



GROWTH PERFORMANCE OF VIGNA RADIATA(L.)R.Wilczek UNDER HYDROPONIC SYSTEM

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Abstract

A system was evaluated for growing for plants at reproducible level of different nutrient contents. Green gram (VIGNA RADIATA(L.)R.Wilczek were grown in coir dust at different conc. Nutrient solution using different type of container(Natural system and plastic pots). Germination rate was evaluated after 7th, 14th, and 21st days in Natural system and plastic pots separately. Then shoot length, root length was evaluated 7th, 14th, and 21st days in Natural system and plastic pots. Using of plastic pots in hydroponic system had better result. Conc.II Nutrient solution (medium level nutrient content) using plants had better growth as well as the plats had better nutrient content. Hydroponic system have a better result when compared to natural system.

Key word:hydroponics,substrate,container,solution medium

I. INTRODUCTION

Plant grown in Natural system may have up and shown in natural ecosystem that why the research has confirmed that plants don't need soil to grow into a healthy line specimens. Rather it is the nutrients supplied to them that will determines whether a plant will live or perished. Soil is merely the root support mechanism from which a plant derives a small portion of it's nutritional needs. By growing plants in hydroponic systems that eliminate soil, there is a marked reduction in the amount of pest problem, soil borne diseases, fungus and other issues that plague many former and gardener's today. Soil-free planter reduce the pollution from our living area and provides a superior growing environment for our cherished house plants and tropical.

Nitrogen plays an important role in hydroponics. It is very important to know a crop-specific nutrient requirement. As a general role, vegetable root crops grown in leaf crops. Pottassium level are generally increased for flowering plants. Plants have two methods of uptake. There is a high nutrient mode and a low nutrient mode depend up the maturity of plant species.

Soilless culture techniques were developed under glass houses in order to overcome major agricultural problems such as defecity of nutrition, plant diseases and environmental pollution. The limited water resources and rapid increase in pollution were major factors that drew the attention towards the use of intensive agriculture in desert. In present study by developing simple low cost system for hydroponic was the main challenge to make soilless culture possible in dessert. Maximizing crops yield per square meter of soil as well as per cubic meter of water could be achieved through the use of hydroponic system. Several efforts have been made to introduce the hydroponic technique in dessert started initially in the tourist villages, where the soil is not in suitable container.

II. REVIEW OF LITERATURE

Coconut is grown commercially in Sri Lanka, The Philippines, Indonesia, Southern India and Latin America. These countries are the main source of coir for use in horticulture. Verdonck et al., (1983) were probably the first to mention the horticultural use of coir in the scientific literature. Characteristics of coir dust relevant to its use as a growth medium were recently reviewed by Prasad

(1997). Anatomically, coir is derived from the mesocarp tissue, Or husk, of the coconut (*Cocos nucifera*) fruit. The husk contains 60-70% pith tissue and the remainder is mainly fibre. The long fibres are used for various industrial purposes. The remaining material can be shredded and separated into a coarse fraction (fibre) and a fine one (dust). Of the two, dust is more stable, while the fibres tend to undergo secondary decomposition in the growth medium.

Production of both fractions involves a period of storage in heaps where they undergo aerobic composting during composting the hemicelluloses, cellulose and to a lesser extent the lignin components are decomposed, causing the C/N ratio to decrease (Yau and Murphy, 2000). These changes are accompanied by an increase in CEC and humic acid content (Yau and Murphy, 2000). Composting also results in modified physical properties: TP, EAW and WBC increase and AFP decrease (Mbab and Odili, 1998).

After composting, the stable material is dehydrated and compressed into a compact form (bricks) for easy transportation. With the addition of water, coir expands to 5 to 9 times its compressed volume. It is noteworthy that the coir is not a uniform material. Different sources and different production procedures result in a large variability of end products. (Evans et al., 1996; Prasad, 1997; Konduru et al., 1999).

III. MATERIALS AND METHODS

In water culture, plants are grown with roots submerged in a nutrient solution, with the stem and upper parts of the plants held above the solution. With this system, the main considerations are: provision of a suitable container, suspension of the plants above the water, provision of a suitable nutrient solution, and proper aeration of the water solution.

3.1 Application (Coir dust as potting medium)

Coir can serve either as a stand-alone medium or as an ingredient in a mix for the cultivation of vegetables and cut flowers, as well as for potted plants, tree saplings and young foliage plants. It is now widely accepted as a peat substitute, showing growth results comparable to that of peat moss (Meerow, 1994; Evans and Iles, 1997). Coir can also serve as a rooting medium for cuttings under mist or in high humidity chambers (Lokesha et al., 1988). Coir dust has been claimed to enhance rooting due to the presence of root-promoting substances. This is supported by chemical analysis, which reveals that cell wall polysaccharides of coconut coir dust are substituted with hydroxybenzoic acids (Suzuki et al., 1998), known in the literature as rooting cofactors.

3.2 Physical characteristics

Coir dust is a lightweight material, having a BD of 0.04-0.08 g cm⁻³ (Evans et al., 1996). The TP of coir dust is in the range of 86-94% and AFP 9-14%. Coir fibre has distinctly different characteristics, with TP around 98% and AFP around 70% (Lemaire et al., 1998). Coir dust is characterized by a relatively high EAW 35% (Prasad, 1997), which resulted in superior growth of foliage plants compared with peat (Stamps and Evans, 1997). Prasad (1997) suggested that predetermined levels of AFP and EAW could be obtained by mixing different proportions of coir dust and fibre. Unsaturated hydraulic conductivity of coir is quite high, which results in high water availability and improved yield of roses, as compared to UC mix (Raviv et al., 2001). A distinct positive property of coir dust is its relatively high elasticity and resistance to compression and height loss over time, as compared to other organic media, such as white and sphagnum peat and wood chips (Wever and van Leeuwen, 1995; Agro and Biernbaum, 1996).

3.3 Chemical characteristics

Crude coir is rich in Na and Cl, which may damage plants. During the production stage, it has to be washed, and usually Ca and Mg are added to facilitate Na removal and to provide nutrients. On the other hand, the content of P and K in coir is very high, which should be taken into account in any fertilization programme. Cation exchange capacity ranged from 320 to 950 mmolc kg⁻¹ and C/N ratio averaged 117 (Noguera et al., 2000; Evans et al., 1996). Using the Nitrogen Drawdown Index

(NDI), Handreck (1993) showed slight N immobilization in coir dust, but not in coir-based media with a conventional fertilization programme (Noguera et al., 2000).

3.4 Sterilization and waste disposal

Sterilization of coir is possible. However, some loss of elasticity may occur. Also, being an organic, non-sterile medium there is a risk of creating a biological vacuum which may permit the rapid development of pathogenic fungi after sterilization. Since it is biodegradable, natural and non-toxic, coir may be disposed without problem. Because of the above reasons coir dust was used in the present investigation.

IV. RESULTS

By comparing of five growing media for characteristic and tomato yield potential, were coconut coir one of the media in its, overview of this results that coconut coir treated plant showed significant results in growth parameter and its yield. This significant results were on par with (Table 1, 2 & 3.) Merle H. Jensen, Patricia A. Rorabaugh and Marcos Garcia A, 2010.

The entire crop could be grown in a nutrients solution container with a single filling and every one can practice this technique with little care to produce fresh vegetables in his court yard, veranda or on a roof top with out using soil as a growing medium (Imai 1987 and Kratky 2002).

By optimizing the concentration of nutrient solution for hydroponic culture of leafy vegetables, were the growth of lettuce plants grown in reducing concentration of nutrient solution. Without loss of growth. (Goto, F; Johkan, M; Hashida, S; Yoshihara, T; Shoji, K 2010.)

A highly demanded medicinal value plant of noni (*morinda citrifolia*) was grown in hydroponic methods which has using of ten different types of substrate. The coconut husk powder also including substrate from this. Therefore, the compounds based on crop residues and animals manure, compained with coconut husk powder has a great potential to become an alternative for noni seedlings production. (Souise, J.A.; Silva neto, P.A.F.S; Ferreira, F.V.M; Arauja, D.B; Sousa, J.C.R. Silva, T.C; Bezerra, F.C....year). Similar to there works, the present study results are become evidence and supportive reference for further works Coir dust/coir pith is the agricultural remaining waste product when long fibers are extracted from coconut husk (*cocos nucifera*. L). The coir constitutes the short fibers and mesocarp pith of coconut (Ma and Nichols, 2004). Coir originates primarily from Sri lanks, India Philippines, Indonesia, Mexico, Costa Rica and Guyana (Evans and Stamps, 1996) and is shipped to the U.S in large bulk bales. Coir dust has many desirable substrate characteristics such as high water holding capacity; excellent drainage; absence of weeds and pathogens; physically resilient; slow decomposition; pH between 6.8 to 7.2, CEC and EC; easily wetttable, and a renewable resource without knowing ecological drawbacks (Abad, et al, 2005; cresswell, 1992); Lennartsson , 1997; Martinez et al., 1997; Pill and Ridley, 1998; Verdonck et al., 1983). Due to these favourable characteristic, coir has been extensively used as an environmentally safe substitute for natural peat in container as consider as substrates.

Researcher have evaluated the potential use of the coir pith as plant growth substrate (or) growth substrate component with successful results growing annual feeding plants (Awang and Ismail, 1997); Evans and stamps, 1996; Handreck, 1993) herbaceous perennials (Pill and Redley, 1998) foliage plants (Stamps and Evans, 1999) vegetables (Cresswell, 1992) and woody plants (Hernandez-Apaolaza et al, 2005) with the success and stability of coir as an organic container substrate it has become a commercially popular material that is now being used around the world as a peat substitute for container- grown crops (Abad et al., 2005; Handreck, 1993c; Noguera et al., 1997). Coir remains a heavily used alternative to peat moss as a substrate but due to the distances it has to be shipped, transporation costs are also becoming a factor in its economical use as a substrate.

V. POTTING MEDIUM

Merle H. Jensen, Patrica A. Rorabaugh and Marcos Garcia A (Tomato) Were grown tomato in five different growth media for physical characteristic and tomato yield potential. In physical characteristic the water holding capacity and air porosity varies a great deal between media but there

was no significant difference in marketable tomato yields. The cost difference between media is great, with coconut coir and perlite far less costly than rockwool and any media containing peat moss.

Commercial hydroponics is a modern technology involving plant growth on inert media in place of the natural soil, in order to uncouple the performance of the crop from problems associated with the ground, such as soil-borne diseases, non arable soil, poor physical properties, etc. Various non-toxic porous materials are used as plant growth substrates, including rockwool, perlite, pumice, expanded clay, various volcanic materials, polyurethane foam, coir dust, etc. A balanced distribution of small and larger pores is required in a substrate to ensure adequate availability of water to the plants without to affect the supply of oxygen to the roots. Hydroponics has no adverse effect on the quality of fruits and flowers produced in such systems. In contrast, the complete control of nutrition via the nutrient solution may enable an enhancement of product quality, particularly in vegetable crops, such as tomato, melon, and lettuce. The switching over from the soil to hydroponics results in a decreased application of pesticides and other toxic agrochemicals, which are necessary in soil-grown crops to disinfect the soil and to control soil-borne pathogens. Moreover, the recycling of the excess nutrient solution that drains off after each watering application may contribute to a considerable reduction of nitrate and phosphate leaching to surface- and groundwater resources. To restrict costs and increase profitability, hydroponics is increasingly based on automation of nutrient and water supply. Future developments in hydroponics are mainly focused on further automation of the nutrient solution management, particularly in closed systems in which the excess nutrient solution is recycled, as well as on a complete standardization of the substrate analysis in order to obtain more reliable results and to facilitate their interpretation. Table 1

The growth of the greenhouse tomato industry is rapidly on the increase in North America. Rockwool and perlite are the most popular growing media. Rockwool is expensive and substitute media containing peat moss are also expensive, especially if transported long distances to the southwestern part of the United States and to Mexico. Coconut coir is abundant in Mexico and of low cost. There is evidence that this medium could be a substitute for peat. This study was to investigate the physical characteristics of five media and the yield response of tomatoes produced in a greenhouse. The water holding capacity and air porosity varies a great deal between media but there was no significant difference in marketable tomato yields. The cost difference between media is great, with coconut coir and perlite far less costly than rockwool and any media containing peat moss. Table 2

In the Netherlands reuse of drainage water is obligatory for all soil less growing systems, to reduce the environmental pollution. However, this strategy has some important bottlenecks. Apart from technical and phytopathogenic aspects, accumulation of Na, Cl or other residual ions could be a problem. Accumulation will occur if the uptake rate is lower than the concentrations in the input. In the recent past a database was established with the maximum acceptable concentrations in the root environment and water sources, for a number of crops. In all cases Na showed to be the bottleneck element. A high tolerance for Na not necessarily means a high uptake rate for this ion, as is found for sweet pepper. Water sources differ highly in Na concentrations. In general, for completely closed growing systems only rainwater or desalinated water is suitable. For some crops (rose, chrysanthemum, sweet pepper) the natural background concentrations of Na in rainwater is sometimes even higher than the average uptake rate. Accumulation above the maximum acceptable concentrations inevitably should be followed by discharge of a fraction of the nutrient solution to prevent yield reduction or decline in produce quality. This will result in lower water and nutrient use efficiencies than required. However, the losses depend highly on the water management strategies accomplished by the grower. Smart strategies are developed for discharge of drainage water as low as possible for N and P. These are based on the uptake dynamics of Na and Cl and minimal required N and P levels observed with different crops. These strategies have been tested for rose and resulted in significant reduction in the nutrient losses. Table 3

The aim of the experiment was to extend the recirculation of nutrient solution in soilless closed system adopting a different concentration of nutrients. Pepper plants were grown in perlite using a closed system. Two nutrient solutions characterised by the same ion ratio, but by a nutrient concentration equal to 100 or 60% were adopted. The total substitutions of nutrient solution were carried out when the EC of recirculating solution increased of 2 dS.m⁻¹ compared with the initial EC. The total yield did not differ between the treatments; however the lower concentration of nutrient determined a significant reduction of incidence of unmarketable fruits (BER) thus a higher marketable productions (+13%). Within the fruit quality characteristics only the dry matter content was significantly higher adopting the full strength nutrient solution. Considering the water use efficiency from a biological point of view, no difference was observed between the treatments. Important differences were found when the agronomic water use efficiency was considered: the kg of marketable pepper produced per m³ of water input was about 30% higher using the reduced concentration treatment according to the lower volume of water released in the environment due to the lower renewal of recirculated nutrient solution. Similar pattern was observed for the use efficiency of main nutrients. Table 4

The results did not reveal any negative effect of biochar on crop production, which indicates a potential as an excellent alternative to the current commercially available growing media in soilless production. Biochar was also tested in an aquaponics system, Biochar with its remarkable stability has an ideal combination of characteristics as both a substrate and water filtration medium in biological productions systems such as aquaponics. Biochar has the added advantage of being carbon neutral, and the potential of being re-useable by further pyrolysis, which will kill any organisms in the medium. Particle size (and particle size distribution) of the biochar are two factors which may require further study. Table 5

Leafy vegetables such as lettuce are produced hydroponically using the Deep Flow Technique (DFT) and the Nutrient Film Technique (NFT) in Japan. A comparatively high density solution is often used to produce vegetables stably and consistently (EC=1.2-3 dS/m). In these cultivation methods, the nutrient solution is changed, when the control of EC and pH becomes difficult or after the harvest of vegetables. The consequent waste water may still have significant concentrations of one or more nutrients and thus presents an environmental hazard or disposal. In this study, we measured EC, PH, and varied the concentrations of Ca, Mg, K, PO₄, NO₃, NH₄ in the nutrient solution, while monitoring the growth of lettuce plants to determine the minimum concentration of each element for optimal growth. Lettuce plants grown in the nutrient solution diluted from 0.2 to 1.0 times (EC = approx. 1.3 dS/m) were harvested approximately 21 days after transplanting to the DFT system. There was little or no difference of the growth or in the amount of absorbed fertilizer among the plants grown in the solutions diluted 0.6, 0.8 and 1.0 times. The plants grown in solutions diluted at 0.4 times and below grew somewhat small and K and PO₄ were almost completely absorbed. No NH₄ remained in the nutrient solution, irrespective of the dilution. The next experiment was carried out to test the hypothesis that fortification of the diluted solution with K and PO₄ leads normal plant growth. The growth of lettuce plants grown in the solution diluted 0.4 times, supplemented with K and PO₄ was comparable to that grown in the regular solution, indicating the possibility of reducing the concentration of the nutrient solution without loss of growth. Table 6

A significant difference for all variables was detected, and the highest values were observed for substrates S2 and S5. Therefore, the compounds based on crop residues and animal manure, combined with coconut husk powder, has a great potential to become an alternative for noni seedlings production. Substrates with best results for noni seedlings production were: S2 = compound 1 [+ CEASA remains of fresh cattle manure (3:1)] + coconut powder (1:2, v / v) and S5 = compound 2 [+ CEASA remains of poultry litter (3:1)] + coconut powder (1:2, v / v). Table 7

T₁ a T₁ b T₁ c T₁ d T₂ a T₂ b T₂ c T₂ d



T₁ - Natural system

T₂ - plastic pots

T₁ a - Concentration I (Coir dust 150 g)
 T₁ b - Concentration II (Coir dust 150 g)
 T₁ c - Concentration III (Coir dust 150 g)
 T₁ d - Control (Soil + Sand, 300 g +100 g)

T₂ a - Concentration I (Coir dust 150 g)
 T₂ b - Concentration II (Coir dust 150 g)
 T₂ c - Concentration III (Coir dust 150 g)
 T₂ d - Control (Soil + Sand, 300 g +100 g)

TABLE: 1 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE GERMINATION PERCENTAGE OF THE SEEDS OF *vigna radiata* (L.) R.wilczek

S.NO		GERMINATION RATE (%)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	60	92	92	76	86	88
2	CONC.I	40	64	66	48	72	74
3	CONC.II	32	86	88	40	80	80
4	CONC.III	64	90	93	60	96	96

TABLE: 2 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE SEEDLING GROWTH(SHOOT LENGTH) OF *vigna radiata* (L.) R.wilczek

S.NO		SHOOT LENGTH (cms)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	7.5	12.5	19.0	6.5	11.8	18.0
2	CONC.I	5.3	10.0	15.0	4.2	8.2	15.2
3	CONC.II	6.2	11.5	16.0	5.8	11.5	17.0
4	CONC.III	9.3	13.0	20.0	8.1	14.9	21.6

TABLE: 3 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE SEEDLING GROWTH(ROOT LENGTH) OF *vigna radiata* (L.) R.wilczek

S.NO		ROOT LENGTH (cms)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	1.0	1.2	1.6	1.3	1.6	1.7
2	CONC.I	2.5	2.8	3.9	3.4	4.5	7.2
3	CONC.II	4.0	4.6	4.9	3.2	4.4	6.0
4	CONC.III	2.2	3.8	4.6	3.5	4.5	5.4

VI. BIO CHEMICAL TEST

CARBOHYDRATE TEST:

In mud pot, highest carbohydrate rate was noted in conc.III. In plastic pot highest carbohydrate rate was noted in conc.II. Among these highest carbohydrate rate was noted in conc.II in plastic pot.[TABLE: 4]

PROTEIN TEST:

In mud pot, highest protein rate was noted in conc. II. In plastic pot highest protein rate was noted in conc.II. Among these highest protein rate was noted in conc. II in plastic pot.[TABLE: 5].

CHLOROPHYLL TEST:

In mud pot, highest chlorophyll rate was noted in conc. II. In plastic pot highest chlorophyll rate was noted in conc. II. Among these highest chlorophyll rate was noted in conc. II in plastic pot.[TABLE: 6]

TABLE: 4 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE CARBOHYDRATE CONTENT OF THE SEEDLING OF *vigna radiata* (L.) R.wilczek

S.NO		CARBOHYDRATE CONTENT (mg\g)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	0.86	1.02	1.30	0.81	1.05	1.32
2	CONC.I	0.87	1.10	1.42	0.84	1.15	1.43
3	CONC.II	0.88	1.25	1.45	0.85	1.25	1.47
4	CONC.III	0.82	1.22	1.40	0.83	1.26	1.35

TABLE: 5 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE PROTEIN CONTENT OF THE SEEDLING OF *vigna radiata* (L.) R.wilczek

S.NO		PROTEIN CONTENT (mg\g)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	1.20	1.35	1.46	1.10	1.36	1.50
2	CONC.I	1.22	1.30	1.42	1.28	1.37	1.48
3	CONC.II	1.30	1.42	1.51	1.32	1.50	1.62
4	CONC.III	1.25	1.36	1.48	1.20	1.35	1.50

TABLE: 6 EFFECT OF HYDROPONIC NUTRIENT CONCENTRATION ON THE CHLOROPHYLL CONTENT OF THE SEEDLING OF *vigna radiata* (L.) R.wilczek

S.NO		CHLOROPHYLL CONTENT (mg\g)					
		MUD POT			PLASTIC POT		
		7 TH DAY	14 TH DAY	21 ST DAY	7 TH DAY	14 TH DAY	21 ST DAY
1	CONTROL	0.0557	0.2608	0.3611	0.0431	0.2576	0.3657
2	CONC.I	0.0615	0.2673	0.3571	0.0512	0.2688	0.3791
3	CONC.II	0.0675	0.2946	0.3781	0.0602	0.2983	0.4076
4	CONC.III	0.0640	0.2561	0.3679	0.0529	0.2722	0.3557

VII. DISCUSSION

Were nutrient solution which is recycled through a hydroponic set-up must be renewed on average every 14th days replacement of nutrient supplements were done. The nutrient depletion duration of a solution varies with the nutrient demand of the plants. A solution feeding a bed of seedling may have a practical life of 3-4 weeks because of the small nutrient usage. By the time the plants reaches fully maturity and maximum productivity potential, the solution may need renewing every 4-7 days. In all hydroponic system it is important that water loss from solution by evaporation

and/or plant transpiration is replaced daily. During hot weather, water usage is extremely high and, if not replaced daily, the volume of the remaining the moisture solution may become concentrated, and in extreme situation could induce a temporary salinity problem and adversely affect plant growth. All hydroponic units using a growing media need to be flushed out with water every so often (1-4 week). This practice prevents the possible build up the concentration of certain salts in the medium to toxic levels. The changing of the old nutrients also removes root exudates do not build up. Fresh water also helps prevent harmful fungi; from growing on the plant roots. And the maximum range of p H values in nutrient solution is from 5 to 8. Plants grown beyond either end of the range make poor growth and may have nutritional problem. (Dimitrios savvas, 2002)

Were nutrient solution can be mixed in small container and added as needed. At the beginning, the container and is filled with solution almost to the level of the litter. Then, at predetermined intervals, the old solution is thrown out and new solution added. The frequency and number of the solution will depend on the size of the container. Just as a starting point for beginners, solution changes might be made at weekly intervals for the first four weeks, then twice weekly for matured plants, should the water level get too low between changes, add only water until time to change solution.(Dimitrios savvas, 2002)

Adjustment of the acidity or alkalinity (measured as p H) of water may be necessary to keep it within an adequate plant growing range of p H 6.0-6.5. this means testing with indicator paper and adding 1 normality sulfuric acid, if needed, to lower p H or an alkaline material such as sodium hydroxide to raise p H.

Moreover, the recycling of the excess nutrient solution that drain off after each watering application may contribute to considerable reduction of nitrate and phosphate leaching to surface- and ground water resources. To restrict cost and increase profitability, hydroponics is increasingly based on automation of nutrient and water supply. Future developments in hydroponics are mainly focused on further automation of the nutrient solution management, particularly in closed system in which the excess nutrient solution is recycled,as well as on a complete standardization of the substrate analysis in order to obtain more reliable results and to facilitate their interpretation.(Giuffrida, F.Leonardi, C.2012)

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