3. Menge, J. A., C. K. Labanauskas, E. L. V. Johnson, and E. G. Platt. 1978. Partial substitution of mycorrhizal fungi for phosphorus fertilization in the greenhouse culture of citrus. Proc. Soil Sci. Soc. Amer. 42:926–930.
4. Mitchell, D. T. and D. J. Read. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of *Vaccinium macrocarpon* and *Rhododendron ponticum*. Trans. Brit. Mycol. Soc. 76:255–260.
5. Mosse, B. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza IV. In soils given additional phosphate. New Phytol. 72:127–136.
6. Pearson, V. and D. J. Read. 1975. The physiology of the mycorrhizal endophyte of *Calluna vulgaris*. Trans. Brit. Mycol. Soc. 64:1–7.
7. Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158–161.
8. Powell, C. L. and P. M. Bates. 1981. Ericoid mycorrhizas stimulate fruit yield of blueberry. HortScience 16:655–656.
9. Read, D. J. 1974. *Pezizella ericae* sp nov., the perfect state of a typical mycorrhizal endophyte of Ericaceae. Trans. Brit. Mycol. Soc. 63:381–382.
10. Read, D. J. and D. P. Stribley. 1973. Effect of mycorrhizal infection on nitrogen and phosphorus nutrition of ericaceous plants. Nature (New Biol.) 224:81–82.
11. Stribley, D. P. and D. J. Read. 1976. The biology of mycorrhiza in the Ericaceae. VI The effects of mycorrhizal infection and concentration of ammonium nitrogen on growth of cranberry (*Vaccinium macrocarpon* Ait) in sand culture. New Phytol. 77:63–72.
12. Stribley, D. P. and D. J. Read. 1980. The biology of mycorrhiza in the Ericaceae. VII The relationship between mycorrhizal infection and the capacity to utilize simple and complex organic nitrogen sources. New Phytol. 86:365–371.
13. Toleman, E. E. 1979. Blueberry production. New Zealand Agr. Sci. 13:127–133.

J. Amer. Soc. Hort. Sci. 107(6):1015–1018. 1982.

Root Initiation in Tomato Seedlings[1]

L. H. Aung
Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Additional index words. *Lycopersicon esculentum*, cultivars, nutrients, growth regulators

Abstract. **The initiation of root primordia on the primary root and hypocotyl of tomato seedlings (*Lycopersicon esculentum* Mill. cvs. Fireball, Epoch, and MD 412-4) was determined under controlled conditions. The first primordium of 'Fireball', 'Epoch,' and MD 412-4 seedlings was initiated on the primary root 5–6, 7, and 8 days, respectively, after seeding. Primordia on the hypocotyl were initiated acropetally, after initiation on the root. Root initiation in 'Epoch' and MD 412-4 lagged behind 'Fireball', but the general pattern was similar. Hoagland's nutrient solution significantly increased the number of primordia on the primary root and hypocotyl. Presoaking seed in 10 μM abscisic acid (ABA) or 6-benzylamino purine (BA significantly increased the number of primordia on the primary root. Application of 50 μl of 1 mM 2,3,5 triiodobenzoic acid (TIBA) in lanolin 1 cm below the cotyledonary node reduced the number of primordia on the hypocotyl.**

The important role of the tomato root system on shoot growth is well-recognized (8, 10, 22) and its development under field and greenhouse conditions has been characterized (18, 20, 21). Explants have been used to investigate root-initiating substances in 10-day-old tomato seedlings (1, 2) and to rapidly screen for root-forming ability of tomato cultivars (3). However, no studies have considered root initiation on the primary root and hypocotyl of tomato seedlings. Zobel (27) observed that the hypocotyl primary root transition zone of tomato possesses unique genetic and biochemical features which influence root development. This study examines the ontogenetic development of secondary roots in the transition zone and adjacent region of 3 tomato cultivars and evaluates the effects of nutrients and growth regulators on root initiation in the primary root and hypocotyl of seedlings.

Materials and Methods

Seeds of 'Fireball', 'Epoch', and MD 412-4 tomato were grown in a growth chamber in 70 ml white quartz sand in 6-cm^3 plastic cups at 26°C day and 20° night. The seedlings received a 12-hr photoperiod with an illuminance of 13 klx, giving a photon flux density of photosynthetically active radiation of 160 $\mu E\ m^{-2}s^{-1}$ from mixed Cool White fluorescent and incandescent lamps.

Hoagland's nutrient solution 1 was prepared according to Hoagland and Arnon (13), except that 5 ppm Fe was applied as NaFeEDTA. Ten ml of 1/4 - strength solution or water was added to each cup; cups contained 20 ml solution at saturation. Cups were not drained, but were never flooded. A randomized complete block design with treatments replicated 4 times was used. For root examination, seedlings were washed in running tap water, and after the cotyledons were removed, seedlings were fixed in 10 ml glacial acetic acid and ethyl alcohol (1:1 by volume) and 40 μl of 1% aqueous acid fuchsin per liter (3). After 48 hr, root primordia were visible as distinct red dots along the central vascular core of the primary root and hypocotyl. Adjacent tissues stained lightly and the primordia were easily counted against a fluorescent light source.

ABA (R. J. Reynolds Tobacco Co., Winston-Salem, N.C.) was prepared in 300 μl of ethanol and diluted to 10 μM concentration with water containing 0.05% Tween 80 (polyoxyethylene sorbitan monooleate). Similarly, TIBA (Sigma Chemical Co., St. Louis, Mo.) at 1 mM was dissolved in 300 μl ethanol and diluted with melted lanolin. The cytokinin BA (Nutritional

[1]Received for publication July 31, 1981.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Biochemicals Corp., Cleveland, Ohio) was prepared by first dissolving in 100 µl of 1 N KOH and then diluting to 10 µM concentration with water containing 0.05% Tween 80.

Results and Discussion

The first primordium on the primary root of 'Fireball' was initiated 5–6 days after seeding, in 'Epoch' on the 7th day and in MD 412-4 on the 8th day. The maximum number of 8–9 primordia on the primary root of 'Fireball' was reached 8 days after seeding. In the hypocotyl of 'Fireball,' the maximum number of primordia was initiated after 12 days. On both the primary root and the hypocotyl, new roots elongated and emerged when the maximum number of primordia was attained (Fig. 1). Epoch initiated fewer primordia than 'Fireball' and these emerged later than those of 'Fireball' (Fig. 2). In MD 412-4, primordia of the primary root emerged 2 days before the maximum number of primordia was initiated; primordia of the hypocotyl emerged 2 days after the maximum number of primordia was initiated (Fig. 3). 'Fireball' seedlings initiated root primordia sooner than 'Epoch' and MD 412-4, perhaps because of its more rapid growth rate.

Hoagland's solution significantly increased the number and accelerated the emergence of roots on the primary root and hypocotyl of 'Fireball' (Table 1). Presoaking 'Fireball' seeds for 24 hr in 10 µM ABA or BA significantly increased the number

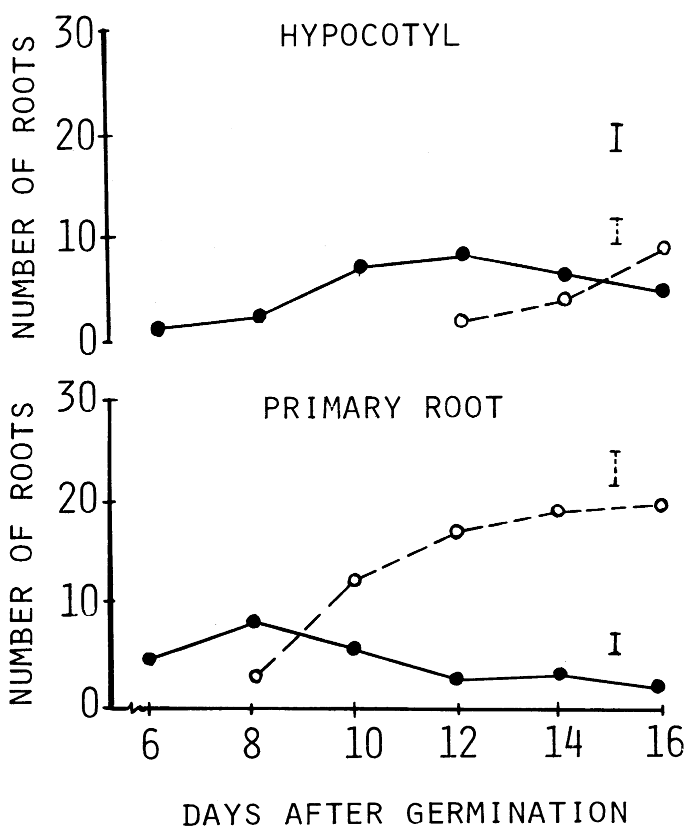

Fig. 1. Root initiation in the primary root and hypocotyl of 'Fieball' tomato seedlings grown in sand-culture under controlled condition; Each value represents a means of 4 replication (8 plants/replicate) and denotes the total number of roots observed on a particular day indicated; solid dots denote nonemergent primordia and open circles denote emergent roots. Solid vertical bar and dotted vertical bar denote HSD at 5% level of probability for nonemergent primordia and emergent roots, respectively.

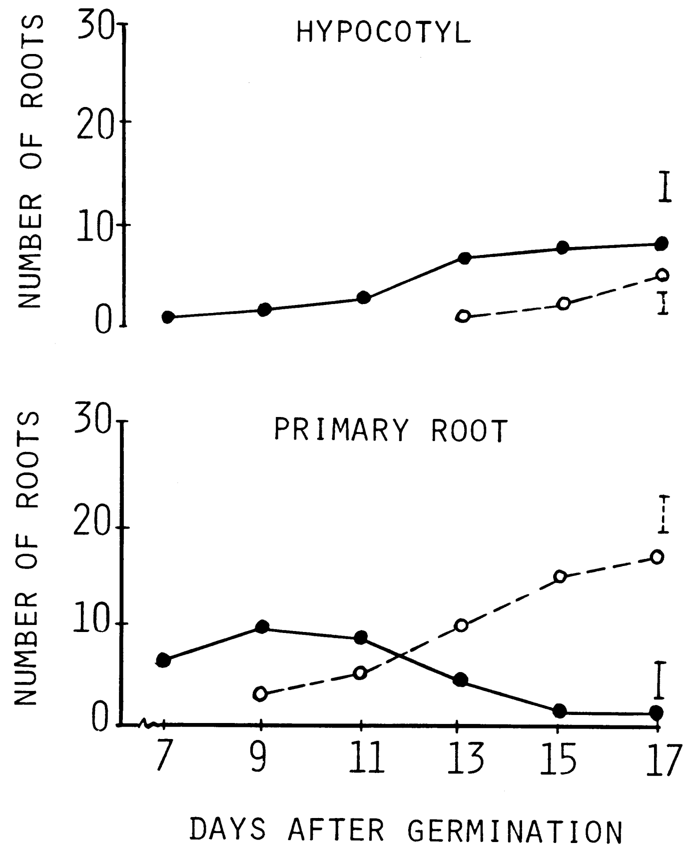

Fig. 2. Root initiation in the primary root and hypocotyl of 'Epoch' tomato seedlings grown in sand-culture under controlled conditions; See Fig. 1 for other details of explanation.

of primordia on the primary root. The number of primordia on the hypocotyl was significantly reduced by BA (Fig. 4). Ringing the hypocotyl of 'Fireball' seedlings below the cotyledonary node with 50 µl of 1 mM TIBA for 24 hr also reduced the number of primordia initiated on the hypocotyl, but did not alter the number on the primary root (Fig. 5).

Incipient root primordia in all cultivars are first evident as localized regions of anticlinal divisions of the pericycle and endodermis (2). Repeated periclinal divisions of the pericycle and its derivatives give rise to organized root primordia (5). The lower region of the hypocotyl from which the first root primordium originated has Casparian strips visible up to 1.4 cm from the hypocotyl base (7). Although secondary roots initiated from the primary root are normally considered laterals and those initiated from other aerial regions adventitious, the distinction is not sharp (12). Secondary roots of the tomato hypocotyl, however, originate from the pericycle and endodermis (5). Therefore, based on tissue of origin they can be considered laterals, based on region of origin they can be considered adventitious. Esau (12) stated that adventitious roots may develop from "more or less old root parts" but did not specify what was meant by that phrase. Based upon the above observations and related genetic and developmental considerations (27), it has been proposed that the roots arising from the hypocotyl primary root transition region should be considered basal roots (6, 27).

The ability of the primary root to form new primordia diminished with age (Figs. 1–3), perhaps due to secretion of an inhibitory substance(s) by the primary root as it ages. It has been shown in peas that following decapitation, the level of an in-

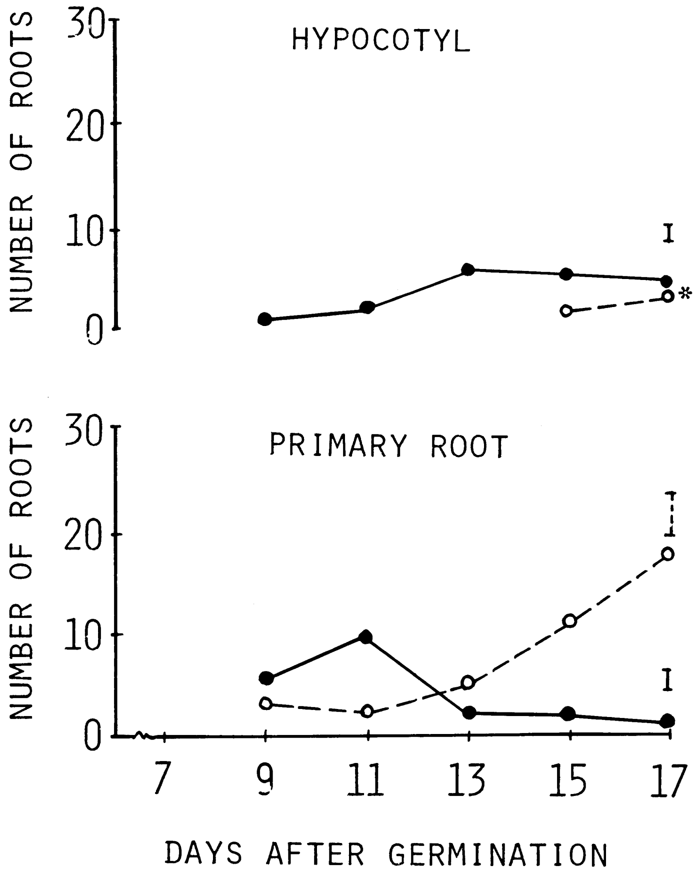

Fig. 3. Root initiation in the primary root and hypocotyl of MD 412-4 tomato seedlings grown in sand-culture under controlled conditions; * denotes significance at 5% level of probability; all other details similar to Fig. 1.

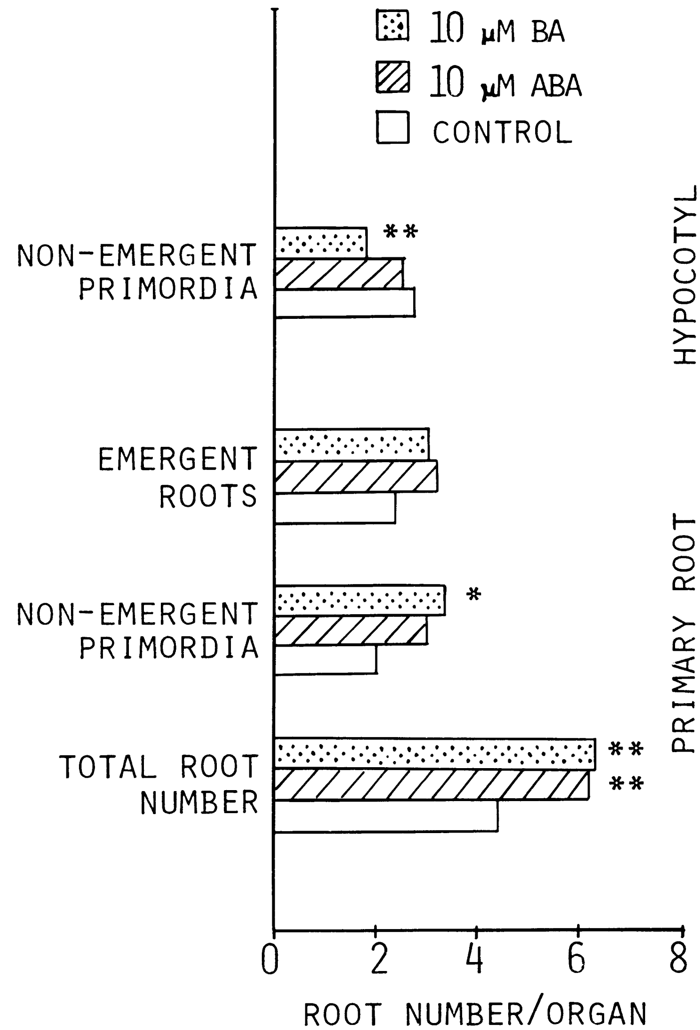

Fig. 4. Effects of ABA and BA on root initiation in the primary root and hypocotyl of 7-day-old 'Fireball' tomato seedlings; each value represents a means of 4 replications (10 plants/replicate); * and ** denote significane from control at 5% and 1% level of probability.

hibitor decreased and the number of lateral roots concurrently increased (4). It is possible that the diminishing ability of tomato seedlings to initiate new primordia with age may be due to secretion of an inhibitor(s).

Root formation of tomato is affected by nutrients and nutritional status (16, 17, 23). Locascio and Warren (14) observed that greenhouse-grown tomatoes fertilized with inorganic nutrients developed visible lateral roots 14 days after seeding. The addition of phosphorus did not significantly enhance root growth, perhaps because of the short duration of the experiment. In 'Fireball' seedlings, complete Hoagland's solution significantly promoted root initiation and emergence (Table 1). Faster germination and root emergence have been reported with similar nutrient solutions (11, 15).

Table. 1. Hoagland's nutrient solution on root initiation of 6-day-old Fireball tomato seedlings grown under controlled conditions.[z]

	Root primordia/primary root			Root primordia/ hypocotyl
Treatment	Emergent	Non emergent	Total	Non emergent
Control (water)	4.0	2.9	6.9	2.7
1/4 Nutrient solution	6.4*	2.5	8.9*	3.5*

[z]Values are means of 4 replicates (10 plants/replicate).
[y]Significance from distilled water control at 5% level.

Certain growth regulators influence tomato root development (1, 5, 19). ABA at 1 µg/ml (4 µM) reduced root growth and number of emergent laterals of *in vitro* tomato roots, but 2.6 ng/ml (10 nM) ABA increased their number (19). In intact 'Fireball' seedlings, 10 µM ABA significantly increased root production (Fig. 4) and similar stimulation by ABA has been reported in mung bean (9) and soybean (26). Thus, although ABA is generally considered to be a growth inhibitor, it promotes root initiation at relatively low concentrations in tomato and some other plants.

The stimulatory effect of BA on tomato root initiation (Fig. 4) has not been reported previously. However, kinetin at 1–5 µg/ml (5–23 µM) reduced rooting of mung bean (9), but at 50 nM it promoted rooting in peas (19). The differential rooting responses may be due to the concentration of cytokinin or to species differences. Wightman et al. (24) found that zeatin, kinetin, isopentyladenine, and kinetin riboside at concentrations higher than 10^{-6}M inhibited the initiation of lateral root primordia of peas, while at 10^{-6} to 10^{-8}M these cytokinins resulted in a 10–20% increase in root primordia.

In previous studies, TIBA interfered with root initiation of tomato explants (1). The present results indicate that TIBA also interferes with root initiation of seedlings (Fig. 5). In explants

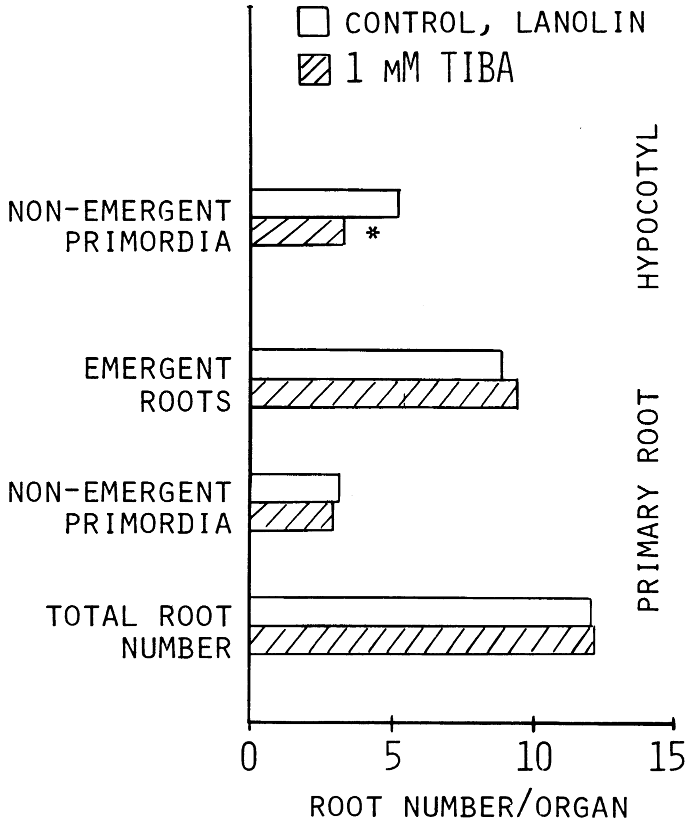

Fig. 5. Effect of TBA on root initiation in the primary root and hypocotyl of 6-day-old 'Fireball' seedlings grown in sand-culture under controlled condition; each value represents a means of 4 replications. (10 plants/replicate); * denotes significance from control at 5% level of probability.

or seedlings TIBA may interfere with root initiation by blocking auxin transport and altering *in vivo* auxin levels (25).

On the basis of the results obtained, it may be concluded that the initiation of primordia in tomato seedlings is under multifactor control involving mineral nutrients, hormonal substances, and probably other as yet unidentified growth inhibitor(s). The delineation of the relative importance of the different factors controlling primordia initiation and the identification of the unknown root inhibitor(s) remain problems for further investigation.

Literature Cited

1. Aung, L. H. 1972. The nature of root-promoting substances in *Lycopersicon esculentum* seedlings. Physiol. Plant. 26:306–309.
2. Aung, L. H., H. H. Bryan, and J. M. Byrne. 1975. Changes in rooting substances of tomato explants. J. Amer. Soc. Hort. Sci. 100:19–22.
3. Aung, L. H. 1976. Adventitious root-forming ability in 55 cultivars of tomato explants. Can. J. Plant Sci. 56:407–409.
4. Bottger, M. 1978. Levels of endogenous indole-3-acetic acid and abscisic acid during the course of the formation of lateral roots. Z. Pflanzenphysiol. 86:283–286.
5. Britton, G., S. Housley, and J. A. Bentley. 1956. Studies in plant growth hormones. V. Chromatography of hormones in excised and intact roots of tomato seedlings. J. Expt. Bot. 7:239–251.
6. Byrne, J. M. and L. H. Aung. 1974. Adventitious root development in the hypocotyl of *Lycopersicon esculentum* Mill. var. Fireball. Amer. J. Bot. 61: suppl. p. 54. (Abstr.)
7. Byrne, J. M., K. A. Collins, P. F. Cashau, and L. H. Aung. 1975. Adventitious root development from the seedling hypocotyl of *Lycopersicon esculentum*. Amer. J. Bot. 62:731–737.
8. Cooper, A. J. 1955. Further observations on the growth of the root and shoot of the tomato plant. Proc. XIV Intern. Hort. Congr.: 589–595.
9. Chin, T. Y., M. M. Meyer, Jr., and L. Beevers. 1969. Abscisic acid stimultated rooting of stem cuttings. Planta 88:192–196.
10. Davis, R. M. and J. C. Lingle. 1961. Basis of shoot response to root temperature in tomato. Plant Physiol. 36:153–162.
11. Ells, J. E. 1963. The influence of treating tomato seed with nutrient solutions on emergence rate and seedling growth. Proc. Amer. Soc. Hort. Sci. 83:684–687.
12. Esau, K. 1977. Anatomy of seed plants, 2nd ed. Wiley, New York.
13. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Cir. 347.
14. Locascio, S. J. and G. F. Warren. 1959. Growth pattern of the roots of tomato seedling. Proc. Amer. Soc. Hort. Sci. 74:494–499.
15. Oyer, E. B. and D. E. Koehler. 1966. A method of treating tomato seeds to hasten germination and emergence at sub-optimal temperatures. Proc. XVII Intern. Hort. Congr. 1:626.
16. Reid, M. E. 1924. Quantitative relations of carbohydrates to nitrogen in determining growth responses in tomato cuttings. Bot. Gaz. 77:404–418.
17. Reid, M. E. 1926. Growth of tomato cuttings in relation to stored carbohydrate and nitrogen compounds. Amer. J. Bot. 13:548–574.
18. Spencer, K. 1951. Tomato root distribution under furrow irrigation. Austr. J. Agr. Res. 2:118–125.
19. Street, H. E. 1969. Factors influencing the initiation and activity of meristems in roots. p. 20–41. In: W. J. Whittington (ed.) Root growth. Butterworths, London.
20. Ward, G. M. 1964. Observations of root growth in the greenhouse tomato. Can. J. Plant Sci. 44:492–494.
21. Weaver, J. E. and W. E. Bruner. 1927. Root development of vegetable crops. McGraw-Hill, New York.
22. Went, F. W. 1943. Effect of the root system on tomato stem growth. Plant Physiol. 18:51–65.
23. White, P. R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. 9:585–600.
24. Wightman, F., E. A. Schneider, and K. V. Thimann. 1980. Hormonal factors controlling the initiation and development of lateral roots. II. Effects of exogenous growth factors on lateral root formation in pea roots. Physiol. Plant. 49:304–314.
25. Winter, A. 1968. 2,3,5-triiodobenzoic acid and the transport of 3-indoleacetic acid. p. 1063–1076. In: F. Wightman and G. Setterfield (eds.) Biochemistry and physiology of plant growth substances. Runge Press, Ottawa, Canada.
26. Yamaguchi, T. and H. E. Street. 1977. Stimulation of the growth of excised cultured roots of soya bean by abscisic acid. Ann. Bot. 41:1129–1133.
27. Zobel, R. W. 1975. The genetics of development. p. 261–275. In: J. G. Torrey and D. T. Clarkson (eds.) The development and function of roots. Academic Press, London.