Effect of nitrogen, phosphorus and calcium fertilizations on the yield and quality of F_1-hybrid tomato seed

Ani Andayani S[1+], Isao Hasegawa[1], Akira Noguchi[1], Yutaka Shinohara[2], Tadashi Ito[2] and Jinya Yazaki[1]

1) Lab. of Plant Nutrition, Coll. of Bioresource Sciences, Nihon University 3-34-1 Shimouma, Setagaya, Tokyo, 154 Japan

2) Lab. of Vegetable Sciences, Fac. of Horticulture, Chiba University 648 Matsudo, Matsudo, Chiba, 271 Japan

Abstract

The effect of nitrogen (N), phosphorus (P), and calcium (Ca) fertilizations on the seed yield and quality of F_1-hybrid tomato seed grown by NFT were investigated. Higher N application (8 me/l) at the onset of flowering increased seed yield. Increasing N level at later growth stage increased seed yield of upper cluster, therefore total seed per plant also high. Four me/l of Ca level combined with 8 me/l of P level increased in the weight of fruit and seed. However, at the case of 8 me/l of Ca, increasing P level declined fruit and seed yield severely. Especially when both P and Ca level were high (8 me/l), fruit and seed yield markedly decreased into about 60 %. Effect of N content in the seed will reflect to the N compounds constituent there and consequently may influence seed germination.

Keywords: *F_1-hybrid tomato seed production - nutrient film technique (NFT) - nitrogen - phosphorus - calcium*

Introduction

Most of the vegetable crops are grown from the seed. It is important therefore that seed is starting point for vegetable crop production. Concerns on detailed technology of F_1-hybrid vegetable seed production has been rising up recently since most of the commercially grown vegetables are F1-hybrids. This condition is resulted that high quality of F_1-hybrid seed is becoming an important aspect.

One of the important things to be considered for improving vegetable seed production is the role of the effect of mineral nutrients. Although the effect of mineral nutrients on vegetable crop production have been widely investigated, but very little work has been done on seed production. Many previous studies on vegetable seed production have been conducted in soil culture so the clear effect of each nutrients on it has not yet been clear due to the complicated system in the soils or antagonistic effects among the nutrients as affected by the activity of various microorganisms in the soils. This is why we conducted with hydroponic culture in this study. Nutrient Film Technique (NFT) is one of the most reasonable one to be adopted because this system will provide the appropriate concentration of nutrient solution to be applied, additional nutrient solution can be maintained precisely and therefore can control the plant nutrient uptake.

Nitrogen (N) as an important element promoting vegetative plant growth and also affect a fruit drop if deficient (Marschner, 1986) . However, excess of vegetative growth due to excess of N affect on many reproductive stages and then causes a fruit drop too. In reproductive growth stage, phosphorus (P) is also the important nutrient for flower formation which will delay plant flowering if deficient (Steward, 1971). As reported by Marschner (1986) P is positively corelated with the number of flower set in. Eventhough the P deficient plants form reproductive organ but their productivity was greatly reduced (Andreeva, 1993). In tomato, increased P was also significantly increased yields and average fruit weights (Al Afifi, 1993) through the strong effect of P to the flower formation and development. However, on the other hand, Kreij *et al.* (1992) were studied that increased P supply also promoted an increasement of plant uptake in calcium (Ca). This element has strong effect to the seed formation and it should be indispensable during pollen germination and the growth of pollen tube (Mengel and Kirby, 1982) especially under artificial pollination which might be affected to the seed formation during fertilization. Therefore, not only the effect of N, P, and Ca, but also combination effect of them on the yield and quality of F_1-Hybrid tomato seed production were investigated in this study.

+ Author responsible for correspondence, phone/ fax. no: (0274) 583639.

Materials and Methods

1. Plant materials

Cultivar "Yubae" was used as maternal stock plant which has a characteristics of pink fruit in weight of 180-200 gram and of heat tolerants. Cultivar "KR-2" was used as paternal stock plant, introduced cultivar from Holland which has a characteristics of thin stem, wide internode, small leaf and sharp pole, produce more pollen and with fruit weight similar to "Yubae". Both cultivars have been using at Nihon Horticultural Production of Japan.

2. Nutrient solution

Modified half strenght of "Enshishoho" Nutrient Solution formulated by National Institute of Vegetables, Ornamental Plants and Tea of Japan was used as a standard solution. It is consisted of: (a) macronutrients: $Ca(NO_3)_2.4H_20$, KNO_3, $NH_4H_2PO_4$, and $MgSO_4.7H_2O$ with their compositions are N : P : K : Ca : Mg = 8 : 2 : 4 : 4 : 2 me/l respectively and (b) micronutrients: Fe-EDTA, H_3BO_3, $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $Na_2MoO_4.2H_2O$ with their compositions are Fe: B : Mn : Zn : Cu : Mo = 3.00 : 0.50 : 0.50 : 0.05 : 0.02 : 0.01 ppm respectively.

2.1. *Experiment I.*

N application for some vegetable crops should be applied gradually (Varis and Raymond, 1985) due to the necessity of the plant for it growth especially for sink and source relationship. In this experiemnt, two levels of N concentration (4 and 8 me/l) were applied at three different regimes, as follows: 4 me/l of N concentration through all stages of plant growth (N4N4), at early growing stage was 4 me/l and when 4th cluster flower bud set it was changed into 8 me/l (N4N8), and at all period of plant growth stage was 8 me/l (N8N8).

Treatment solution containing 4 me/l of N application (N4N4) was prepared by 2 me/l of $Ca(NO_3)_2.4H_2O$ and 2 me/l of KNO_3. Other potassium was added by 2 me/l of KCl. Whereas other nutrients was provided as standard solution. Treatment solution containing 8 me/l of N application (N8N8) was provided as same as standard solution. In the case of treatment N4N8, N concentration in the treatment solution was changed during renewal solution as were done for 4 me/l and followed by 8 me/l.

2.2. *Experiment II*

Kreij *et al.* (1992) reported that P and Ca have a linkage influence in between them for tomato crop production through their effect for reproductive organ formation. How their effect on seed production, therefore, three levels of P concentration (2, 4 and 8 me/l) as P2, P4 and P8 respectively combined with two levels of Ca concentration (4 and 8 me/l) as Ca4 and Ca8 were set in this experiment. Total treatments in this experiment were six: P2Ca4, P2a8, P4Ca4, P4Ca8, P8Ca4 and P8Ca8. Increasement of P level in this treatment solution adjusted by KH_2PO_4 and increasement of Ca level adjusted by CaCl2. In this experiment, P and Ca applications were treated for whole period of plant growth during seed production period.

Renewing solution in both experiment was done every two weeks while the additional solution was done by adjusting the Electric Conductivity (EC) every 3-4 days. Expected EC in each different treatments were maintained in 1.5-2.5 ms/cm. The flow rate of nutrient solution was maintained at 2-3 liters/minutes at the slope of NFT channel equal into 0.01.

3. Cultivation methods

"Yubae" was cultivated in NFT culture whereas "KR-2" was in soil culture at the separated greenhouses to avoid cross pollination. "KR-2" seed was sown three weeks earlier to provide a pollen sufficiently for pollination.

"Yubae" stock seed was sown in the nursery tray using carbonized rice husk as a medium. When two true leaves were set, the seedlings transplanted into plastic pots put rockwool media for about 3 weeks until eight true leaves set. Seedlings were transplanted in the NFT channel which had 45 cm and 70 cm distance in between plants and rows respectively. A treatment was adapted at the channel of NFT with 16 plants.

"KR-2" stock seedlings were about 20-25% of the total amount of "Yubae" were cultivated in the distance of 60 cm and 150 cm between plant and rows respectively. All plants maintained only single main stem by removing lateral shoots and topped setting 5 and 4 clusters in Exp.I and Exp.II respectively. Treatment solution was applied just after new rooting system developed (about 7 days after transplanting).

4. Seed Production Methods

Emasculation was done to the "Yubae" flowerbuds 2 days before anthesis (Shinohara, 1989). Artificial pollination was done by putting fresh pollen of "KR-2" into stigma carefully at the blooming day of emasculated flower (Leopold, 1986). Fruits were harvested 48-60 days after pollination as estimated by the total accumulated of average day temperature about 1800° C or about 12.5% longer than harvesting period of tomato fruit production for fresh marketing or processing. Post harvest ripening for fruit was done for 3-5 days at 28-29° C naturally (Liptay, 1989).

5. Seed Processing Methods

Extracted seed was fermented naturally at 28-29° C for 48 hours (Liptay, 1989) to peel off the gel sorrounding seed. Cleaned seeds were air dried for 24 hours followed by oven dried at 35° C for about 48 hours as estimated by seed water content reached 9-11%.

Germination test done as the rule of ISTA (International Seed Testing Association) standard method. Seeds germinated above 2 layers of wetted filter paper, 100 seeds in each replication and in a total of 4 replications, incubated at 25° C in the darkness for 14 days. Germination percentage was counted as the total normal germinated seed until 14 days after sowing. Germination date was calculated as an average of germination speed everyday.

6. Nutrient analysis

6.1. *N,P,K,Ca and Mg*

0.5 gram dried sample seed for each treatment was ground and ashed in 575° C for about 48 hours. After adding 2 ml of 50% chloric acid, all samples were filled up exactly into 100 ml with ultra pure water (UPW). Prepared solution is ready for seed analysis. Content of P, K, Ca, and Mg were determined by Atomic Absorption Spectrophotometry and N content was determined by C/N corder as a total nitrogen in the seed.

6.2. *Protein*

Protein content was analyzed by the following methods: (a) Well ground seed was extracted by 10OmM phosphate buffer solution (pH 7.6) at 4° C and was centrifuged at 4.000 rpm for 15 minutes to get a *soluble protein* from supernatant; (b) The precipitation from *ad* (a) was extracted by 3% SDS (Sodium Dodecyle Sulfate) solution to obtain a *membrane binding protein* and *cell wall composition protein* from supernatant and precipitate respectively. Each protein was determined as the total amonium-nitrogen constituent (Protein = total amonium nitrogen x 1.6) using Spectrophotometry.

Results and Discussions

1. Seed yield and germination

Experiment I. N application was not significantly affected the seed yield harvested from middle cluster (Figure 1), but it was significantly affected to the upper cluster seed. When N application was increased from 4 to 8 me/l at the onset of flowering when 4 th cluster flowerbud setted (Treatment N4N8), increased seed yield of upper cluster. In consequent, total seed yield per plant found from this treatment was highest among others.

Four me/l of N application at all plant growth stage (N4N4) resulted a smaller seed estimated from 1000 seed weight (Figure 2). Oh the other hand, 8 me/l of N application have a tendency produced bigger seed, i.e especially at the later growth stage, increased N concentration from 4 into 8 me/l (N4N8) produced bigger seed. This finding supported the results of Mengel and Kirby (1982), higher concentration of N was needed at the time of flowerbud set and seed maturity which have strong effect to particular relationship between sink and source.

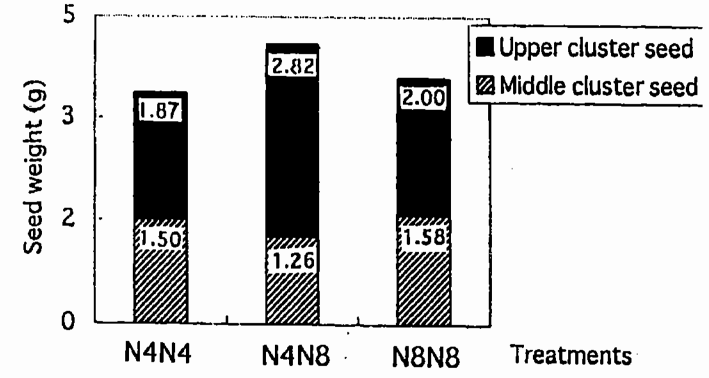

Figure 1. Effect of nitrogen regimes on F1-hybrid tomato seed yield

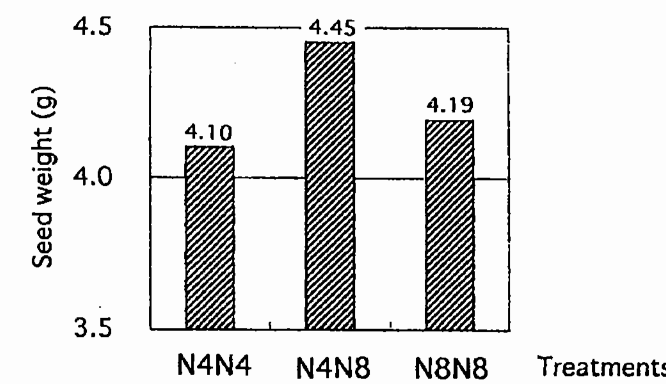

Figure 2. Effect of nitrogen regimes on 1000 seed weight of F1-hybrid tomato seed

Increasement of N concentration from 4 into 8 me/l in the nutrient solution at the onset of flowering when flowerbud set could increased seed yield of upper cluster and made a total seed yield per plant also high consequently.

From this experiment, all of the treatments have a good germination percentage (above 90%). Accordance to the ISTA rules which regulated if seed germination percentage more than 90% is categorized as a good seed. Hence, high seed yield obtained from N4N8 treatment with germination percentage 90.1% was a best treatment for tomato seed production. Eventhough low application of N at early plant growth stage was applied, but when at the later stage especially when the reproductive organ formation is very active high application of N is very important for producing better seed yield.

Experiment II. P and Ca were significantly affected the weight of fruit either that harvested from lower cluster or upper cluster (Figure 3). At 4 me/l of Ca concentration, increased P concentration resulted to the increasement of fruit weight. However in the contrary, at 8 me/l of Ca concentration, increased P concentration decreased fruit weight. At the same concentration of P (8 me/l), increased Ca from 4 into 8 me/l resulted to the decreasing fruit weight into 64.8%.

P also recognizable affected the stability of fruit formation up to the upper cluster. These can be compared from the treatment P4Ca4 and treatment P8Ca4. When P concentration increased from 4 to 8 me/l, although fruit weight from lower cluster almost same in both treatments (1.48 and 1.50 kg/cluster) but this tendency was disappeared for upper cluster fruits that only could harvested 1.19 kg/cluster (P4Ca4) whereas P8Ca4 was 1.35 kg/cluster. From this datas we could cite that under the high concentration of P. fruit formation in upper cluster could be maintained better and it was correspondingly resulted the higher yield of total seed per plant (Figure 4). Fruit and seed weight have a similar pattern as shown in Figures 3 and 4. This is also reported by Marschner (1988) that increased P related to the flower formation and development but from this result cited that it has to be noticed if undertow concentration of Ca.

The effect of P and Ca and the balance of them on seed production was resumed in Figure 4. Under low concentration of Ca (4 me/l), increasing concentration of P was resulted to the increasement of seed yield. Highest seed yield obtained from P8Ca4 (6.75 g/plant). In the contrary, if Ca concentration 8 me/l, increasing P concentration was significantly decreased seed yield. This is suggested caused by the increasement of Ca concentration in the nutrient solution which may influence to the transport of P to

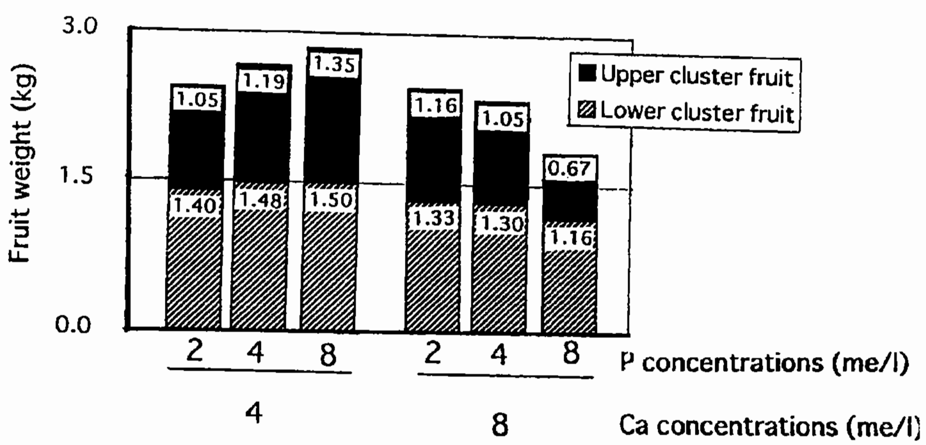

Figure 3. Effect of phosphorus and calcium fertilizations on the fruit yield of F_1-hybrid tomato

the upper parts of the plant was inhibited and fruit formation become worst. Especially at the treatment P8Ca8, not only P transport was inhibited but also to the Ca transport that resulted to the high BER incident of fruit harvested from this treatment. In conclusion, treatment P8Ca4 was the best treatment for producing F_1-hybrid tomato seed under artificial hand pollination system.

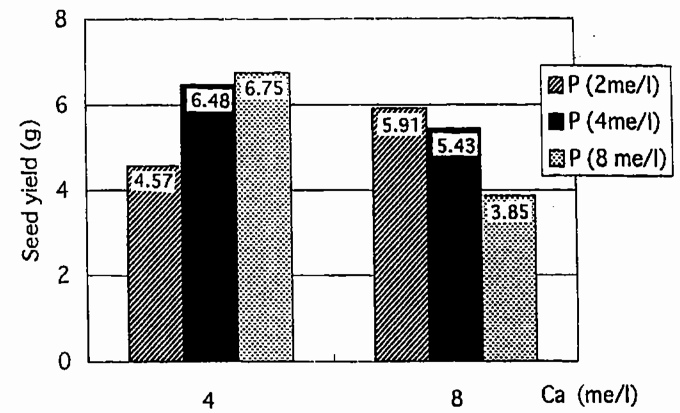

Figure 4. Effect of phosphorus and calcium fertilizations on the seed yield of F_1-hybrid tomato

2. Nutrient analysis and seed quality

The seed was used for nutrient analysis of N, P, K, Ca and Mg constituent in the seed was taken from the seed harvested from treatment P8Ca4 (Exp.II).

Correlation between N, P, K, and Mg constituents in the seed with germination date presented in Figure 5. High content of N, P, and K in the seed were found from the seed with early germination date. From this figure also showed the seed with higher N content (4.2 %DW) germinated at 4.5 days after sowing, but seed with low N content (3.7 %DW) germinated at 5.6 days after sowing or about 1-2 days longer to be germinated. Similar tendencies were not found for Ca content in the seed, therefore Ca content in the seed was not significantly affected the germination date.

Effect of N and P to the germination process were recognizable from their content in the seed. This results may lead that N constituent in the seed also has a certain effect to the nitrogen compounds in the seed.

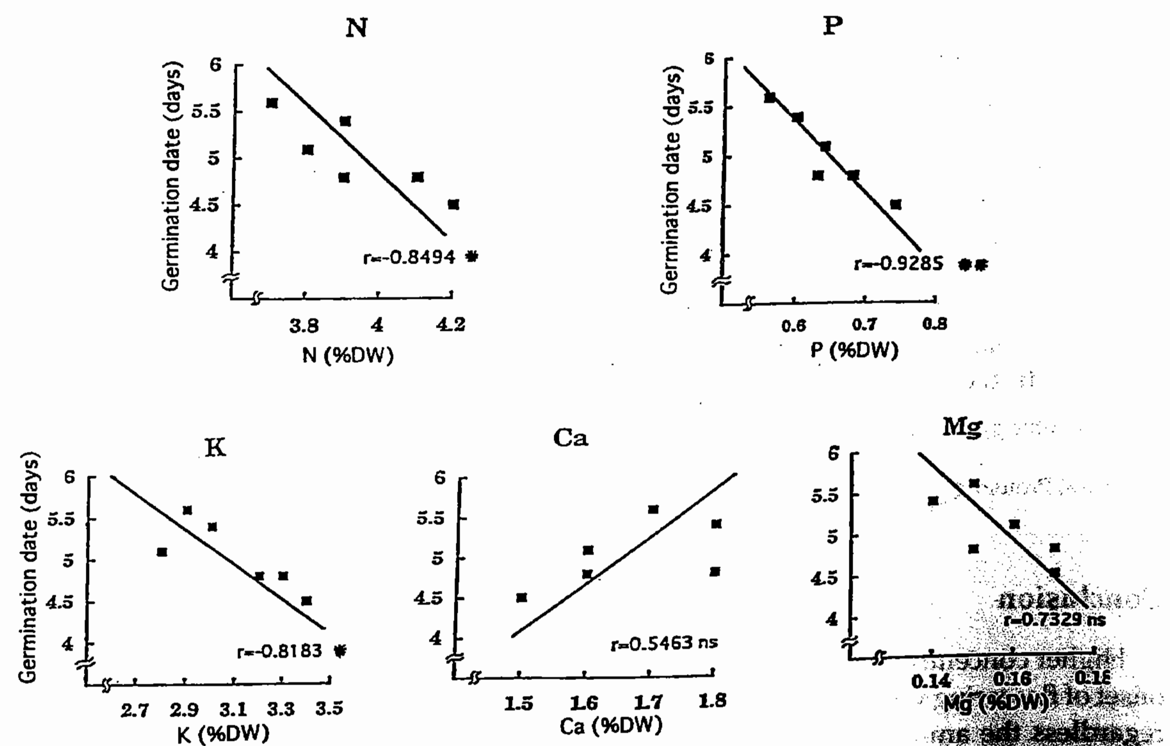

Figure 5. Correlation between mineral nutrients in the seed and average germination date

Figure 6 presented the distribution between protein in high quality seed (early germination date) and low quality seed (late germination date). High quality seed has a higher total protein than that of low quality one, especially on the distribution of soluble protein and cell wall composition protein. Soluble protein is may related to the enzyme protein, peptide, etc that maybe influence to the acceleration of seed germination process. Cell wall composition protein of high quality seed also higher and might have a strong correlation with water imbibition during seed germination process. Mayer (1974), reported if some enzyme systems will become active instantaneously as soon as water is imbibed. This phenomenon was related to the importance of soluble protein content and cell wall composition binding protein in high quality seed on water imbibition and enzymatic activities accelerating germination process.

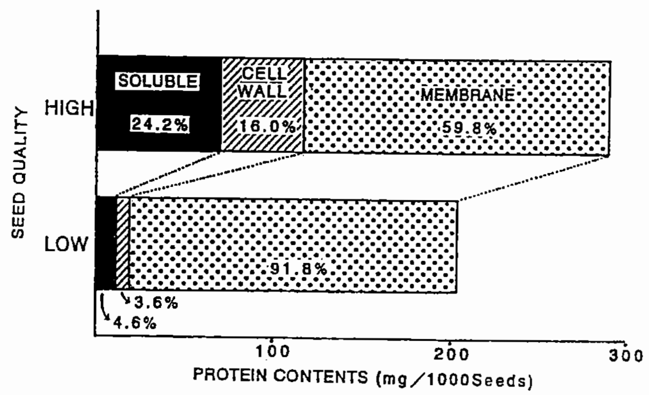

Figure 6. Protein contents in tomato seed tissues from different seed quality.

Notes: Soluble protein; as enzyme, peptide, etc.

Structural Protein → Membrane binding-protein; → Cell wall composition-protein

Conclusion

Higher concentration of N applied at the onset of flowering was increased seed yield, regardless the amount of N application at the early stage of plant growth.

Application of high concentration of P or about 2 times higher than for standard application for crop production, combined with low concentration of Ca produced better seed yield and improved seed quality.

N content in the seed might reflect to the nitrogen compounds constituent in the seed that may influence seed germination process

Acknowledgment

We thank the Director and staff of Nihon Horticultural Production of Japan for parental seed used in this study and Prof. Kazuo Yoneda for valuable suggestions in preparing this manuscript.

References

Al Afifi, *et al.* (1993) Response of processing tomato to N and P application at moderate and low temperature. *Egyptian Journal of Hort.* 18 (1): 45-G2.

Adams, P and Holder, R.(1992) Effect of humidity, Ca and salinity on the accumulation of dry matter and Ca by the leaves and fruit of tomato. *Journal of Hort. Sci.*67(1): 137-142.

A. Liptay (1989) Extraction procedure for optimal tomato seed quality. *Acta Hort.* 253:163-170.

Bando, K (1991) Growing tomatoes in flowing culture solution. *Agric. and Hort.* 66(6) 731-736

Bewley, J. D.and Black, M. (1978) *Physiology and biochemistry of seed in relation to germination*. Vol.I Springer-Verlag, Berlin Heidelberg New York. 306 p.

Bould, C. and Hewitt, E.J (1963) Mineral nutrition of plants in soils and in culture media. *In Plant Physiology*, F.C. Steward (1963) Academic Press, New York, London vol.3, p 15-133.5.

El Behairy, *et al.* (1991) Intermittent circulation for earlier tomato yield under NFT. *Acta Hort.* 287: 267-272.

Hoagland, D.R and D.I. Arnon (1950) The water culture method for growing plant without soil. *Calif. Agric. Exp. Sta Circ.* 347.

Howard, M.R (1981) *Hydroponic Food Production.* Wood Bridge Press Publishing Co. California 327p.

Khan, A.A (1977) *Biology and biochemistry of seed dormancy and germination.* North Holland.447 p.

Kreij, C.D. *et al.* (1992) The incidence of calcium oxalate crystals in fruit walls of tomato as affected by humidity, phosphate and Ca supply. *Journal of Hort. Sci.* 67 (1): 45-50.3.

Leopold, A.C. and Kriedemann P.E. (1975) *Plant Growth and Development.* Mc.Graw Hill Book Co 545 p.

Marschner, H. (1986) *Mineral nutrition of higher plant.* Academic Press. London. 647 p.

________. (1995) *Mineral nutrition of higher plant.* Second Edition, Academic Press. London.889 p.

Matthews, S. (1982) Evaluation of techniques for germination and vigor studies. *Seed Sci. and Technology* 9: 543-551.

Maguire, J.M. (1977) Seed quality and germination. *In The Physiology of seed dormancy and germination.* Edited by Khan, A A, North Holland. p 77-109.

Mayer, A.M. and Shain, Y. (1974) Germination of the seed. Ann. Rev. Plant Physiol. 25, 167-193. *In The Physiology and Biochemistry of seed. Edited by Khan, A.A.,* North Holland.

Mengel, K. and Kirkby, E.A. (1982) Principles of plant nutrition. Third edition. *Int. Potash Inst.* Bern, Switzerland.

Minamide, R.T., Ho, L.C. (1993) Deposition of Ca compounds in tomato fruit in relation to Ca transport. *J. of Hort. Sci.* 68 (5): 755-762.

Powell A.A. (1979) The influence of testa conditionon the imbibition and vigor of pea seeds. *J. of Exp. Botany* 30: 193-197.

Powell, A.A. and Matthews, S. (1981) Evaluation of controlled deterioration a new vigor test for small seeded vegetables. *Seed Sci. and Technology* 12: 649-657.

Quijada, J.S. (1990) Effects of various nitrogen concentrations on growth and development of young tomato plants. *Acta Hort.* 277: 167-177.

Raymond,A.G. (1984) *Vegetable seed production.* Univ. of Bath. London. N.Y.308 p.

Sanches, E.C. *et al.* (1992) Effect of timing of N application on nitrogen partitioning between vegetative, reproductive and structural component of mature *comicee peara. J. of Hort Sci.* 67 (1) 51-58.

Shinohara, S. (1989) Vegetable seed production technology of Japan elucidated with respective variety development histories. Vol. II. *Shinohara's Auth. Agric. Consulting Eng. Office.* 317 p.

Steward, F.C. (1963) *Plant Physiology. A treatise.* Academic Press. N. Y. 811 p.

Varis, S. and Raymond, A.G. (1985) The influence of mineral nutrition on fruit yield, seed yield and quality in tomato. *J. of Hort. Sci.* 60 (3): 373-376.