also induced shoot differentiation. Whole plant regeneration occurred in media with hormone concentrations and this technique facilitated massive propagation of virus-free stock of tomato plants within 35

days (Kartha et al., 1977). Similarly, the citrus exocortis viroid (CEV) was eradicated from infected tomato (cv. Rutgers) plants by shoot tip culture of axillary buds excised from greenhouse grown plants. Plantlets developed from 23 out of 67 shoot tips excised showed the same characteristics as the seedling plants (Duranvilla and Semancik, 1986).

2.3 (i) Factors affecting propagation by tissue culture:

Apart from basic factors, such as nutrient media composition, hygiene or the necessity for a sterile environment and environmental conditions like temperature and photoperiod (light duration and intensity, pH, etc), factors within the plant material are known to influence the requirements and success of micropropagation procedures. Some of these include, the part of plant from which the tissue explant is derived. For example, in Irish potatoes (Solanum tuberosum, Cv. Ruma) differences were observed in the nutritional and hormonal requirement both for callus induction and proliferation and for differentiation and regeneration of complete plants from tuber, leaf and stem pith tissues. Differences in light intensity requirements for the 3 tissue sources were also detected. Whereas tuber tissues needed a high light intensity, leaf tissues grew best in the dark and stem-pith tissues preferred low light intensity. NAA at 2.0mg/l proved best for tuber and leaf tissues, while IAA yielded better results with stem-pith cultures. Tuber cultures needed BAP (Benzylaminopurine) whereas the other 2 sources grew better with kinetin.

A sequence on MS formula + 0.1mg/l IBA + 1.0mg/l BAP + 0.1mg/l GA_3 followed by MS + 0.5mg/l GA_3 in liquid shaken culture yielded best regeneration responses regardless of tissue source (Oschalt and Caso, 1986). In Solanum melongena, Saxena et al. (1987) found that leaf tissue obtained from axenic shoot cultures grown in controlled environment was the best source for isolating viable protoplasts. Protoplast-derived cell colonies developed calli on a medium of low osmotic potential and differentiated shoots on a cytokinin supplemented medium. Regenerated shoots were able to develop into whole plants on a hormone free culture medium.

One other factor that is important in the morphogenetic response of plant tissues is the age of plant tissue from which the explant is derived. For example, results of Osifo (1988) showed that, in Solanum brevidens young leaflet explants proliferated more callus prior to shoot initiation than old leaves. The frequency of regenerated shoots was also higher from young leaf explants than old leaves. It was suggested that this may be related to the level of cellular differentiation between young and old leaves (Raju and Mann, 1970). Furthermore, it has been found that basal explants of Echeveria elegans were more organogenetic than distal explants from entire leaves (Raju and Mann, 197). This observation agrees with that of Cheng (1977) in Douglas fir seedling cotyledon tissues.

2.3 (ii) Physiological and Developmental considerations in Organogenesis in tissue cultures

The minimal quantitative changes in the ratio of certain components of the nutrient media are decisive and determine whether organs are initiated or not. Normally, the critical components of the media are auxins and cytokinins. These two substances determine not only whether the tissue grows, but also how it grows (Street, 1977). Generally, high auxin to low kinetin levels lead to root initiation, while lower levels of auxin: kinetin lead to shoot formation while root formation is suppressed. When levels of both auxin and kinetin are intermediate, only an undifferentiated mass of callus is formed (Skoog and Miller, 1957). Other compounds modify this interaction. Raising the phosphate concentration of the medium can reinforce the shoot forming and suppress the root forming effect of auxin. Adenine and amino acids such as tyrosine act similarly (Street, 1977). In other tissues, it is the absolute concentration rather than the ratio of auxin to kinetin that controls root or shoot formation. Apart from this well established relationship, a balanced ratio of gibberellic acid and cytokinin is involved in leaf and shoot development in tobacco (Engelke, et al., 1973). In Jerusalem artichoke, differentiation can be regulated by balancing the sucrose-glucose ratio in the medium (Minocha and Halperin, 1974).

It is quite possible that the mechanisms of organogenesis in the cells of higher plants are alike, differing only in that different limiting factors are decisive for different tissue

sources, varieties, species or families (Street, 1977). In many of these cases of organogenesis, it is not clear whether inorganic nitrogen compounds or hormones like auxins and cytokinins induce their effects in tissue cultures de novo or whether they merely enhance a process initiated by the isolation of cells. However the 'rhizocaline' concept of Bouillene and Bouillene-Walrand could be extended to include the synthesis of other organ-specific substances involved in shoot and leaf production, such as caulocaline and phyllocaline (Street, 1977).

The foregoing is a purely physiological approach to organogenesis. As a complement to this, developmental biologist such as Christianson and Warnick (1988) consider organogenesis to involve a series of discrete steps. Leaf explants on shoot inducing medium undergo an initial 'de-differentiation' process during which competent callus (callus with the ability to respond to induction effects of the medium) is formed along the cut edges of the explant. The second step is shoot induction, which proceeds under the influence of phytohormone balance in the medium. The end result of this process is a group of cells fated or determined for shoot production, which then undergo morphological differentiation and growth to result in shoots. The developmental process leading to the determined state involves some kind of gene action, and specific m-RNAs have been associated with the induction state in both shoot and root regenerating cultures (Hagen et al., 1984; Theologis and Ray, 1982).

2.4 INFLUENCE OF PROPAGATION METHOD ON GROWTH CHARACTERISTICS

Vegetative propagation can change the growth habit of plants depending on the method used. For example, Meyer (1976) found that tissue culture techniques can be used to rapidly propagate tetraploid day lilies and speed the commercial release of new daylily cultivars by several years. It was also found that callus pieces transfered to a growth medium with 0.5mg/l NAA and 0.1mg/l kinetin produced plantlets and produced true-to-type flowering plants in the greenhouse. In young apple trees, budded trees made more rapid growth than grafted trees, but grafted trees made 39% more total growth in the second season. Two seasons later, the trunk circumference, total shoot growth, tree height and spread, numbers of shoots and root suckers were greater on grafted trees than on budded trees. Transplanted trees on the other hand had less trunk circumference, total shoot growth, tree height and spread, numbers of shoot and root suckers than stationary trees at the end of the fourth season. Grafted trees had more flower clusters than budded trees and stationary trees had more clusters than transplanted trees (Ferree, 1976). Lyrene (1981) found in Blueberry that shoots from tissue culture of 2 rabbit eye blueberry cultivars had smaller leaves, thinner stems, shorter internodes and rooted faster than shoots from field grown plants of the same cultivars. Rooting of cuttings taken directly from tissue culture or from 3-month-old tissue culture derived plants averaged 95% for 'Beckyblue' and 96% for 'Bluebelle' after 19 days compared to 7% for 'Beckyblue' and 0% for 'Bluebelle' softwood cuttings

taken from field-grown plants. At 41 days, 100% of the cuttings taken from tissue culture or from tissue-culture-derived plants had roots as compared to 54% for cuttings from field grown plants.

Field planting of corms of Liatris pycnostachya (Michx.) cv. Eureka, produced superior plants for cut flower production than seeds, seedlings, or rooted stem cuttings. Transplanted seedlings produced larger corms with more buds than corms harvested from rooted stem cuttings or direct-seeded-plants. Direct seeded plants and seedling transplants remained vegetative. Rooted stem cuttings developed flowering stems, but spikes were small and stems were weak and lodged. Corms of the plant produced by transplanted seedlings and rooted stem cuttings were significantly heavier and bigger than those of direct-seeded plants (Salao and Fitzgerald, 1983). Similarly, Ettinger and Preece (1985) found that in Rhododendron hybrids, stem-cutting-macropropagated plants generally were more variable in height, had less basal branching and were more erect than plants from micro-culture. Although micropropagated plants grew as fast as or faster, they soon bent and assumed a horizontal growth habit, bending generally occuring before basal branches were well formed. Stem-cutting propagated plants appeared to have more lignified stems than from microculture. In Chickpea, Bassiri et al. (1985) found that plants from cuttings were productive provided they were not overwatered, similarly, rooted cuttings of potato (Ipomoea sp.) were successfully established in the field and grown to maturity with an average of 230g and 18 tubers per plant (Manandhar and Raybhadary, 1986).

2.5 GROWTH, DEVELOPMENT AND YIELD OF TOMATOES AND OTHER VEGETABLES AS AFFECTED BY NPSK NUTRITION.

Nitrogen an inert gas though most abundant on this planet, is the most easily deficient mineral element in the mineral nutrition of plants. Due to its importance as part of biomolecules especially proteins, deficiency of this element causes far reaching effects in plant growth and development. It is therefore a common practice to supplement soil nitrogen supply to cultivated plants through nitrogen fertilizer application. For example, tomato plants in the greenhouse developed over a 5 week period from seedlings to early bloom stage, increased in fresh and dry weight of shoots and roots, plant height, and internal NO_3-N concentration in relation to the NO_3 supply of the culture solution (Gomez-lepe and Ulrich, 1974). In a related study, Weston and Zandstra (1989) found that moderate (100mg/l) rates of N, produced the larger root: shoot ratio than either of higher (400mg/l) and lower (^{0} mg/l) rates. Also plants fertilized with moderate and high N levels produced larger early yields. Similarly, increases in fruit yield, seed yield as well as early flowering and early ripening have been recorded (Lorenz et al., 1970; Varis and George, 1985; Asi and Amjad, 1985). On the other hand, no consistent increases in marketable yield, plant growth, dry matter content of fruits and nitrogen uptake were observed in tomatoes with N-treatments (Omar et al., 1979; Doss et al., 1981; Manson and Wilcox, 1982).

Qualitatively, N-treatment has been found to increase K and N content of whole plants up to about 400% in 7 days (Ward, 1969). Also, nitrogen recovery rate of up to 95% has been recorded and an average of 65% of the N in the plant and fruit originated from fertilizer N (Sweeney et al., 1987). While NO_3-N tended to decrease P uptake, Mg uptake was enhanced by leaf concentrations of NO_3-N and Ca (Agbede and Aduayi, 1985).

In other vegetables, similar results have been obtained. For instance, increasing levels of N-fertilizer have been found to increase the protein and total fresh weight of the edible portion of lettuce, mustard, collards, cabbage, beets, corn, tomatoes and peppers (Splittstoesser et al., 1974). In celery and cabbage, increasing N-treatments have been shown to consistently increase early season crop, total dry matter and leaf growth rate as well as hasten maturity (Stark et al., 1982; Scrinivas, 1982). Similarly, in Okra, N-fertilization has increased plant height, number of branches per plant, number of fruits per unit area and per plant, seed yield, green pod yield, pod diameter and pod weight (Zanin and Kimoto, 1980; Shresta; 1983; Majantu et al., 1985). Similar increases in yield characteristics have been reported in red peppers (Khan et al., 1982) onions (Subbiah et al., 1982) Spinach (Kansal et al., 1981; Olday et al., 1976) eggplant (Rajveen and Rao, 1980) and Ogarlie (Escaff and Al Daro, 1982).

Apart from yield increases applied N has also been found to improve the quality and characteristics of other vegetables. For example, Subbiah and Ramanathan (1982) found that added nitrogen enhanced crude protein, carotene and chlorophyll

in Amaranthus sp. it however, decreased ascorbic acid content. In Chillipeppers (Subbiah et al., 1982) and okra (Mani and Ramanathan, 1981) have found that N treatments increased total tissue N, K, P and Ca contents. On the other hand, N and Cu levels increased but P, Ca and Zn levels decreased with N-applications in Okra (Asif and Greig, 1972). Similarly, Taylor et al. (1983) observed that N-application had no significant effect on accumulation of Ca, K, Na, Cu and Zn in any of 5 vegetables studied and the P and Mg levels of Telfaira, Solanum aethiopicum or S. microcarpum. Nitrogen depressed the Fe content of all the vegetables studied and the Mg content of Amaranthus and Cucurbita, but increased the Mn and Ce contents of all vegetables and P content of Amaranthus and Cucurbita (Taylor et al., Ibid.).

In cucumber however, Farouk et al. (1979) observed no significant effect of applied N on plant growth, fruit yield, dry matter contents of fruits and nitrogen uptake.

In some crops the source of nitrogen may affect the response. For example, higher yields of tomato were obtained by 100% NH_3NO_3 than with 50% NH_3NO_3 and 50% sulfur-coated urea (SCU), (Sweeney et al., 1987). Similarly, in peppers, Urea-N significantly decreased the dry matter yield of leaves by about 25% and 32% as compared to NH_4-N and NO_3-N respectively. Generally, urea-N retarded the growth and anthesis of pepper more drastically than NO_3-N and NH_4-N (Gabal, 1982). Leaf nitrate reductase activity in maize was found to decrease significantly when N was supplied as NH_4-N and amide-N rather than NO_3-N (Mengel et al. 1983). On the other hand, Lorenz et al. (1970) found that N from all sources increased tomato yields significantly. Yields with $(NH_4)_2SO_4$ or urea were approximately the same, but both exceeded those from Nitroform.

In Potatoes, McCollum (1978a) found that when soil phosphorus (P) was low (35kg) or medium (66kg) row applied P increased yields by 38 and 22% respectively, but no response was obtained if initial soil P was high (10kg). On low P regimes, rates of dry matter and P accumulation were still positive at 84 days after emergence, but plants on other treatments had lost most of their leaves at 79 days. About 75% of the total accumulated P and 70-75% of total dry matter was in tubers at harvest. Also row-applied enchanced early growth but hastened leaf senescence and if its initial soil P was low, shortened the duration of the tuber bulking. Leaf growth rate was maximal with 0.35% P in foliar tissue. Phosphorus deficiency reduced mean net assimilation rate during early growth, but it markedly increased this during late growth periods (McCollum, 1978b). In maize, anthocyanin content of leaves decreased with increasing P concentration, but there were marked differences among hybrids in anthocyanin content at a given shoot P concentration. There were also marked differences in degree of purpling among hybrids with similar anthocyanin concentration (Cobbina and Miller, 1987).

In tomato, the relative growth rate and shoot dry matter accumulation were correlated with the rate of K accumulation (Widders and Lorenz, 1982). Tissue K concentrations increased with K applications, while calcium (Ca) and Magnesium (Mg) decreased with increased K applications (Reneau et al., 1983). About 70% of the K accumulated in the plant at harvest was taken up during fruiting (Widders and Lorenz, 1982). Trudel and Ozbun (1971) found that most of the carotenoid especially lycopene, generally increased with increasing K concentration except B-carotene which decreased.

Nitrogen, Phosphorus and Potassium when applied in combinations may or may not modify the response of crops. For example, Majambu et al. (1985) found that in okra, applications of both N and P increased yield parameters like green pod yield, pod diameter, number of fruits per plant, number of seeds per pod and pod weight. Similarly, in three varieties of chilli peppers, the crop yield per hectare and number of fruits per plant were increased by increasing doses of N and P. In NPK combinations however, addition of K did not significantly increase yield and number of fruits per plant (Khan et al., 1982). In tomatoes, highest early yields were obtained in field grown transplants fertilized with Low N-low P or high N-high P, but yields were reduced with high N-low P (Jaworski and Web, 1966). Also transplants fertilized with high N-low P produced up to 35% greater early yields than transplants fertilized with low N-high P (Weston and Zandstra, 1989). Nitrogen at 400mg lit^{-1} and P at 30mg lit^{-1} produced the largest transplants at 5 weeks after sowing N at 100mg lit^{-1} produced the largest root:shoot ratio, had no effect on root:shoot ratios (Weston and Zandstra, 1989).

Other factors that affect tomato growth, yield and quality are temperature (Trudel and Gosselin, 1982; Maletta and Jones, 1987) water stress (Bar Yosef and Sagiv, 1982; De Konning and Hurd, 1983) soil salinity (Perez and Sartain, 1982; Orabi et al., 1982) and diseases (Erinle, 1979a, b).

2.6 CHANGES IN NUTRITIONAL VALUE OF VEGETABLES WITH AGE OF PLANT TISSUE

The nutritional value of vegetables (both leafy and fruiting) seem to be profoundly influenced by age of the plant tissue. For instance, in a study of Amaranthus cultivars, plants harvested between 5 and 7 weeks after sowing contained higher percentage crude protein, total vitamin C (dry wt.) and gave higher leaf/stem ratio than plants harvested between 9 and 11 weeks after sowing for all cultivars (Omueti, 1977). However, marketable, edible leaf dry matter and total crude protein yields were higher in plants harvested between 9 and 11 weeks after sowing than in the 5-7 week old plants (Omueti, 1977). Similarly, phosphorus and potassium declined significantly with age, the highest percentage occuring in 5 week old leaves and the lowest in 11 weeks of age (Omueti, 1982).

Wood and McMeans (1982) found that in Pecan fruit, during endospermic expansion, fructose and glucose rapidly accumulated and fatty acids were present in small concentrations. During embryo and cotyledon expansion, fatty acids accumulated and reducing sugars and inositol declined while sucrose increased. In pawpaw, Salvaraj et al. (1982) found a steady decline in starch and several mineral elements and increases in total sugars. The concentration of citric and malic acids decreased to a minimum at the ripe stage. Similarly the concentration of seventeen amino acids identified changed from anthesis to ripening. The colour break stage was characterised by increases

in Vitamin A and protein. Potassium, phosphorus and Calcium content were high at first and no clear trend was observed in the later stages of development. Iron content showed little change during development. In a similar study with mango pulp, glucose and fructose were more plentiful than sucrose, until maturity. Later sucrose was predominant. There was an increase in total sugar content after 72 days from fruit set. Starch accumulation was slow at first but later increased with a concommitant increase of amylase activity, a decline in both was noted after 91 days growth. The protein content decreased until 44 days after fruit set and then increased during 96 days of further development (Tandon and Kalra, 1983). Handley et al. (1983) found in cucumber fruits that fruit maturity, was correlated with an increase in citric acid content, also the concentration of glucose and fructose in seeds decreased during development.

Tomatoes like other fruiting vegetables, show changes in quality of fruit at various stages of development. For instance, Widders and Lorenz (1982) found that maximum rates of K accumulation in the whole plant as well as fruits occured at 90 days and this was correlated with the relative growth rate. K content in vegetative parts decreased by 15-40% during fruiting. Starch content of tomato fruits increased from 10-16 days after anthesis. It reached a maximum at about 42 days followed by a decline. Depletion of starch content was associated with an increase in α-amylase activity and an accumulation of reducing sugars like fructose and glucose (Dinar and Stevens, 1981; Hewitt et al., 1982).

Fructose and glucose concentration were found to increase from the immature green to the table-ripe stage with fructose being the primary sugar. Sucrose concentration was higher in the immature green than the table ripe stage. Citric acid was the primary organic acid and it increased with maturity. Malic acid was found to decrease with maturity (Pichia, 1987).

2.7 POST-HARVEST PHYSIOLOGY

Ripening of fruits indicates the termination of fruit growth and the onset of physiological qualitative changes which include marked increases in the amount of carotenoids in the fruit (Ulrich, 1958) Lycopene is usually the predominant carotenoid in old fruits and this increases by as much as ten-fold in tomatoes from the ninth to twelfth day of ripening at 30°C. Phytofluene may increase by up to two-fold.[s.] Pigment changes may be due mainly to modifications in carotene content rather than to variations in the amount of carotenoids or chlorophyll (Ulrich, 1958). Red pigmentation is dependent on stage of maturity of fruit at harvest (Tigchelaer and McGlasson, 1978). The manifestation of other fruit ripening characteristics, such as respiration levels and ethylene gas evolution also depend on the level of maturity of fruits at harvest (Kamellis et al., 1986).

Varis and George (1985) found that in tomatoes, seed weight decreases with fruit size. This has an effect on the germination capacity of the seed. For example, Vadivelu (1983) reported that seeds from large fruits produced seeds that germinated better than small fruits. Khudairi (1972) found that fruit ripening characteristics

are associated with seed maturation and this may have an adaptive value in seed dispersal.

Other workers have reported that until the attainment of full maturity, full germination capacity and viability are not realized. In carrots Austin and Longden (1967b) found that for four progressive harvest dates, percentage germination and field emergence increased with seed weight. Studies with fleshy fruits have also shown that the harvesting and extraction of seed before full maturity is detrimental to viability. For example, in Capsicum frutescens ('Pimiento pepper') seeds extracted from 30 day old fruits (before full maturity) had an emergence of 5-6%. Those stored for 30 days further, had 95%, while those left to ripen fully had 92% (Cochran, 1943). In tomatoes, however, Kerr (1963) found that provided fruits were at least at the mature green stage, maturity had little effect until they are over-ripe. Luckwill (1939) and Hatcher (1940) found no general correlation between inherent vigour (the relative growth rate at the exponential phase) with seed or embryo weight.

Seed viability after harvest is also subject to a variety of factors to which the parent plant is exposed during seed formation and ripening (Meyer and Poljakoff-Mayber, 1975).

CHAPTER THREE

MATERIALS AND METHODS

3 (i) SEED PROPAGATION: PLANTING AND CULTURAL PRACTICES

Two seed boxes each measuring about 1.35m^2 were used as nursery beds. These were filled with seived soil to about an inch of the brim. Fertilizer (NPK 15:15:15) that has been ground was applied at the rate of 25g per sq. meter (about 34g as recommended by AERLS) and mixed thoroughly with the soil. Seed drills were drawn at about 7-10cm apart and about 1cm deep. Seeds of tomato (Roma-VF) were sown in drills and covered thinly with soil. The boxes were then watered and kept in a cool but sufficiently lighted place. Seedlings emerged after about 6 days and by 2 weeks after emergence (WAE) they had developed at least 4 true leaves and were ready for transplanting.

Forty-five earthware pots (each measuring 38cm depth by 30cm diameter) were filled with about 135cm^3 of sieved garden soil to about 3cm of the brim.+ A week before transplanting, NPK (15:15:15) was applied at the rate of 600kg/ha (about 12g/pot) to 15 pots; 300kg/ha (6g/pot) to 15 pots and to the remaining 15 pots no fertilizer was applied. The pots were labelled 'O' (no fertilizer) 'R' (recommended rate = 300kg/ha) and 'H' (high = twice the recommended rate of fertilizer).* The pots were arranged randomly in the garden and watered to field capacity.

About two hours before transplanting, the seed boxes were watered and seedlings were lifted with a garden trowel with some soil attached to the root zone and inserted into

+ Appendix A
* Appendix B

holes made in the soil-filled pots at the rate of three plants per pot. Plants were watered immediately after transplanting. Transplanting was done in the late afternoon (4-6pm). Seedlings of about the same height, and leaf area were used. Transplanted plants were watered regularly (once a day) throughout the period of the experiment.

3 (ii) SAMPLING FOR GROWTH ATTRIBUTES, AUXIN CONTENT AND CHO:N RATIO

For each fertilizer treatment, plant height was measured from soil surface to tip of topmost leaf with a ruler for 5 plants at three stages of growth. Three plants per treatment were cut from the soil level for shoot dry weight measurements.* Plant materials were dried in an oven at 80°C to constant weight and final weight was taken.

Phyllotaxy was determined by observing whether the second branching of the plant was to the left or right. If the first branch was to the left and the second branching to the right, the plant was said to show a right-handed phyllotaxy and vice-versa.

After flowering and fruiting, the number of fruits per plant were counted for each nitrogen treatment at four subsequent harvests. The fruit weights were also taken for each treatment. Fruit samples were taken for post-harvest characteristics determination.

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* One plant from each of three pots/treatment.

3 (iii) TOTAL SOLUBLE CARBOHYDRATE (TSC)

Clegg-Anthrone Method:

Principle: The plant material is digested with perchloric acid and hydrolysed starches together with soluble sugars are determined colorimetrically by anthrone method.

Procedure: 0.35g of dried ground sample was weighed and transferred into a graduated 100ml measuring cylinder. Ten millilitres of distilled water were added and stirred with a glass rod to thoroughly disperse the sample. Thirteen millilitres of 52% perchloric acid reagent were then added and stirred frequently over a period of 20 minutes. The glass rod was washed down with distilled water and the contents of the cylinder were diluted to 100mls. This was then transferred to a 250ml volumetric flask. The measuring cylinder was washed out and the washings transferred to the volumetric flask to make up to 250ml. The flask was thoroughly shaken and approximately 5mls were filtered into a test tube. One millilitre of filtrate was transferred into each of 2 test tubes. A blank of distilled water (1 ml) was also prepared. Five millilitres of freshly prepared anthrone reagent (2g anthrone in 1 litre cold 95% H_2SO_4), were pipetted into the test tubes and mixed thoroughly. The flasks were then capped and placed in a boiling water bath for 12 minutes. The test-tubes were then removed and quickly cooled to room temperature under a running tap. The solutions were transferred into a glass cuvette and the absorbance of the green coloured complex was read at 620nm in a corning FEL colorimeter.

Total soluble carbohydrate (as % glucose) was calculated using the formula:

$$\frac{2.5 \times b}{w}$$

where 2.5 - constant

b - glucose concentration of diluted sample

w - weight (g) of sample taken.

Standard curve for estimation of TSC

A serial dilution of 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20mg/ml of glucose were made and 1 ml of each was transferred into test-tubes in duplicate. Five millilitres of freshly prepared authrone reagent were added to each tube and the tubes were capped and placed in a boiling water bath for 12 minutes. The test-tubes were then cooled and the absorbance read in a corning-EEL colorimeter at 620nm. A curve of absorbance against glucose concentration was obtained and regressed to get the line of best-fit.

3 (iv) % NITROGEN

This was done by Kjeldahl method on a Markham distillation apparatus.

Principles:

(a) Organic matter is oxidized by sulphuric acid in the presence of a suitable catalyst and nitrogen converted quantitatively to ammonia.

(b) The ammonia is librated by distillation in a Markham steam distillation apparatus in the presence of caustic soda and collected in boric acid.

(c) The ammonia is then titrated directly with standard hydrochloric acid using a mixed indicator.

Digestion:

(a) 10mg of dry powdered sample were weighed out and transferred to a small Kjeldahl flask. A small amount of catalyst (a mixture of 1g copper sulphate, 8g potassium sulphate, 1g selenium dioxide) on the tip of a small spatula was added and 2ml of nitrogen-free concentrated sulphuric acid were added. This was then heated gently on a digestion stand until fumes of sulphuric acid were freely evolved. Further stronger heating was done until the digests were apple-green in colour. Digestion was continued for a further half hour. Blanks of the reagents alone were run at the same time. Care was taken to avoid particles of indigested carbon sticking to the sides of the flasks. Digests were then allowed to cool to a point when crystals started to form.

(b) Distillation:

A Markham distillationstill was used. Since the apparatus had not been used for a long time, it was steamed out for about 30 minutes before use.

Two millilitres of distilled waterwere added to Kjeldahl flasks containing digests to rinse out the sample. This was then placed in the distillation flask through the funnel. The ground glass stopper was then replaced and excess (about 11-12mls) of 40% sodium

hydroxide was added to the funnel. This was then allowed to run into the digest gently through the manipulation of glass stopper. The clip was then closed to start the reaction. The ammonia liberated was distilled into 5ml of 2 percent boric acid contained in a 50ml conical flask. The tip of the condenser was immersed in the acid until after the first few drops had distilled, and then raised above the surface of the boric acid. Distillation was continued until 20ml of distillate had collected (making a total of 25ml). The distillation was then stopped by loosening the clip and the system flushed out with distilled water.

(c) Titration:

Four drops of indicator, a mixture of 0ml methyl red (0.16% in 95% alcohol); 12ml Bromo-cresol green (0.04% in water); 6ml 95% alcohol, were added to the distillate previously made up to 50mls in a 100ml beaker, and titrated with 0.02N hydrochloric acid to a faint pink colour. The analysis of each sample was done in duplicate.

Percentage nitrogen was calculated for each sample using the formula:

$$\% \text{ Nitrogen} = \frac{(A-B)\ N \times 14.007}{W} \times 100$$

where A – quantity of HCl used to neutralize test sample

B – quantity of HCl used to neutralize blank

N – Normality of HCl (0.020)

14.007 – relative atomic mass of nitrogen in ammonia

W – weight of sample used in mg.

% protein – % N x nitrogen–protein factor of 6.25.

3 (v) QUANTITATIVE DETERMINATION OF AUXIN CONTENT

(a) Extraction:

Three plants of equal height were taken from each nitrogen treatment and washed to remove soil particles. Leaves were removed and the stems were homogenised in a mortar. 30mls of ethyl alcohol (95%) were added and mixed thoroughly with the homogenised plant material at 25°C. The mixture was strained to remove remains of lignified tissue. Extracts were then centrifuged at 200xg for 5 minutes to remove cell wall debris. The supernatant solutions were decanted and kept in storage bottles at 4°C. The residues were washed in two changes of 5ml distilled water. The water was decanted into storage bottles and kept at 4°C. The undissolved material was then discarded.

(b) Assay:

Standard curve: Two standard curves were prepared from serial dilutions of authentic IAA at 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1mg/l; by the methods of

Tang and Bonner (1947) and Gorden and Weber (1951). In Leopold (1955) the two curves were regressed and compared. It was found that the method of Gordon and Weber was more sensitive. This was then adopted and used for the assays.

The acid reagent was prepared by taking 1ml of 0.5m $FeCl_3$ and then adding 50ml of 35% $HClO_4$. This was kept in a 50ml flask for IAA assay.

1ml of plant extract was taken and 2ml of acid reagent were added. The mixture was swirled gently in the test-tube and left to stand for 25 minutes at room temperature ($25^{\circ}C$). After this time a pink colour complex was developed from the reaction of the ferric chloride reagent with IAA. The absorbance of the solution was read in a Bausch and Lomb spectronic 20 set at maximum absorbance at 530nm.

Both alcoholic and aqueous extracts were tested and it was found that the colour complex was formed only with the alcoholic extracts. It was then concluded that the aqueous extract had negligible amounts of IAA and were thus discarded.

IAA extraction and assay was carried out at weekly intervals from 2 to 6 WAT (flowering).

3(vi) MACROPROPAGATION (STEM CUTTINGS)

A propagation case (chamber measuring 120cm long by 50cm wide and 50cm high was constructed. Washed river sand was placed in two wooden crates each measuring 60cm long and 40cm wide. This was used as the rooting medium. The sand was watered regularly and kept at temperatures between $26-28^{\circ}C$.

Half strength Hoagland's solution was used to supply the essential mineral nutrients. The rooting medium was kept moist and well-drained. The propagation chamber was covered with one layer of transparent polythene sheet to maintain a constant temperature and allow sufficient light penetration.

Axillary shoots were taken from plants when the first flower buds had appeared but not opened (for plants with recommended and high N-treatments). For plants with no fertilizer treatment, axillary shoots were not available, so the main terminal shoots were used as cuttings. Five leafy cuttings of about equal length were used. The bases were dipped in freshly prepared hormone solutions at the following concentrations, for each treatment:

IAA 0.025, 0.05, 0.1g/l

IAA/NAA 0.025, 0.05, 0.1g/l

NAA 0.025, 0.05, 0.1g/l

and control (no hormonal treatment).

After 30 minutes the cuttings were transferred to the rooting medium and watered regularly to maintain a high humidity.

Rooting was monitored by visual observations every and recorded for each fertilizer and auxin treatment. After 10 days in the rooting medium, adventitious roots had developed at the base of cuttings and these were counted and their lengths measured. Photographs of rooted cuttings were taken and some cuttings from each hormonal and fertilizer treatment were used for anatomical studies to determine the origin of adventitious roots. Percentage of rooted cuttings per treatment were also recorded.

3 (vi) ANATOMICAL STUDIES (ORIGIN OF ADVENTTITIOUS ROOTS)

Plant material was collected, cut into pieces about 3mm long and fixed in F.A.A. (Formalin-Acetic-Alcohol, 0.5:0.5:9) for 24 hours. They were dehydrated by transferring to several changes of chloroform/alcohol at increasing/decreasing concentrations thus:

70% alcohol for 2 hours

Absolute alcohol for 2 hours

25% chloroform/alcohol 2 hours

50% chloroform/alcohol 2 hours

75% chloroform/alcohol 2 hours

pure chloroform (two changes) 2 hours each

Materials were infilterated by adding flakes of 58-60°C paraffin wax until the wax started to jel in the chloroform. The specimens were then left overnight for proper infilteration. Chloroform was evaporated by pouring the material with jelled paraffin wax into a shallow dish and leaving on top of embedding oven at 60°C for half an hour. Specimens were transferred into the embedding oven at the same temperature for more evaporation for another half an hour. Specimens were then transferred into pure molten wax in the embedding oven at 60°C and left for half an hour.

L-angle pieces were used as embedding moulds. The inner surface of the embedding moulds was smeared with dilute glycerine. Molten wax slightly heated above 60°C was poured into the mould to about half-full. Specimens were transferred into the wax in the embedding block and oriented into their cutting angles. A thin film was allowed to form on the surface

of the molten wax after the specimens were properly oriented. The mould was then submerged in cold water until the wax blocked. The mould was then removed and the angles separated from the embedded block. The block was then kept in a cold place until needed for sectioning.

For sectioning, pieces of the wax block containing the specimens, were cut in equal sides so as to get a straight ribbon when sectioning. The trimmed blocks were then mounted on a microtome specimen holder using a warm blunt scalpel and submerged in cold water to stick the block firmly on the specimen holder. Sections were then cut in both transverse section (T.S) and longitudinal section (L.S).

To mount the specimens, clean slides were smeared with drops of Mayer's egg albumen and allowed to dry for a few minutes. Portions of the sections were cut from the ribbons and mounted on the albumenised slides with few drops of water. The sections were stretched on a warm hot plate. Excess water was drained off after stretching and the sections were stuck firmly on slides with the aid of several thickness of filter paper. The sections were then left to dry for three to four days before staining.

For staining, slides were de-waxed in two changes of xylene for 5 minutes each. Specimens were then dehydrated in absolute alcohol for 2 minutes. Further dehydration was done in 95% and 70% alcohol for two minutes each consecutively. Specimens were stained in safranin for 5 to 10 minutes, then washed in 50%, 70%, 95% and absolute alcohol for 1 minute each consecutively. Specimens were then counterstained in fast green in 95% alcohol for 1 minute . Specimens were then

differentiated in 95% alcohol for ½ a minute then transferred to absolute alcohol for 1 minute. Specimens were cleared in two changes of xylene and dried on a warm hot plate. Slides were mounted on a light microscope viewed and labelled. Photomicrographs were taken of the various stages of adventitious root development.

3 (iii) TRANSPLANTING AND CULTURAL PRACTICES

Sixty earthenware pots of about 30cm diameter and 30cm depth were filled with 135cm^3 of sieved garden soil to about 3cm of the brim. Fertilizer (NPK 15:15:15) was also applied at the same rates as that of seed propagated plants (O, R and H). These were watered until saturated and holes made in the soils in the pots. Pots were then labelled according to fertilizer treatment and hormone type and arranged in completely randomised design (two factorial) (15 for 'control'; 15 for IAA alone; 15 for IAA/NAA and 15 for NAA alone) for each of these there were 5 pots for each of the fertilizer treatments. Rooted cuttings were then transplanted into pots at the rate of 3 plants per pot. These were left in a shadded place for two days, for acclimatization before transferring to exposed field. Vegetative growth characteristics were measured as was done with seed-propagated plants. After flowering and fruiting, fruit counting and weighing were done as with seed-propagated plants. Fruit samples were also taken for post-harvest studies. (Ascorbic acid and mineral elements could not be determined due to lack of material for the analysis).

3 (ix) POST-HARVEST PHYSIOLOGY

FRUIT COLOUR:

Twenty fruits of about the same size were selected randomly from both stem-cutting and seed-propagated plants and placed on the shelf for 12 days at 25 ± 1°C. These were observed 48 hourly and the results are presented in Table 13 (Plate 11).

SEED WEIGHT/GERMINATION

One hundred seeds extracted from fruits of seed-propagated as well as stem-cutting propagated plants were fermented for two days and thoroughly washed in several changes of water to remove the pulp around the seeds. Seeds were then sun-dried for three days and weighed on a chemical balance, in three seed lots of 100 seeds each. Results are presented on Table 14.

Seed germination tests were carried out with seeds from seed-propagated as well as stem-cutting propagated plants. Thirty seeds from each mode of propagation were used in each of four replicate petri-dishes containing constantly moistened filter papers. Seeds were treated with 2% sodium hypochlorite solution before testing to prevent microbial growth. Tests were carried out at 26 ± 1°C. Results obtained are presented in Table 15.

3 (x) PLANT TISSUE CULTURE

Media preparation:

Packets of powdered Murashige and Skoog (1962) basal medium and hormones for tissue culture were obtained from Carolina Biological Supplies Company, Carolina, U.S.A.

Approximately 800ml double-distilled water at room temperature were put in a 2 litre flask. Powdered medium was added to the water while gently stirring. The packet was rinsed out to remove all traces of residual powder. Medium was thoroughly dissolved and 30 grams of analytical grade sucrose were added and dissolved. IN sodium Hydroxide or IN Hydrochloric acid were used to adjust the pH to 5.8. Additional water was added to bring the total volume to 1 litre. Eight grams of Difco-Noble agar were added and thoroughly dispersed. The medium was then heated to boiling when the medium was clear. The hot medium was then dispensed into test-tubes (20.0mls each) and hormones added. The tubes were then plugged with absorbent cotton wool and capped with aluminium foil. The media were then autoclaved at 15Psi and 121°C for 15 minutes, then cooled at room temperature.

Shoot segments containing young shoot tips (about 0.5cm length) were taken from 6 week old tomato plants growing in the glasshouse and transported to the lab in distilled water and disinfected in 1.2% sodium hypochlorite for 15-20 minutes.

Sterile environment was obtained by thoroughly wiping the working bench and the inner surfaces of a previously switched on laminar air flow cabinet with 70% alcohol.

The cabinet was again sprayed with a solution of 5% Cetyltrimethyl ammonium bromide in 70% alcohol. All materials to be used in the cabinet such as petri-dishes, test-tubes containing media and test-tube baskets were similarly treated before being placed in the cabinet.

Dissecting instruments to be used were placed in a plastic dish containing 70% alcohol and regularly flamed on a spirit lamp during explanting.

Shoot tips (about 0.5cm) were obtained from plant material and thoroughly rinsed in 3 changes of sterile double-distilled water before being transferred into tubes containing agar solidified media with various concentrations and combinations of hormones. The concentrations and combinations used were as follows (in micromoles uM):

IBA	0.01, 0.1, 1.0, 10.0
K	0.01, 0.1, 1.0, 10.0
2,4-D	0.01, 0.1, 1.0, 10.0
K	0.01, 0.1, 1.0, 10.0
IBA	0.01, 0.1, 1.0, 10.0
K	10.0, 1.0, 0.1, 0.01
2,4-D	0.01, 0.1, 1.0, 10.0
K	10.0, 1.0, 0.1, 0.01

Controls had no hormone supplement. Each treatment had 5 replicates. After innoculation, the cultures was tightly covered and placed in an incubator at 27°C and a 16 hour photoperiod. 4000 lux light intensity,

Rooted plantlets were transferred to media without auxin to allow for root development and plantlets that showed adequate root development were transplanted into clay pots filled with garden soil in the glasshouse and placed under observation.

CHAPTER FOUR

R E S U L T S

4.1 Life cycle:

Seed-propagated plants in the field had a life-span of 7-9 weeks (after transplanting) before the onset of senescence. Flower buds appeared first in plants-treated with 300kg/ha of NPK fertilizer. This occured at about 6 weeks after transplanting. Plants that received 600kgNPK/ha and those that received no fertilizer at all had their flowering delayed by 6 and 13 days respectively. By the 8th week after transplanting the first fruits had been harvested in the plants given 300kgNPK/ha. In fertilizer-treated plants, there was axillary branching while there was none in untreated plants.

The life span of stem-cutting propagated plants, was between 6 and 7 weeks after transplanting. Flower buds first appeared at 5 weeks after transplanting and this occured in both 300 and 600kgNPK/ha treated plants simultaneously. Plants with no fertilizer application had longer life span of approximately 7 weeks. By 6 weeks after transplanting, the first fruits had been harvested and this also occurred in both 300 and 600kgNPK/ha treated plants. Those that received no additional fertilizer treatments, but propagated by stem-cuttings had their delayed by as much as 8 days.

4.2 VEGETATIVE GROWTH CHARACTERISTICS

4.2 (i) Plant height:

Plant height generally increased significantly ($P < 0.05$) with age of plants for both modes of propagation (Table 1). Except for 0' levels fertilizer, the

Table 1: Effect of levels of NPK fertilizer on height (cm) and plant dry weight (g/plant) of seed-propagated tomato.

Time of measurement (DAT)	Height+ Levels of fertilizer (kg/ha)			Plant Dry Weight+ Levels of fertilizer (kg/ha)		
	0	300	600	0	300	600
14	10.82a	10.78a	10.80a	0.058a	0.57a	0.56a
*	0.24	0.38	0.74	0.02	0.05	0.08
28	14.20	16.08	21.10	0.32	0.73	1.15
*	0.38	1.06	1.39	0.04	0.05	0.04
42	19.62	30.76	40.54	0.93	1.40	1.72

* Rate of increase in height (cm/day) and in weight (g/day)

\+ Means in a row under each parameter, followed by the same letters are not significantly different (P 0.05) of Duncan's multiple range test (DMRT).

DAT = days after transplanting.

Table 2: Effects of growth hormones and levels of NPK fertilizer on height (cm) of stem-cutting propagated tomato.

Time of Measurement (DAT)	Hormone Treatments											
	No hormone			IAA			IAA/NAA			NAA		
	Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)		
	0	300	600	0	300	600	0	300	600	0	300	600
14	9.72b	9.27b	9.20b	9.42b	9.40b	9.36b	9.26h	9.18b	9.30b	9.52b	9.14b	9.28b
*	0.13	0.74	0.81	0.17	0.62	0.86	0.14	0.60	0.79	0.16	0.80	0.85
28	11.55f	19.67e,c	20.50e	11.80f	18.20e	21.34e	11.54f	17.38c	20.34e	11.82f	20.36e	21.11e
*	0.12	0.32	0.36	0.12	0.37	0.38	0.10	0.32	0.29	0.13	0.33	0.34
42	13.25d	24.10h	25.60h	13.56d	23.33i	26.60h	12.89d,f	21.80e	24.48h,i	13.50d	25.01h	25.90h

* Rate of increase in plant height (cm/day)

\+ Means in a row followed by the same letters and not significantly different at 5% level of Duncan's multiple range test.

DAT = days after transplanting.

rate of plant height increase was higher for stem cutting propagated plants than seed propagated plants during the first 14 days of growth in the field. In the 0' fertilizer level treated plants, this was lower for stem-cutting propagated plants than seed propagated plants during the same period. For all NPK levels, seed-propagated plants had higher mean plant height at 6WAT than stem-cutting propagated plants of the same age. Regardless of mode of propagation, plant height at 42 days after transplanting varied according to the level of fertiliser treatment and it was in the order 600kgNPK/ha>300kgNPK/ha> '0'level of fertilizer.

For stem-cutting propagated plants, regardless of auxin and fertiliser treatments, rate of plant height increase was higher during the first two weeks of transplanting than during the subsequent 2 weeks. Seed-propagated plants on the other hand, generally had a significantly higher rate of plant height increase between 4 and 6 WAT than in the first two weeks of transplanting. Also during this period, the rate of increase in height in seed-propagated plants was higher than that of stem-cutting propagated plants.

Plant heights for all fertilizer levels were generally lower for IAA/NAA treated plants than in other hormone treatments throughout the growth period. Rates of increase in height were also lower for IAA/NAA treatments. These differences were however not significant (P - 0.05) (Table 12).

4.2 (ii) Shoot dry weight:

Shoot dry matter increased significantly (P - 0.05) with age, regardless of auxin or fertilizer treatment (Table 23) Dry matter at 42 days was lower for seed-propagated plants than stem-cutting propagated plants (Table 3) except in the non-additional fertilizer levels, when this was higher for seed-propagated plants than stem-cutting propagated plants. Rate of dry matter increase was generally higher in seed-propagated plants between 28 and 42 days than stem cutting propagated plants, for all fertilizer levels. Between 14 and 28 days of growth however, rate of dry matter production was generally higher for stem-cutting propagated plants than seed propagated plants for all auxin levels (Table 23). The rate of dry matter increase was lowest between 28-42 days in stem cutting propagated plants. Differences among stem-cutting propagated plants were not significant (P - 0.05). Treatments generally affected dry matter production significantly, with higher rates of having more dry matter production.

4.2 (iii) Phyllotaxy:

Except for unfertilized seed propagated plants and stem-cutting propagated plants, all other fertilizer and auxin treated plants showed a right-handed phyllotaxy. This probably influenced the yield of the plants as NPK treated plants with right-handed phyllotaxy had higher yields than un-fertilized plants (Table 10).

Table 3: Effects of growth hormones and levels of NPK fertilizer on shoot dry weight (g) of stem-cutting propagated tomato.

Time of measurement (DAT)	Hormone treatments											
	No Hormone			IAA			IAA/NAA			NAA		
	Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)			Fertilizer levels (kg/ha)		
	0	300	600	0	300	600	0	300	600	0	300	600
14	0.15b	0.16b	0.18b	0.15b	0.16b	0.17b	0.15b	0.16b	0.17b	0.15b	0.16b	0.17b
*	0.005	0.13	0.15	0.007	0.14	0.18	0.05	0.13	0.15	0.01	0.15	0.18
28	0.23a	1.96c	2.25f	0.25a	2.10c,f	2.63d	0.23a	1.99c	2.27f	0.29	2.25f	2.63d
*	0.002	0.05	0.04	0.002	0.04	0.03	0.001	0.05	0.04	0.002	0.03	0.03
42	0.26g	2.61d	2.80d	0.28g	2.60d	3.01e	0.27g	2.63d	2.83e	0.30g,h	2.73d	3.00e

* Rate of increase in plant height (cm/day)

\+ Means in a row followed by the same letters are not significantly different at 5% level of Duncan's multiple range test.

DAT = days after transplanting.

4.3 (i) Native auxin activity:

Native auxin extracted from terminal shoot portions of seed-propagated plants over a period of six weeks showed the following trends: For zero fertilizer levels, the tissue auxin activity increased steadily and significantly (P - 0.05) with age of plant tissue (6 WAT) up to the time of cutting. For plants treated with 300kgNPK/ha and 600kgNPK/ha however, the activity increased to a peak at 3 WAT then declined to the lowest point at 4 WAT. It further increased at 5 WAT and decreased again at 6 WAT (Table 4; Fig. 1).

A comparison of auxin activity for the various NPK treatments with the rooting response of cuttings taken from such plants showed that tissue auxin activity was highest in plants with zero NPK levels and lowest in plants with highest NPK levels. The mean number and length of roots however, were highest at highest NPK levels and lowest at lowest NPK levels. Rate of root growth were however lower at high NPK levels than at lower NPK levels (Table 5).

4.3 (ii) Total soluble carbohydrate, percentage nitrogen and CHO:N ratio.

Total soluble carbohydrate (TSC) was generally higher in plants given no fertilizer than for either of 300kgNPK/ha and 600kgNPK/ha-treated plants throughout the period of sampling (Table 6; Fig. 2).

Table 4: Levels of endogenous auxin (g/lit^9) in seed propagated tomato as affected by levels of NPK fertilizer and age of plant.

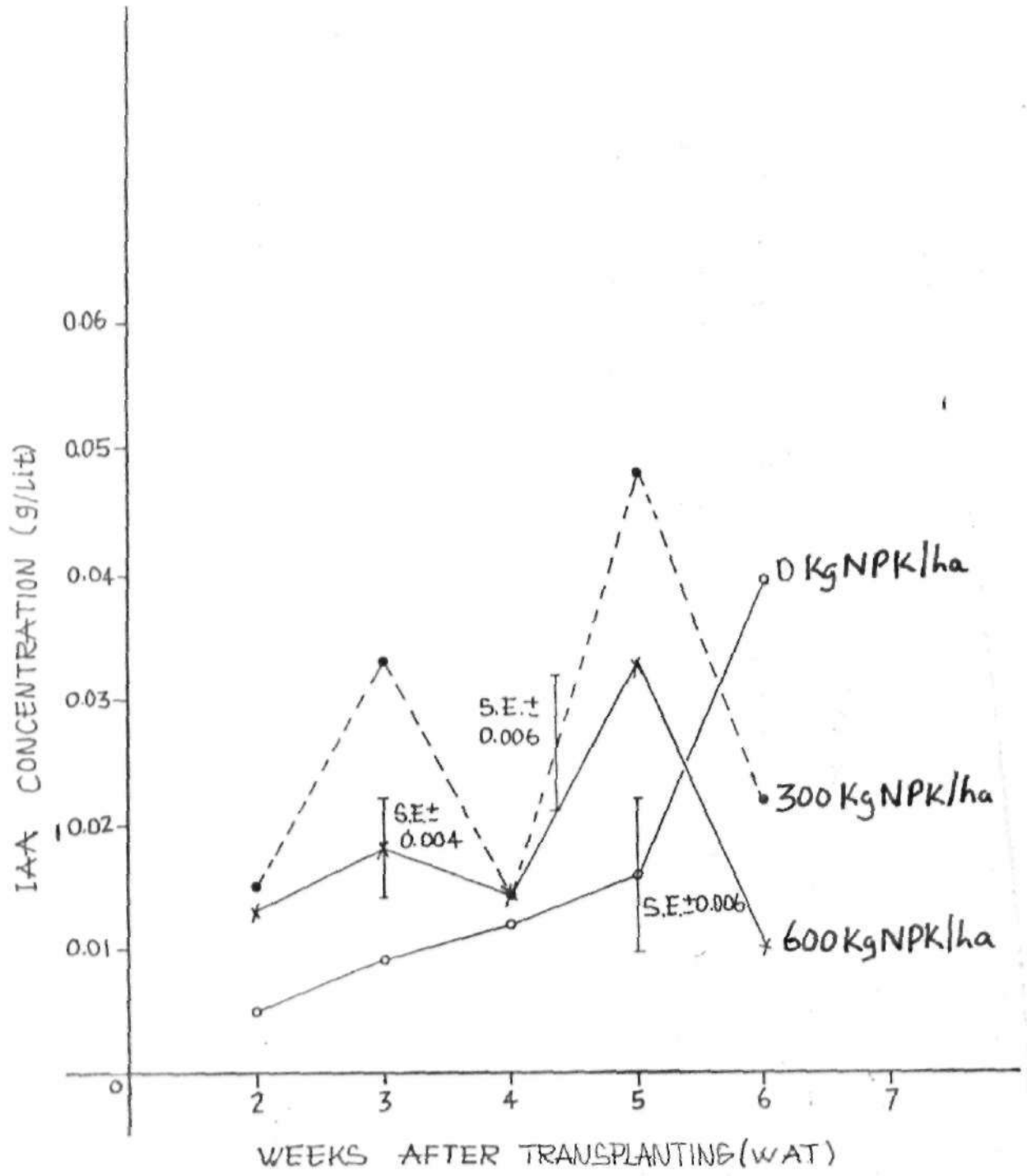
Age of Plant	Levels of NPK fertilizer (kg/ha)		
	0	300	600
2	0.005	0.015	0.013
3	0.009	0.033	0.018
4	0.012	0.014	0.014
5	0.016	0.048	0.093
6	0.040	0.022	0.010
MEAN	0.026	0.026	0.018
SE ±	0.006	0.006	0.004

Table 5: Number and length of roots as affected by tissue auxin activity and nitrogen level

NPK (kg/ha) LEVEL	AUXIN CONCN. (g/lit. IAA)	NUMBER OF ROOTS	LENGTH OF ROOTS(CM)
0	0.040	12.0	1.12
*		7.6	0.64
300	0.022	19.6	1.76
*		3.4	0.06
600	0.010	23.0	1.83

* = Rate of increase in number and length of roots

FIG. 1 CHANGES IN AUXIN ACTIVITY IN PLANT TISSUE AS INFLUENCED BY FERTILIZER TREATMENT AND AGE



For no fertilizer-treated plants, TSC increased steadily and significantly with age and had the highest level at 6 WAT. For 300kgNPK/ha and 600kgNPK/ha-treatments however, TSC had the lowest levels during the third week of transplanting followed by significant increases until the 6th week (Table 6; Fig. 2). The level of nitrogen (as percentage N) was lowest in plants given no fertilizer. The level of N also increased steadily and significantly with age until the 6th week in the untreated plants (Table 6; Fig. 3). In plants given 300kgNPK/ha however, % N fluctuated with age. It increased significantly (P 0.05) from the 2nd to the 3rd WAT and declined thereafter until the 5th week during which its lowest level was recorded. It then increased sharply during the 6th week (Table 6; Fig. 3). In plants given 600kgNPK/ha, the % N level increased steadily and significantly with age to a peak at 6 WAT.

Carbohydrate:nitrogen ratio (CHO:N) in plants given no fertilizer treatments remained steady during the first four weeks of transplanting. It then increased gradually but not significantly at the 6th week (Table 6; Fig. 4). For both 300kgNPK/ha and 600kgNPK/ha treatments, this declined significantly (P - 0.05) to its lowest point at 3 WAT. After this there was a steady significant increase (P - 0.05) up to the 6th week for 600kgNPK/ha treatments and up to the 5th week for 300kgNPK/ha treated plants after which it declined again significantly (P - 0.05) at the 6th week (Table 6; Fig. 4). Carbohydrate was lowest for 600kgNPK/ha-treated plants, followed by 300kgNPK/ha-treated plants and highest for plants with no fertilizer throughout the period of study (Table 6).

Table 6: Showing the level of total soluble carbohydrate (TSC) as % glucose: percentage nitrogen and carbohydrate:nitrogen ratio (CHO:N) in seed-propagated tomato shoots as affected by nitrogen level and age.

	Level of NPK fertilizer (kg/ha)								
	0			300			600		
AGE (WAT)	TSC	N	CHO:N	TSC	N	CHO:N	TSC	N	CHO:N
2	1.14	3.64	0.31	1.06	4.69	0.23	0.93	5.74	0.16
3	1.18	3.85	0.31	0.48	10.92	0.04	0.44	11.34	0.04
4	1.22	3.99	0.31	1.00	8.26	0.12	0.56	12.32	0.05
5	1.40	4.40	0.32	1.06	5.32	0.20	0.77	12.78	0.06
6	1.65	5.00	0.33	1.11	11.34	0.10	1.02	13.72	0.07
MEAN	1.32	4.18	0.32	0.94	8.11	0.14	0.74	11.18	0.08
SE ±	0.09	0.24	0.004	0.12	1.38	0.03	0.11	1.41	0.02

FIGURE 2. TOTAL SOLUBLE CARBOHYDRATES (TSC) AS % GLUCOSE CONTENT OF TOMATO SHOOTS AS AFFECTED BY FERTILIZER LEVEL AND AGE.

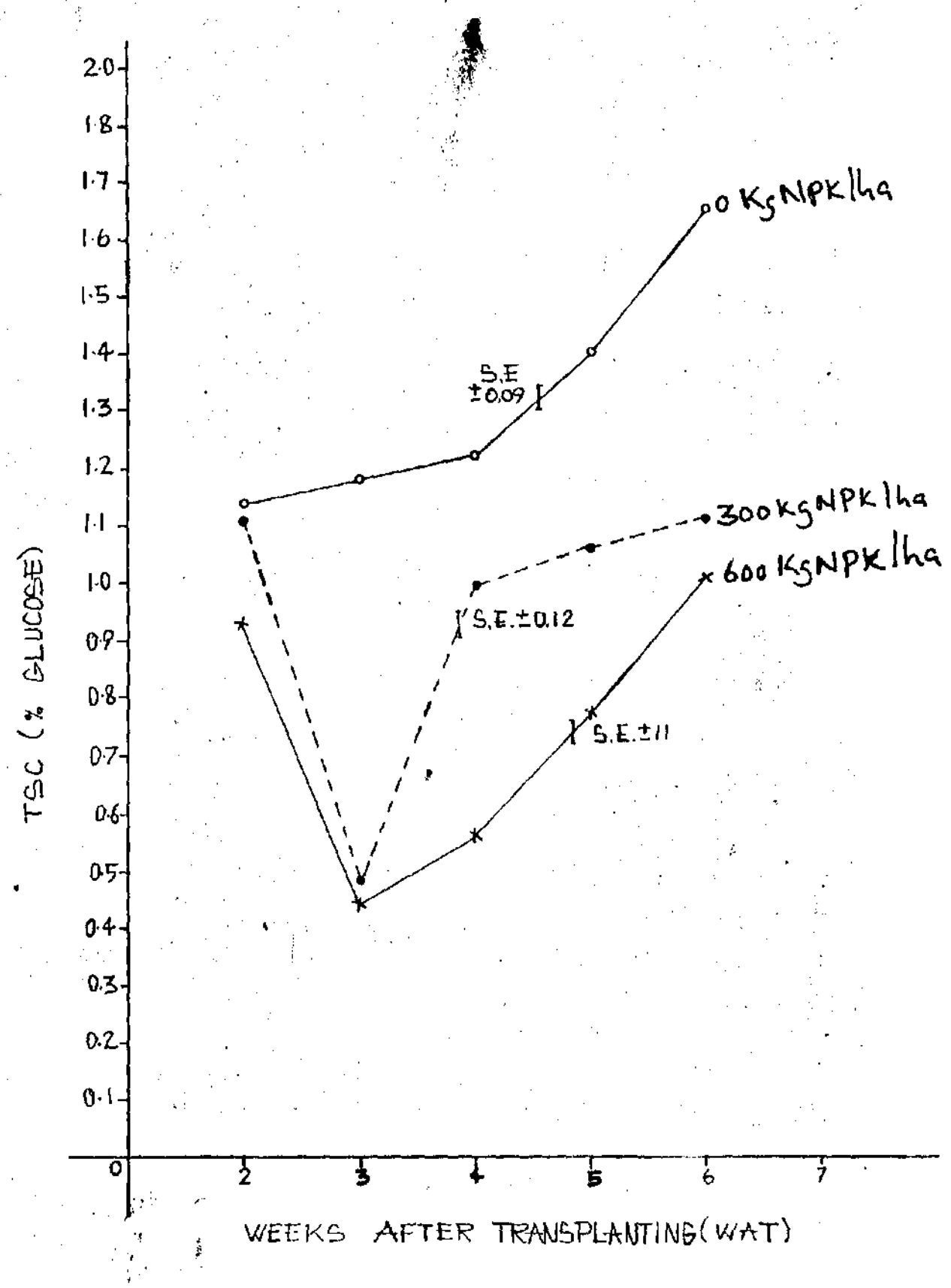


FIGURE 3 % NITROGEN IN SHOOTS OF TOMATO PLANT AS AFFECTED BY FERTILIZER TREATMENT AND AGE.

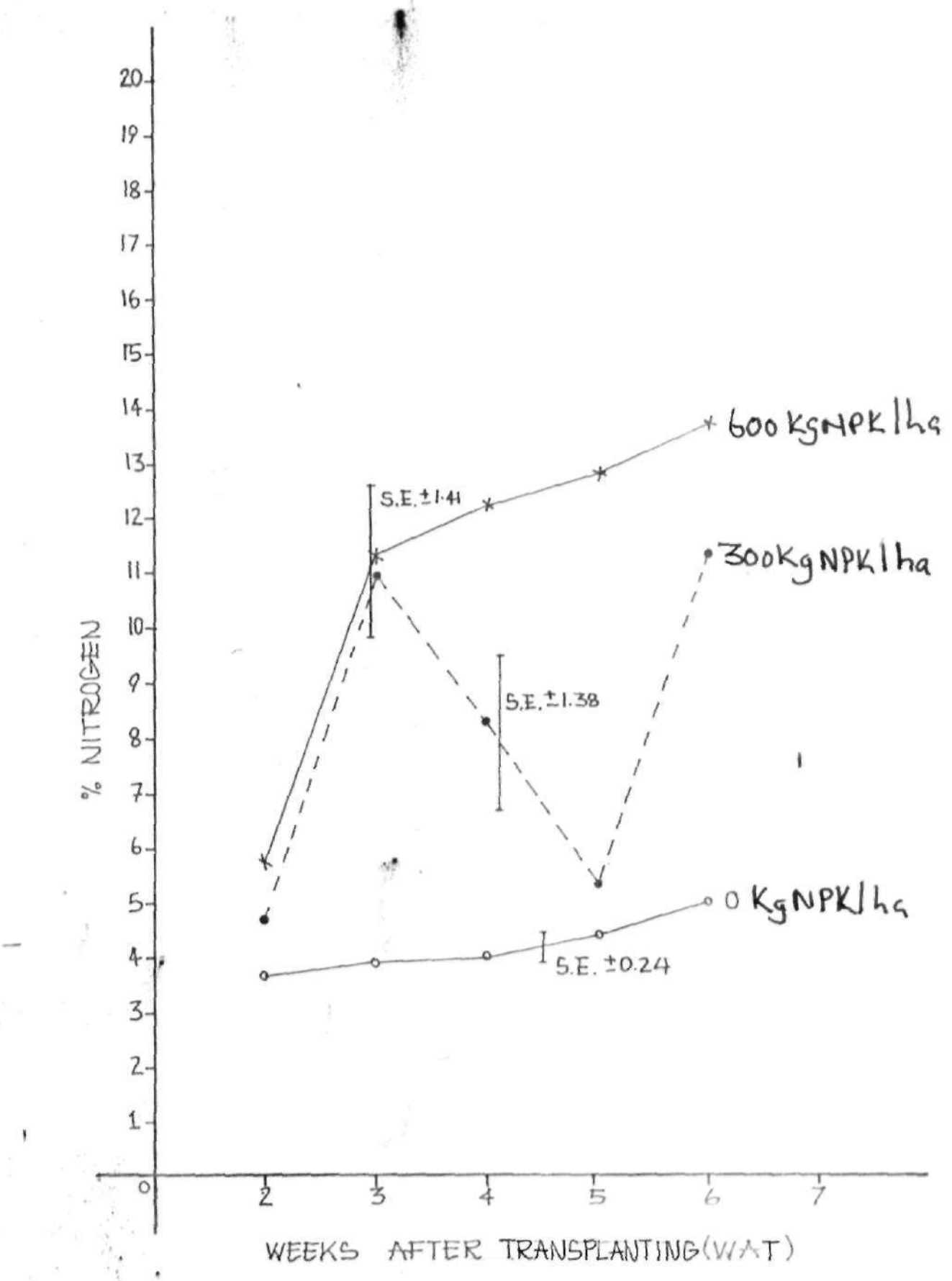
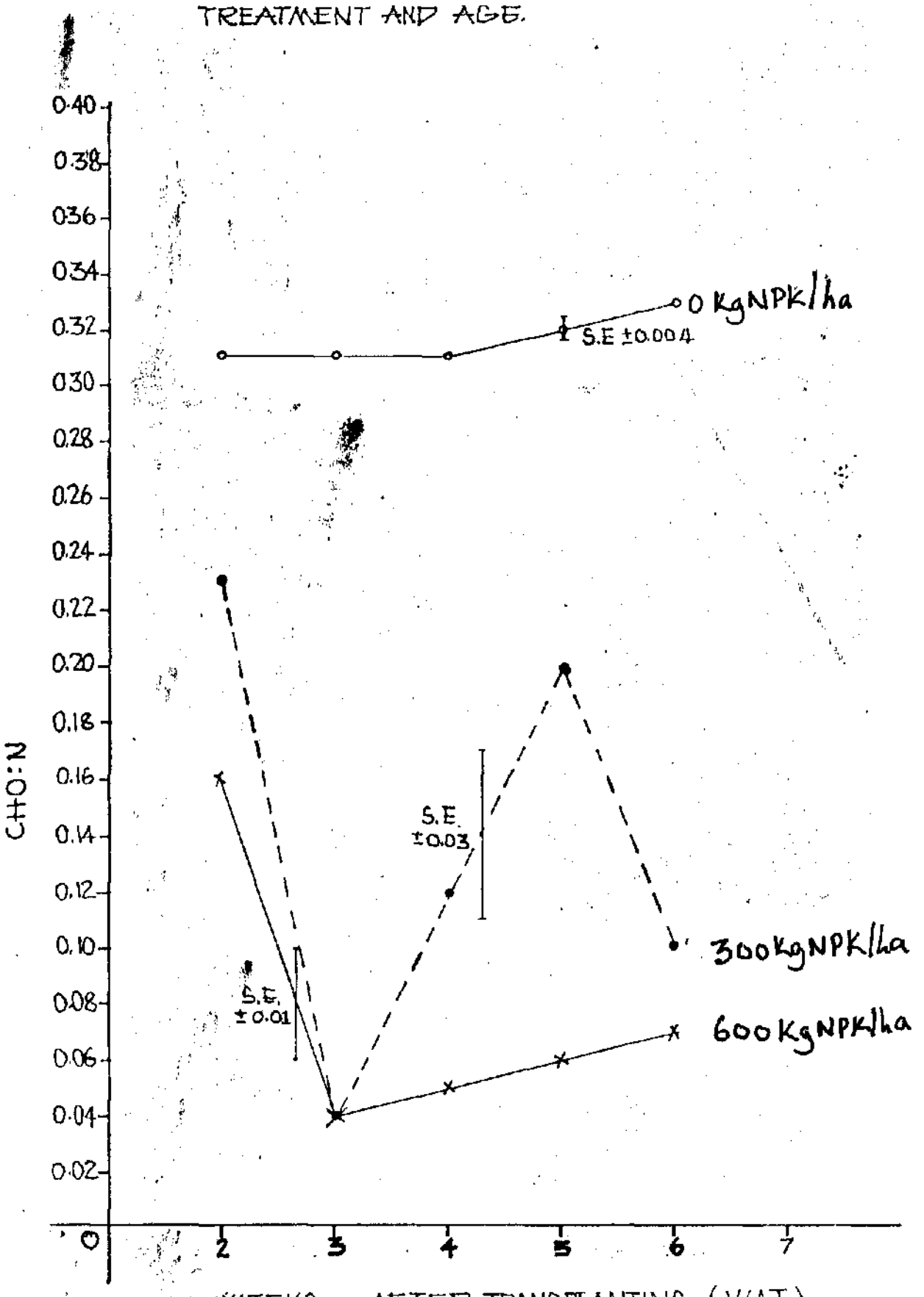


FIGURE 4 SHOWING THE CARBOHYDRATE: NITROGEN RATIO (CHO:N) IN TOMATO SHOOTS AS AFFECTED BY NITROGEN TREATMENT AND AGE.



4.4 ROOTING RESPONSES

4.4 (i) Earliness and rate of rooting:

Generally, IAA gave the earliest and most uniform rooting response than other auxin treatments and the control regardless of fertilizer levels (Table 7).

For all fertilizer levels, IAA at 0.05kg/l gave the earliest rooting and highest number of roots, followed by IAA at 0.1g/l. IAA at 0.05g/l for 300kgNPK/ha-treated plants was next. IAA at 0.025g/l for no fertilizer and the 600kgNPK/ha treatments took the longest time to root, but had higher number of roots at 10 days than their respective controls. Time of rooting was similar for all controls, but the controls for 600kgNPK/ha treatment cuttings had higher mean number of roots than the other two treatments. Generally, 300kgNPK/ha treatments gave better rooting responses regardless of auxin treatments (Table 7).

For IAA/NAA-treated cuttings, rooting responses were rearliest at 0.025g/l and 0.05g/l for zero fertilizer levels (Table 7). However, the number of roots was higher in 300kgNPK/ha treatments. Rooting responses of 600kgNPK/ha-treated cuttings were similar to their controls at 0.05g/l and 0.1g/l. These were both lower than cuttings treated with IAA/NAA at 0.025g/l. At 0.05g/l for 300kgNPK/ha rooting responses were poorest. Generally however, this level of fertilizer gave the best rooting response in cuttings regardless of auxin treatments (Table 7).

Table 7: Time of rooting of stem cuttings treated with indole-acetic acid (IAA): Naphthalene acetic acid (NAA) and a combination of both as affected by nitrogen nutrition of stock plant and auxin concentration (g/lit.)

DAYS IN ROOTING MEDIUM	Levels of NPK fertilizer (Kg/ha)											
	0				300				600			
	CONCENTRATION				INDOLEACETIC ACID IAA				CONCENTRATION			
	CONTROL	0.025	0.05	0.1	CONTROL	0.025	0.05	0.1	CONTROL	0.025	0.05	0.1
2	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	**	-	-	-	**	-	-	-	**	-
6	*	-	***	*	*	*	***	**	*	-	***	**
8	*	**	***	**	*	**	***	***	**	*	***	***
10	*	***	***	**	**	***	***	***	***	***	***	***
	INDOLE-ACETIC ACID + NAPHTHALENE ACETIC ACID (IAA/NAA)											
2	-	-	-	-	-	-	-	-	-	-	-	-
4	-	*	*	-	-	**	-	**	-	-	-	-
6	*	**	**	*	*	***	-	***	*	**	*	*
8	*	**	**	**	*	***	**	***	**	**	**	**
10	*	***	***	***	**	***	**	***	***	***	***	***
	NAPHTHALENE ACETIC ACID (NAA)											
2	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	*	-	-	-	-	**	-	-	-	-
6	*	*	*	*	*	*	-	***	*	*	*	**
8	*	*	**	**	*	**	*	***	**	*	**	**
10	*	***	***	**	**	***	**	***	***	***	***	***

* = less than 10 roots/cutting/treatment
** = 11-20 roots/cutting/treatment
*** = More than 20 roots/cutting/treatment

For NAA treatments, rooting responses were earliest and highest in 300kgNPK/ha at 0.1g/l. This was followed by zero level fertilizer treated cuttings at 0.05g/l NAA treatments and then by 600kgNPK/ha cuttings at 0.1g/l, although the number of roots were higher in the latter than the former category. Least rooting response was obtained in 300kgNPK/Ha treated cuttings given 0.5g/l NAA. Time of rooting was similar in cuttings given 0.025g/l of NAA in all fertilizer levels. However, the number of roots were higher for the cuttings from plants given 300kgNPK/ha (Table V).

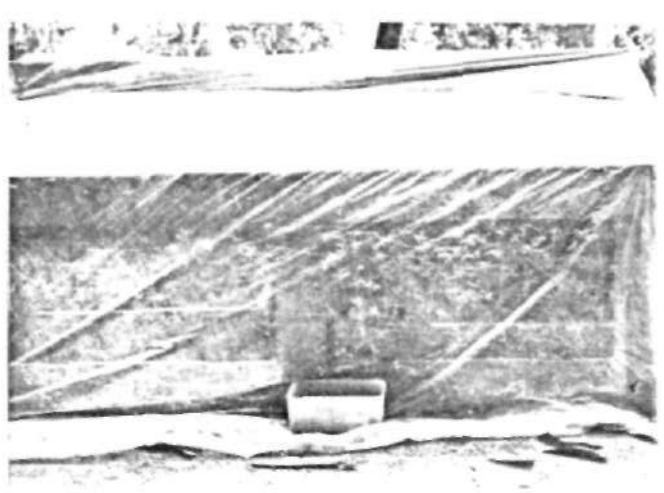
4.4 (ii) - Mean number and length of roots per cutting:

Visual observations on rooting in stem cutting-propagated plants are presented in Plates 1-6. IAA generally gave higher mean number and length of roots at all fertilizer levels than other auxin treatments (Table 8). IAA at 0.05g/l gave the highest mean number and length of roots at all levels of fertilizer followed by 0.1g/l and then 0.025g/l IAA. For IAA/NAA treated cuttings, 0.1g/l gave the highest mean number of roots per cutting than other concentrations except in the cuttings from 600kgNPK/ha treated plants at 0.025g/l. IAA/NAA at 0.05g/l gave the least mean number and length of roots, and these were even lower than controls. For 600kgNPK/ha treated cuttings, at all concentration IAA/NAA gave a slightly lower mean number of roots per cutting than controls. At this level of fertilizer mean root lengths were also lowest (Table 8).

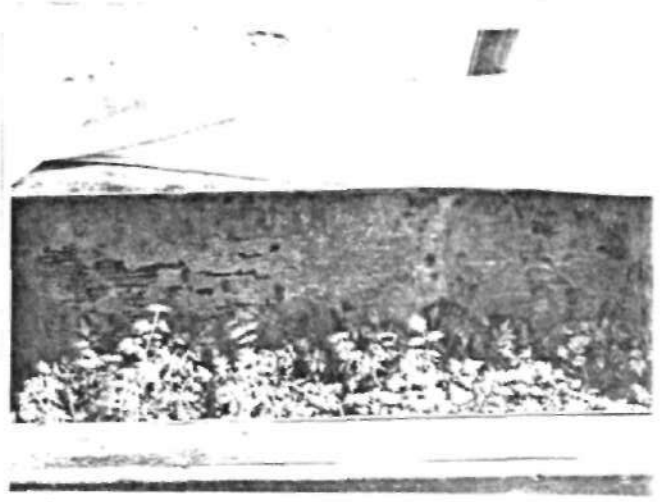
Table 8: Mean number and length (cm) of roots per cutting as affected by auxin type and concentration (g/lit.) NPK fertilizer level.

AUXIN TYPE	IAA								IAA/NAA								NAA					
CONCEN-TRATION	CONTROL		0.025		0.05		0.1		0.025		0.05		0.1		0.025		0.05		0.1			
	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH	NO.	LENGTH		
NITROGEN LEVEL																						
0	12.0	1.12	27.0	0.71	46.2	2.53	38.8	1.05	20.0	1.45	23.0	2.14	28.0	1.22	23.2	1.04	31.4	2.21	10.6	0.81		
	c	e	b	f		h	d	e	a	g	a	h	b	e	d	e	b	d	c	f		
R	19.6	1.76	28.0	0.88	92.0	2.70	52.4	1.53	29.0	1.92	11.0	1.06	49.6	2.52	26.0	1.51	11.4	1.13	35.0	2.13		
	a	g	b	f		h		g	b	g	e	e		f	b a	g	c	e	d	b h		
H	23.0	1.83	35.0	0.91	59.8	2.98	42.6	1.77	22.0	1.56	21.4	1.80	21.2	1.13	22.0	1.14	27.0	2.68	29.0	1.82		
	a	g	d	f		h		g	a	g	a	g	a	e	a	e g	b	h	b	g h		

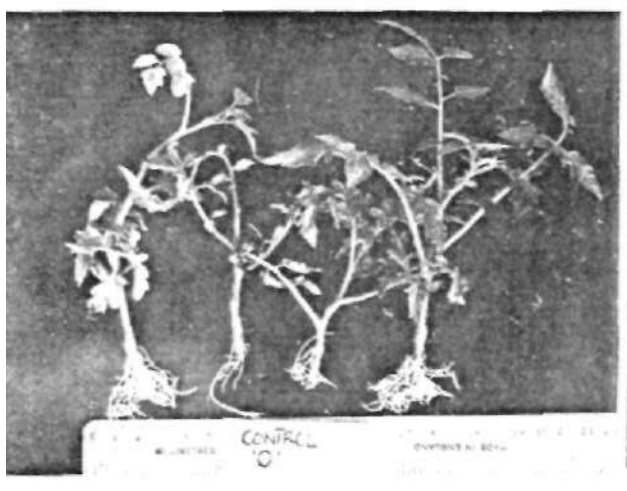
Differences are significant by (DMRT) Duncans multiple range test except where indicated by let(in 5/4.4 column).



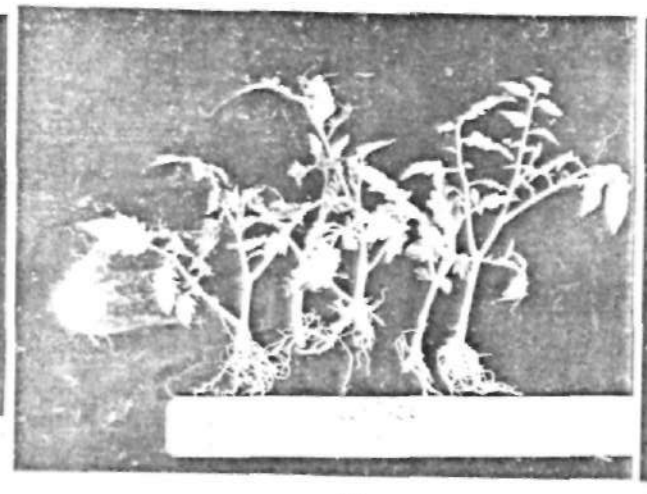
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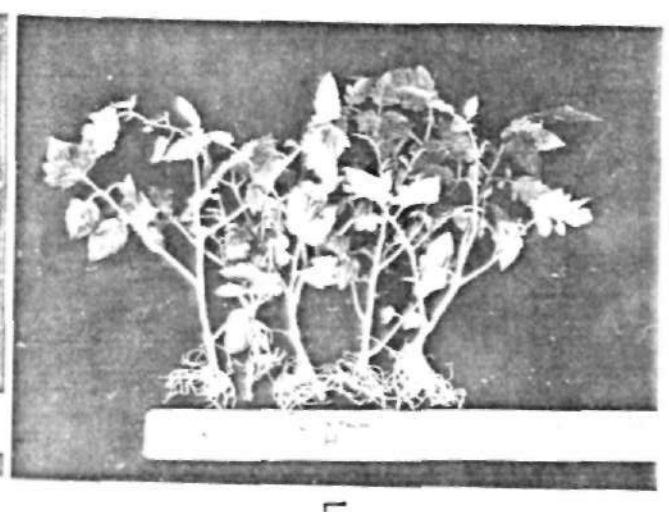
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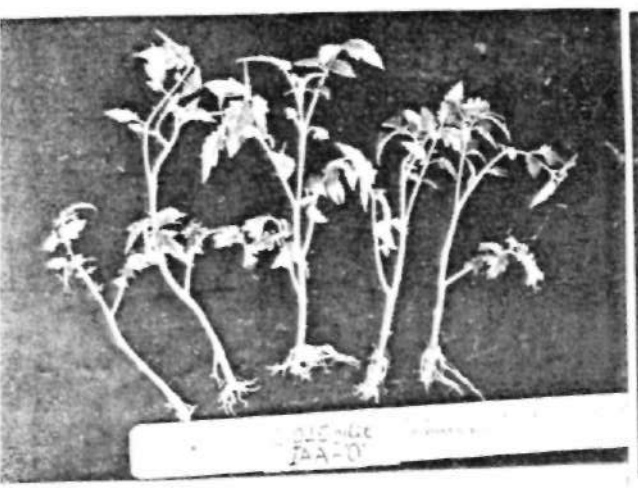
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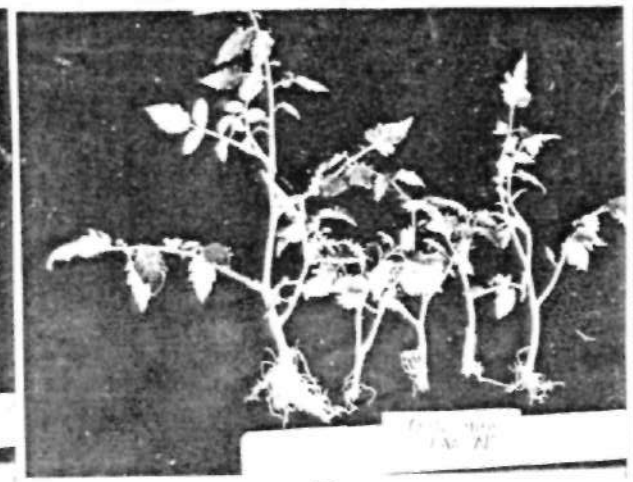
D



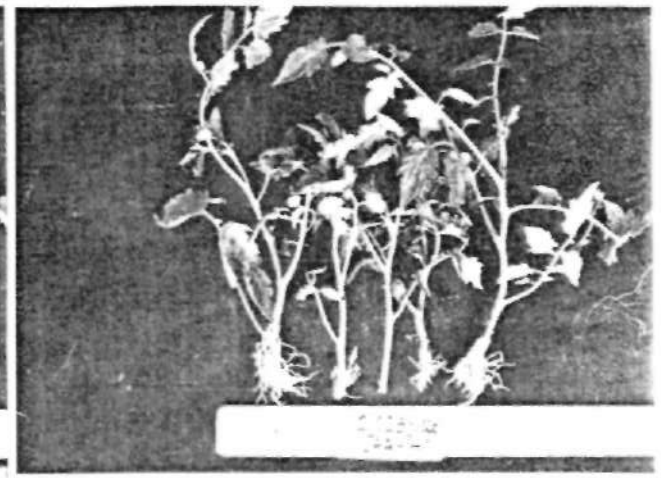
E



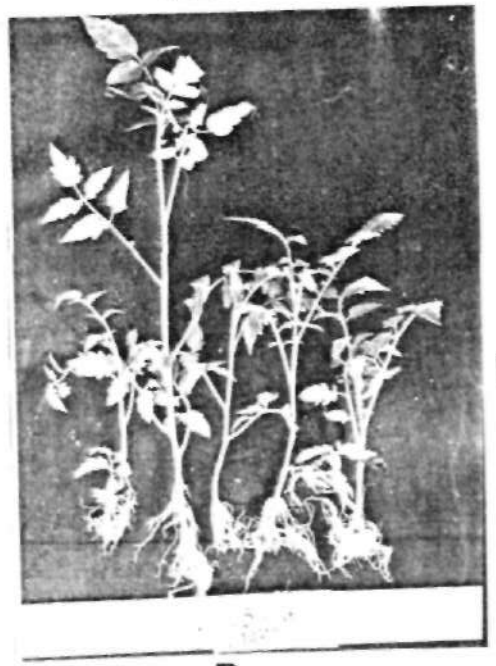
A



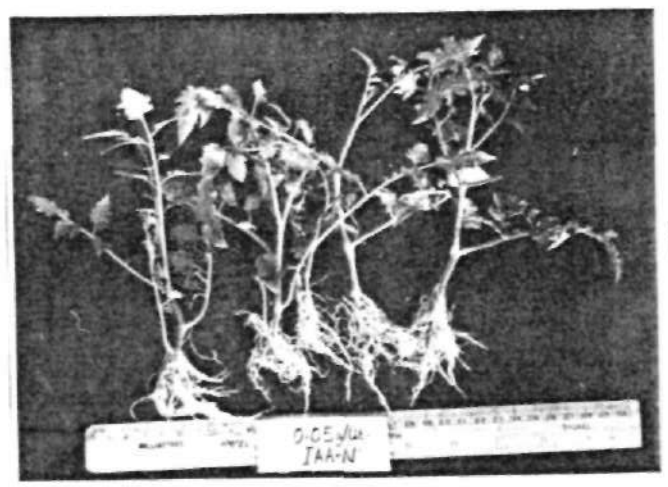
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C



D



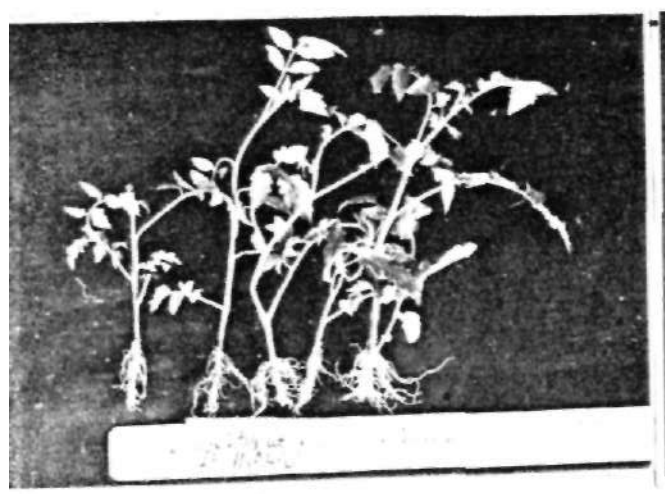
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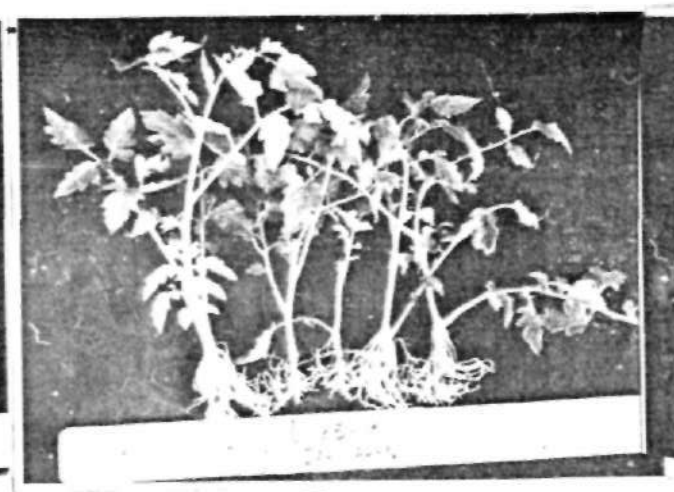
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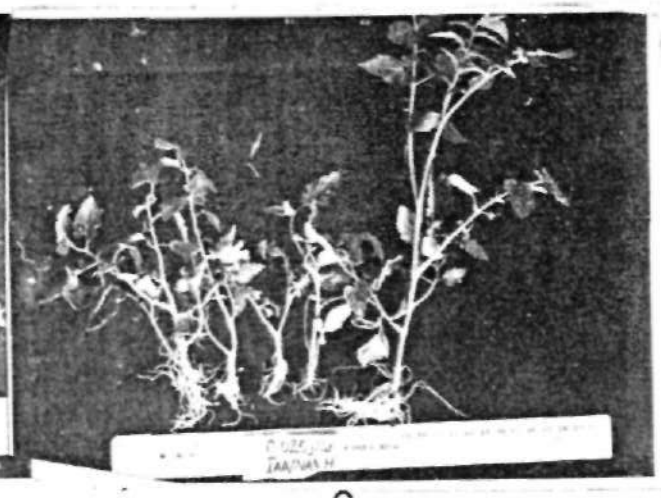

A
B
C
0·1g/Lt-
IAA/NAA-0
D
E
F



A



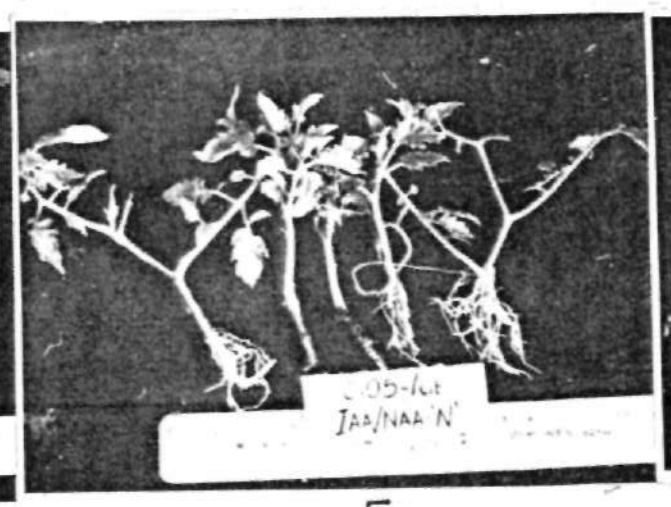
B



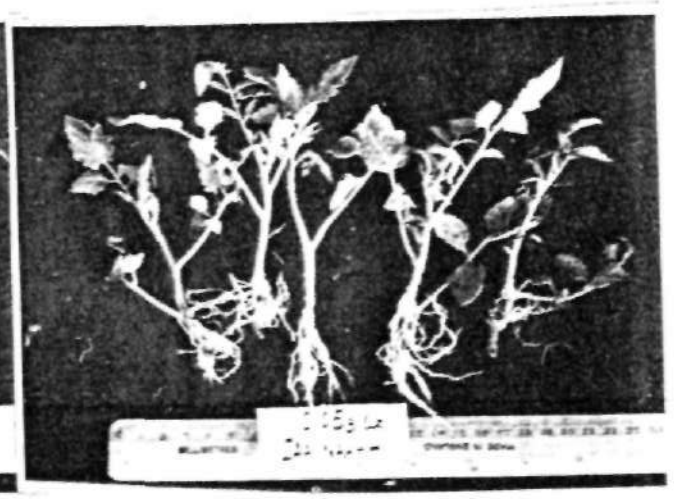
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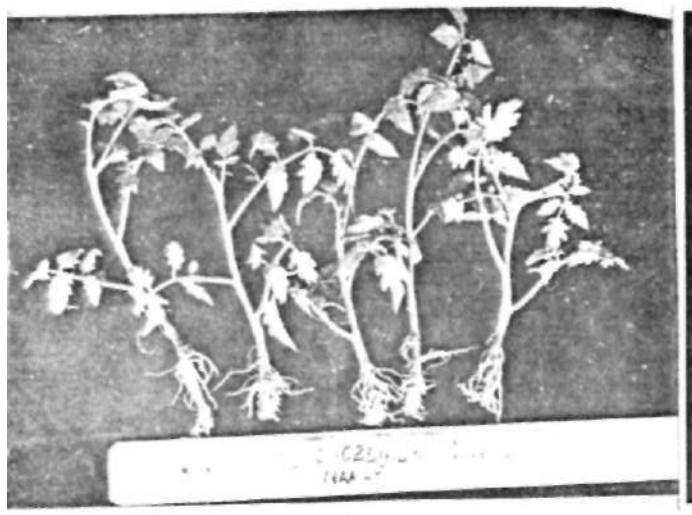
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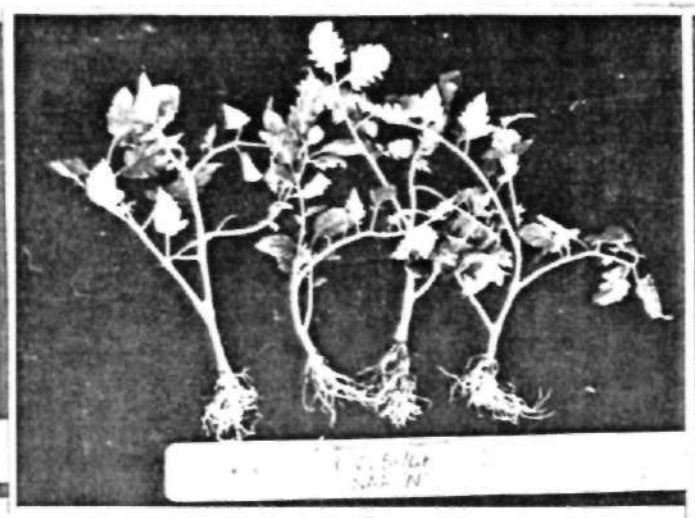
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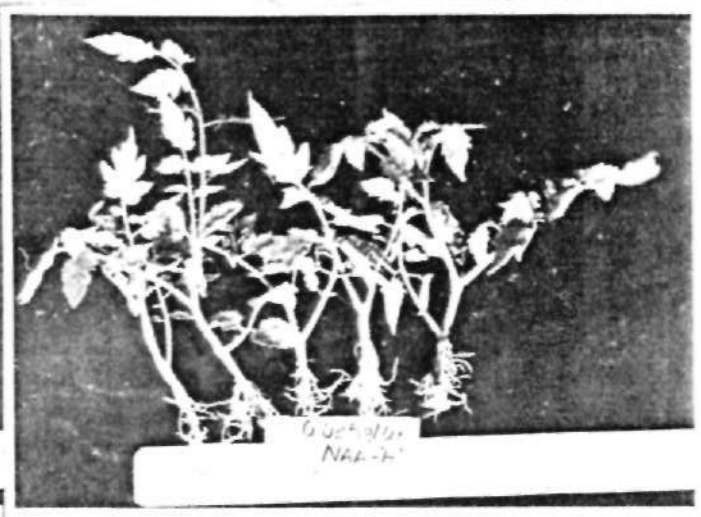
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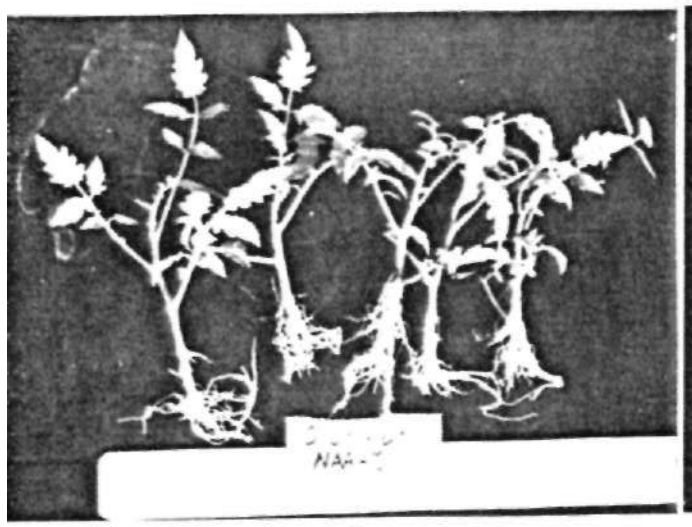
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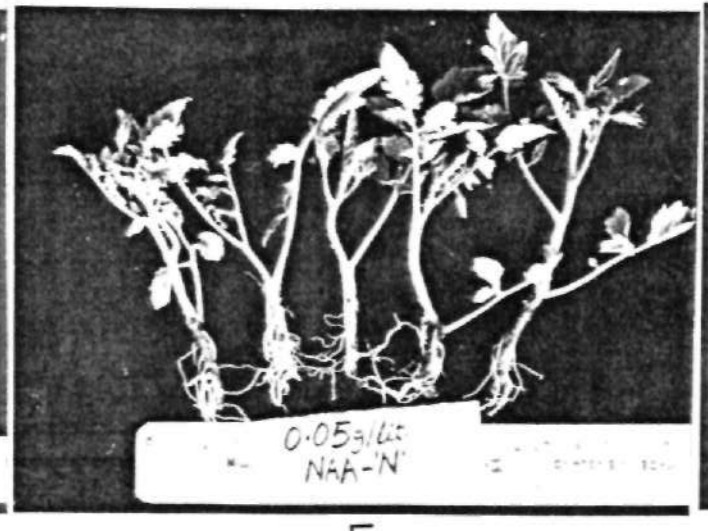
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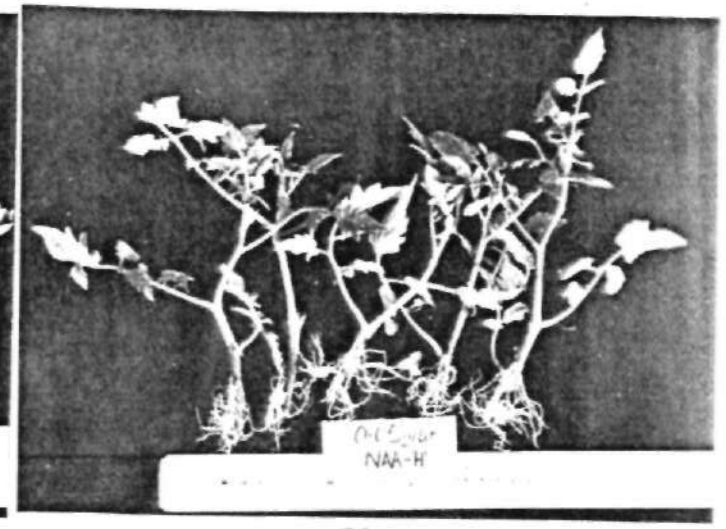
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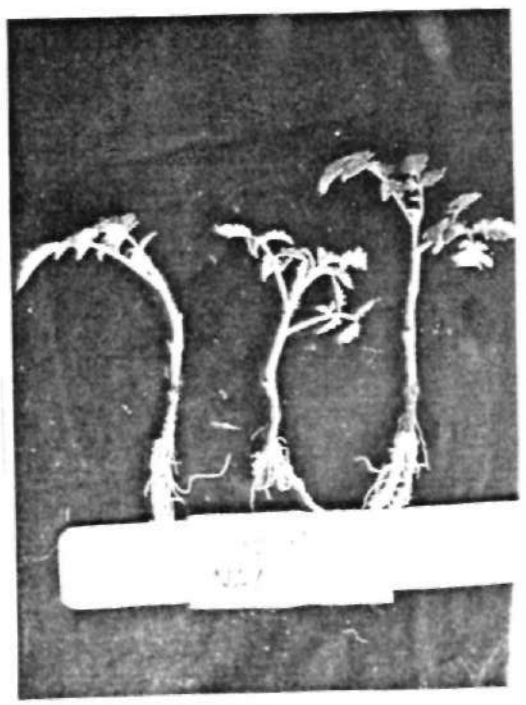
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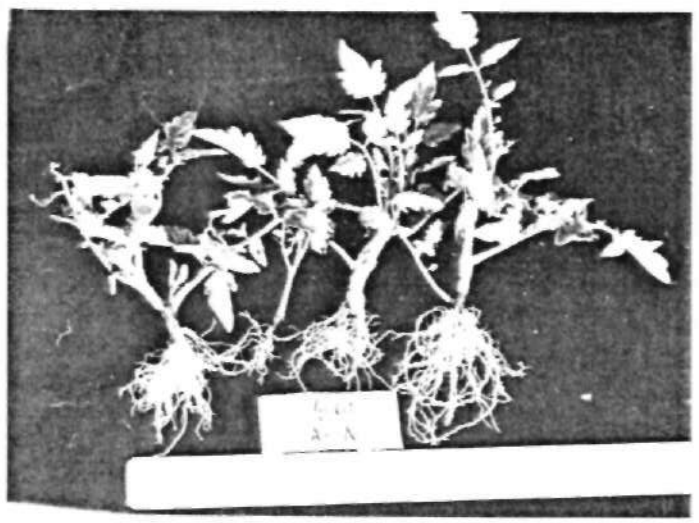
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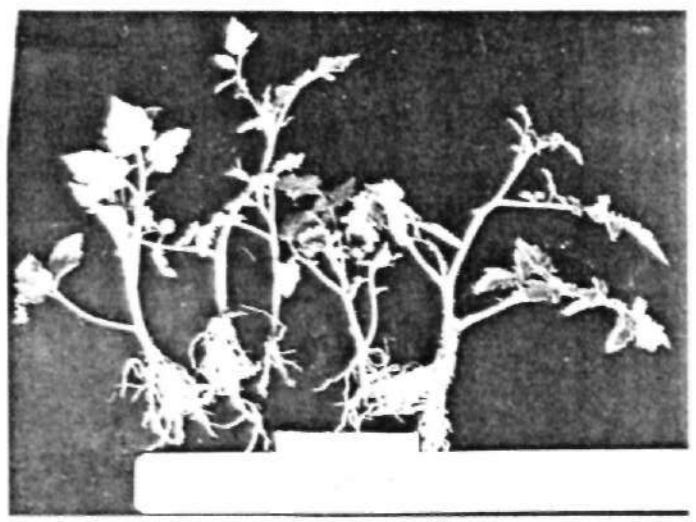
F



A



B



C

6

For NAA treated cuttings, the highest root number was obtained at 300kgNPK/ha and 0.1g/l NAA, while the highest root length was obtained at 0.05g/l NAA and at 300kgNPK/ha. The least number of roots were obtained with NAA at 0.1g/l at zero levels of NPK fertiliser. NAA at 0.025g/l had a mean root length lower than cuttings not treated with NAA.(Table 8).

Except for controls, IAA at 0.025g/l, IAA/NAA at 0.05g/l and NAA at 0.05g/l had higher mean number and length of roots in stem cutting taken from plants treated with 300kgNPK/ha than in cuttings taken from plants given 600kgNPK/ha and zero level of NPK/ha.

4.4 (iii) Number of rooted cuttings per treatment (% rooting):

Rooting percentages ranged between 80-100% despite NPK fertilizer and auxin treatments (Table 9). Cuttings from plants with no fertilizer treatments had 90.0% rooting. Those with 300kgNPK/ha had 92.0% while those with 600kgNPK/ha had 96.0% rooting. Cuttings from plants given no fertilizer but treated with IAA had an overall mean rooting of 86.67%; those with IAA/NAA had 100% while those with NAA alone had 96.67%. Cuttings from plants given no fertilizer and not treated with growth hormones had 80% rooting. Cuttings from 300kgNPK/ha treated plants, treated with IAA had overall percentage of 100.0%; while those treated with IAA/NAA and NAA had 86.67% and 86.67% rooting respectively. The untreated plants had 100%. 600kgNPK/ha-treated cuttings with IAA gave 93.33%; IAA/NAA 93.33% while NAA had 100%. The untreated plants had 93.33% (Table 9).

Within auxin concentrations, IAA at 0.05g/l gave the highest rooting of 100% followed by 0.025g/l (93.33%) and 0.1g/l (86.67%). For IAA/NAA, 0.025g/l gave highest rooting of 100% followed by 0.1g/l with 93.33% and 0.05g/l with 86.67%.

Table 9: Percentage rooting per treatment of 5 cuttings each as affected by auxin type and concentration and fertilizer level.

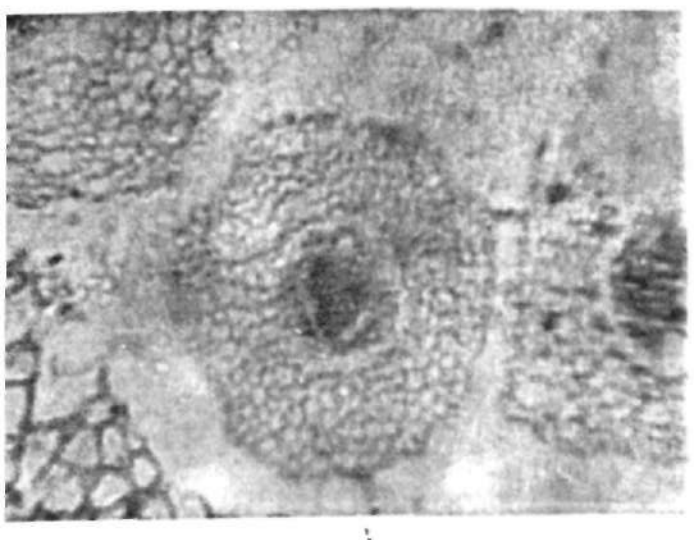
Fertilizer Level		AUXIN TYPE									
		IAA conc.(g/lit.)			IAA/NAA conc.(g/lit.)			NAA conc. (g/lit.)			
KgNPK/ha	Untreated	0.025	0.05	0.1	0.025	0.05	0.1	0.025	0.05	0.1	Mean
0	4 80%	5	5	3	5	5	5	5	5	3	90%
		86.67%			100%			86.67%			
300	5 100%	5	5	5	5	3	5	4	5	4	92%
		100%			86.67%			86.67%			
600	5 100%	4 93.33%	5 100%	5 86.67%	5 100%	5 86.67%	4 93.33%	5 93.33%	5 100%	5 100%	96%
	93.33%	933.33%			93.33%			100%			
Mean	93.33%	93.33%			93.33%			88.88%			

NAA at 0.05g/l gave highest rooting percentage of 100 followed by 0.025g/l with 93.33% and 0.1g/l least with 80% (Table 8).

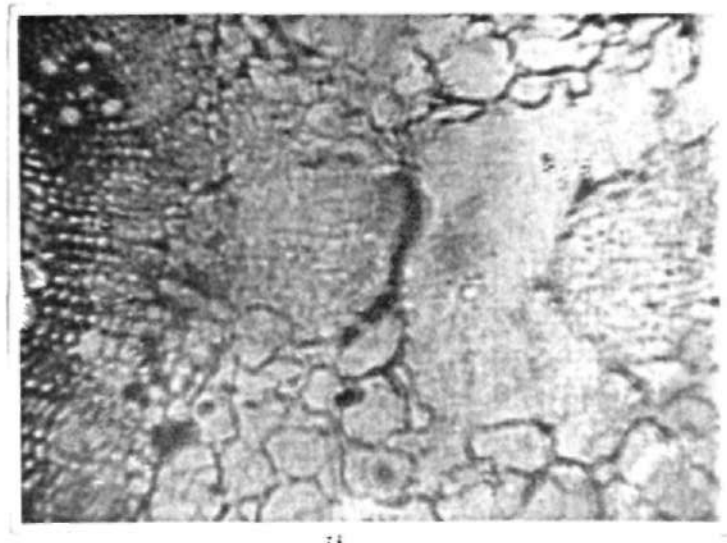
4.4 (iv) Origin and development of adventitious roots in rooted stem cuttings.

In both transverse and longitudinal sections of rooted stem cuttings, adventitious root initials are seen as a spherical clump of cells, which are parenchymatous in nature and located in the cortex (Plate 7(i)). These appear to be activated and *activated rapid division and subsequent elongation* at the portion of the primordium distal to the vascular cylinder (Plate 7ii, iii(a)). As cell division proceeds, the extension of the root primordium continued in both directions. Contact was soon made with the vascular cylinder and the inner boundary of the epidermis (Plate 7iii(b) and iv. The emerging root soon developed a vascular connection with the cylinder and the root emerged through the epidermis (Plate 7(v)). This pattern of development was similar for both fertilizer(Plate 8 (i-v)) and hormone treatments, except that the number of root initial cells was higher and they were larger in plants given fertilizer treatments than in those without fertilizer treatments (Plate 8(i)). Plate 9 shows the stages of root development in longitudinal section (L.S). Plate 9(i&ii) show dormant and activated root initial cells. Plate 9(iii) shows root primordium before emergence from the stem. Plate 9(iv) shows fully emerged root without complete vascular connection and 9(v) shows fully emerged root with complete vascular connection.

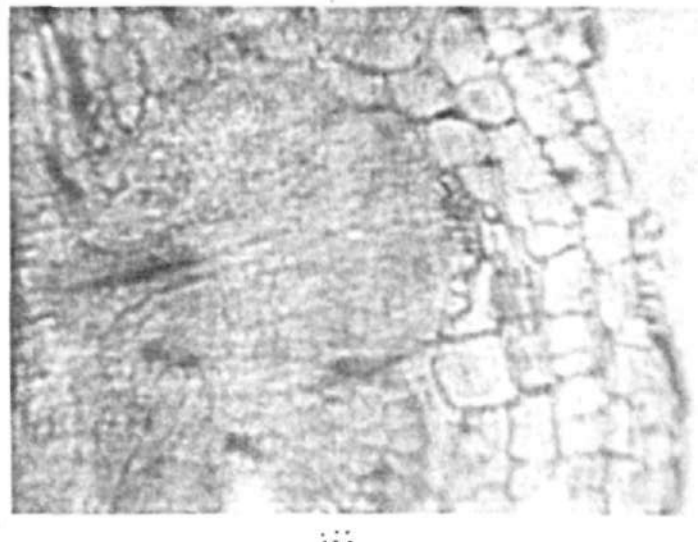
iiia
v
ii
iv
i
iiib



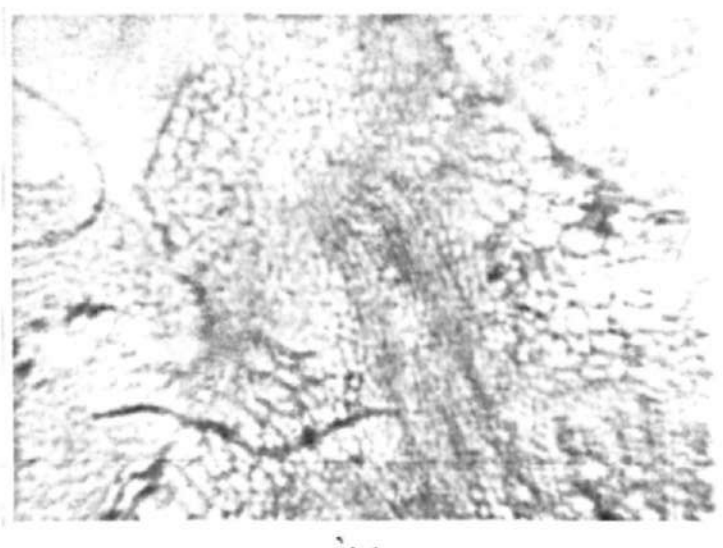
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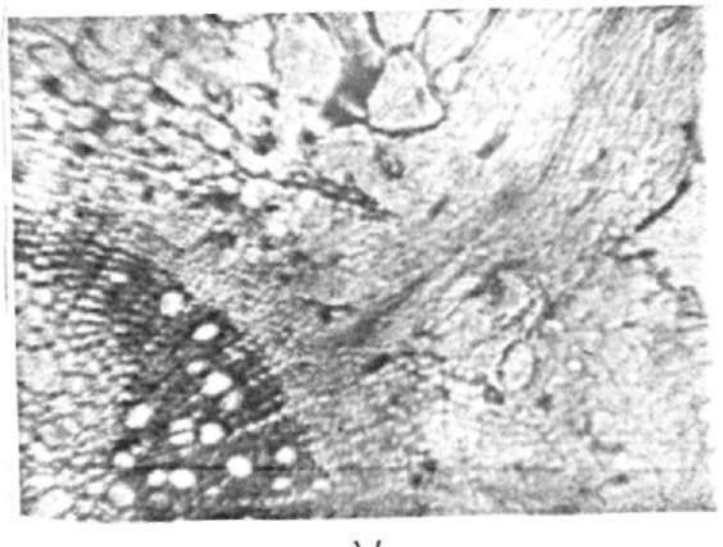
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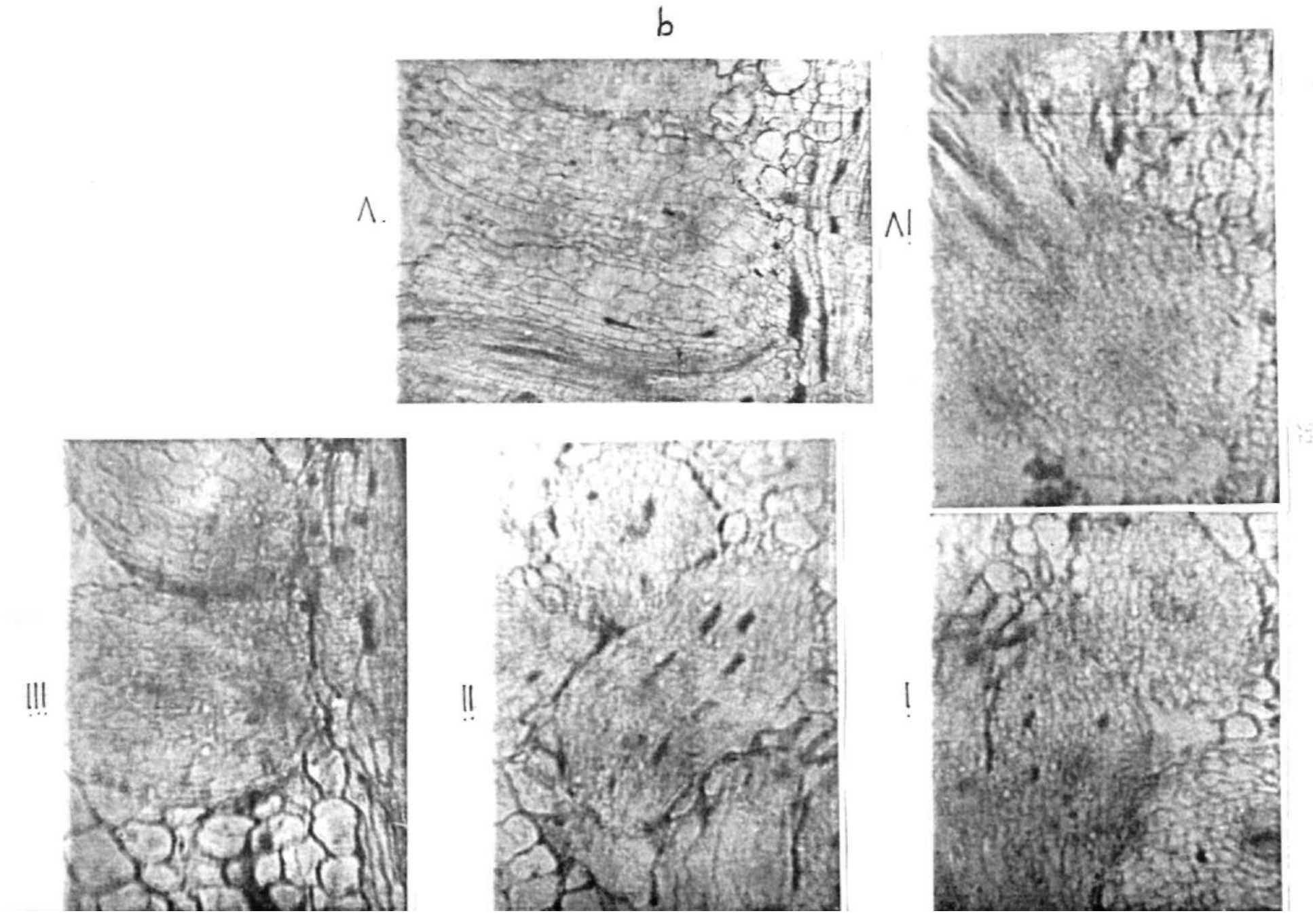
iii



iv



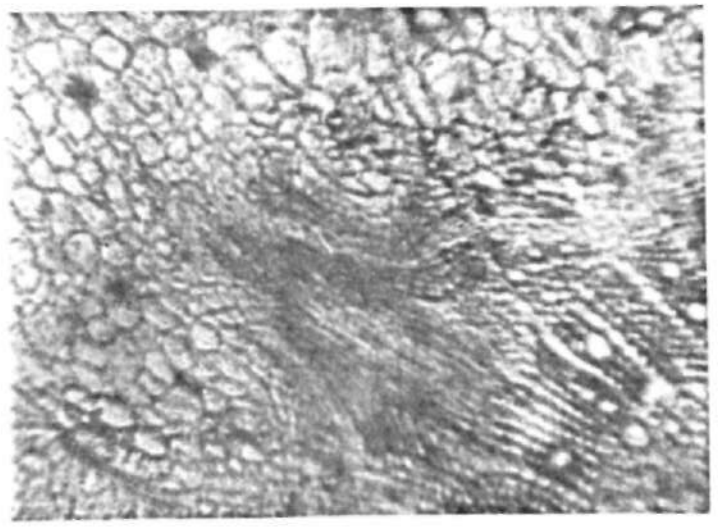
v


b
A.
IV
III
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I

II



I



4.5 YIELD AND YIELD COMPONENTS

Seed-propagated plants generally had higher mean number of fruits per plant than stem-cutting propagated plants, for all levels of NPK fertilizer and auxin treatments. Seed-propagated plants given high levels of NPK fertilizer (600kg/ha) had higher mean number of fruits per plant (9 ± 0.92g) than those given 300kgNPK/ha (6 ± 0.31g) and the zero fertilizer levels (2 ± 0.025g) (Table 9).

In stem-cutting propagated plants, auxin treatments did not significantly affect fruit number, which ranged from 1-6 fruits per plant. Plants given no NPK fertilizer had between 1 ± 0.38 to 1 ± 0.67 fruits per plant. Those given 300kgNKP/ha had between 4 ± 0.25 to 4 ± 0.55 fruits per plant, while those given 600kgNPK/h had between 6 ± 0.66 to 6 ± 1.03 fruits per plant (Table 9).

Mean fruit weight per plant at first harvest was generally significantly ($P = 0.05$) higher in seed-propagated plants than in stem-cutting propagated plants (Table 9), for all fertilizer treatments. Among seed-propagated plants, mean fruit weight per plant was 17.14 ± 0.67g for zero fertilizer level, 49.42 ± 2.28g for 300kgNPK/ha and 40.67 ± 1.37g for 600KgNPK/ha. Among stem-cutting propagated plants, plants given no fertilizer had the least mean fruit weights ranging from 1.89 ± 1.89g to 3.53 ± 1.85g. Plants given 300kgNPK/ha had 17.59 ± 0.84g to 18.34 ± 0.75g, while plants given 600kgNPK/ha had 18.16 ± 0.77g to 19.34 ± 1.22g (Table 9). Auxin types did not influence fruit weight significantly.

Table 10: Number and weight (g) of fruits per plant at first harvest as affected by mode of propagation of tomato, auxin type and fertilizer.

MODE OF PROPAGATION	SEED PROPAGATED			STEM-CUTTING PROPAGATED											
AUXIN TYPE	-			CONTROLS			IAA			IAA/NAA			NAA		
NITROGEN LEVEL	0	R	H	0	R	H	0	R	H	0	R	H	0	R	H
PLANT No.															
*	2	5	7	1	5	8	0	3	8	0	4	7	0	3	5
1	16.60	52.70	41.30	4.35	16.28	22.27	0	17.90	21.80	0	14.20	20.20	0	17.30	21.40
*	1	6	12	0	4	6	2	4	8	0	4	8	1	4	4
2	14.80	48.90	39.65	0	18.69	17.53	5.68	17.52	22.40	0	18.80	19.60	3.80	16.20	23.00
*	1	6	7	1	4	6	0	4	8	0	5	5	1	3	6
3	18.20	42.50	36.35	6.25	20.26	16.80	0	16.58	17.60	0	19.60	17.30	4.60	18.10	17.20
*	2	7	9	-	5	7	-	3	3	-	3	4	-	5	6
4	17.50	55.80	44.80	-	15.58	16.35	-	18.69	16.90	-	19.50	16.00	-	20.30	16.80
*	2	6	9	-	4	3	-	6	5	-	3	4	-	4	8
5	18.60	47.20	41.25	-	17.15	18.70	-	21.02	17.80	-	17.30	17.70	-	19.30	18.30
*	2.00	6.0	9.0	1.0	4.0	6.0	1.0	4.0	6.0	0.00	4.0	6.0	1.0	4.0	6.0
MEAN	17.14	49.42	40.67	3.53	17.59	18.33	1.89	18.34	19.30	0.00	17.88	18.16	2.80	18.24	19.34
*	0.25	0.31	0.92	0.33	0.25	0.84	0.67	0.55	1.03	0.00	0.37	0.81	0.33	0.37	0.66
S.E ±	0.67,	2.28/	1.37/	1.85	0.84	1.06	1.89	0.75	1.16	0.00	1.01	0.77	1.42	0.72	1.22

* = Figures for number of fruits per plant (to the nearest whole number).

The variation in fruit weight for seed-propagated plants resulting from fertiliser application showed the following trend: 300kgNPK/ha > 600kgNPK/ha > 0' fertilizer levels, while for stem-cutting propagated plants it was 600kgNPK/ha >300kgNPK/ha >0' fertilizer levels.

Mean weight of fruits per plant at four subsequent harvests decreased with harvest date for both seed-propagated and stem-cutting propagated plants, but the decrease was more aparent in seed-propagated plants than in stem-cutting propagated plants (Table 10).

Total fruit weight from the four harvests was higher in seed-propagated plants than in stem-cutting propagated plants (Table 10). In seed-propagated plants, 300kgNPK/ha treatments had the highest overall mean fruit weight of 43.91 ± 2.09g followed by 600kgNPK/ha with 37.52 ± 1.80g than zero fertilizer levels had the least with 15.95 ± 0.60g. For stem-cutting propagated plants, 600kgNPK/ha treatments had the highest overall means of 14.24 ± 1.54g per plant followed by 300kgNPK/ha with 13.40 ± 1.28g per plant and then zero level fertiliser with 5.06 ± 0.00g/plant. Fruits from 300 and 600kgNPK/ha treatments of stem-cutting propagated plants were also significantly heavier than the no-fertilizer level treatments, but there was no significant difference between the two fertiliser levels. While the trend was 300gkNPK/ha >600kgNPK/ha >0' levels for seed propagated plants, it was 600kgNPK/ha >300kgNPK/ha> 0' levels for stem-cutting propagated plants.

4.6 PERCENTAGE SURVIVAL

Transplanted cuttings generally had high percentage of survival at the end of the experiment. Survival percentage ranged between 61-91.67% based on auxin and fertilizer treatments (Table 12).

Table 1[illegible]: Mean weight of fruits from seed propagated and stem-cutting propagated plants at four subsequent harvests as affected by nitrogen levels (gms)

Harvest No.	MODE OF PROPAGATION					
	SEED-PROPAGATED			STEM-CUTTING PROPAGATED		
	Fertilizer levels (kgNPK/ha)			Fertilizer levels (kgNPK/ha)		
	0	300	600	0	300	600
1	17.14 a	49.42 d	40.67 c	5.06f	17.21a	18.08a
2	16.62 a	44.82 e	39.68 c	-	12.26b	14.18ba
3	15.62 a	41.22 e	37.10 e	-	12.18b	14.16b,a
4	14.42 a.b	40.18eg	32.62 e	-	11.90b	10.54b
MEAN	15.95	43.91	37.52	5.06	13.40	14.24
S.E. ±	0.60	2.09	1.80	0.00	1.28	1.54

Mean followed by the same letters in a column are not significantly (P = 0.05) different by (DMRT) Duncans multiple range test.

Table 12: Percentage of survival of transplanted cuttings at 6 weeks after transplanting as affected by auxin type and fertilizer levels.

Fertilizer Levels (kgNPK/ha)	Untreated Control	AUXIN TYPE IAA	IAA/NAA	NAA	MEAN
0	66.67%	100%	75%	91.67%	87.18%
300	100%	83.33%	83.33%	83.33%	84.62%
600	33.33%	91.67%	33.33%	66.67%	61.54%
MEAN	66.67%	91.67%	63.89%	80.56%	

Survival percentage was highest in IAA-treated cuttings (91.67%) followed by NAA and IAA/NAA treatments with 80.56% and 63.89%, respectively. The untreated plants had 66.67%. Cuttings without fertilizer application had the highest survival % of 87.18% and then 300kgNPK/ha had 84.62% and 600kgNPK/ha-treated cuttings had 61.54%.

4.7 POST-HARVEST PHYSIOLOGY

Fruit colour:

The average number of days taken for fifty percent ripening (full red colour) was 7 ± 1 days for fruits from seed propagated plants. For fruits from stem cutting propagated plants, however, this was not achieved up to the end of the observation period. Fruits from this latter mode of propagation remained orange/yellow even at 12 days (Table 13; Plate 11).

Seed weight and Germination:

Seeds from seed-propagated plants were about twice as heavy as those from stem-cutting propagated plants (Table 14). Seed germination tests showed an average of 86.7% and 23.33% germination for seed-propagated and stem-cutting propagated plants, respectively after 25 days in petri-dishes (Table 15). The germination of seeds from seed-propagated plants exhibited a sigmoid pattern of response with an initial lag phase, an exponential (log) phase and a final plateau. Seeds from stem-cutting propagated plants however did not show this pattern of germination (Figure 5).

FIGURE 5. PERCENTAGE GERMINATION OF TOMATO SEEDS OBTAINED FROM SEED PROPAGATED AND STEM-CUTTING PROPAGATED PLANTS IN PETRI-DISHES INCUBATED AT 26± 1°C.

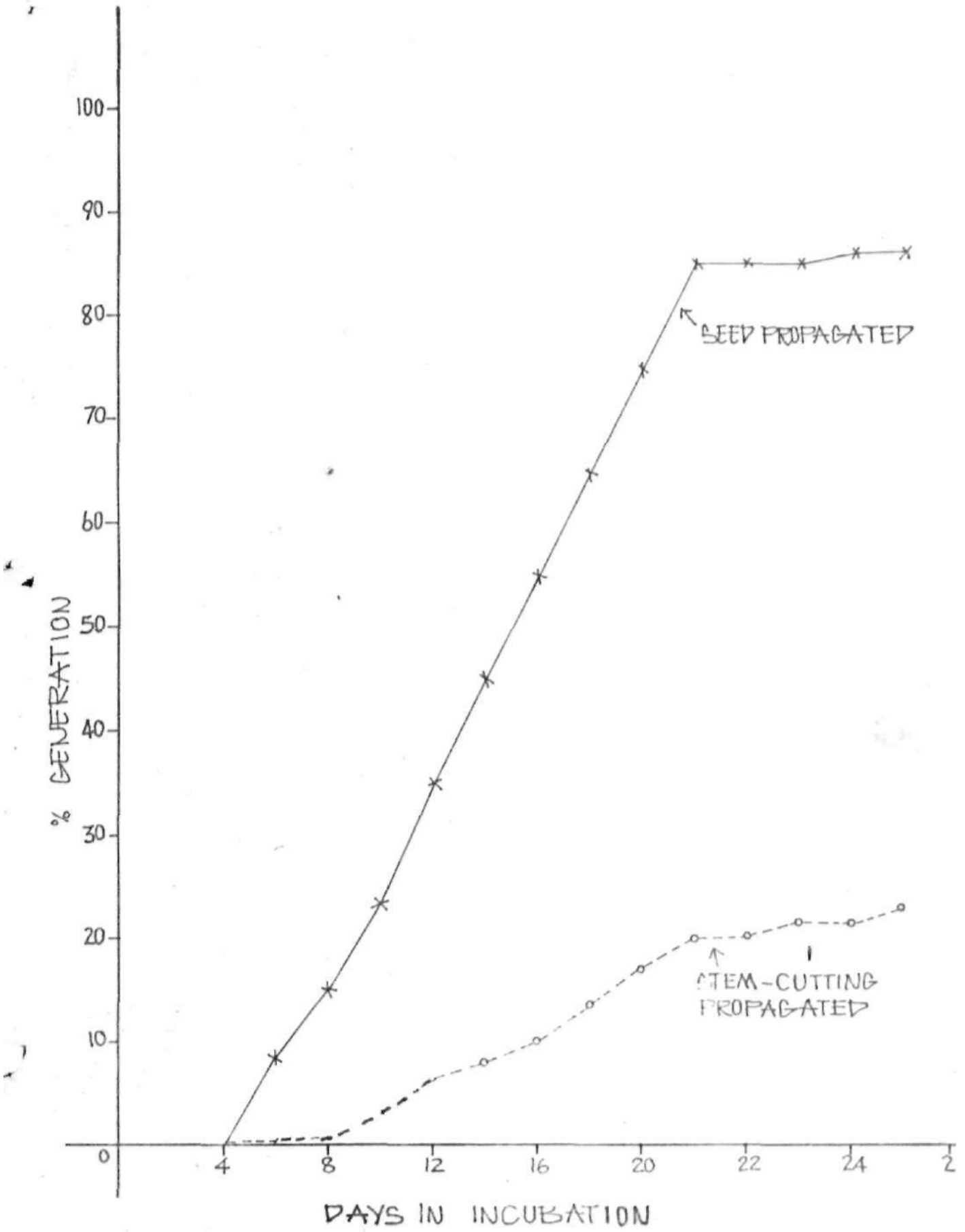


Table 13: Percentage surface skin colour of fruits from seed-propagated and stem-cutting propagated plants.

Mode of Propagation	Seed propagated			Stem-cutting propagated		
Skin colour	Green	Orange/ Yellow	Red	Green	Orange/ Yellow	Red
DAYS AFTER HARVEST						
2	100	0	0	100	0	0
4	60	30	10	100	0	0
6	30	40	30	80	20	0
8	0	40	60	60	40	0
10	0	10	90	40	60	0
12	0	0	100	0	100	0

* Based on observation of skin colour of 20 fruits from each mode of propagation placed on the shelf for 12 days at 25 ± 1°C.

Table 14: Weight of one hundred seeds from seed-propagated and stem-cutting propagated plants.

Seed propagated		Stem-cutting propagated
LOT I	0.21	0.12
LOT II	0.19	0.11
LOT III	0.20	0.09
MEAN	0.20	0.11
S.E. = ± 0.006		0.009


A
B

Table 15: Percentage germination of seeds from seed-propagated and stem cutting propagated tomato incubated at 26 ± 1°C for 25 days.

DAYS IN INCUBATION	NO. OF GERMINATED SEEDS		% GERMINATION	
	SEED PROPAGATED	STEM-CUTTING PROPAGATED	SEED PROPAGATED	STEM-CUTTING PROPAGATED
2	0	0	-	-
4	0	0	-	-
6	2.5	0	8.33	-
8	4.5	0	15.0	-
10	7.0	2.0	23.33	6.67
12	10.5	2.0	35.0	6.67
14	13.5	2.5	45.0	8.33
16	16.5	3.0	55.0	10.00
18	19.5	4.0	65.0	13.33
20	22.5	5.0	75.0	16.67
21	25.5	6.0	85.0	20.00
22	25.5	6.0	85.0	20.00
23	25.5	6.5	85.0	21.67
24	26.0	6.5	86.7	21.67
25	26.0	7.0	86.7	23.33

4.8 SHOOT-TIP CULTURE

Shoot-tip explants placed in increasing concentrations of both auxin and kinetin (0.01 - 10.0uM) did not show any organogenesis throughout the period of observation lasting 40 days. Explants started to die after about 2 weeks in culture and this was random for all auxins and kinetin combinations and concentrations. Explants on reversed combinations and concentrations of auxins and kinetin however exhibited callus, root and shoot formation and growth. Results are presented on Table 16. Plate 12 shows three stages of organogenesis. Tube A shows an explant that has undergone shoot growth without either callus or root formation. Tube B shows moderate callus formation with slight root regeneration and growth. Tube C shows a plantlet, with well formed callus and well developed roots. Shoot growth is also well marked.

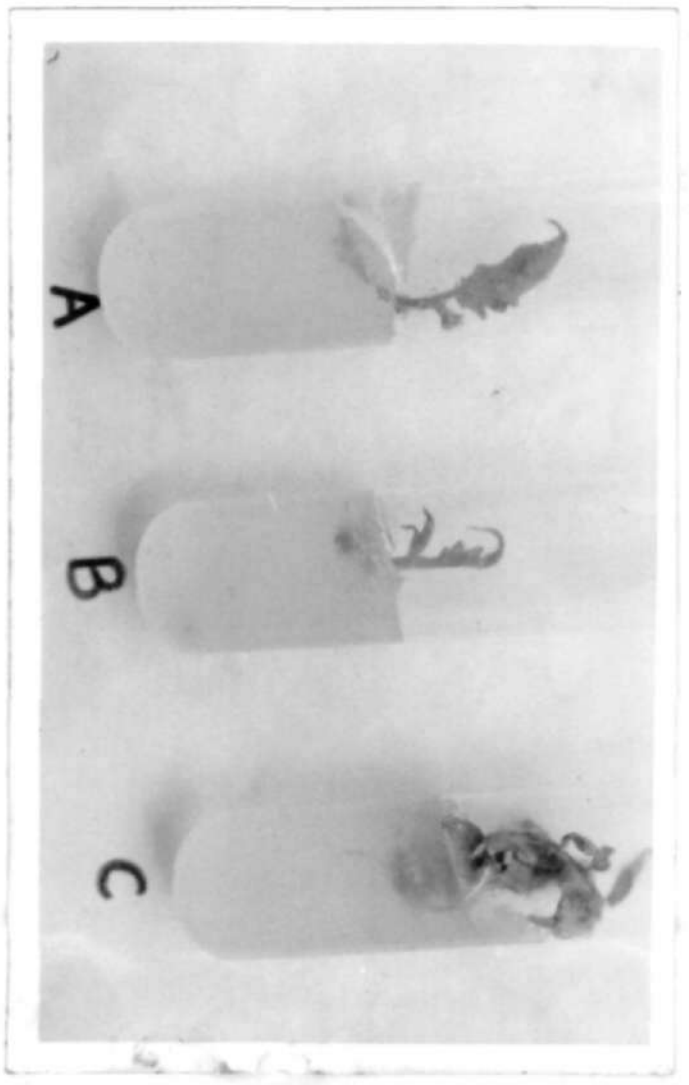
Indole Butyric Acid (IBA)/Kinetin (K) at 10.0/0.01uM ratio gave the best root regeneration and shoot growth response (Table 16) throughout the culture period.
At 1.0/0.1uM IBA/K callus formation was most prominent. Root regeneration and shoot growth were poor and by 15 days of culture, explants had started to die. At 0.1/1.0uM IBA/K, both root regeneration and callus formation were poor. Shoot growth was however good (Table 16).

2,4-dichlorophenoxy acetic acid/kinetin 10.0/1.0uM was detrimental to callus formation, root regeneration and shoot growth (Table 16). Death of explants was recorded as from 15 days of culture. At 1.0/0.1uM, 2,4-D/K induced callus

Table 16: The effect of various concentrations and combinations of two auxins (Indole-butyric acid, 2,4-dichlorophenoxyacetic acid) and kinetin on root regeneration and shoot growth of tomato shoot-tip explants in vitro.

		CONTROL			INDOLE-BUTYRIC ACID/KINETIN									2,4-DICHLOROPHENOXYACETIC ACID/KINETIN					
		0.0/0.0 (uM)			10.0/0.01 (uM)			1.0/0.1 (uM)			0.1/1.0 (uM)			10.0/0.01 (uM)			1.0/0.1 (uM)			0.1/1.0 (uM)		
		Callus	Roots	Size	Callus	Roots	Size	Callus	Roots	Size	Callus	Roots	Size	Callus	Roots	Size	Callus	Roots	Size	Callus	Roots	Size
DAYS OF CULTURE:																						
10	Tube no.																					
	a	0	3	x2	++	4	x2	++	0	x2	0	2	x2	0	0	x1	++	0	x2	0	0	x2
	b	0	0	x2	+	0	x2	++	0	x2	0	0	x2	0	0	x1	++	0	x2	+	0	x2
	c	0	0	x2	0	0	x2	++	0	x2	0	0	x2	0	0	x1	++	0	x2	0	0	x4
	d	0	0	x2	0	0	x2	++	0	x2	++	0	x2	0	0	x2	0	0	x1	0	0	x1
	e	0	0	x2	0	0	x2	0	0	x2	++	0	x2	0	0	x2	0	0	x1	0	0	x1
15	a	0	3	x2	++	8	x3	++	0	x3	0	2	x3	0	0	x1	++	0	x2	0	0	x1
	b	0	0	x2	+	3	x2	++	1	x3	0	0	x3	0	0	x1	++	0	x2	+	0	x3
	c	0	0	x2	0	3	x3	-	-	D	0	0	x3	0	0	x2	-	-	D	0	0	x2
	d	-	-	D	0	0	x2	-	-	D	++	0	x2	-	-	D	0	0	x1	0	0	x2
	e	-	-	D	0	0	x2	-	-	D	-	-	D	-	-	D	0	0	x2	0	0	x4
20	a	0	3	x2	++	10	x3	+++	1	x3	0	2	x3	-	-	D	++	0	D	-	-	D
	b	0	0	x2	+	4	x3	++	0	D	0	0	x3	-	-	D	++	0	D	+	0	D
	c	0	0	x2	+	3	x2	-	-	D	0	0	x3	-	-	D	-	-	D	-	-	D
	d	-	-	D	-	-	D	-	-	D	++	0	x3	-	-	D	-	-	D	-	-	D
	e	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D
25	a	0	3	x2	+++	10	x4	+++	1	x4	0	2	x4	-	-	D	++	0	D	-	-	D
	b	0	0	x2	+	8	x4	++	0	D	-	-	D	-	-	D	++	0	D	+	0	D
	c	0	0	x2	+++	3	x6	-	-	D	+	0	x4	-	-	D	-	-	D	-	-	D
	d	-	-	D	-	-	D	-	-	D	++	0	x6	-	-	D	-	-	D	-	-	D
	e	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D	-	-	D

+ = 1-2mm diameter; ++ = 3-4mm diameter; +++ = 5mm diameter; D = Dead.



12

formation at earlier stages of culture. Root regeneration was however impeded. Shoot growth was also poor. At 0.1/1.0uM, 2,4-D/K induced only slight callus formation in a few explants and root regeneration was also impeded. Shoot growth was favoured at earlier stages of culture and this was better than at 1.0/0.1uM 2,4-D/K. There was uniform mortality of explants at various concentrations of 2,4-D/K combination as from the 20th day of culture. Death was preceeded by a curling of the shoot and subsequent browning of the tissues at the onset of senescence.

Controls (no growth hormones) showed poor callus and root formation throughout the culture period. Shoot growth was however slightly but uniformly encouraged (Table 16). Plantlets transplanted into pots filled with garden soil died after one week in the glasshouse.

CHAPTER FIVE

DISCUSSIONS

5.1 Life Cycle:

That the seed-propagated plants generally had a longer life span (7-9 weeks after transplanting (WAT)) than the stem-cutting propagated plants (6-7 WAT) could be due to juvenility effect. While seed-propagated plants were started as seedlings which had great growth potentials, stem-cuttings, were taken from older tissues (parts of parent plants about 6 weeks old) which had undergone a much higher degree of physiological differentiation. This could hve resulted in the earlier senescence of the stem cutting-propagated plants. Flower formation in the unfertilized seed-propagated plants was delayed by as much as 13 days. This was probably due to poor plant growth. This corroborates the findings of Lang (1952) that early flower initiation is associated with increase in dry weight, leaf area and shoot height. He postulated that the time of flower initiation depends on the proceeding vegetative growth rate. Similarly Klapwijk and de Lint (1972) found that in tomatoes, stages of reproductive development were closely correlated with rate of growth in the fresh weight of the plant. Olarewaju (1980) also confirmed these findings in sweet pepper in which he observed that the growth rate of the whole plant was an important factor in determining the time of flower initiation. The delay of flowering by up to 6 days observed in the plants treated in 600kgNPK/ha could be due to

the prolonging of the vegetative growth period caused by high nitrogen level. High levels of nitrogen in the soil have been shown to promote excess vegetative growth in tomatoes (Salisbury and Ross, 1969).

In stem-cutting propagated plants, however, flowering was delayed by a maximum of eight days in unmanured plants, while flowering occured concurrently in plants treated with 300 and 600kgNPK/ha. This was probably because in stem-cutting propagated plants, the stage of 'determination' had been achieved before propagation. Christianson and Warnick (1988) proposed that during organogenesis, the stage of determination was a stage at which tissues have been destined to form a particular organ, and organ formation would proceed even in the absence of the conditions that favour such an activity. Since plants treated with 300kgNPK/ha and 600kgNPK/ha had attained the minimum vegetative growth and physiological maturity necessary for flower initiation, this proceeded even in the presence of excess nitrogen in the 600kgNPK/ha treated stem-cutting propagated plants. Vegetatively propagated plants have been reported to come to bearing earlier than seed-propagated plants (Hartmann and Kester, 1975). These results, particularly for seed-propagated plants seem to be at variance with the works of Saito et al. (1963 (b)) in which high N levels induced earlier flowering and greater flower numbers in tomato. Wittwer and Teubner (1956) reported that high N promoted flowering and reduced the number of nodes before first cluster in tomatoes. However, Eguchi et al. (1958) found that the

mean number of days from seeding to floral bud formation varied from 28, 27, 31 and 33 days for plants treated with 4X (NPK); 2X (NPK); NPK and NP only respectively. In this respect they concluded that tomato was a 'nutritional type' as opposed to a photoperiodic plant in its flowering behaviour. Furthermore, the apparent discrepancy is eliminated when one considers that the results of some of these workers particularly Saito et al. (1963 (b)) were obtained under high light intensity, as opposed to low medium light intensity during the hazy harmattan period, under which the present experiments were carried out.

5.2 Vegetative growth characteristics:

The increase in vegetative growth with age observed in the present experiments agrees with the observations of Rhoads and Bloodworth (1964), Watson (1937); Zrust et al. (1974) in cotton and potato. In their works correlations were found between vegetative growth parameters like leaf area, leaf fresh or dry weight and plant age. Leaf area and dry matter were also highly correlated in soybean (Shilbes and Weber, 1965) and Cowpea (Rhoden and Croy, 1988).

That nitrogen treatments elicited more growth responses in terms of plant height, and dry matter in treated plants over-controls in both modes of propagation is a manifestation of the importance of nitrogen in the synthetic processes of ppants generally. Nitrogen is a key component of both structural and enzymatic proteins which regulate metabolism as well as make up parts of plant tissues. Nitrogen applications have similarly been found to increase leaf dry

weight and plant height over zero N levels in ginger (Musa, 1986) although differences between the recommended rate (100kgN/ha) and higher rate (200kgN/ha) were not significant.

That plant height for the IAA/NAA treated stem-cutting propagated plants was lower than other treatments, could have been a result of a delay in the establishment of these cuttings on transplanting due to their generally poorer rooting (in terms of number, length and speed of rooting) as compared to other auxin treatments. This is more clearly seen in the 300kgNPK and 600kgNPK-treated plants given IAA/NAA at 0.5g/l (Table 8; Plate 4). It is possible that materials derived from synthetic activities were channelled towards root growth rather than plant height increase.

That plant height in stem-cutting propagated plants had a higher rate of growth during the first 2 weeks (0.13 - 0.86cm/day) than in the subsequent two weeks (0.10 - 0.38cm/day) could be explained as follows: In those first two weeks, a lot of the metabolites and energy produced in the cuttings had to be diverted to the initiation/ regeneration of tissues. The decline in the rate of vegetative growth in subsequent samplings was however, probably due to the onset of the reproductive phase. Yunana (1982) found a similar trend in sweet peppers, where it was observed that during the first 87 days of growth, plant height increased at 0.4cm/day, but declined to 0.2cm/day for the subsequent 105 days.

That the rates of dry matter production for both modes of propagation declined with age for all fertilizer and auxin levels could also be attributed to ageing. However,

the rates of dry matter increase were generally higher for seed-propagated than stem-cutting propagated plants especially from 28-42 days. This is probably because at that age, while seed-propagated plants were just approaching a stage of physiological maturity, stem cutting propagated plants (which were parts of mature plants) had already achieved this state with a concomittant decrease in the rate of synthetic activities.

Phyllotaxy:

That plants with right-handed phyllotaxy and fertilizer treatments gave higher fruit yields than left-handed plants with no fertilizertreatments agrees with the work of Bernard (1976) in which it was found that total yields in terms of fruit weight and number was related to phyllotaxy. He also observed that in four of five tomato and two of three pepper cultivars, total yield was higher in right-handed plants than in the left-handed ones.

5.3 Tissue auxin activity, total soluble carbohydrate, % nitrogen and rooting of cuttings.

The progressive increase in the level of tissue auxin in the no fertilizer-level treated plants could explain the complete lack of axillary branching observed in these plants up to the time of cutting. Auxin is known to be the main growth factor used to impose apical dominance in plants. The fact that cuttings taken from these plants also rooted readily even in the absence of exogenous auxin (controls) supply notwithstanding poor growth conditions of the plant, also

support the high level of auxin activity detected in the cuttings from these plants. For 300kgNPK/ha and 600kgNPK/ha-treated plants however, the level of endogenous auxin activity fluctuated more or less regularly during the growth period. This can be explained in terms of auxin induced apical dominance.

The initial increase in plant height was probably related to the continual increase in auxin activity (at 3 weeks). Auxin levels declined leading to lateral branching of the plants as apical dominance was temporarily broken (at about the 4th week). Vertical growth was however, soon restored and apical dominance was re-established as a result of the higher auxin levels at the 5th week. The decline in auxin levels at the sixty week corresponded with copious axillary branching as the dominance of the apex was broken. It was these axillary branches that were taken as cuttings. Fluctuations in the level of endogenous auxin activity has been implicated in a similar manner in various investigations by Grunkel and Thimann (1949); Hemberg (1949); Zimmermann (1936) Avery et al. (1937) and reviewed by Leopold (1955). That tissue auxin activity was highest in plants treated with no fertilizer levels and lowest in 600kgNPK/ha-treatments while it was intermediate at 300kgNPK/ha, could mean that plants with no additional fertilizer had more free-auxin. Auxin in the plants at higher levelsof fertilizer could have been bound to protein complexes and as such were not available for reaction with the acid reagent. It is also noted that 600kgNPK/ha had less free auxin

than 300kgNPK/ha. It is also quite possible that the peaks of auxin activity that occurred in treated plants were points of highest free auxin level. This was probably soon bound to proteins due to the rapid rate of protein synthesis, thereby causing a decline. Moore (1979) presented evidence that auxin could be a bound to proteins and that the binding is an adsorptive type by weak hydrogen bonds. Furthermore, the regulation of 'free IAA' levels has been found to be by conjugation with amino-acids like asportic acid (Jacobs, 1972; Andraeae and Good, 1955).

The consistently higher level of soluble carbohydrate (TSC) in the stem of plants given no additional fertilizer than the level in plants which received fertilizer treatments throughout the period of the study was probably because most of the synthetic activity in these plants was directed towards the manufacture and accumulation of soluble carbohydrates to provide energy for tissue respiration (Salisbury and Ross, 1969). It may also indicate a high rate of starch hydrolysis for the same purpose, rather than nitrogenous compounds for protein synthesis at sites of organ formation. The level of N as well as CHO:N ratio in these plants with no fertilizer treatment also increased with age, but was lower than that of either 300kgNPK/ha or 600kgNPK/ha treatments.

Plants treated with 300kgNPK/ha had the next higher level of tissue TSC followed by 600kgNPK/ha treatments. The level of N in 300kgNPK/ha-treatment of plants was conversely lower than that of 600kgNPK/ha-treated plants, and this could be related to the amount of N available for absorption in the soil.

That for both 300 and 600kgNPK/ha treatments, the level of TSC was lowest at the 3rd week of growth, when the level of N was highest, could mean that during this period, most of the soluble carbohydrates were used up as a respiratory substrate to provide energy for the manufacture of nitrogenous compounds that were used to build up cellular materials and enzymes. For 600kgNPK/ha-treated plants a rise in TSC level at the 4th week was associated with a decline in N levels up to the 5th week. This could mean that, as the plants matured, less protein was synthesized, so the need for nitrogenous materials was reduced and since carbohydrates provide the carbon skeleton for protein synthesis (Goss, 1975), there was an accumulation of soluble carbohydrates. A sharp rise in nitrogenous material was noted at the 6th week for 300kgNPK/ha treatments and this could be due to resumed meristematic activity during protein synthesis in the floral primordia during flower bud formation. This was however, not associated with a proportionate rise in TSC.

For plants treated with 600kgNPK/ha, tissue nitrogen levels increased steadily with age all through the period of sampling. This could have led to the excessive vegetative growth noticed in these plants. It should be expected that the excessive leaf area produced by these plants should lead to a high rate of photosynthesis and thus a much higher level of carbohydrates; but these carbohydrates could have been rapidly incorporated into cell wall materials or rapidly converted to nitrogenous materials. Salisbury and Ross (1969) have observed that more organic acids are converted to amino-acids for protein synthesis at rapidly growing sites. These could

even have been rapidly broken down during tissue respiration due to high metabolic rate and this led to the low levels of TSC in tissues with a concomittant low CHO:N ratio throughout the growth period.

That cuttings without fertilizer treatment rooted at all could be due to the fact that in these plants, levels of endogenous auxin, TSC and CHO:N were highest at the time of cutting and the high CHO:low N requirement was fulfilled, thus favouring rooting. However, the roots in these cuttings were thinner and shorter and this could have been a result of N deficiency the untreated plants as observed in Plates 1, 2, 3, 4. For 300 and 600kgNPK/ha-treated cuttings, native auxin activity was lowest at the time of cutting but rooting was good. This could have been due to the relative level of TSC in the cuttings which were highest at this point. In addition, the low levels of native auxin activity could have been augmented by exogenous applications of auxin. This is clearly seen in the cases of cuttings treated with IAA at 0.05g/l (300kgNPK/ and 600kgNPK/ha) IAA/NAA 0.1g/l (300kgNPK/ha) and NAA 0.1g/l (300kgNPK/ha) (Table 8).

For most of the cuttings from 600kgNPK/ha however,external auxin did not have such pronounced effects and this could be because these cuttings had the lowest auxin levels of all cuttings at this time and even exogenous auxin could not get the tissue concentration of auxin to a favourable level for root initiation. It was however, also seen that the mean number and length of roots were generally higher in cuttings from 300 and 600kgNPK/ha treated plants and this could have been due to the amount of

nitrogen available for synthetic processes. That the rate of root growth was lower at 600kgNPK/ha than 300kgNPK/ha (Table 5) could indicate that high NPK levels particularly its N content are not favourable for root development even though it is required for root formation. The implication of carbohydrate and nitrogen levels as well as the ratio of these to one another as well as to auxins have been found to be true for many plant species as had been found in the present experiment. Workers that have reported similar findings include Reuveni and Adato (1974); Howard (1965); Gautheret (1969); Oliéman et al. (1971); Heuserand Hess (1972); Reuveni and Raviv (1981); Odegbaro and Adedipe (1986); Davis and Porter (1981); Cheffins and Howard (1982 a & b) where the importance of carbohydrate reserves to rooting have been emphasized. Low proportion of nitrogen in relation to carbohydrate stockplants recorded in this study is also favourable to rooting (Lowe and Ries, 1972); Hartmann and Kester (1975). Extreme nitrogen deficiency, however, decreases rather than increase rooting (Pearse, in Hartmann and Kester, 1975). It is not clear why high levels of nitrogen are detrimental to good rooting, however, for root initiation to take place, it is necessary to supply N for nucleic acid and protein syntheses.

It appears that auxins stimulate the formation of roots by an interaction involving organic materials in the plants, particularly carbohydrates and nitrogenous materials. This interaction apparently controls the basic step of morphological differentiation at the cellular level. This contention corraborates the 'rhizocaline' concept of Bouillene and Bouillene-Walrand in Hartmann and Kester (1975).

5.4 Rooting responses to exogenous auxin:

That IAA generally gave earliest and most uniform rooting response and also highest number and longest roots for all nitrogen levels and auxin types suggests that IAA was better at stimulation of rooting than NAA or a combination of both even though IAA is said to be a weak auxin (Leopold, 1955).

In selecting auxins for rooting it seems that in general, the strong auxins (such as NAA) are undesirable because they inhibit bud and root development. Weak auxins are generally selected (Leopold, 1955). The earlier rooting response for IAA could have also been due to the fact that this auxin is a naturally occurring one as such it more readily increased the tissue auxin level to threshold level, where rooting was stimulated. Rooting response to auxin is known to be a quantitative phenomenon (Leopold, 1955). Better rooting responses to IAA than other auxins have also been reported by Torre et al. (1980) in 'red-raspberry' and Negi (1982) in Dalbergia latifolia cuttings; Khan and Khan (1982) in apple cuttings.

Within IAA concentrations, that 0.05g/l gave the earliest as well as the highest mean number and length of roots followed by 0.1g/l and 0.025g/l IAA, could mean that 0.05g/l was the optimum level required for root initiation and growth . While at 0.1g/l this auxin could have been inhibitory, it was sub-optimal at 0.025g/l. It is known that the active range of IAA is narrow (Torre et al., 1980).

That the combination of IAA/NAA gave a better rooting response at a lower (0.025g/l) concentration than either of IAA or NAA alone, suggests that a combination of these two hormones stimulates the formation of foot primordia much more than either of the two alone and this occurs at a lower concentration. However, at higher concentrations (0.05g/l) IAA/NAA was detrimental to number and length of roots as well as the speed of rooting. Similar reports of more roots and higher percentage of rooted cuttings have been given by Malstede and Haber (In: Hartmann and Kester 1975) on Thuja sp. and Celastrus sp. However, IBA up to 4000ppm and IBA/NAA at up to 2000ppm have also been found to give similar results in the rooting of Popolus tremula (Al-Khamsy, 1981).

That NAA at 0.1g/l gave the earliest rooting response as well as the highest number of roots than other concentrations, but the lowest length of roots than other concentration emphasizes the fact that the strong auxins (such as NAA) may stimulate the formation of root primordia at relatively high concentrations, but inhibit the later growth and development of roots (Leopold, 1955).

Rooting percentages (no. of rooted cuttings per treatment) were generally high (80-100%) for all auxin and nitrogen treatment. This could be attributed to the ease with which tomato cuttings are known to root (Cobley and Steele, 1976). That rooting percentages between nitrogen levels were lowest for zero NPK levels (90%) and highest for 600kgNPK/ha treated cuttings (96%) could be due to the fact that nitrogenous compounds are not only essential during the initiation of root

primordia, but are also required as a substrate for actual root growth. Cuttings treated with 300kgNPK/ha had an intermediate (92%) percentage rooting.

Survival percentages of rooted cuttings at the time of last fruit harvest were best for plants treated with IAA (Table 12). These were followed by NAA and IAA/NAA treatments respectively. This could be linked to the rooting responses of these cuttings. It was seen that IAA/NAA treatments generally had the poorest rooting response in terms of number and lengths. With regards to fertilizer levels, zero levels of fertilizer treated plants survived longer than either of 300 or 600kgNPK/ha treatments followed by 300kgNPK/ha then 600kgNPK/ha treated plants where plants reached the end of the observation period. This could be an effect of the 'physiological exhaustion' of the plant tissues due to the pattern of growth experienced in each case.

5.5 ORIGIN AND DEVELOPMENT OF ADVENTITIOUS ROOTS

Adventitious roots were seen to originate from a group of cells located in the cortex (Plates 8 and 9). This agrees with the findings of Priestly and Swingle (1929) that in herbaceous plants, adventitious roots originate outside the vascular bundles. Other workers have however found that the type of tissues involved at the site of origin depends on plant species and these range from the vascular rays, the cambium or the pith (Shella and Roberts, 1975; El-Toni and Galal, 1980).

The group of cells that form root initials (dormant initials) apparently resume meristimmatic activity which lead to the extension in two directions towards the central core of the vascular cylinder as well as outwards towards the epidermis. This pattern of development agrees with the description given by Esau (1965). Upon emergence from the stem, the adventitious roots have developed a root cap and the usual root tissues. A vascular connection is seen seen to develop in the fully emerged root and a functional root is obtained.

No differences were observed between auxins and this shows that auxin type does not affect the formation and/or activation of root initials in tomato stem cuttings. However, low fertilizer levels (zero levels) seemed to reduce the number of such root initial cells that appear in the cortex. This could be related to the involvement of nitrogen in protein synthesis during mitotic activities that take place in these initial cells during the activation process. Theologis and Ray (1982), Hagen et al. (1984) have earlier observed that organogenesis involves gene action and specific m-RNAs have been associated with both root and shoot regeneration. In addition, certain enzymatic proteins play specific roles in root regeneration and very low nitrogen levels may not favour the formation of these enzymes (Hartmann and Koster, 1975).

5.6 YIELD AND YIELD COMPONENTS

The observation that both number and weight of fruits produced at first harvest were higher for seed-propagated than stem-cutting propagated plants, could be a result of the physiological stress that the stem-cutting propagated plants

were subjected to. While the seed-propagated plants were allowed to grow uninterrupted from germination to fruiting, stem-cutting propagated plants were forced to extend their life-span by cutting. These cuttings which were the same age as their parent plants had to divert energy to processes leading to regeneration of parts and initiation of roots which could have resulted in their low productivity. This is similar to the findings of Salac and Fitzgerald (1983) in Liatris pycnostachya in which rooted stem-cuttings produced flowering stems. Spikes from such plants however, were very small compared to seed-propagated plants. Also their stems were weak and lodged. However, in several species, the vegetatively propagated plants have been known to have good attributes. These include Rhododendron (Ettinger and Preece, 1985). Chickpea (Bassiri et al., 1985) and Potato (Manandhar and Raybhadary, 1986).

That seed-propagated plants treated with 600kgNPK/ha had more fruits per plant at first harvest than either of '0' level and 300kgNPK/ha treated plants could be as a result of the higher number of branches that were available for the formation of flowers on the 600kgNPK/ha treatments than in either of '0' level and 300kgNPK/ha treatments. This is similar to the findings of Zanin and Kimoto (1980), Shresta (1983) in tomatoes, Majanbu et al. (1985) in okra, where N-fertilization increased plant height, number of branches per plant and number of fruits per unit area and per plant. Also in other species such as red peppers (Khan et al., 1982); Onions (Subbiah et al., 1982); Spinach (Kansal et al., 1981); eggplant (Rajveen and Rao, 1980), and garlic (Escaff and Al Daro, 1982). However fruit weight was significantly highest in the 300kgNPK/ha

treatments than both of the other treatments and this could be because these plants had less fruits to which to partition assimilates. While for [illegible]kgNPK/ha treatments assimilates where shared among a greater number of fruits, in the zero level treatments even though they had less fruits, there was not enough assimilate production. In a similar study, Weston and Zandstra (1989) found that high N levels produced larger early yields, but total fruit yields were higher for moderate than high rates of N in tomatoes.

The mean fruit weight at four subsequent harvests decreased with number of harvest for both seed-propagated and stem-cutting propagated plants. This decline could be associated with the onset of senescence, which could have been associated with a reduction in the level of plants hormones like auxins and gibberellic acid that are involved in fruit growth and development (Salisbury and Ross, 1969). Senescence could have also resulted in the reduced uptake of mineral elements which are important in plant growth from the soil. Downes and Miller (1966) made a similar observation in tomatoes where average fruit size decreased with harvest date.

Mean fruit weights recorded for seed-propagated plants treated with 300kgNPK/ha(recommended rate) of 43.91 ± 2.09g/fr. weight agree with the findings of Quinn (1971) who recorded a mean fruit weight of 42.53g/fruit (± 1.50g) for the same variety ('Roma VF') of tomatoes in Samaru during the dry season.

5.7 POST-HARVEST PHYSIOLOGY

The observation that fruits from stem-cutting propagated plants did not achieve the full red colouration observed in fruits from seed-propagated plants, but remained at the orange/

yellow stage throughout the period of observation (12 days) could have resulted from the fact that plants that produced these fruits suffered the physiological shock of having had to regenerate vegetative parts when they should have been flowering. This is also evidenced by their much smaller sizes. Furthermore, the fruits probably had not achieved the necessary level of physiological maturity (Mature green stage) before the onset of ripening. Red pigmentation is dependent on stage of maturity of fruit at harvest (Tigehler and McGlasson, 1978). The manifestation of other fruit ripening characteristics, such as respiration levels and ethylene gas evolution also depend on the level of maturity of fruits at harvest (Kanellis et al., 1986). Ripening of fruits indicate the termination of fruit growth and the onset of physiological qualitative changes which include marked increases in the amount of carotenoids in the fruit (Ulrich, 1958). Lycopene is the predominant carotenoid in old fruits and this increases by as much as ten-fold in tomatoes from the ninth to twelfth day of ripening at 30°C (as observed in this study). Phytofluene may increase by up to two-fold. Pigment changes may be due mainly to modifications in carotene content rather than to modifications in the amount of carotenoids or chlorophyll (Ulrich, 1958).

The low seed weight recorded for stem-cutting propagated plants is also a function of the fruit size. This agrees with the work of Varis and George (1985) in which the seed weight of tomatoes decreased with fruit size.

The low percentage germination (23.33%) of seeds from stem-cutting propagated plants as opposed to high percentage germination (>86%) recorded for seeds from seed-propagated plants could also be related to the fruit size. Vadivelu (1983) reported similar trends in tomatoes, in which seeds from bigger fruits germinated better than seeds from the smaller fruits. Both the low percentage germination and irregular pattern of germination could be due to the fact that the physical and physiological levels of maturity required for good germination had not been achieved prior to extraction from the fruit. The growth habit of the parent plants (shorter life-span) did not allow for adequate formation and accumulation of food reserves in the seed that would have provided energy for respiration during germination. Furthermore, the formation of structural as well as enzymatic proteins that participate in germination processes could have been disturbed. Fruit maturation and ripening is known to be associated with seed maturation in tomatoes (Khudairi, 1972). Many workers have found that until the attainment of full maturity, full germination capacity and viability are not realized. In carrots, Austin and Longden (1967(b)) found that for four progressive harvest dates, percentage germination and field emergence increased with seed weight. Studies with fleshy fruits have also shown that the harvesting and extraction of seed before full maturity is detrimental to viability. For example in Capsicum frutescens ('Pimiento pepper') seeds extracted from fully ripe fruits had 92% emergence while those extracted before full maturity had only 5.6% emergence.

Seed viability after harvest is also subject to a variety of factors to which the parent plant is exposed during seed formation and ripening (Meyer and Poljakoff-Mayber, 1975). As was seen, plants propagated from stem cuttings were subjected to physiological stress.

5.8 SHOOT-TIP CULTURE

The observation that shoot-tips cultured on media with continuously increasing concentrations of auxins IBA, 2,4-D and kinetin did not elicit either root regeneration or shoot growth could have been because these hormones were not present in the appropriate ratios to one another. This also indicates that absolute concentrations of these hormones alone in culture media may not be favourable to organ formation. While in certain tissues, it is the absolute concentration of auxin to kinetin that is important, in others, it is the ratio of these hormones in the nutrient media that are decisive for organogenesis. These two substances in the appropriate ratios determine not only whether the tissue grows but also how it grows (Street, 1977).

A reversal of concentrations of both auxins and kinetin however, induced organogenesis. Shoot growth, root regeneration and callus formation were all observed. That IBA/K at 10.0uM/ 0.01uM gave the best root regeneration response and shoot growth agrees with Murashige *et al.* (1972) and Murashige (1977) that high auxin:low kinetin levels favour root initiation. The good shoot growth obtained with this combination could have resulted from the adequate absorption of nutrient

materials from the medium as a result of good root formation. It has also been observed in Asparagus officinalis that NAA concentrations of 0.3mg/l and kinetin 0.1mg/l encouraged good rooting without inhibiting spear formation within 6 weeks of culture (Murashige et al., 1972; Yang and Clore, 1971). Furthermore, IAA (5.7uM/l) and kinetin (4.7uM/l) have been found to be highly efficient in shoot regeneration of tomato and reduced the time required for shoot production (Uddin et al., 1988).

At 1.0/0.1uM IBA/K, callus formation was most prominent, while root regeneration and shoot growth were poor and this could also have been as a result of the ratio auxin to kinetin. This combination and concentration was the intermediate concentration of all used. Murashige (1977) has also reported that intermediate levels of auxin to kinetin favour callus formation most. However, the low degree of differentiation observed (root formation and shoot growth) with these combinations and concentration could have been as a result of absolute concentration of these hormones in the culture media. Similar observations have been made in garlic, where IAA and kinetin at 1mg/l did not permit extensive callus growth but shifted growth towards differentiation (Kehr and Shaeffer, 1976). Similarly, best sprout formation from tomato leaves has been obtained when concentrations of both auxin and kinetin were high (4mg/l) in the medium (1:1) (Santana, 1985). That at 0.1/1.0uM IBA/K, both root regeneration and callus formation were poor while shoot growth was favoured agrees

with the findings of Murrashige (1977); Asokan et al. (1984) and Liu and Sanford (1988). For instance, Liu and Sanford (1988) reported that optimal shoot regeneration in blue berry was found to occur in media containing 2.5mg/l, 0.5mg/l, BA/IBA; 8.0mg/0.1mg/l; BA/IBA for one variety and 5.0mg/l BA/0.5mg/l IBA for another.

Organogenesis was generally poor in cultures that contained 2,4-D especially at higher concentrations (10.0uM). This was probably due to the phytotoxic effects of this auxin. 2,4-D is commonly employed as a potent herbicide particularly for broad-leaved plant species (Akobundu, 1987), hence the occurrence of slight callus formation observed at earlier stages of culture, at lower concentration of 1.0/0.1uM 2,4-D/K, could not be sustained for longer periods. A similar observation was made for 2,4-D at 0.1uM and kinetin at 1.0uM, where shoot growth was slightly favoured. That death of explants was preceeded by a curling of leaves before senescence is characteristic of the mode of phytotoxicity of phenoxy acetic acid herbicides on leaves of susceptible plants (Akobundu, 1987).

The observation that IBA generally performed better as an auxin for organogenesis than 2,4-D emphasized the concept that auxin types differ in their organogenetic effects of plant tissues in culture. Nagasawa and Finer (1988) had earlier observed that in garlic, the morphogenic ability of five auxins differed. In order of decreasing effectiveness, this was 2,4-D > 2,4,5-T > 'dicamba' > 'Picloram', while NAA did not induce morphogenic callus formation over a wide range of concentrations.

CONCLUSION

This investigation revealed that tomato plants can be propagated by rooting stem cuttings. Stem cuttings generally root very easily regardless of nitrogen nutrition of stock plant or auxin treatment of cuttings. Nitrogen however, was found to affect the number and length of roots. Medium N (300kgN/ha) gave the best response for these parameters. IAA (0.05g/l) was found to be most suitable for rooting than NAA or a combination of the two auxins.

Relatively high levels of tissue auxin activity were detected in tomato stems. This however, fluctuated in those plants given N treatments, but increased steadily for non-additional N treatments probably due to auxin binding by nitrogenous compounds. High carbohydrate:nitrogen ratios were also observed in tomato stems and this combined with the native auxin activity could be responsible for the ready rooting of tomato stem cuttings. Ready rooting may however, also be linked to the presence of preformed root initials, which undergo the various stages of development on appropriate stimulation.

Tomato plants can be grown to maturity (fruiting) by stem cuttings. Such plants, however, have a short life span (6-7 weeks) and a high vegetative growth rate. This leads to an early senescence with a concomittant reduced yield both in terms of fruit number and weight.

Ripening of fruits is also retarded due probably to arrested development of the fruit as a result of early senescence. Seed yield and germination are also poor from such plants.

Tomato plants can also be propagated by shoot-tip culture. Best medium for root regeneration was found to be MS basal, medium + 10.0uM IBA + 0.01uM kinetin. Shoot growth was also good at 0.1uM IBA + 1.0uM K.

2,4-D was seen to be unsuitable for tomato shoot-tip culture due to its toxicity to this plant.

REFERENCES

Agricultural Extension and Research Liaison Services (AERLS) Extension Guide, No. 1, 1985. Ahmadu Bello University, Zaria.

Agbede, O.O. and E.A. Aduayi (1985). Phosphorus-Magnesium interaction in tomato nutrition in 3 soil types of southern Nigeria. Niger. Jour. Agric. 19/20, pp. 102-113.

Akobundu, I.O. (1987). Weed Science in the tropics: Principles and Practices. IITA, Ibadan, Nigeria. John Wiley and Sons Ltd., pp. 110-126.

Al Barazi, Z. and W.W. Schwabe (1984). The possible involvement of polyphenol-oxidase and the auxin-oxidase system in root formation and development in cuttings of Pistachia vera. Jour. Hort. Sci. 59(3): 453-461.

Al-Kinamy, A. (1981). Effects of auxins on root formation in the vegetative propagation of Populus alba, Populus tremula. Picea abies and Juniperus communis. Indian Forester, 107(9): 537-550.

Andreae, W.A. and N.E. Good (1955). The formation of indole acetylaspartic acid in pea seedlings. Plant Physiol. 30: 380-382.

Asi, Abdur-Rahim and M. Amjad (1985). Response of various tomato cultivars to different levels of fertilizers. Jour. Agric. Res. 23(2): 93-96.

Asif, M.T. and J.R. Greig (1972). Effects of N, P and K fertilization on fruit yield, macro and micro-nutrient levels and nitrate accumulation in Okra (Abelmoschus esculentus (L.) Monech). J. Amer. Soc. Hort. Sci. 97(4): 440-442.

Asokan, M.P., S.K. O'Hair and R.E. Litz (1984). In vitro plant regeneration from leaf discs of 'Hausa potato' (Coleus parviflorus). Hortscience 19(1): 75-76.

Austin, R.B. and P.C. Longden (1967b): Some effects of seed size and maturity on the yield of carrot crops. J. Hort. Sci. 42: 335-353.

Bansal, M.P. and K.K. Nanda (1981). IAA-oxidase activity in relation to adventitious root formation on stem cuttings of some forest tree species. Experientia. 37(12): 1273.

Bar Yosef, B. and Sagiv, B. (1982). Response of tomato to N and water applied via a trickle irrigation system 1:N. Agronomy Journal 74(4): 633-637.

Bassiri, A., A.E. Shinkard and F. Ahmad (1985). Rooting of stem cuttings in Cicer sp. International Chickpea newsletter 13: pp. 10-11.

Bassuk, N.L., Hunter, L.D. and B.H. Howard (1981). The apparent involvement of polyphenol oxidase and phloridzin in the production of apple rooting co-factors. Jour. Hort. Sci. 56(4): 313-322.

Basu, R.N., B.N. Roy and T.K. Bose (1970). Interaction of abscissic acid and auxin in rooting of cuttings. Plant and cell physiol. 11: 681-684.

Bernard, B.B. (1976). Non-equivalence of left-hand and right-handed phyllotary in tomato and peppers. Hort. Sci. 11: 601-604.

Bhella, H.S. and A.N. Roberts (1975). Seasonal changes in origin and rate of development of root initials in Douglas fir stem cuttings. J. Amer. Soc. Hort. Sci. 100(6): 643-646.

Bose, F.K., B.N. Roy and R.N. Basu (1972). Synergism between auxins and Phenolic compounds in rooting of cuttings. Indian Agric. 16: 171-176.

Blocksberg, L.N. and M.E. Saltveit Jr. (1986). Regeneration of plants from axillary buds of harvested and stored heads of field-grown 'Iceberg' lettuce. Hortscience 21(5): 1201-1203.

Breen, P.J. and T. Muroaka (1973). Effect of indolebutyric acid on distribution of ^{14}C photosynthate in softwood cuttings of Marianna 2624 Plum. Jour. Amer. Soc. Hort. Sci. 98(5): 436-439.

Bryant, S.D. and F.E. Lane (1979). Indole-acetic acid oxidase from peas. I. Occurrence and distribution of peroxidative and non-peroxidative forms. Plant Physiol. 63: 696-699.

Cheffins, N.J. and B.H. Howard (1982a). Carbohydrate changes in leafless winter apple cutting. I. The influence of level and duration of bottom heat. Jour. Hort. Sci. 57(1): 1-8.

_____ (1982b). Carbohydrate changes in leafless winter apple cuttings. II. Effects of ambient air temperature during rooting. Jour. Hort. Sci. 57(1): 9-

Cheng, T. (1977). Factors affecting adventitious bud formation of cytoledon cultures of Douglas fir. Pl. Sci. Lett. 9: 179-187.

Christianson, M.L. and D.A. Warmick (1988). Organogenesis in vitro as a developmental process. Hortscience. 23(3): 515-519.

Cobbina, J. and M.B. Miller (1987). Purpling in maize hybrids as influenced by temperature and soil phosphorus. Agron. Jour. 79: 576-582.

Cobley, L.S. and W.M. Steele (1976). An introduction to the Botany of tropical crops. English Language Book Society and Longman 1979 (2nd ed.).

Cochran, H.L. (1943). Effect of stage of fruit maturity at time of harvest and method of drying on the germination of pimiento seed. Proc. Am. Soc. Hort. Sci. 43: 229-234.

Cooper, D.C. (1927). Anatomy and development of tomato flower. Bot. Gaz. 83: 399.

Cooper, A.J. (1962). Relations between day length and annual patterns of plant development. Nature, 195: 1218.

Crow, W.D., W. Nicholls and M. Sterns (1971). Root inhibitors in Eucalyptus grandis: naturally occurring derivatives of the 2,3-dioxabicyclo (4,4,0) decane system. Tetrahedron Letters 18. London: Pergamon Press. pp. 1353-1356.

Csizinszky, A.A. and D.J. Schuster (1982). Yield response of staked, fresh-market tomatoes to reduced use of fertilizers and Insecticides. J. Amer. Soc. Hort. Sci. 107(4): 648-652.

Davis, T.D. and J.R. Potter (1981). Current photosynthate as a limiting factor in adventitious root formation on Pea cuttings. J. Amer. Soc. Hort. Sci. 106(3): 278-282.

DeKoning, A. and R.G. Hurd (1983). A comparison of winter-sown tomato plants grown with restricted and unlimited water supply. Jour. Hort. Sci. 58(4): 575-581.

Dinar, M. and M.A. Stevens (1981). The relationship between starch accumulation and soluble solid content of tomato fruits. J. Amer. Soc. Hort. Sci. 106(4): 415-418.

Doss, E.B., J.L. Turner and C.E. Evans (1981). Influence of tillage, nitrogen and Rye cover crop on growth and yield of tomatoes. J. Amer. Soc. Hort. Sci. 106(1): 95-97.

Downes, J.D. and K.J. Miller (1966). Nitrogen and spacing of tomatoes in relation to earliness, yields market quality and petiole concentration of NPK Proc. Int. Hort. Cong. 319-321.

Duranvilla, N. and J.S. Semancik (1986). Shoot-tip culture and eradication of viroid-RNA, Scientia Horticulturae 29(3): 199-203.

Eguchi, T., T. Matsumura and M. Ashizawa (1958). The effect of nutrition on flower formation in vegetable crops. Proc. Amer. Soc. Hort. Sci. 72: 343.

El-Kholei, M.S., F.M. Salem and Elsaid Omran, M. (1982). The combined effect of salinity and Melodogyne javanica infection on dry matter and nutrient content in tomato plants cutivated in different soil types. Res. Bull. Faculty of Agric. Aim Shams Univ. Egypt. No. 1755, p. 16.

El-Toni, A.L. and M.A. Galal (1980). Origin and development of adventitious roots in semi-hardwood cuttingsof sweet lemon. Egyptian Jour. Hort. 7(1): 79-80.

Engelke, A.L., H.Q. Hamzi and F. Skoog (1973). Cytokinin, Gibberellin regulation of shoot development and leaf form in tobacco plantlets. Am. J. Bot. 60: 491-495.

Erinle, I.D. (1979a). Tomato disease in the Northern State of Nigeria. Ext. Bull. No. 31, AERLS, Zaria. 3-19.

Erinle, I.D. (1979b). Evaluation of tomato cultivars for resistance to foliar diseases in Northern Nigeria. Paper presented at the 9th annual conference of the Nigerian Society for Plant Protection, Ibadan.

Ernst, A.A. and L.C. Holtzhaussen (1982). Rooting of avocado (Persea americane) cuttings. Hort. Abst. 52(2): 1153.

Esau, K. (1965). Plant Anatomy. John Wiley and Sons Inc. (2nd ed.) 1965, pp. 513-514.

Escaff, G.M. and U.A. Al-Daro (1982). Two experiments on the effects of N and P on Garlic. Agricultura Technice 42(2): 143-147.

Ettinger, T.L. and J.E. Breece (1985). Aseptic micro-propagation of Rhododendron 'P.J.M.' hybrids. Jour. Hort. Sci. 60(2): 269-274.

Evans, L.T. (1969). The induction of flowering. Some case histories. Evans, L.T. (ed). MacMillan of Australia. 1969.

Food and Agricultural Organisation (F.A.O) (1975). Food Bulletin. Rome.

Farouk, A.U., E.A. Arafa and H.H. Shaheem (1979). Recovery percentage of N supplied to cucumber. Agric. Res. Rev. 57(3): 193-201.

Ferree, D.C. (1976). Effect of roots tocks, propagation method and transplanting on growth and flowering of young apple trees. J. Amer. Soc. Hort. Sci. 101(6): 676-678.

Gabal, M.R. (1982). Effect of urea nutrition on seedlings plant growth and flowering time and yield of sweet pepper (Capsicum annum L.). Acta Agronomica (Hungary) 31(3-4): 378-385.

Gautheret, R.J. (1969). Investigations on the root formation in the tissues of Helianthus tuberosus culture in vitro. Amer. Jour. Bot. 56(7): 702-717.

Geneve, R.L. and C.W. Heuser (1982). The effect of IAA, IBA, NAA and 2,4-D on root promotion and ethylene evolution in Vigna radiata cuttings. J. Amer. Soc. Hort. Sci. 107(2): 202-205.

Gomez-lepe, B.E. and A. Ulrich (1974). Influence of NO_3 on tomato growth. J. Amer. Soc. Hort. Sci. 99(1): 45-49.

Gorst, J.R., R.A. Fossard and M. Slaytor (1982). The influence of auxins and minerals on root morphogenesis of Eucalyptus ficifolia F. Muell in vitro. Combined proceedings. International plant propagation society. 1981. (Publ. 1982). 81: 286-295. New England Univ. Armidale New South Wales, Australia.

Goss, J.A. (1973). Physiology of plants and their cells. Pergamon Press Inc. New York, Toronto Oxford. Sydney.

Greenwood, M.S. and G.P. Berlyn (1973). Sucrose-indole-acetic acid interactions on root regeneration by Pinus lambertiana embryo cuttings. Amer. Jour. Bot. 60(1): 42-47.

Hagen, G.A. Kleinschmidt and T. Guifoyle (1984). Auxin regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections. Planta: 162: 147-153.

Haissig, B.E. (1970). Influence of IAA on adventitious root primordia of Brittle willow. Planta 95: 27-35.

Handley, L.W., D.M. Pharr and R.F. McFeeters (1983). Carbohydrates changes during maturation of cucumber fruit. Plant Physiol. 72(2): 498-502.

Hartmann, H.T. and D.E. Kester (1975). Plant propagation: Principles and Practices. (3rd ed.). Prentice-Hall Inc. N. Jersey.

Hatcher, E.S. (1940). Studies on the inheritance of Physiological characters. V. Hybrid vigour in tomato. Pt. III. A critical examination of the relation of embryo development to the manifestation of hybrid vigour. Ann. Bot. 4: 735-764.

Hess, C.E. (1962). Characterization of the rooting co-factors extracted from Hedera helix and Hibiscus rosasinensis. Proc. 16th Intern. Hort. Cong. p. 382-388.

Heuser, C.W. and E.E. Hess (1972). Endogenous regulators of root initiation in Mung bean hypocotyl. J. Amer. Soc. Hort. Sci. 97(3): 392-396.

Hewitt, J.D., M. Dinar and M.A. Stevens (1982). Sink strength of fruits of two tomato genotypes differing in total fruit solids content. J. Amer. Soc. Hort. Sci. 107(5): 898-900.

Howard, B.H. and J.Y. Sykes (1966). Regeneration of the hop plant (Humulus lupulus L.) from softwood cuttings. II. Modification of the carbohydrate resources within the cutting. Jour. Hort. Sci. 41: 155-163.

Hoyle, M.C. (1972). Indole-acetic-acid oxidase: a dual catalytic enzyme? Plant Physiol. 50: 15-18.

Jacobs, W.P. (1972). The movement of plant hormones: auxins, gibberellins and cytokinins. In: Plant growth substances. 1970. D.J. Carr (ed). pp. 701-709. New York, Springer-Verlag

Jain, M.K. and K.K. Nanda (1972). Effect of temperature and some antimetabolites on the interaction effects of auxin and nutrition in rooting etiolated stem segments of Salix tetrasperma. Physiol. Plant. 27: 169-172.

Jona, R. and I. Gribaudo (1987). Adventitious bud formation from leaf explants of Ficus lyrata. Hortscience 22(4): 651-653.

Kansal, B.D., Singh, B. and K.L. Bajaj (1981). Effect of different levels of N and farmyard manure on yield and quality of spinach. Qualitas Plantarum. 31(2): 163-170.

Kanellis, A.K., L.L. Morris and M.E. Saltveit Jr. (1986). Effects of stage of development on post-harvest behaviour of cucumber. Hortscience 21(5): 1165-1167.

Karikari, S.K. (1988). Vegetable seed production. Unpublished paper at the Department of Agronomy, Ahmadu Bello University, Zaria.

Kartha, K.K., S. Champeux, O.L. Gamborg and K. Pahl (1977). In vitro propagation of tomato by shoot apical meristem culture. J. Amer. Soc. Hort. Sci. 102(3): 346-349.

Kay Ryugo and P.J. Breen (1974). Indoleacetic acid metabolism in cuttings of Plum (Prunus cerasifera X. P. munsoniana cv. Mariana 2624). J. Amer. Soc. Hort. Sci. 99(9): 247-251.

Kefford, N.P. (1973). Effect of a hormone antagonist on the rooting of shoot cuttings. Plant Physiol. 51: 214-216.

Kehr, A.E. and C.W. Schaeffer (1976). Tissue culture and differentiation of Garlic (Allium sativum (L.)) Hortscience 11(4): 422-423.

Kelly, R.L. and B.C. Moser (1983). Influence of temperature and auxin on root regeneration by seedlings of Liriodendron tulipfera (L.). Hortscience 18(6): 891-892.

Kerr, E.A. and F.I. Cook (1983; 1984). Germplasm and cultivar releases. Can. Jour. Plant. Sci. 63: 769-770. 1103-1105; 1107-1109; 64: 25-30.

Kerr, E.A. (1963). Germination of tomato seed as affected by fermentation time, variety, fruit maturity, plant maturity and harvest date. Repp Hort. Exp. Stn. Prod. Lab. Vineland. 1962, pp. 79-85.

Khan, M.A. (1982). Rapid vegetative propagation of Ficus sp. J. Agric. Res. 20(3): 141-144.

Khan, S.F.A.F. and J. Khan (1982). Effects of hormones on root initiation of Apple cuttings cv. 'Red delicious'. J. Agric. Res. 20(2): 99-102.

Khan, S., H. Inayatullah and M. Ishtiaq (1982). Effect of nitrogen alone and in combination with P and K on yield of red peppers. Pakistan Jour. of Agric. Res. 3(1): 21-25.

Khoon, C.B. and S.S. Talib (1985). Effects of NAA and two Phenolic compounds on rooting of pepper shoots cultured in vitro. NARDI Res. Bull. 13(1): 108-110.

Khudairi, A.K. (1972). The ripening of tomatoes. Amer. Sci. 60(696-707).

Klapwijk, D. and P.J.A.L. de Lint (1972). Freshweight and flowering of tomato plants as influenced by container type and watering conditions. Acta Hort. 39: 237-247.

Kling, G.J. and M.M. Meyer Jr. (1983). Effects of Phenolic compounds and IAA on adventitious root initiation in cuttings of Phaseolus aureus. Acer, saccharinum and Acer griseum. Hortscience 18(3): 352-354.

Lang, A. (1952). Physiology of flowering. Ann. Rev. Plant Physiol. 3: 305-306.

Lee, L.W., K.C. Sanderson and J.G. Williams (1983). Effect of fungicides applied to polyurethane propagation block on rooting of poinsettia cuttings. Hortscience 18(3): 359-360.

Leopold, A.C. (1955). Auxins and Plant growth. Univ. of Calif. Press. Los Angeles.

Li, J. and G.W. Eaton (1984). Growth and rooting of Grape shoot apices in vitro. Hortscience. 19(1): 64-66.

Litz, R.E. and R.A. Conover (1978). In vitro propagation of sweet potato. Hortscience. 13(6): 659-660.

Liu, Z.R. and J.C. Sanford (1988). Plant regeneration by organogenesis from strawberry leaf and runner tissue. Hortscience. 23(6): 1057-1059.

Lorenz, O.A., B.L. Weir and J.C. Bishop (1970). Effect of controlled-release N-fertilizers on yield and N-application on potatoes, cantaloupes and tomatoes. Jour. Amer. Soc. Hort. Sci. 97(3): 334-337.

Lowe, L.B. and S.K. Ries (1972). Growth of vegetatively propagated tomato plants in relation to crude protein content of the stock plant. Hortscience 7(3): 280.

Luckwill, L.C. (1939). Observations on heterosis in Lycopesicon. J. Genet. 37: 421-440.

Lyrene, P.M. (1981). Juvenility and production of fast-rooting cuttings from blueberry shoot cultures. J. Amer. Soc. Hort. Sci. 106(3): 396-398.

Majenbu, I.S., V.B. Ogunlela, M.K. Ahmed and J.D. Olarewaju (1985). Response of two okra (Abelmoschus esculentus L. Moench) varieties to fertilizers. Yield and yield components as influenced by N and P application. Fertilizer Research. 6(3): 257-267.

Maletta, M. and H.W. Jones (1987). Inter-relation of root and shoot temperatures on dry matter accumulation and root growth in tomato seedlings. Jour. Hort. Sci. 62(1): 49-54.

Manandher, A. and Raybhandary, S.B. (1986). Rooting in non-sterile potting mix of in vitro potato and its field establishment. Indian Jour. of Hort. 43(3-4): 235-238.

Mann, S. and K.M. Ramanathan (1981). Studies on the effects of nitrogen and potassium on the nitrogen uptake by bhendi ('okra') fruits at successive stages of picking. Madras Agric. Jour. 68(8): 530-536.

Manson, S.C. and C.E. Wilcox (1982). Nitrogen status evaluation of tomato plants. J. Amer. Soc. Hort. Sci. 107(3): 483-486.

Marini, P.R. (1983). Rooting of semi-hardwood peach cuttings as affected by shoot position and thickness. Hortscience 18(5): 718-719.

Maynard, D.N. and A.V. Barker (1972). Nitrate content of vegetable crops. Hortscience 7(3): Section I, pp. 224-226.

Mayol, P.S. (1980). Inter-relationship of soil microflora and root-knot nematode in roots of tomato. Philippine Jour. Sci. 109(3-4): 73-76.

McCollum, R.E. (1978a). Analysis of potato growth under differing P regimes, I: Tuber yields and allocation of dry matter and P. Agron. Jour. 70: 51-57.

_____ (1978b). Analysis of potato growth under differing P regimes, II: Time by P status interactions for growth and leaf efficiency. Agron. Jour. 70: 58-67.

Menendez, T. (1973). Application of mutation methods to banana breeding. In: Induced Mutations in vegetatively propagated plants. Proc. International Atomic Energy Agency (I.A.E.A)/ Food and Agric. Organisation (F.A.O.) I.A.E.A-PL-501/8 1973, pp. 75-84.

Mengel, K., P. Robin and L. Salsac (1983). NRA in shoots and roots of maize seedlings as affected by the form of N-nutrition and the pH of the nutrient solution. Plant Physiol. 71(3): 618-622.

Meyer, A.M. and A. Poljakoff-Mayber (1975). The germination of seeds. (2nd ed.). Pergamon Press, Oxford, N. York, Toronto.

Meyer Jr. M.M. (1976). Propagation of Daylilies by tissue culture. Hortscience 11(5): 485-487.

Minocha, S.C. and Halperin, W. (1974). Hormones and metabolities which control tracheid differentiation with or without concomittant effects on growth in cultured tuber tissues of Helianthus tuberosus L. Plants. 116: 319-331.

Moh, C.C. and J.J. Alan (1973). Methods for inducing mutations in cassava and the possible use of the mutants. In: Induced mutations in vegetatively propagated plants. Proc. I.A.E.A./F.A.O. I.A.E.A-PL-501/7 1973: pp. 67-74.

Molnar, J.M. and L.J. Lacroix (1972a). Studies of the rooting of cuttings of Hydrangea macrophylla: enzyme changes. Canad. Jour. Bot. 50(2): 315-322.

_____ (1972b). Studies of the rooting of cuttings of Hydrangea macrophylla: DNA and protein changes. Canad. Jour. Bot. 50(3): 387-392.

Moore, T.C. (1979). Biochemistry and Physiology of plant hormones. Springer-Verlag. New York. Berlin (1979), pp. 47.

Murashige, T. (1977). Clonal crops through tissue culture in: Plant tissue culture and its Biotechnological Applications. Borz. W. Reinhard E. and M.H. Zank (eds.), pp. 392-403. Springer-Verlag Berlin, New York.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Murashige, T., M.N. Shabde, P.M. Hasegaroa, F.H., Takatori and J.B. Jones (1972). Propagation of asparagus through shoot apex culture. I. Nutrient medium for formation of plantlets. J. Am. Soc. Hort. Sci. 97: 158-161.

Murneek, A.E. (1926). Effects of correlation between vegetative and reproductive functions in tomato. Plant Physiol. 1, 3.

Musa, B.U. (1986). The effects of N and P on the growth and yield of Ginger (Zingiber officinale. Roscoe). A thesis submitted in partial fulfilment for M.Sc. Agron. A.B.U., ZARIA.

Nagasawa, A. and J.J. Finer (1988). Induction of morphogenic callus cultures from leaf tissue of garlic. Hortscience 23(6): 1068-1070.

Negi, K.S. (1982). A note on rooting response in Dalbergia latifolia Roxb. cuttings by plant hormones. Indian forester 108(4): 300-301.

Ng, S.Y.C. (1987). Using tissue culture materials to meet worldwide sweet potato responsibilities. I.I.T.A. Research Briefs. 8(1): 1-2.

Nyomora, A.M.S. and N.A. Mnzawa (1982). Rooting response of juvenile and adult cuttings of apple and peach to IBA and season in Tanzania. Beitiage zue tropischen Landwirtschaft und veterinarmedizin 20(2): 135-140.

Ockerse, R. and J. Waber (1970). The promotion of indole-3-acetic acid oxidation in pea buds by G.A. Treatment. Plant Physiol. 46: 821-824.

Odegbaro, D.A. and O.N. Adedipe (1986). Rooting classification and culture responses of cacao hybrids. Ministry of Science and Tech., Lagos, Nig. (IICA). 36(2): 197-204.

Okezie, C.E.A. (1985). Indole butyric acid as a pre-sprouting agent for growth and tuberization in Dioscorea rotundata Poir. Tropical Agric. 62(4): 297-301.

Olarewaju, J.D. (1980). Effects of environment on flowering and fruit set in sweet pepper (Capsicum annum). Ph.D. Thesis, Dept. of Agric. and Hort., Univ. of Reading.

Olday, F.C., A.V. Barker and D.N. Maynard (1976). A physiological basis for different patterns of nitrate accumulation in two spinach cultivars. J. Amer. Soc. Hort. Sci. 101(3): 217-219.

Olieman, van der Meer, R.L.M. Pierik and S. Roest (1971). Effects of sugar, auxin and light on adventitious root formation in isolated stem explants of Phaseolus and Rhododendron. Med. Facult. Landbonw, Weten Crent. 36(1): 511-518.

Omar, F.A., A.E. Arafa, H.H. Shaheen (1979). Recovery percentage of N supplied to tomato. Agric. Res. Rev. (Egypt): 57(3): 223-231.

Omueti, O. (1977). Comparative studies on cultivars of Nigerian local leaf vegetables. I. Effects of age and season on yield, components of yield and nutrient contents of Amaranthus cultivars. Niger. Agric. Jour. 14(1): 12-20.

Omueti, O. (1982). Comparative studies on cultivars of Nigerian local leaf vegetables. II: Effects of age on the elemental nutrients of Amaranthus cultivars. Niger. Agric. Jour. 17/18: 141-148.

Omura, M., Matsute, T.M. and I. Kozaki (1987). Adventitious shoot and plantlet formation from cultured pomegranate leaf explants. Hortscience 02(1): 133-134.

Ooishi, A., Y. Shiobara, H. Machida and T. Hosoi (1982). Role of light in rooting of softwood Euonymus japonicus cuttings. Jour. Jap. Soc. Hort. Sci. 50(4): 511-515.

Orabi, A.A., A.S. Ishmail and A. Mashadi (1982). Zinc-phosphorus relationship in the nutrition of tomato plants as affected both by the soil and by the rate of applied Zinc. Plant and Soil 69(1): 67-72.

Oshalt, S.J. and O.H. Caso (1986). Differential requirements among tissue source in Solanum tuberosum L. B.S.P. andigene callus cultures. Nat. Coun. Sci. and Tech. Res. Argentina. 36(3): 363-368.

Osifo, E.O. (1988). Influence of leaf age on shoot regeneration from callus culture of potato. Solanum brevidens. Nig. Jour. Bot. 1(1): 43-46.

Paton, D.M., R.R. Willing, W. Nicholas and L.D. Pryor (1976). Rooting of stem cuttings of eucalyptus a rooting inhibitor in adult tissue. Austral. Jour. Bot. 18: 175-183.

Pereguine, W.T.H. and K.B. Ahmad (1982). Grafting a simple technique for overcoming bacterial wilt in tomato. Tropical Pest Management 28(1): 71-76.

Perez, D.E. and J.B. Sartain (1982). Influence of zinc sources and P fertilization on tomatoes (L. esculentum). Proc. Soil and Crop. Sci. Soc. of Florida. 41: 171-175.

Pichia, D.H. (1987). Sugar and organic acid content of cherry tomato fruit at different ripening stages. Hortscience 22(1): 94-96.

Pillay, V.S., A.B.M. Ali and K.C. Chandy (1982). Effect of IBA on root initiation and development in stem cuttings of Pepper (Piper nigrum). Indian Cocoa Arcanant and Spices Jour. 6(1): 7-9.

Pokorny, F.A. and M.E. Austin (1982). Propagation of Blueberry by softwood terminal cuttings in pinebark and pea media. Hortscience 17(4): 640-642.

Priestly, J.H. and C.F. Swingle (1929). Vegetative propagation from the stand point of plant anatomy. SDA Tech. Bul. 151.

Quinn, J.G. (1971). Factors affecting the production of tomatoes for the processing industry during different seasons in the Northern States of Nigeria. Unpublished M.Sc. Thesis, IAR, A.B.U. Zaria.

Quinn, J.G. (1980). A Review of tomato cultivar trials in the Northern States of Nigeria. Samaru Misc. Paper. 84, IAR, Zaria. pp. 4.

Raju, M.U.S. and Mann, H.E. (1970). Regeneration studies on detached leaves of Echeveria elegans. Anatomy and regeneration of leaves in sterile culture. Can. J. Bot. 48: 1887-1891.

_____ (1971). Regeneration studies on detached leaves of E. elegans. Patterns of regeneration of leaves in sterile culture. Can. J. Bot. 49: 2015-2021.

Rajveen, P.K. and N.S. Rao (1980). Effect of soil and foliar applied N on the growth and yield of Brinjal (Solanum melongina L.) under rainfed conditions. Agric. Res. Jour. Kerala 18(1): 45-50.

Reboul, J.A., Taeactua and R. YauAkui (1981). Tomato grafting in French Polynesia. Agronomic Tropicale 36(3): 299-306.

Reinecke, D.M. and R.S. Bandurski (1983). Oxindole-3-acetic acid, an indole-3-acetic and catabolite in Zea mays. Plant Physiol. 71(1): 211-213.

Reuveni, O. and I. Adato (1974). Endogenous carbohydrates, root promoters and root inhibitors in easy and difficult-to-root date palm (Phoenix dactylifera) off-shoots. J. Amer. Soc. Hort. Sci. 99(1): 361-363.

Reneau, R.B. Jr. G.D. Jones and J.B. Friedricks (1983). Effect of P and K on yield and chemical composition of forage sorghum. Agron. Jour. 75(1): 5-8.

Ritzert, R.W., D.D. Hemphill, H.M. Sell and M.J. Bukare (1972). Indole-3-acetic and oxidase activity from developing peach seeds and its inhibition by extratible catechin. J. Amer. Soc. Hort. Sci. 97(1): 48-50.

Rhoads, F.M. and M.E. Bloodworth (1984). Area management of cotton leaves by a dry weight method. Agron. Jour. 56: 520-522.

Rhoden, E.G. and Croy, L.I. (1988). Relationship between leaf area and dry matter in southern peas (Vigna unguiculata). Hortscience 23(4): 726-728.

Roy, B.N., N. Roychoudhury and T.K. Bose (1972). Endogenous phenolic compounds as regulators of rooting in cuttings. Phyton. 30: 147-151.

Saito, T., T. Hatayama and H. Ito (1963a). Studies on growth and fruiting in the tomato. C: Effects of early environment on growth and fruiting.2. Light. J. Jarp. Soc. Hort. Sci. 32: 49.

Saito, T., Y. Konao and H. Ito (1963b). Studies on growth and fruiting of tomato (iv). Tertility of the bed soil, watering and spacing. J. Jap. Soc. Hort. Sci. 32: 38.

Salac, S.S. and J.B. Fitzgerald (1983). Influence of propagation method and fertilizer rate on growth and development of Liatris pycnostachya. Hortscience. 18(2): 198-199.

Salama, F.M., Khodany, S.E.A. and M.M. Heikal (1981). Effect of soil salinity and IAA on growth, photosynthetic pigments and mineral composition of tomato and rocket (Eruca sativa) Plants. Phyton (Austria). 21(2): 177-188.

Salisbury, F.B. and C. Ross (1969). Plant Physiology. Wadsworth Publishing Company Inc., Belinont, California.

Salvaraj, Y., D.K. Pal, M.D. Subramayan and C.P.A. Iyer (1982). Changes in the chemical composition of four cultivars of Papaya during growth and development. Jour. Hort. Sci. 52(1): 135-143.

Sandhu, A.S., Raghbir, S. and C.S. Malhi (1982). Root forming capacity of pear hardwood cuttings taken from different regions of the shoot. Jour. of Res. India 19(2): 116-117.

Santana, N. (1985). A study on sprouting of tomato leaves in vitro culture. Cultives tropicales (Cuba). 7(2): 117-122.

Satomi, U. and J.C. Preece (1987). Micro and cutting propagation of 'crimson pygmy' Berberry. Hortscience. 22(3): 488-490.

Saxena, P.K., R. Gill and A. Rashid (1987). Optimal conditions for plant regeneration from mesophyll. Scientia Hort. 31(3-4).

Scott, E.N., P.M. Hasegawa and R.A. Brensaan (1982). Stimulation of root initiation from cultured rose shoots through the use of reduced concentrations of mineral salts. Hortscience 17(1): 82-83.

Scrinivas, K. (1982). Growth and yield of cabbage as affected by spacing and fertilizers. Singapore Jour. of Prim. Industries. 10(1): 58-63.

Shilbes, R.M. and C.R. Weber (1965). Leaf Area, Solar interaction and dry matter production of soybeans. Crop. Sci. 5: 573-577

Shresta, G.K. (1983). Effects of spacing and N-fertilizer on Okra (Abelmoschus esculentus L.) in Nepal. Experimental Agric. 19(3): 239-242.

Skoog, F. and C.O. Miller (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. exp. Biol. 11: 118-130.

Smagula, J.M. and P.R. Hepler (1980). Effect of nitrogen status of dormant rooted lowbush blueberry cuttings on rhizome production. J. Amer. Soc. Hort. Sci. 105(2): 283-285.

Smith, N.M. and H. Chiu (1980). Seasonal changes in the rooting of juvenile and adult peach cuttings. Hortscience. 15(5): 594-595.

Smith, Y.E. and C.W. Lee (1983). Propagation of Arizona Rosewood by stem cuttings. Hortscience 18(5): 764-765.

Spiegel, Y.E., Cohn and U. Kafkafi (1982). The influence of ammonium and nitrate nutrition of tomato plants on parasitism by the root knot nematode. Phytoparasitica. 10(1): 33-40.

Splittstoesser, W.E., J.S. Vandemark and S.M.A. Khan (1974). Infle Influence of nitrogen fertilization on protein and nitrate concentration in some vegetable crops. Hortscience. 9(2): 124-125.

Stark, J.C., N.M. Jarell and J. Letey (1982). Relationship between growth and N-fertilization of celery. Hortscience. 17(5): 754-755.

Stoltz, L.P. and C.E. Hess (1968). Factors influencing root initiation in an essay and difficult-to-root Chrysanthemum. Proc. Amer. Soc. Hort. Sci. 92: 622-626.

Street, H.E. (1977). Plant tissue and cell culture. Botanical monographs II (2nd ed.) H.E. Street (ed). Blackwell Scientific Publications (1977).

Subbiah, K. and K.M. Ramanathan (1982). Influence of N and K_2O on crude protein, carotene and ascorbic acid and chlorophyll contents of Amaranthus, South Indian Horticulture. 30(2): 82-86.

Subbiah, K., J. Helkiah and V. Ravikumar (1982). Effect of combined application of organic and inorganic fertilizers on the yield and nutrient uptake of MDU.I. Chilli. South Indian Horticulture. 301(1): 45-47.

Sweeney, D.W., D.A. Graetz, A.B. Bottdher, S.J. Locascio and K.L. Campbell (1987). Tomato yield and N-recovery as influenced by irrigation method, N-source and Mulch. Hortscience. 22(1): 27-29.

Tandon, D.K. and S.K. Kalra (1983). Changes in sugars, starch and amylas activity during development of Mango fruit. cv. Dashehari. Jour. Hort. Sci. 58(3): 449-453.

Taylor, O.O.A., B.L. Fatuga and V.A. Oyenuga (1983). Accumulation of mineral elements in five tropical leafy vegetables as influenced by N-fertilisation and age. Scientia Horticulturae 18(4): 313-322.

Theologis, J.C. and P. Ray (1982). Early auxin-regulated polyadenylated mRNA sequences in pea stem tissue. Proc. Natl. Acad. Sci. U.S.A. 79: 418-421.

Thorpe, H.J. and W.R. Jarvis (1981). Grafted tomatoes escape fusarium foot and root rot. Can. J. Plant. Sci. 61: 1027-1028.

Tigeheler, E.C. and W.B. McGlasson (1978). Genetic regulation of tomato fruit ripening Horticultural Science. 13: 505-513.

Tognoni, F. and R. Lorenzi (1972). Acidic root-promoting growth inhibitors found in Picea and Chamaecyparis. J. Amer. Soc. Hort. Sci. 97(5): 574-578.

Torre, L.C., R.P. Doss and B.H. Baritt (1980). Rooting of young root-shoots of Red-Raspberry in auxin solutions. Hortscience 15(2): 153-154.

Trudel, J.M.J. and J.L. Osbun (1971). Influence of K on carotenoid content of tomato fruit. Jour. Amer. Soc. Hort. Sci. 96(6): 763-765.

Trudel, M.J. and A. Gosselin (1982). Influence of soil temperature in green house tomato production. Hortscience 17(6): 923-929.

Uddin, M.R., S.Z. Berry and A.D. Bisges (1988). An improved shoot regeneration system for somaclone production in tomatoes. Hortscience. 23(6): 1062-1064.

Ulrich, R. (1958). Postharvest physiology of fruits. Ann. Rev. Plant Physiol. 9: 385- 416.

Vadivelu, K.K. (1983). Seed quality estimation in relation to size of the fruits in tomato varieties, Col. 1 and Col. 2. In: Proceedings of the National Seminar on Production Technology of tomato and Chillies. Coimbatore, India. Tamil Nedu Agric. Univ. 100-101.

Varis, S. and R.A.T. George (1985). The influence of mineral nutrition on fruit yield, seed yield and quality in tomato. Jour. Hort. Sci. 60(3): 373-376.

Villareal, R.L. (1980). Tomato in the tropics. Westview Press Boulder Colorado (1980).

Ward, G.M. (1969). A study of the rate and efficiency of nutrient absorption (KNO_3) by tomato seedlings. J. Amer. Soc. Hort. Sci. 94: 128-130.

Watson, D.T. (1937). The estimation of leaf area in field crops. J. Agric. Sci. 27: 474-483.

Weston, L.A. and B.H. Zandstra (1989). Transplant age and N and P nutrition effects on growth and yield of tomatoes (Pik-Red'). Hortscience 24(1): 88-90.

Widders, I.E. and O.A. Lorenz (1982). Potassium nutrition during tomato plant development. J. Amer. Soc. Hort. Sci. 107(6): 960-964.

Wittwer, S.H. and F.G. Teubner (1956). New practices for increasing the fruit crop of greenhouse grown tomatoes. Michigan Agr. Expt. St. Quart. Bul. 39: 198.

Wood, B.W. and J.L. McMeans (1982). Carbohydrates and fatty acids in developing pecan fruit (Carya illinoensis Wang.). J. Amer. Soc. Hort. Sci. 107(1): 47-50.

Yang, H. and W.J. Clore (1974). Development of complete plantlets from moderately vigorous shoots of stock plants of Asparagus in vitro. Hortscience. 9(2): 138-139.

Yunana, R. (1982). The effect of mixed cropping and seedling age on the performance of peppers (Capsicum species). M.Sc. Thesis, Department of Agronomy, A.B.U., Zaria.

Zanin, A.C.W. and T. Kimoto (1980). Effects of plant spacing and fertilizers on okra seed production. Revista Brassileira de Sementes (Brazil). 2(3): 105-121.

Zeven, A.C. and P.M. Zhukovsky (1975). Dictionary of cultivated plants and their centres of diversity. Centre for Agricultural Publishing and Documentation. Wageningen 1975, pp. 158.

Zrust, J.E., E. Pantykova and J. Necas (1974). Relationships of leaf area to leaf weight and length in potato plants. Photosynthetica (Prague). 8: 118-124.

APPENDIX A

Measurement of soil volume

The seived garden soil was put in a transparent polythene bag and immersed in a caliberated beaker which contained water of a known volume. The displacement of the water by the soil was taken as the approximate volume of the soil.

APPENDIX B

Calculation of approximate rate of fertilizer application

The recommended rate of fertilizer application was 300kgNPK/ha.

This was reduced to square meter by the relationship:

$$1\ ha = 10{,}000m^2$$

$$300kg/ha = 300{,}000g/ha$$

$$= 300{,}000g/10{,}000m^2$$

$$= \frac{300{,}000}{10{,}000} = 30g/m^2$$

The estimated number of pots that will fit into an area of $1m^2$ = 5 pots, therefore if we divide 30g by 5 we have 6g per pot.