Original Article

Calcium, potassium and nitrate in plant nutrient solutions by measuring of with ion-selective electrodes in hydroponic green house of some vegetables

Running title: Plant development tracked with biosensors

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Abstract

Life cycle of plants in a healthy way depends on being taken essential plant nutrients in a balanced manner and toxic elements under a certain concentration. Lack of control of nutrient levels in nutrient solution can result in reduced plant growth levels and undesired conditions such as blossom-end rot. In this study, sensitivity and selectivity tests for various PVC based ion-selective membranes were conducted to identify those suitable for measuring typical concentration ranges of macronutrients, i.e., NO^{3-}, K^{+} and Ca^{2+}, in hydroponic solutions. The sensitivity and selectivity of PVC membrane based ion-selective sensors prepared with tetradodecylammoniumnitrate for NO^{3-}, valinomycin for K^{+} and Ca ionophore IV for Ca^{2+} were found satisfactory for measuring NO^{3-}, K^{+}, and Ca^{2+} ions in nutrient solutions over typical ranges of hydroponic concentrations. Potassium, calcium, and nitrate levels that were utilized by cucumber and tomato seedlings in the greenhouse were different. The findings show that tomato plant was consumed less amounts of nitrate than cucumber plant over the first two months period of growing. We also found that the potassium intake was more over other nutritional elements tested for all plants.

Keywords: Plant, nutrient elements, hydrophobic system, chemical, biosensor

1. Introduction

In recent years, hydroponic plant growing systems increased attention in the industrial world especially in the world's arid and semi-arid regions of safe food supply for the growing population, and developed regions are gaining off-season vegetables, like cucumber and tomato [1]. In protected cultivation, the adoption of soilless culture with technical practices together such as integrated plant protection, fertigation, drip irrigation and climate control increases the yield potential and water-use efficiency [2], while decreasing adverse effects of agro-chemicals on environment [3-5].

Many greenhouse operators now employ hydroponics, where plants are grown in a soilless or inert support substrate with the vast majority of nutritional requirements supplied via the irrigation water. Hydroponic crop production systems allow for much tighter control over nutrient inputs than soil-based systems [6]. Since the reserves of nutrients for the plants are limited in hydroponic greenhouse cultivation, an inaccurately balanced nutrient solution may result in an unbalanced nutrient composition in the root environment [7]. In closed hydroponic systems that reuse the drainage solution, it is important to determine the nutrient concentrations in the reused solution to regenerate a nutrient solution of optimal composition [8].

Studies over the last few decades have therefore focused on the development and rehabilitation of new or readily available systems especially which aim to provide more water and nutrient saving, increase yield and decrease waste nutrients [9]. Plant growth can be influenced by the amount of any one nutrient element. So that, in the hydroponic system to control nutrients with sensors is important for effective plant growth. The need for such fast and continuous monitoring has led to the application of ion-selective electrode (ISE) technology to measure hydroponic macronutrients [8,10,11].

In this study, NO^{3-}, K^{+}, and Ca^{2+} concentrations in hydroponic nutrient solutions was simultaneously and directly measured by potentiometric sensor array for cucumber and tomato vegetables those were growth in hydrophobic green house. Optimization of NO^{3-}, K^{+}, and Ca^{2+} concentrations in nutrient solution were also tried to reuse of the nutrients by measuring such ions in the drainage using the sensor array. Overall we have produced vegetables in the greenhouse without disadvantages arising from soilless culture techniques, by establishing a plant feed medium combined with the organic farming rules.. Healthy g rowing tomato and cucumber plants can be achieved with administration plant nutrients in a controlled manner and faster.

2. Materials and Methods

2.1. Chemicals

Ionophores for K^+, Ca^{2+} and NO^{3-}, polyvinylchloride (PVC), dioctylsebacate (DOS), potassium tetrakischloroaphenylborate (KTpClPB), nitrophenyloctylether (NPOE), tetradodecylammonium nitrate (TDA-NO_3), dibuthylphthalate (DBF), tetrahydofuran (THF), graphite, epoxy and all other salts used for the sensor performance tests were of analytical grade and provided from Sigma-Aldrich. Commercial grade forms of potassium nitrate, calcium nitrate potassium phosphate, magnesium sulfate iron(II)nitrate, manganese(II)chloride, copper(II)nitrate, zinc(II)nitrate and sodiumborate as essential macro and micronutrients provided from different local markets were used for preparation of the nutrient solutions of the vegetables in the green house. The seedlings were purchased commercially from the local sellers in Turkey.

2.2. Apparatus

Potentiometric measurements were carried out at room temperature (25±1 ^oC) by using a laboratory-made computer-controlled high-input impedance multi-channel potentiometric measurement system. Throughout the measurements, a micro-sized solid silver / silver chloride electrode was used as reference electrode (RE) with the ion-selective electrodes. The reference electrode was obtained from BiomArge. Ltd. Sti. (Istanbul, Turkey). To investigate the potentiometric characteristics of the prepared sensors, the emf measurements were taken in the following cell assembly:

Micro sized solid silver / silver chloride RE │ test solution │ sensor membrane / ion-selective electrode│ all solid state contact material │ Cu wire

The steady-state measurements were taken while stirring at a constant rate by immersing the sensor and reference electrodes to the same depth in 10 mL of tested solution. Reference electrode and sensor were washed with deionized water and dried with adsorbent tissue before each measurement of test solution.

The pH of the buffer solutions was adjusted by using a glass pH electrode (Russell) with a Jenway 3040 model Ion Analyser. Solutions at required concentrations were homogenized using Ultrasonic LC30 (Germany) stirrer.

A high technological greenhouse which is located at the Yıldız Technical University campus was applied. It has plastic reservoirs and fully otomated environment to carry out continuous-flow solution culture that does not use a solid medium for the roots. The temperature, humidity, and pH level of the greenhouse was tightly controlled and monitored constantly for maximum efficiency. A shallow stream of water containing all the dissolved nutrients required for plant growth was constantly flowed at almost 1 LMin-1 flow-rate and recirculated within the plastic reservoirs past the bare roots of plants. A picture of the greenhouse applied in the present study is given in Figure 1.

2.3. Preparation of NO_3, K, Ca ion-selective electrodes

For the preparation of the all solid state PVC membrane sensors, the similar steps described in our previous works were followed [12,13].

An interface (solid contact) was used for holding PVC membranes on metal surface and coherent membrane to prepare PVC membrane sensors. Mixture of conductive material consisting of 50% (w/w) graphite, 35% (w/w) epoxy and 15% (w/w) hardener was prepared by solving in sufficient THF. The sandpapered tip of the copper wire was dipped into conductive material mixture for a few times to obtain a solid-state contact with coating thickness about 1 mm. The surface of the conductive materials obtained at the first step coated with the membrane by dipping into the cocktails of nitrate (NO^{3-}), potassium (K^+), and calcium (Ca^{2+}) in THF for 1-3 times to get membrane thickness about 0.1 mm. The membrane compositions of the sensors prepared are summarized in Table 1. The coated electrode surface was left to be dried at laboratory conditions for 12 hours. The dried micro sized membrane sensors were conditioned by soaking in 10^{-1} M main ion solutions of each ion for at least 6 hours.

2.4. Preparation of solutions

The potentiometric performance characteristics of the sensors were characterized by measuring them in standard solutions of main ions with concentrations ranging from 10^{-5} to 10^{-1} M. Distilled deionized water was used in the preparation of the aqueous solutions. 10^{-1} M stock solutions were prepared for each ion. The diluted solutions were prepared from ion stock solution by using deionized water in sufficient amounts. Standard KNO_3 and $CaCl_2$ solutions in the range of 10^{-1} - 10^{-5} M were used for calibration of K^+, NO_3^- and Ca^{2+} sensors.

3. Results and Discussion

3.1. Potentiometric measurement of electrodes

The potentiometric performance characteristics of the all solid-state ion-selective membrane sensors are summarized in Table 2. From the potentiometric performance, it can be seen that the sensors had a near-Nernstian responses in the concentration range of 0. 1-100 mM ($R^{(2)}$ = 0.9981 for K^+, 0.9981 for NO_3^-- and 0.9981 for Ca^{2+}). The NO_3^--selective sensor exhibited a detection limit of $5x10^{-6}$ M with a 41±7 mV slope between $1x10^{-1}$ and $1x10^{-5}$ M concentration levels of nitrate. The K^+-selective sensor exhibited almost a 44±2 mV slope between $1x10^{-1}$ and $5x10^{-5}$ M concentration levels of potassium and a detection limit of $4x10^{-5}$ M. The Ca^{2+}-selective sensor exhibited almost a 25±6 mV slope between $1x10^{-1}$ and $1x10^{-5}$ M concentration levels of nitrate and detection limit of $1x10^{-6}$ M. To simulate the application of the ion-selective sensor to a real sample, the selectivity of the sensor membrane to entities other has to be considered. Therefore, the interference effects from different ions were studied. However the selectivity coefficients of the sensors evaluated by the separate solution method according to the IUPAC recommendation [14] are shown in Table 2.

The sensors exhibited a reasonable response time of 15-30 s, working pH range of 5.0-9.0 for K^+-selective sensor, 2.0-7.0 for NO_3^--selective sensor and 3.0-7.0 for Ca^{2+}-selective sensor, and lifetime of 30 d in daily use. With respect to potentiometric sensitivity, selectivity, pH range and response time, all the sensors performances can be decided useful for the detection of nutrients in the green house solution

3.2. Determination of plant nutrients

The sensors calibrated with standard solutions were then applied to measure nutrients in the green house solution. Nutrient solutions used in this study were obtained from "Biotechnology Greenhouse", which is located in Yıldız Technical University. Plant beds water nutrient levels were detected with micro-sized chemical sensors and controlled with a computer controlled potentiometer for continuous measurements. The pH of the nutrient solutions were monitored daily and maintained between 5.5 and 6.5. Potassium, calcium, and nitrate levels that were utilized by cucumber and tomato seedlings in the greenhouse are summarized in Table 3.

Nitrate is an important macronutrient and also serves as an important signal for growth as plants respond to nitrate by altering their metabolism and by inducing genes in the nitrate assimilation pathway. Variation in soil nitrate levels affects the rate of plant growth and the concentration of nitrate stored in plants [15].

Results obtained show that the nitrate amounts which used by plants is vary for each type of the vegetables for each new additional week (Fig.2). Similar amount of nitrate was used by cucumber and tomato. Tomato was consumed less amount of nitrate than cucumber (Fig.2). Although tomato used same amount of nitrate for first two weeks. After first two weeks, nitrate amount used by tomato had increased in small amounts. So that, changes in the amount of nitrate was based on plants nitrate requirements and amount of the nitrate consumptions.

Potassium is an essential plant nutrient for plant growth and the most abundant cation in plants, constituting up to 10% of plant dry matter [16]. Together with nitrogen (N) and phosphorous (P), K^+ belongs to the top three elements, the availability of which strongly determines crop yield.

In our study we found that the potassium more obtained than other nutritional elements by plants. Study shows the potassium content which used by vegetables was different for cucumber and tomato (Fig. 2). When we look at the consumed content of potassium for these two vegetables; cucumbers constitute more potassium than tomato. The potassium amount was used by cucumber for first 4 weeks showed a rapid increase; but in between sixth and eighth weeks was increased constantly (Fig. 2).

Calcium is a macronutrient ion and its concentration within hydroponic nutrient solution can significantly influence plant growth. Insufficient and excess calcium concentrations in the nutrient solution can result in reduced plant growth. Calcium can constitute more than 10% of dry weight of mature leaves [17]. Our experimental data for the consumed calcium showed that the same amounts of calcium were taken by cucumber and tomato (Fig. 2).

Finally, compared to the structural characteristics of tomatoes with the cucumber, tomato found grow better and easier than cucumber in a short time. So that, tomato needs least amount of the nutrients and grow faster than cucumber.

4. Conclusions

In this study, sensitivity and selectivity tests for various PVC based ion-selective membranes were conducted to identify those suitable for measuring typical concentration ranges of macronutrients, i.e., NO^{3-}, K^+ and Ca^{2+} in hydroponic solutions. The sensitivity and selectivity of PVC membrane based ion-selective sensors with TDA for NO^{3-}, K ionophore I for K^+ and Ca^{2+} ionophore IV for Ca^{2+} were satisfactory for measuring NO^{3-}, K^+, and Ca^{2+} ions in nutrient solutions over typical ranges of hydroponic concentrations.

This study produced vegetables in greenhouses without problems arising from the soil and minimizes the disadvantages of soilless culture techniques combined with the continuous measurement system consisted of ion-selective sensors. Growing plants can efficiently be achieved by plant nutrients in a controlled manner and faster with administration. There are also similar sensing devices for macro and micronutrients commercially available of those could be useful for the follow up the nutrient solution continuously, and application to the hydroponic greenhouse system to increase the growth, quality and yield of the tomato plant.

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Table 1. Cation and anion-selective membrane compositions of the sensors

Sensor	**Ionophore (w/w)**	**Polymer (w/w)**	**Plasticizer (w/w)**	**Ionicity (w/w)**
K^+	K ionophore I (% 1)	PVC (% 33.5)	DOS (% 64)	KTpClPB (% 0.5)
Ca^{2+}	Ca ionophore IV (%2)	PVC (% 29)	NPOE (% 68)	KTpClPB (% 1)
NO_3^-	TDA-NO_3 (% 4)	PVC (% 30)	DBF (% 65)	KTpClPB (% 1)

Table 2. Potentiometric performances of the sensors tested under standard conditions

Sensor	Linear range, mol.L^{-1}	Selectivity $-\log K_{K^+,B^{n+}}$	Response time for 10^{-2} mol.L^{-1}, s	Detection limit, mol.L^{-1}	Lifetime, week	pH working range
K^+ selective	$5x10^{-5} – 10^{-1}$	Li^+:3.89 Mg^{2+}:6.83 Na^+:4.11 Ba^{2+}:4.98 NH_4^+:2.79 Sr^{2+}:6.01 Ca^{2+}:4.97	< 15	$4x10^{-5}$	10	5-9
NO_3^- selective	$10^{-6} – 10^{-1}$	SO_4^{2-}:2 I^-:1.84 Br^-:1 SCN^-:2.67 Cl^-:2.7 F^-:3.3	< 5	$5x10^{-7}$	18	2-7
Ca^{2+} selective	$10^{-5} – 10^{-1}$	Li^+:4.2 Mg^{2+}:5.6 Na^+:3.2 Ba^{2+}:4.98 NH_4^+:4.2 K^+:4.2	< 10	$1x10^{-6}$	10	3-7

Table 3. Nitrate, potassium and calcium levels

Crop (a plant way)	NO^{3-} (M)	K^{+} (M)	Ca^{2+} (M)
Cucumber	0.020	0.06	0.02
Tomato	0.014	0.02	0.01

Figure 1. The hydroponic greenhouse house control systems and the growing medium where the sensor were applied for the follow up of nutrients.

Figure 2. Mineral consumption of both plants in nutrition reservoirs. Experimental conditions were 25 ^{o}C and pH 7.0. Each data point represents the average value of three independent experiments.