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ELECTROCHEMICAL DETECTION OF SMALL VOLUMES OF GLYPHOSATE WITH MASS-PRODUCED NON-MODIFIED GOLD CHIPS

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Mass-produced printed circuit board (PCB) electrodes were used as electrochemical cells to detect the widely-used herbicide glyphosate. Square wave voltammetry (SWV) was used to determine the presence of glyphosate in aqueous $Cu(NO_3)_2$ solution. Optimal measurement conditions for the detection of glyphosate with PCB electrodes were found. It was determined that glyphosate was able to soak into the growing plants from the substrate. Glyphosate-contaminated plant juice was distinguished from control samples using the PCB electrode. Glyphosate-contaminated plants were found to have DNA mutations.

Keywords: *electrochemical, glyphosate, printed circuit board, sensor, square wave voltammetry*

1. INTRODUCTION

Glyphosate is the widely-used herbicide considered to have low toxicity, and it is widely used in agriculture [1]. However, ingestion of glyphosate leads to a large number of health issues, including respiratory malfunction, altered consciousness, increased cancer risk, and death [2]–[4].

It is necessary to develop reliable glyphosate detection methods since modern laboratories frequently fail to detect glyphosate in food products [5]. Electrochemical methods are widely used to determine various chemical substances present in solutions, ranging from simple ion detection [6] to complex biological molecule detection [7]. Several electrochemical approaches have been developed in the past two decades [8]. Copper electrodes have shown potential for selective detection of glyphosate in liquids [9]–[11]. Several researchers have suggested that glyphosate can form water-soluble complexes with copper ions

[12]–[13]. Since copper ions by themselves can be detected with electrochemical methods, it is possible to use Cu^{2+} containing buffer solutions to indirectly detect glyphosate from the change in copper ions electrochemical activity.

Since glyphosate is a widely-used herbicide, it is necessary to develop an easy-to-use and cheap method for controlling glyphosate levels at farming sites. An easily available approach for electrode mass production is printed circuit boards (PCBs) since they can be produced at factories throughout the world in large quantities for low cost, and most of the factories can offer electrode-surface coating with gold, making them chemically neutral. PCB electrodes can also be suitable for small liquid volumes, making it possible to run an electrochemical analysis of a 10-µL drop. This greatly reduces the number of the necessary sample preparation steps and the amount of chemicals used in the analysis. There is no need for electrode-surface modification since glyphosate can be detected indirectly by electrochemical quantification of Cu^{2+} ions. In this paper, PCB gold-coated electrodes were used to indirectly detect glyphosate in solution using the described method.

2. EXPERIMENTAL

2.1. Reagents and Materials

Reagents used in the research were the following: copper nitrate trihydrate (99.9 % pure, purchased from Lach-Ner), commercial glyphosate-based herbicide (containing 360 g/L of glyphosate izopropylamine), 97 % ethanol, and distilled water.

2.2. Mass-Produced Au Chip

In place of an electrochemical cell, a mass-produced PCB chip was used. The working area of the chip consisted of 8 copper electrodes covered in gold via the ENIG process, on an FR-4 glass-reinforced epoxy laminate. Each of the electrodes had an individual copper track connected to it, allowing for versatile electrode configuration. All tracks were covered in a protective dielectric polymer. The final product can be seen in Fig. 1. The electrodes were designed to each have a rectangular-shaped 150 x 125-µm exposed gold-covered area. The electrochemical measurements were made using a Zanher Zennium Electrochemical Workstation in square wave voltammetry (SWV) mode, using chip as a three-electrode electrochemical cell.

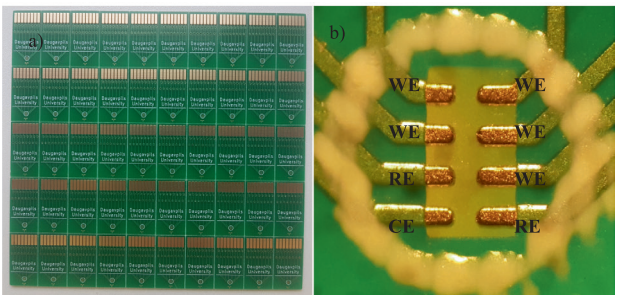

Fig. 1. a) Mass-produced PCB chips with Au-coated electrodes. b) Chip electrode configuration. WE=working electrode, CE=counter electrode, and RE=reference electrode. The diameter of the white ring is 1 mm.

2.3. Sample Preparation

Prior to electrochemical measurements, PCB chips were washed in ethanol and cleaned with a nitrogen jet to free the working surface of any production-process residue. In the first experiment, commercial glyphosate was mixed in copper nitrate buffer to the desired concentration. Then, after a delay of 30 minutes, 10 μL drops of the resulting solution were placed on the chip working-area and measured via SWV. A new chip was used for each separate drop; however, all used chips were from the same batch. In the second experiment, plant juice was extracted by crushing plant tissue and filtering the resulting mass through coarse mesh. The extracted juice was then mixed with copper nitrate buffer, centrifuged after a 30-minute delay, and the resulting solution was separated from the solid residue. This solution was then placed on the chip working area in 10-μL drops and measured via SWV.

2.4. Molecular Studies

Genomic DNA was extracted from samples (n=60) of fresh, 7-day-old rye plant seedlings treated with 1/10 of the working concentration of glyphosate (n=30) and without it (n=30). Extraction was done with slight modification using the purification of total DNA from plant tissue Mini Protocol (DNeasy Plant Mini Kit, Qiagen GmbH, Hilden, Germany). The genomic DNA was quantified using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Waltham, the USA) to measure absorbance at 260 nm, and the purity of the DNA was determined. Stock DNA was diluted to make a working solution of 50 ng/μL for further PCR analysis.

Five random amplified polymorphic DNA (RAPD) primers, OPA-02, OPA-07, OPA-11, OPD-18, and OPN-15 (Table 1), were selected for the study. PCR reactions were carried out in a thermocycler (Veriti 96-WellThermal Cycler, Applied Biosystems, Foster City, the USA). All PCR reactions were prepared as described in [14]. RAPD fragments were separated, and the product length was detected using the QIAxcel (Qiagen GmbH, Hilden, Germany) capillary automated electrophoresis system. The amplification reaction for each primer was repeated twice for each sample to ensure reproducibility. Only clear and reproducible bands were considered for the analysis.

Table 1. Sequences of the 10-mer Primers (5'–3') Used in the Experiments

Primer ID	**Sequence (5'–3')**
OPA-02	5'- TGCCGAGCTG-3'
OPA-07	5'-GAAACGGGTG-3'
OPA-11	5'-CAATCGCCGT-3'
OPD-18	5'-GAGAGCCAAC-3'
OPN-15	5'-CAGCGACTGT-3'

3. RESULTS AND DISCUSSION

3.1. Glyphosate Detection in Copper Nitrate Buffer Solution

Due to the extremely small electrode surface area (approximately 1.88×10^{-8} m^2 for a single electrode), to obtain significant current through the cell, a buffer with relatively high copper nitrate concentration is required. However, higher copper nitrate concentration leads to a higher glyphosate detection threshold since a less relative change in Cu^{2+} ion concentration is induced by the same glyphosate concentration. The optimal buffer was determined to be a 15 mmol/L $Cu(NO_3)_2$ solution in deionized water.

The SWV results for the detection of glyphosate in 15 mmol/L $Cu(NO_3)_2$ buffer can be seen in Fig. 2. It should be noted that the current was normalised to the maximum current value. As the measurements have shown, the maximum-current peak-amplitude corresponds to the 0 mmol/L glyphosate control solution. The potential of the main peak from the SWV curve drifts with changes in glyphosate concentration. This could be caused by the change in the pH value of the solution, as the glyphosate is acidic, and measurements are done in a non-controlled pH environment. The peak at 50–300 mV is reported to be caused by the reduction of Cu^{2+} ions [9], and it is easy to notice since this peak has the biggest amplitude. Figure 2b shows the main peak amplitude versus glyphosate concentration. In the 0–1.5 mmol/L range, the peak amplitude drops linearly as the concentration increases and the cell becomes saturated. There is no change in the peak amplitude for concentrations greater than 1.5 mmol/L. This behaviour is approximated by the line in Fig. 2b. The reduction in the main-peak amplitude can be explained by passivation of the electrode surface by the glyphosate/Cu^{2+} complex [9].

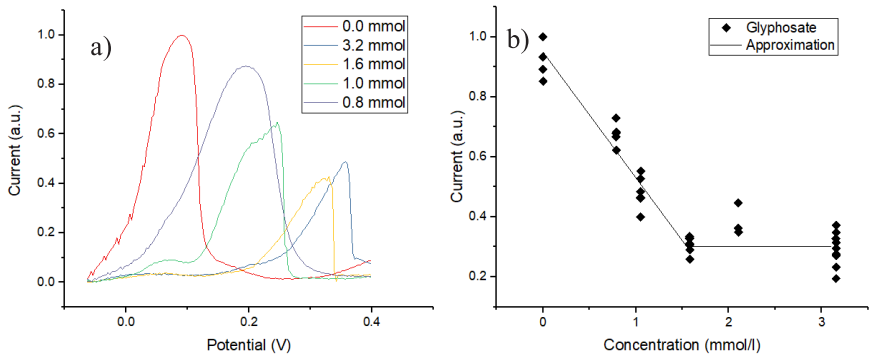

Fig. 2. a) The square wave voltammetry (SWV) results for the detection of glyphosate in copper nitrate solution. b) The main-peak maximum from SWV versus glyphosate concentration. The potential was measured relative to the on-chip Au reference electrode.

3.2. Glyphosate Detection in Grown Plants

Rye, wheat, and barley plants were grown to 6-cm long grass, then treated with a 1:10 dose of glyphosate and allowed to grow for additional 5 days. Plant juice was collected and mixed with a small amount of highly-concentrated copper nitrate buf-

fer to achieve 15 mmol/L resulting copper nitrate concentration in the solution. The SWV measurement results of the samples can be seen in Fig. 3. The control plant juice did not change the SWV curve shape, and the SWV curve for the control plants with no glyphosate treatment was similar to the curve obtained from copper nitrate solution in deionized water. However, plants that underwent glyphosate treatment showed a slight reduction in the main-peak amplitude of the SWV curve. As can be seen from Fig. 3b, samples from the control and glyphosate groups formed two distinct distribution clouds. It is possible to distinguish glyphosate-containing plant juice since the main peak value is less than 0.9 a.u.

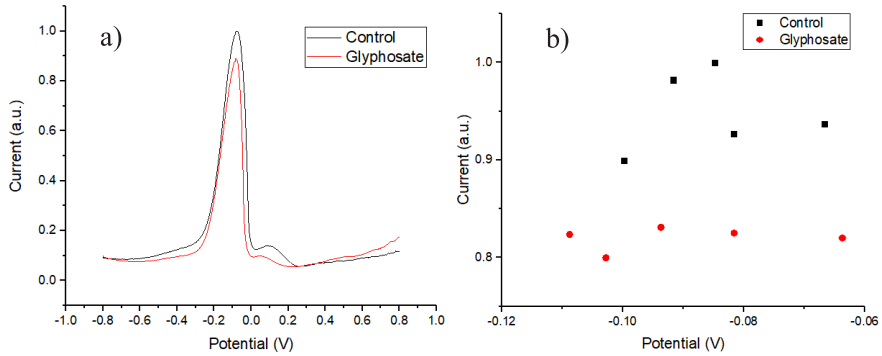

Fig. 3. a) Averaged SWV curve for rye samples.
b) The main-peak potential and current for rye samples.

3.3. Molecular Studies

The RAPD technique, a PCR-based technique, has been shown to successfully detect genotoxicity from DNA in plants [15]–[17]. RAPD analysis is capable of detecting temporary DNA changes and is considered more sensitive than classic genotoxic tests, i.e., the comet or micronucleus assay [18]. Moreover, RAPD profiling is successfully used for evaluating the genetic effects of glyphosate on plants [19], [20]. The evident changes observed in the RAPD profiles, such as disappearance and/or appearance of bands in comparison with untreated control samples, were evaluated and considered to be genotoxic changes. Treatment of rye plant seedlings with 1/10 of the working concentration of glyphosate for 5 days resulted in changes in the RAPD profiles obtained from the exposed plants. RAPD profiles of the plants showed the disappearance of a normal band and the appearance of a new band in comparison with the control. Differences in the DNA banding pattern between control samples and samples treated with glyphosate were significant and were detected at different places with all utilized primers. All obtained DNA bands were polymorphic. The RAPD profiles obtained from the five oligonucleotide primers are presented in Table 2.

In total, among all primers in the control samples, 27 fragments were detected. Samples treated with glyphosate showed 15 new bands, and 12 bands were eliminated in comparison with the samples without treatment. According to the literature, the disappearance of normal bands can probably be designated as DNA damage through modified bases, point and deletion mutations, and single and double strand breaks, whereas

new bands generally reveal a change in some oligonucleotide priming sites due to mutations, large deletion, and/or homologous recombination [18], [21], [22]. Overall, the RAPD results indicate that 1/10 of the working concentration of glyphosate caused significant changes in the genome of the rye plant seedlings.

Table 2. RAPD Profiles from Rye Plant Seedlings without Treatment (Control) and Treated with Glyphosate

Primers ID	Fragment length, bp		Primers ID	Fragment length, bp	
	Control	Glyphosate		Control	Glyphosate
OPD-18	_ 345 355 424 _ 445 _ 561 _ _ 972 _ 1203 1217 _ _ 1314 _ _ 2612 2716	339 349 358 419 434 441 549 559 827 873 _ 1049 1209 _ 1275 1290 1305 2573 2590 _ _	OPA-11	512 2650 2736	514 _ 2740
			OPN-15	_ 2741	285 _
			OPA-07	213 276 505 514 _ _ _ 2593	_ _ 507 524 1264 1280 2762 _
			OPA-02	_ 616 659 1060 1094 1449 2911 2924	602 615 659 _ 1099 _ 2913 _

4. CONCLUSION

Glyphosate can be successfully detected in both distilled water and plant juice indirectly by adding a Cu^{2+} ion source to the solution and detecting changes in ion electrochemical activity. Glyphosate presence in plant juice was also confirmed by investigation of the plant DNA. The described method provides a fast and cheap way to control glyphosate usage in the field. Since PCB electrodes are easy to mass produce, electrochemical analysis can be done in a mobile lab, and required chemicals are common. Due to consistent reduction in the main peak amplitude from the SWV, glyphosate-contaminated samples can be easily distinguished from non-contaminated samples, and the glyphosate tests can be automated.

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