Development of a Dual-Label Time-Resolved Fluorometric Immunoassay for the Simultaneous Detection of Two Recombinant Proteins in Potato

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Immunological methods such as ELISA have been traditionally employed to quantify protein levels in plants improved through modern biotechnology. Combined trait products (i.e., plants producing multiple recombinant proteins) created by introducing multiple genetic traits by transformation or traditional breeding methods have prompted the need for the development of analytical assay technologies capable of detecting and quantifying multiple proteins in a single assay. The development of a two-site, sandwich, dual-label, time-resolved fluorometry-based immunoassay (TRFIA) capable of simultaneously quantitating two recombinant proteins (CP4 EPSPS and Cry3A) in plant sample extracts of genetically improved potato cultivars is reported here. The performance characteristics of TRFIA were similar to or exceeded those of current ELISA methods used to detect and quantitate these proteins. TRFIA is a practical and reliable assay for the quantitation of proteins in genetically improved potato plants and offers an alternative approach to conventional ELISA methods with the added benefit of multiple analyte detection.

Keywords: *ELISA; time-resolved fluorometry (TRFIA); DELFIA; fluoroimmunoassay; CP4 EPSPS; Cry3A; agricultural biotechnology*

INTRODUCTION

At least 40 different plants derived through modern agricultural biotechnology methods have been approved by regulatory agencies and are available commercially (James, 1999). In many cases, these plants were produced through the use of single genes whose gene product (mRNA or protein) conferred a desired phenotype. As the knowledge of genes and their functions continues to increase, the number of genes and gene combinations inserted into plants is also expected to increase. In conjunction with the techniques of gene introduction through modern plant biotechnology and gene combinations created during conventional plant breeding, future plant products are likely to contain numerous combinations of introduced genes.

The detection and quantitation of proteins in plants derived from biotechnology has up to now been dominated almost exclusively by two immunochemical methodologies: the Western blot (Gleave et al., 1998; Witcher et al., 1998; Hashimoto et al., 1999) and ELISA (Rogan et al., 1992; Reed et al., 1995; Wunn et al., 1996). Although these methods are useful, they suffer from limitations that do not readily allow for multi-analyte detection. Time-resolved fluorescence immunoassay offers an alternative to ELISA and western blot for use in the characterization of plants improved through biotechnology. TRFIA offers increased sensitivity over comparable immunoassay techniques [radioimmunoassay (RIA) and ELISA (Halonen et al., 1984; Walls et al., 1986; Hierholzer et al., 1987)] without sacrificing specificity and with the additional benefit of simultaneous detection of multiple analytes in one reaction vessel; thus increasing the efficiency and reducing the resources needed for analysis of plants containing multiple transgenes.

Immunoassays based on lanthanide chelates and time-resolved fluorometry (TRFIA) have been developed for detection of several important compounds (Soini and Kojola, 1983; Barnard et al., 1989; Helsingius et al., 1986; Hemmilä et al., 1987; Mitrunen et al., 1995; Papanastasiou-Diamandi et al., 1992; Tolvonen et al., 1986; Xu *et al.*, 1992). This technology is based on the labeling of antibody with stable but nonfluorescent chelates, and on the use of a dissociative fluorescence enhancement solution (Hemmilä et al., 1984). Among the commercialized TRFIA technologies, DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay, E. G. & G. Wallac, Gaithersburg, MD), is widely used in clinical applications and offers readily available reagents. This technique relies on the unique fluorescent properties of the chelates as characterized by the broad excitation in the absorption region of the ligand (250−360 nm), long Stokes shift (> 250 nm), narrow-band emission lines, high quantum yields, and long fluorescence lifetimes, ranging from microseconds to milliseconds (Xu *et al.*, 1991). The long emission duration results in a specific signal distinct from background signals. Following reaction in the assay system, the lanthanide (usually europium or samarium) is released from the solid phase and chelated to a highly fluorescent compound in solution. This is accomplished by a low pH solution which dissociates the lanthanide from the bound antibody and promotes micelle formation. The

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lanthanide favors the hydrophobic environment of the micelle and bonds to chelating agents (TOPO, 2-NTA) within the micelle which promote fluorescence. Specific fluorescence can be measured after allowing a short decay (400 μs) during which background fluorescence of specimen, reagents, and plastic wells of the assay plate disappears (Roberts et al., 1991).

Analysis of combined trait plants has highlighted the need for development of more efficient technologies capable of multi-analyte detection and quantitation. Dual-label assays have been used for determining various analyte pairs (Hemmilä et al., 1987). The fluorescent lanthanides (Eu^{3+}, Tb^{3+}, Sm^{3+}, and Dy^{3+}) are all excellent candidates for multiple-label assays because of their characteristics described previously (Hemmilä, 1985). Eu^{3+}/Sm^{3+} is a commonly labeled pair that has been used for the detection of plant viruses, yielding increased sensitivity and decreased background fluorescence (Saarma et al., 1989). Although application of this technology to agricultural biotechnology has been limited, a few laboratories are beginning to develop methods (Reimer et al., 1998). This report describes the development and validation of a dual label TRFIA using the lanthanides Eu^{3+}/Sm^{3+}. The assay described in this report is capable of simultaneously quantifying the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and Cry3A protein in leaf tissue of genetically improved potato (*Solanum tuberosum*) plants. The proteins are products of the CP4 EPSPS coding sequence that directs the synthesis of an enzyme that confers tolerance to the herbicide Roundup and the cry3A coding sequence which encodes for an insecticidal protein (δ-endotoxin) that confers resistance to the Colorado potato beetle (*Leptinotarsa decemlineata*).

MATERIALS AND METHODS

Instruments. The fluorometric determination of the lanthanide ions and the calculation of results were performed using a Victor 1420 multi-label counter (Wallac Oy, Turku, Finland) equipped with narrow-band (6−8 nm) emission filters suitable for Eu^{3+} (615 nm), Sm^{3+} (642 nm), Tb^{3+} (545 nm), and Dy^{3+} (572 nm) and factory-installed parameters: 400-μs counting delay, 400-μs counting window, 200-μs recovery time, and powered by a xenon-flash lamp as an excitation source of approximately 1000 cycles per second. A Bio-Rad model 3550 spectrophotometer was used to analyze the levels of the two proteins using conventional ELISA methodologies.

Antibodies. Antibodies were produced against Cry3A in rabbits and against CP4 EPSPS in rabbits and mice by immunization with bacterially produced Cry3A or CP4 EPSPS protein, respectively (Perlak et al., 1993; Harrison et al., 1996). These antibodies were previously characterized and used in validated ELISAs to analyze the levels of CP4 EPSPS and Cry3A protein present in potato tissues.

Reagents. DELFIA Eu^{3+} and Sm^{3+} labeling kits were obtained from Wallac Oy (Turku, Finland). Because the quantum yield of Eu^{3+} is greater than Sm^{3+}, and the observation that levels of Cry3A protein are usually lower than those of CP4 EPSPS protein, the Eu^{3+} chelator complex was conjugated to anti-Cry3A polyclonal antibodies and the Sm^{3+} chelator complex was selected for conjugation to anti-CP4 EPSPS polyclonal antibodies. The labeling was performed by overnight incubation at room temperature using a 50 mM carbonate buffer containing 0.9% NaCl, pH 8.5. The labeled antibodies were separated from both the free chelates and protein aggregates by gel filtration on a combined column of Sephadex G-50 and Sepharose 6B (both from Sigma, St. Louis, MO) and eluted using a 50 mM Tris-HCl (pH 7.8) buffer containing 0.9% NaCl and 0.1% NaN_3.

Buffer Solutions. The TRFIA plate-coating buffer was 0.035 M $NaHCO_3$, 0.015 M Na_2CO_3, pH 9.6. The blocking/drying solution was 5.0% sucrose and 0.2% bovine serum albumin (BSA) in deionized water (dH_2O). As the assay buffer, 0.05 M Tris-HCl, pH 7.75, 0.15 M NaCl, 8.0 mM NaN_3, 1.0% BSA, 0.05% bovine gamma globulin, 0.01% Tween-40, 20 μM diethylenetriamine-pentaacetic acid (DTPA), 0.5% poly(ethylene glycol) 8,000 (PEG), and 0.8 μM danazol was used. The washing solution contained 0.15 M NaCl, 0.05 M Tris-HCl, pH to 7.8, 0.05% Tween-20 and 9.0 mM NaN_3. The enhancement solution was purchased from Wallac Oy (Turku, Finland). Sample extraction buffer was 1X phosphate-buffered saline (PBS), 0.05% Tween-20, and 0.2% BSA.

Sample Preparation. Samples were extracted in 1X potato extraction buffer (PEB) which contained 1.38 M NaCl, 0.081 M $Na_2HPO_4 \cdot 7H_2O$, 0.015 M KH_2PO_4, 0.027 M KCl, and 2% Tween 20 at a fresh tissue weight-to-buffer ratio of 1:15 using a Brinkmann Polytron PT3000. Three 15-s bursts at 17 500 rpm with a 10-s interval between bursts were used. Following extraction, samples were centrifuged for 10 min at 22 619*g* in a Sorvall RC5B model centrifuge (Newtown, CT). The supernatant was dispensed in single-use aliquots and stored at −80 °C.

Immunoassay. The TRFIA assay format was a one-step, two-site sandwich, dual-label time-resolved fluorometry-based immunoassay performed in a 96-well microtiter plate. The TRFIA assay was performed in five steps:

(1) 200 μL of protein A affinity-purified monoclonal anti-CP4 EPSPS antibody (Mab) and rabbit anti-Cry3A polyclonal antibodies were diluted in coating buffer and co-immoblized onto microtiter plates at 1.0 μg/mL and 0.625 μg/mL for the CP4 EPSPS and Cry3A antibodies, respectively.

(2) Microtiter plates were stored overnight at 4 °C to allow antibody binding, then aspirated, and washed three times with TRFIA wash buffer.

(3) Blocking solution was added to each well at 350 μL per well and incubated for 1 h at room temperature. After manually decanting the blocking solution, plates were dried overnight in an incubator at 37 °C, and each plate was stored at 4 °C in a sealed package containing a desiccant pouch until use.

(4) For each well, 100 μL of a cocktail that contained standards or plant extract, and 100 μL of lanthanide-labeled polyclonal antibodies (rabbit anti-CP4 EPSPS diluted 1:100 and rabbit anti-Cry3A diluted 1:1500) were added. Plates were covered with adhesive sealer and incubated for 1 h at room temperature with continuous agitation at 600 rpm on an orbital microtiter plate shaker.

(5) Plates were washed five times, quickly, with TRFIA wash buffer and enhancement solution was added (200 μL per well). Plates were covered and incubated for an additional 10 min with shaking. Plates were analyzed and fluorescence counts per second (counts/s) collected on a Victor model #1420 fluorometer (EG&G Wallac, Gaithersburg, MD). Data were exported into winSeLecT data analysis software (Tecan, Research Triangle Park, NC) for data reduction.

Assay Validation: Accuracy, Precision, Limit of Quantitation, and Limit of Detection. The accuracy of the TRFIA was evaluated by performing experiments to assess the recovery of CP4 EPSPS and Cry3A proteins, parallelism, correlation of dual-label TRFIA with ELISA and single-label TRFIA, and matrix effects. To evaluate the analytical accuracy of dual-label TRFIA, leaf extracts prepared from conventional potato plants were diluted 1:20 with assay buffer and spiked with known quantities of CP4 EPSPS and Cry3A proteins. Spike concentrations ranged from 1 to 40 ng/mL and 0.333 to 20 ng/mL for the CP4 EPSPS or Cry3A proteins, respectively. The recovery was expressed as the percent of the measured protein concentration versus the expected protein concentration.

A parallelism experiment was performed to determine if bacterially produced CP4 EPSPS and Cry3A were immunologically equivalent to plant produced CP4 EPSPS and Cry3A and to determine if the TRFIA was free from matrix effects. As defined by Rodbard et al. (1978), parallelism of the dual-label TRFIA was assessed by serially diluting, with assay buffer, two sample extracts previously identified as containing

high concentrations of CP4 EPSPS and Cry3A proteins. The amount of CP4 EPSPS and Cry3A protein was determined and results were corrected for the dilution factor. These data were plotted and the slope of the lines determined.

Matrix effects were further assessed by preparing standard curves in the presence or absence of conventional potato plant sample extract. Separate standard curves prepared in leaf extract or assay buffer (dilution ranged from 1:5 to 1:80) were plotted and compared to the standard curve prepared in assay buffer alone.

A correlation study was performed to evaluate the agreement between conventional ELISA and TRFIA technologies. The CP4 EPSPS and Cry3A protein ELISA methods employed in this study were routinely used by Monsanto for measurement of Cry3A and CP4 EPSPS proteins. Both assays have been validated, are direct, one-step, sandwich ELISAs employing polyclonal antibodies (rabbit anti-Cry3A) or a monoclonal antibody (anti-CP4 EPSPS) for capture followed by co-incubation of sample or standard with a polyclonal antibody conjugated to HRP. ELISA procedures and validation were similar to that described by Rogan et al., 1992. For comparison of the data derived from ELISA and TRFIA, extracts prepared from potato leaf were assayed by dual-label TRFIA and CP4 EPSPS or Cry3A ELISA. Linear-regression analysis was performed to assess the correlation between protein concentrations estimated by both methods.

To evaluate intra-assay precision, a precision profile was constructed using data derived from five standard curves with the % CV calculated for each standard concentration (Ekins, 1978). As mentioned below, this precision profile was also used to establish the upper and lower limits of quantitation. Inter-assay precision was determined by calculating the variation in Cry3A or CP4 EPSPS protein content in the inter-assay quality control (QC) sample which was routinely analyzed on every 96-well plate. The CP4 EPSPS and Cry3A values for the QC sample derived from 10 separate assays were used to determine inter-assay variation.

The limits of quantitation (LOQ), or working range of the assay, was derived from a precision profile (described above). Quantitation limits were established by calculating the percent coefficient of variation (% CV) associated with the fluorescence counts per second (counts/s) values associated with standards run in triplicate over five separate assays. Concentrations of CP4 EPSPS or Cry3A protein with an imprecision of < 20% CV were chosen to represent the range of quantitation.

The limit of detection (LOD) for the CP4 EPSPS and Cry3A dual-label TRFIA was calculated by analysis of 25 individual leaf sample extracts obtained from conventional potato plants comprising several potato cultivars grown in several geographical locations. For this analysis, leaf extracts were diluted 1:5, 1:10, and 1:20 in TRFIA assay buffer. The LOD was calculated for each dilution based on the mean background counts/s derived from analysis of 25 individual leaf samples. To establish the LOD, the standard deviation (SD) associated with the counts/s was multiplied by three and added back to the mean counts/s associated with each diluted sample to achieve three standard deviations from the mean. The concentration of CP4 EPSPS or Cry3A associated with this value was then calculated by extrapolation from the standard curve. This was performed for each dilution.

RESULTS AND DISCUSSION

Polyclonal anti-CP4 EPSPS or Cry3A antibodies were labeled with Sm^{3+} or Eu^{3+} as described previously. Polyclonal and monoclonal antibodies, previously used in conventional ELISAs, were readily adapted for use in the TRFIA assay. The antibodies showed excellent specificity; no signal above background (buffer blank) was observed for samples derived from conventional potato plants. Capture and detection antibodies were identical to those used in the CP4 EPSPS and Cry3A ELISAs.

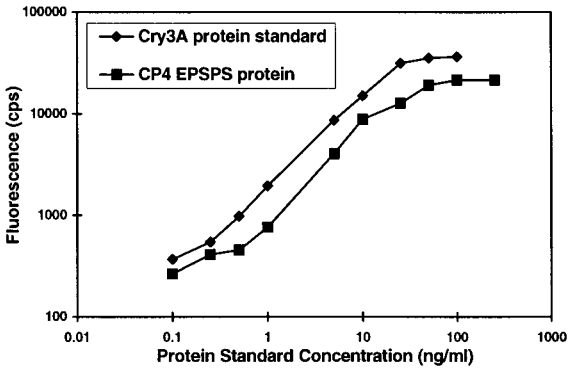

Figure 1. Standard curves of CP4 EPSPS and Cry3A proteins. Standard curve of CP4 EPSPS protein was obtained using a coating antibody concentration of 1.0 μg/mL and anti-CP4 EPSPS labeled with Sm^{3+} at a dilution of 1:100. Standard curve of Cry3A protein was obtained using a coating antibody concentration of 0.625 μg/mL and anti-Cry3A labeled with Eu^{3+} at a dilution of 1:1500.

Standard curves for the CP4 EPSPS and Cry3A assays are presented in Figure 1. When counts/s were plotted against the dose of CP4 EPSPS protein or Cry3A protein standard assayed, a curvilinear dose-response curve was generated. For data reduction, the amount of CP4 EPSPS or Cry3A protein and the response (counts/s) were logarithmically transformed and data fit to a four-parameter logistic model. The concentration of the proteins in unknown sample extracts was determined by reference to an 8-point standard curve. The working range was from 0.094 to 30 ng/mL and 0.25 to 80 ng/mL for the Cry3A and CP4 EPSPS proteins, respectively (Figure 1). During assay development, it was observed that both the CP4 EPSPS and Cry3A assay performed comparably in single or dual-label analyte mode (data not shown).

The accuracy of the dual-label TRFIA was excellent for both CP4 EPSPS and Cry3A proteins. For instance, mean percent recovery of CP4 EPSPS or Cry3A spiked into conventional potato leaf extract was 104.1 and 95.1% for CP4 EPSPS and Cry3A proteins, respectively (Table 1).

According to Rodbard et al. (1978), a linear regression performed using the log microliters assayed vs log nanograms observed should yield a slope of 1.0 if parallelism exists. Linear regression performed on the log−log transformed data from potato leaf extracts assayed from 1.9 to 125 μL satisfied the parallelism test for both Cry3A and CP4 EPSPS proteins (Figures 2 and 3). For CP4 EPSPS protein in samples 420 and 2217, the slopes of the log−log regression line were 0.99 for both samples. For Cry3A protein in samples 420 and 2217, the slopes of the log−log regression line were 0.98 and 1.02, respectively. The correlation coefficients (R^2) of the lines generated from these data were 0.99. These data establish that the assay was free from matrix interferences and that the Cry3A and CP4 EPSPS proteins produced by transgenic plants were immunologically equivalent to the bacterially produced standards. On the basis of these data, it is concluded that parallelism exists for the TRFIA CP4 EPSPS and Cry3A assays.

Further, standard curves of CP4 EPSPS and Cry3A proteins prepared in TRFIA assay buffer and in potato leaf extracts at dilutions of 1:5 (tissue-to-buffer ratio) or greater were nearly superimposable, thus confirming the lack of matrix effects (data not shown).

Table 1. Overview of the Test Performance Characteristics Exhibited by Dual-Label TRFIA Compared to ELISA Methods

	CP4 EPSPS		Cry3A	
test performance criteria	TRFIA	ELISA	TRFIA	ELISA
limit of detection (ng/mL)	0.17	0.23[a]	0.16	0.47[a]
limit of quantitation (ng/mL)	0.50 to 80.0	0.50 to 40.0	0.19 to 30.0	1.25 to 80.0[b]
analytical recovery (%)	104.1	88.1	95.1	129.5
intra-assay variation (% CV)	<15.0	<15.0	<15.0	6.5
inter-assay variation (% CV)	13.1	<15.0	12.5	8.3
QC positive (mean ng/mL ± 3 SD)	0.91 to 1.33	NA	0.81 to 1.47	NA
QC negative (ng/mL)	<0.25	NA	<0.094	NA
method comparison	$y = 1.14x - 0.37$	$R^2 = 0.97$	$y = 0.74 + 1.33$	$R^2 = 0.95$

[a] LOD was calculated for CP4 EPSPS and Cry3A ELISAs using the same samples tested in the TRFIA. LOD value reported here for the ELISA was obtained at a 1:20 matrix dilution. [b] LOQ was calculated in this study for CP4 EPSPS and Cry3A ELISAs for purposes of method comparison.

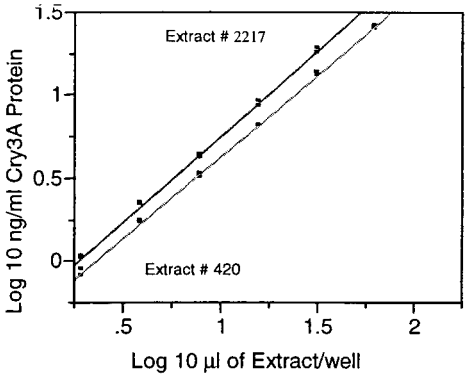

Figure 2. Parallelism and linearity of dual-label TRFIA for Cry3A protein in leaf extracts of potato: Sample 420, mean Cry3A protein = 106.3 ng/mL, standard deviation = 2.9, range = 102.1 to 109.8 ng/mL; sample 2217, mean Cry3A protein = 140.0 ng/mL, standard deviation = 4.8, range = 135.0 to 146.9 ng/mL.

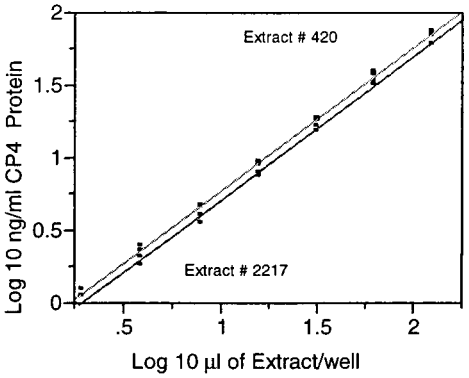

Figure 3. Parallelism and linearity of dual-label TRFIA for CP4 EPSPS protein in leaf extracts of potato: Sample 420, mean CP4 EPSPS protein = 148.7 ng/mL, standard deviation = 3.4, range = 145.1 to 152.7 ng/mL; sample 2217, mean CP4 EPSPS protein = 126.0 ng/mL, standard deviation = 3.9, range = 120.9 to 130.2 ng/mL.

Method Comparison (ELISA vs TRFIA). Overall, there was good agreement between the dual-label TRFIA and ELISA. As expected, some minor bias was noticed between the two methods. Eighty-one CP4 EPSPS positive sample extracts were analyzed to determine CP4 EPSPS protein concentrations. There was a slight proportional error of 14.2% between the two methods as illustrated by the slope of the line generated by plotting the results derived from both methods (Figure 4). Using a Student's *t* test, the 95% confidence interval of the slope, which ranged from 1.095 to 1.190, showed that the slope value of 1.142 was statistically different from a 1.0 value. The 95% confidence interval of the *y* intercept ranged from −1.238 to 0.499, and the intercept value of −0.370 was not statistically different from 0. On the basis of these data, it is concluded that the TRFIA yields comparable results when compared to the conventional ELISA for quantitation of CP4 EPSPS protein in potato leaf extracts.

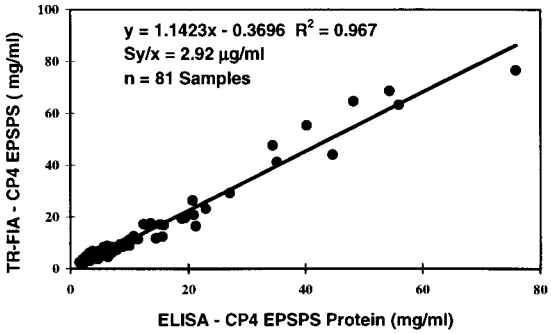

Figure 4. Comparison between CP4 EPSPS protein concentrations in leaf extracts of potato as measured by dual-label TRFIA and ELISA. Regression analysis of the concentration of CP4 EPSPS protein was determined by both analytical methods.

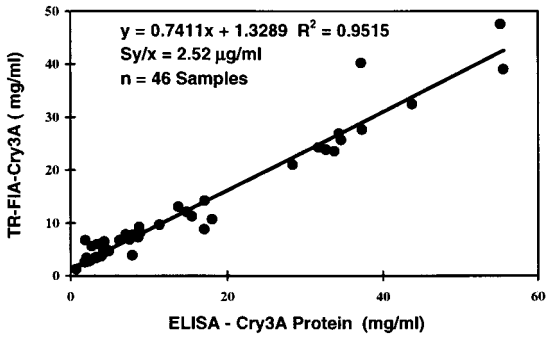

Figure 5. Comparison between Cry3A protein concentrations in leaf extracts of potato as measured by dual-label TRFIA and ELISA. Regression analysis of the concentration of Cry3A protein was determined by both analytical methods.

For the Cry3A protein, 46 samples were fitted into a regression analysis (Figure 5). Using a Student's *t* test, the 95% confidence interval of the slope ranged from 0.69 to 0.792. The slope value of 0.74 was statistically different from 1.0. The 95% confidence interval of the *y* intercept ranged from 0.274 to 2.383. The intercept value of 1.33 was also statistically different from 0. On the basis of the slope and intercept values, it is concluded that a bias exists between the results generated by the two assays. Experiments describing the analytical recovery of Cry3A protein measured by ELISA report an over-recovery of 29.5% (data not

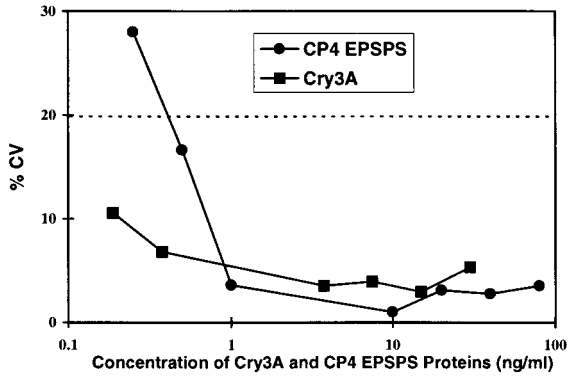

Figure 6. Intra-assay precision profile of CP4 EPSPS and Cry3A proteins in the dual-label TRFIA (n = five sets of duplicate wells across the microtiter plate). The LOQs for CP4 EPSPS protein ranged from 0.5 to 80 ng/mL and from 0.19 to 30 ng/mL for Cry3A protein.

shown). Therefore, it is concluded that the observed differences between the TRFIA and ELISA are due to an overestimate of the Cry3A protein concentration attributable to bias associated with the ELISA.

Precision of the Assay. Precision of the dual-label TRFIA was excellent for both CP4 EPSPS and Cry3A proteins. The intra-assay precision % CV ranged from 1.0 to 16.6% for CP4 EPSPS protein and from 2.9 to 10.6% for Cry3A protein (Figure 6). Inter-assay precision for the dual-label TRFIA was 13.1% and 12.5% for the CP4 EPSPS and Cry3A proteins, respectively.

The limits of quantitation (LOQ) or quantitative range was 0.5 to 80.0 ng/mL and 0.19 to 30.0 ng/mL for CP4 EPSPS and Cry3A proteins, respectively (Figure 6, Table 1). The limits of detection (LOD) of TRFIA for CP4 EPSPS and Cry3A proteins were constant at all three dilutions of leaf matrix tested. The LOD of the TRFIA for CP4 EPSPS protein at 1:5, 1:10, and 1:20 dilutions of conventional leaf extracts with assay buffer were 0.16, 0.17, and 0.18 ng/mL, respectively, with an overall mean of 0.17 ng/mL. The LOD of TRFIA for Cry3A protein at matrix dilutions of 1:5, 1:10, and 1:20 were 0.17, 0.15, and 0.17 ng/mL, respectively, with an overall mean of 0.16 ng/mL. All samples exceeding the mean LOD of 0.17 and 0.16 ng/mL for CP4 EPSPS and Cry3A proteins, respectively, were considered positive. The implication of a constant LOD over different sample extract dilutions as demonstrated in this study permits the broad use of one single LOD across extracts that may have been tested in the dual-label TRFIA at different dilutions.

In conclusion, we report the successful development and validation of a dual-label, time-resolved fluorometric immunoassay that permits simultaneous detection and quantitation of two agricultural biotechnology derived proteins (CP4 EPSPS and Cry3A) in leaf extracts of potato. The performance characteristics of this TRFIA are either similar to or exceed those of ELISA and we speculate that this technology could be readily adaptable to numerous plant matrixes.

This TRFIA is more accurate, has a greater working range, and is a more flexible analytical method compared to two conventional ELISAs routinely used for quantitation of CP4 EPSPS and Cry3A proteins in potato (Table 1). This technology is and will be a critical component for the development, safety assessment, and commercialization of plants improved through agricultural biotechnology.

ABBREVIATIONS

CP4 EPSPS, CP4 5-enol-pyruvyl-shikimate-3-phosphate; CPS, counts per second; ELISA, Enzyme-Linked Immunosorbent Assay; Eu^{3+}, europium metal; HRP, horseradish peroxidase; LOD, limit of detection; LOQ, limit of quantitation; Mab, monoclonal antibody; % CV, percent coefficient of variation; SD, standard deviation; Sm^{3+}, samarium metal; TRFIA, Time Resolved Fluoroimmunoassay.

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