IN-PLANTA NITRATE DETECTION USING INSERTABLE PLANT MICROSENSOR

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ABSTRACT

This paper reports a nutrient microsensor for in-situ detection of nitrate concentration inside plants. The sensor is inserted into the stalk of maize plant for continuous monitoring of dynamic nitrate uptake of the plant. The inserted part of the sensor consists of a nitrate sensing unit that works on the principle of chemical sensitive field effect transistor (chemFET), an integrated metallic thin-film thermorist, and a microscale reference electrode. The sensor enables measurement of nitrate concentration variations under different environmental conditions (e.g., light condition) and irrigation and fertigtation management. This device offers a new method to continuously detect and quantify nitrate levels inside the plants.

KEYWORDS

Plant sensor, Nitrate sensor, in-planta measurement

INTRODUCTION

Sustainable agriculture and plant phenomics is an attractive area at the global level, aiming at increasing both productivity and sustainability of agriculture and food systems to meet the needs of food and textile in the present and future [1][2]. Nitrogen fertilizer is one of the most expensive inputs for crops. Nitrate levels in soils are used to prescribe nitrogen fertilizer inputs and monitor environmental outcomes. Yet, the success of laboratory-based soil nitrate measurement has been limited by high cost and long-time lags between sampling and analyses. Recently, in-situ soil sensors have been reported for monitoring of soil health conditions [3][4].

Rapid and accurate measurement of corn stalk nitrate concentration can be a powerful indicator of crop N dynamics [5]. The vast majority of corn N uptake occurs in the nitrate form and is reduced after transport to the leaves [6]. As a result, it is likely that this measurement can be directly related to corn N uptake rate and amount. Indeed, measurement of corn stalk nitrate is a standard agronomic test for soil N supply sufficiency [7]. Generally, to measure stalk nitrate, stalks are manually sampled, treated, and analyzed using expensive and bulky instruments such as spectrophotometry and ion chromatography in laboratories [8]. Low-cost, high-resolution sensing has great potential to improve the stalk nitrate test and better inform plant N status because the current stalk N test is limited to a one-time post-senescence measurement due to cost and analysis time. Also, implementation is relatively low due to the sample number required to overcome spatial variability [9][10]. The broad ranges of N sufficiency in the current test are a result of low spatiotemporal data resolution. Stalk nitrate sensors can overcome these challenges. However, no in-situ stalk sensors are available to continuously provide nitrate information during the growth of plant. This has limited our ability to not only test stalk nitrate but also understanding of nitrate uptake and utilization process in the plants.

We report a miniature sensor that works on the principle of chemFET to realize continuous measurement of nitrate inside corn plants. We demonstrate the ability of the sensor to track nitrate dynamics under different growth conditions (e.g., light conditions), and management practices (e.g., irrigation and fertigation).

SYSTEM DESCRIPTION

The sensor is an integrated silicon chip consisting of with three major components: a nitrate-selective field effect transistor, a temperature sensing unit, and a silver/silver chloride (Ag/AgCl) reference electrode (RE). In Fig. 1c-d, the sensor chip is wire-bonded to a printed circuit board (PCB). All connections between the chip and PCB was sealed by waterproof epoxy. After assembling, the gate region of the transistor was treated with saline before coating a layer of poly(2-hydroxyethyl methacrylate (poly-HEMA) and a nitrate-specific ISM membrane.

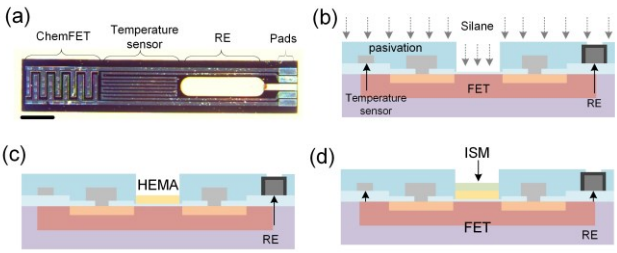

Figure 1: (a) Photo of a fabricated chemFET-based nitrate plant sensor. (b-d) Major fabrication steps for the sensor.

The plant nitrate sensor system includes the assembled sensor chip (inserted into the stalk of plant) and a readout circuit. The readout circuit collects current signals from the transistor and then converts the current to voltage. The circuit also includes a voltage amplifier and a filter. The voltage output is stored into an SD card (Fig. 2) or can be wirelessly transmitted to a data center.

EXPERIMENTAL

Standard solutions were prepared by dissolving $NaNO_3$ powders in deionized water to obtain a series of nitrate concentrations. To calibrate the sensor, the sensor was dipped into different standard solutions. A SU8-based passivation layer protects other regions than the gate from being exposed to the solutions. The gate voltage Vg was applied to the RE while the drain-source voltage Vd was applied to the drain of the transistor. The voltage potential at the gate depends on the concentration of test solution, influencing the drain current Id. The characterization was conducted on a digital hotplate at different temperatures. The real solution temperature was measured with a commercial thermal couple.

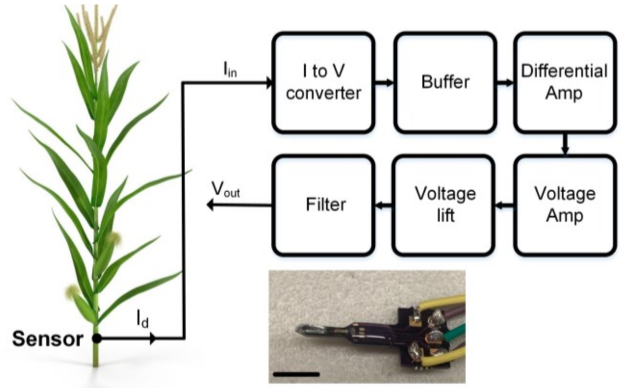

Figure 2: Schematic of the plant sensor system.

RESULTS AND DISCUSSIONS

The sensor chip was characterized with the standard nitrate solutions. Figure 3 shows the drain current (Id) response of the sensor to nitrate concentrations from 0.1 to 1000 ppm NO_3^--N. As the nitrate concentration increased, the drain current decreased. At the gate voltage Vg = 10V, the slope of the calibration plot was found to be 0.37 mA/dec (see the inset of Fig. 3).

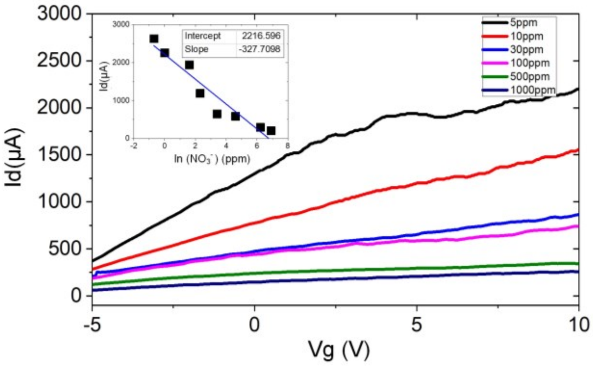

Figure 3: Drain current response of the sensor chip to different nitrate concentrations of standard solutions. The inset shows the calibration plot at Vg = 5 V.

Figure 4a shows the output voltage Vout of the sensor as a function of nitrate concentrations at different temperatures ranging from 20 ºC to 40 ºC. This range of temperature was comparable to that of the growing season for corn. The sensitivity of the sensor in this temperature range was found to be almost consistent at the level of 5.7 mV/dec, while the output voltage increased with increasing temperature. Figure 4b shows the electrical resistance response of the integrated temperature sensing unit to environmental temperature variations. The result shows that the resistance could linearly increase with increasing temperature with the sensitivity of 70 mΩ/ºC.

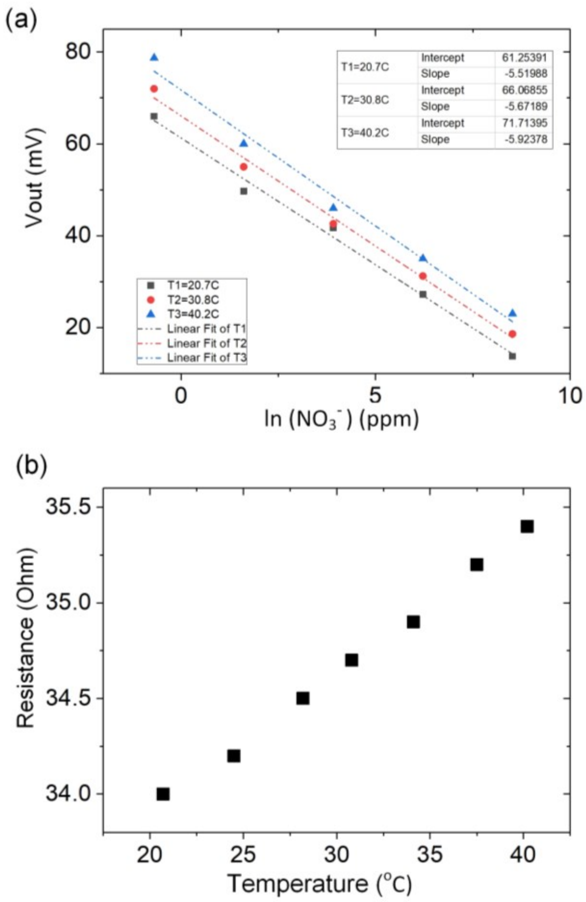

Figure. 4: (a) Calibration plots of the sensor at different environmental temperatures. (b) Calibration plot of the temperature sensing unit.

The repeatability of the sensor (Fig. 5a) was evaluated with multiple measurement cycles of alternating high (500 ppm NO_3^--N) and low concentration (1 ppm NO_3^--N) solutions. The result shows that the sensor has a good repeatability as evident by a low relative standard deviation of Vout in response to the same concentration at 10 mM. In addition, the selectivity of the sensor was tested by mixing different interference ions such as SO_4^{2-}, Cl^- and HCO_3^- (each 0.01 M) with the standard 0.01 M NO_3^--N solution at 1:1 molar ratio (Fig. 5b). These interference ions were chosen because they often appear in corn plants. The selectivity coefficient of the sensor was calculated using the separate solution method based on the measurement results [11]. The result in Table 1 shows that chloride ion has a higher influence on the sensor reading, compared to the other two interference ions.

Table 1: Ion selectivity coefficients of the nitrate sensor

Interference ions (0.01 M each)	SO_4^{2-}	Cl^-	HCO_3^-
Selectivity coefficient	0.3244	0.4768	0.282

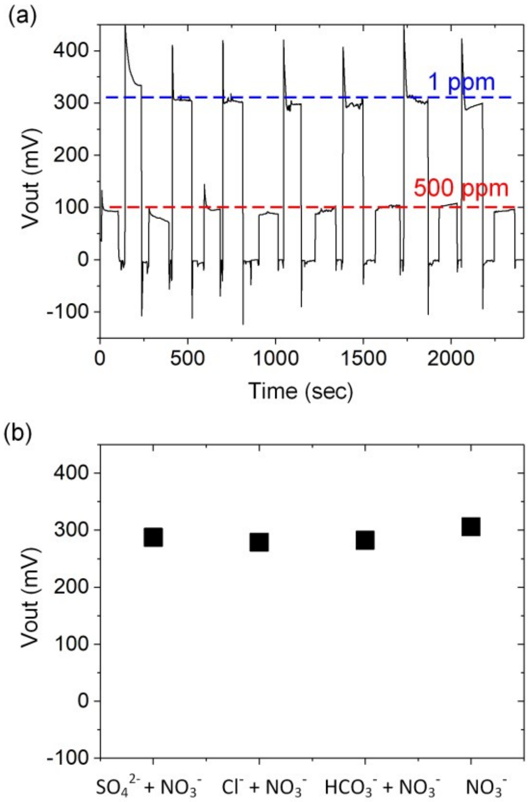

Figure. 5: (a) Repeatability test of the sensor in response to alternating 1 ppm and 500 ppm nitrate concentrations for multiple cycles. (b) Selectivity test of the sensor in different sample solutions with mixtures of interference and nitrate ions and nitrate with 1:1 molar ratio.

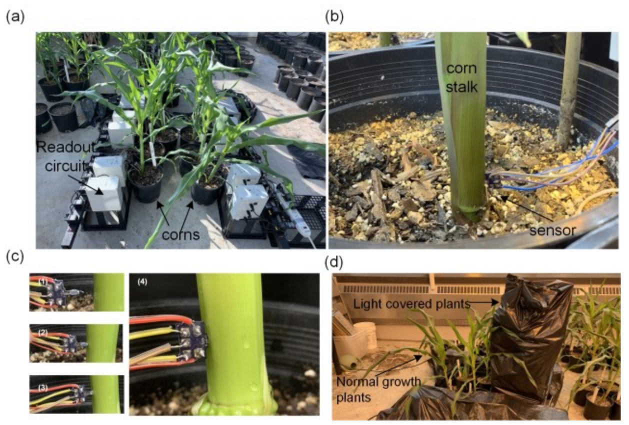

Figure. 6: (a) Experimental setup for in-planta nitrate measurement using the fabricated sensors. (b-c) Photos of the plant sensor inserted to the stalk of corn plants. (d) Several plants were covered by black bags to shield lights.

Next, continuous in-situ nitrate measurements for corn plants were performed in the greenhouse (Fig. 6a). The plants were grown in pots with Ironite Mineral Supplement (containing 1% urea nitrogen). Slow release fertilizer (Harrell's 17-5-12 6M FS) was applied to the soil surface of pot. To study the influence of N fertilization rate on accumulative nitrate concentration in plant tissues, extra 150 kgN/ha urea was applied to the pots. To study the influence of irrigation on nitrate uptake of plants, the plants were watered multiple times during the measurement. For studying how light affects nitrate uptake, some plants were covered with a black bag to block light (Fig. 6d), while other plants were exposed to light as usual. At the V6 stage, the sensors were inserted vertically to the stalk of plant at the internode between leaf 2 and leaf 3 at 1 cm above the lower node (Fig. 6b). The readout circuit recorded the sensor output continuously.

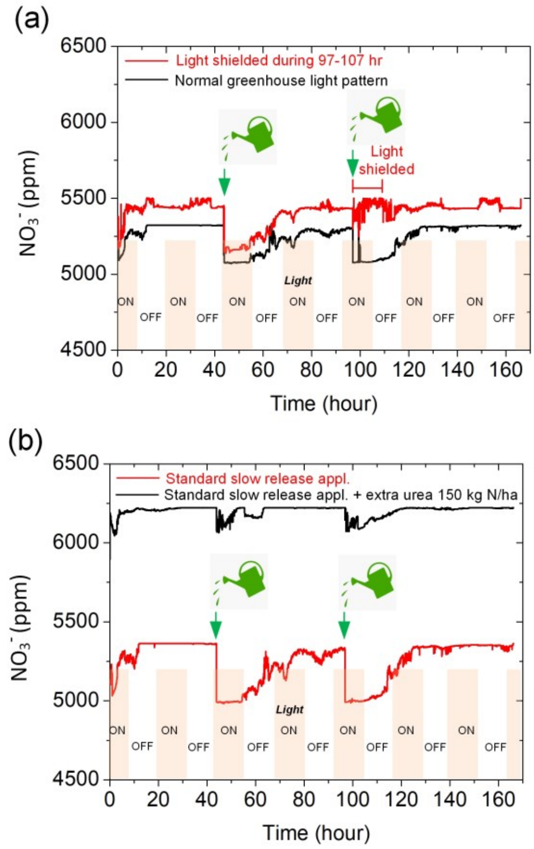

Figure 7: (a) Response of the accumulative nitrate level in the stalk of corn plants to light and water irrigation. All the plants were fertigated using slow release nitrogen fertilizer. Light was shielded for one group of plants at 97-107 hr (see Fig. 6d), while remaining on for the other group. (b) Response of the accumulative nitrate level in the stalk of corn plants to light and water irrgation, where two different N fertilizer rates were aplied to two groups of plants.

Figure 7a demonstrates the continuous measurement of accumulative nitrate inside the plants grown in the pots that were applied with the aforementioned slow release fertilizer. When no irrigation was applied to the plants, the accumulative nitrate kept at a relatively stable level, although the plant-to-plant variation was found significant. The plants were watered twice. Upon irrigation at 41 hr, the stalk nitrate level began with an immediate drop, and then gradually increased. A plausible reason is as follows. The accumulative nitrate in the plant is higher than that in the soil water. When the plant uptakes water, the accumulative nitrate in the plants may be diluted by the absorbed water.

As time went, the nitrate ions are accumulated in the plant tissue, thus lifting the *in-planta* nitrate back to a high level. At 97 hr, the plants were watered again. At the same time, one group of plants was covered by a black bag to block light, while the other group of plants remained uncovered. Interestingly, the accumulative stalk nitrate in the covered plants was found not to change as much as that appeared in the uncovered plants. This may be due to the limited water uptake ability of the plants when they stay in the dark.

Figure 7b shows that the plant sensor can differentiate the stalk nitrate concentration in the plants received a low N-fertilizer rate from that in the plants received a high rate. Similar responses of the accumulative nitrate level to water irrigation were observed in both the plants.

CONCLUSIONS

We have developed a chemFET-based nitrate sensor for continuous monitoring of accumulative nitrate level inside the plants. The device consists of a nitrate sensing unit, a thermorist, and a miniature reference electrode. The sensor could be partially inserted into the stalk of plants. This minimally invasive device has demonstrated its sensing ability that could facilitate studying the effect sof water irrigation, fertilizer rate, and environmental conditions such as light on the nitrate uptake and accumulation inside the plants.

By applying different ion-selective membranes onto the sensors, it is possible to adopt the presented plant sensor technology to continuously monitor fluxes of many other nutrient ions in the plants under different environments and agricultural management practices. Further, in addition to measuring nutrients inside corn plants, this sensor could also be applied to other plants through appropriate modifications to the dimensions, materials, and geometries of the sensors. Therefore, this sensor method will pose a significant impact to nutrient management in agriculture and plant science.

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