Supporting Information

Antibody Array in a Multiwell Plate Format for the Sensitive and Multiplexed Detection of Important Plant Pathogens

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ABSTRACT

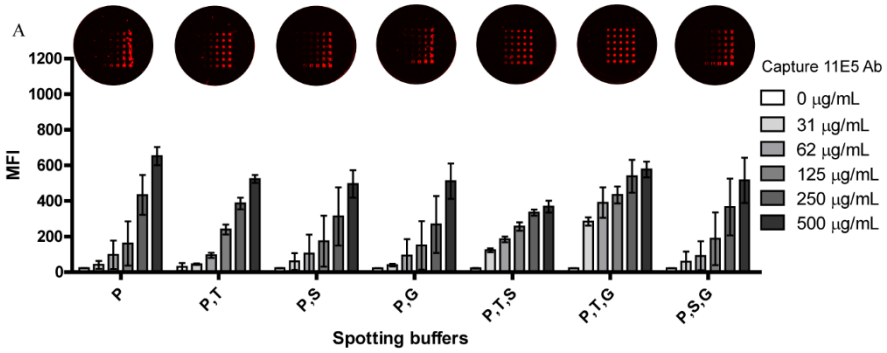
The global seed market is considered to be an important industry with a total value of $10,543 million US dollars in 2012. Because plant pathogens such as bacteria and viruses cause an economic loss to both producers and exporters, the seed export industry urgently requires rapid, sensitive, and inexpensive testing for the pathogens to prevent disease spreading worldwide. This study developed an antibody array in a multiwell plate format to simultaneously detect four crucial plant pathogens, namely, a bacterial fruit blotch bacterium *Acidovorax avenae* subsp. *citrulli* (Aac), *Chilli veinal mottle virus* (ChiVMV, potyvirus), *Watermelon silver mottle virus* (WSMoV, tospovirus serogroup IV), and Melon yellow spot virus (MYSV, tospovirus). The capture antibodies specific to the pathogens were immobilized on each well at preassigned positions by an automatic microarrayer. The antibodies on the arrays specifically captured the corresponding pathogens present in the sample extracts. The presence of pathogens bound on the capture antibodies was subsequently detected by a cocktail of fluorescently conjugated secondary antibodies. The limits of detection of the developed antibody array for the detection of Aac, ChiMV, WSMoV, and MYSV were 5×10^5 CFU/mL, 30 ng/mL, 1000 ng/mL, and 160 ng/mL, respectively, which were very similar to those of conventional ELISA method. The antibody array in a multiwell plate format accurately detected plant pathogens in single and multiple detections. Moreover, this format enables easy handling of the assay at a higher speed of operation.

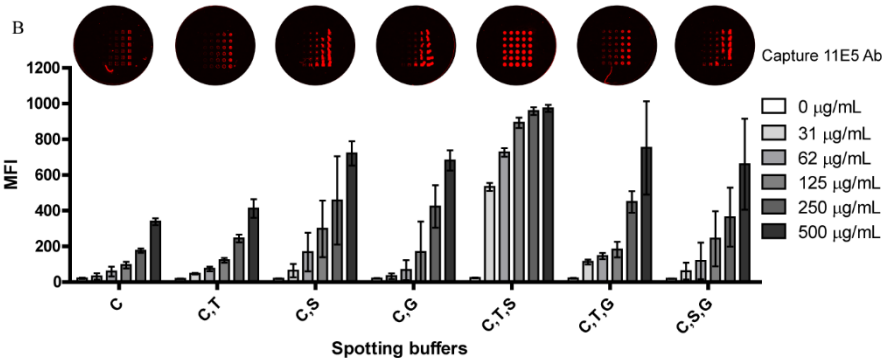
Supporting Information (SI) contains the following details:

Figure S-1. Optimization of spotting buffers for antibody array fabrication on 96-well plate. Fourteen spotting buffers based on (A) phosphate buffered saline pH 7.4 (P) and (B) carbonate-bicarbonate buffer pH 9.6 (C) supplemented with combination of Tween20 (T), sucrose (S) and/or glycerol (G) were test with different concentrations (0, 31, 62, 125, 250 and 500 µg/mL) of capture antibody against *Acidovorax avenae* subsp. *citrulli* (Aac) (11E5 Ab). (C) Image zoom in each spotting buffers at 500 µg/mL of capture antibody. (D) The percentages of coefficient of variation (CV) were calculated for each spotting buffer (n = 6, 6 spots). The system was tested with Aac at 10^9 CFU/mL. The fluorescent signals obtained from detecting molecules (Cy5 labeled MPC, 50 µg/mL) reported in term of mean fluorescent intensity (MFI). Each data set was plotted as a mean of six replicates ± standard deviation.

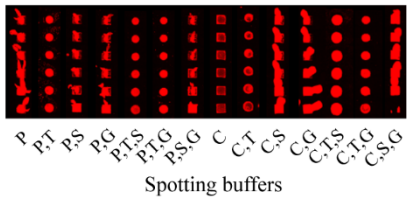
Figure S-2. Optimization of the blocking buffer. Three blocking buffers: 1% gelatin, 2% bovine serum albumin (BSA) and 3% skim milk were tested by detecting *Acidovorax avenae* subsp. *citrulli* (Aac) in PBST and spiked in healthy watermelon extract with different concentrations (0, 31, 62, 125, 250 and 500 µg/mL) of capture antibody against Aac. (A) Image of antibody array acquired from a fluorescence scanner. (B) The fluorescent signals reported in term of mean fluorescent intensity (MFI). Each data set was plotted as a mean of six replicates ± standard deviation.

Figure S-3. Optimization of concentrations for capture and secondary antibodies. Four capture antibodies (11E5, 1B4, 2D6, and 5E7) were spotted in a well at five different concentrations (0, 31, 63, 125, and 250 µg/mL for 11E5 antibody; 0, 125, 250, 500, 100 µg/mL for 1B4 antibody; 0, 250, 500, 1000, 1500 µg/mL for 2D6 and 5E7 antibodies). Each antigen was added to the fabricated antibody array before each Cy5-labeled antibody was used to detect the presence of the antigen. Four concentrations of secondary antibodies (5, 10, 20, and 40 µg/mL) were optimized for each antigen. (A) Aac detection: Aac (10^9 CFU/mL) and Cy5-MPC, (B) ChiVMV detection: ChiVMV (0.5 µg/mL) and Cy5-1G8, (C) WSMoV detection: WSMoV (50 µg/mL) and Cy5-MYSV6 (C), and (D) MYSV detection: MYSV (10 µg/mL) and Cy5-MYSV6. Six replicates were performed for each data set and the mean fluorescent intensity was reported. The arrow symbol indicates the optimal condition for each pathogen detection.





C



D

Spotting buffer	CV (%) (n=36)	Spotting buffer	CV (%) (n=36)
P	75.4	C	32.9
P,T	9.9	C,T	11.7
P,S	99.6	C,S	60.1
P,G	81.7	C,G	78.1
P,T,S	8.5	C,T,S	3.0
P,T,G	13.2	C,T,G	20.0
P,S,G	98.6	C,S,G	79.8

P = phosphate buffered saline pH 7.4, C = carbonate buffer pH 9.6, T = Tween20, S = sucrose, G = glycerol

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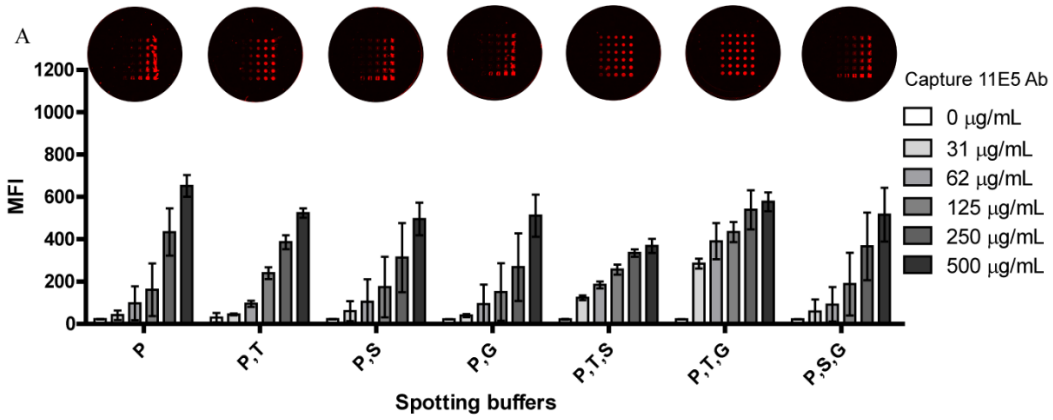
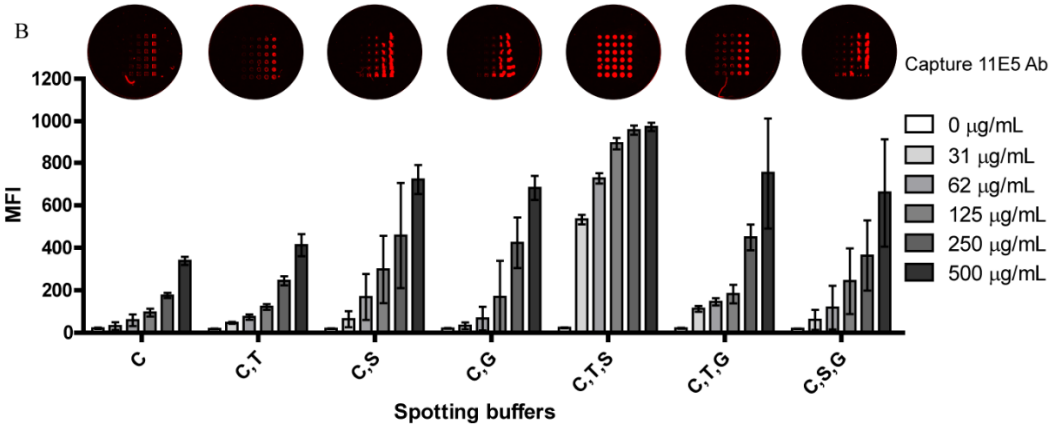
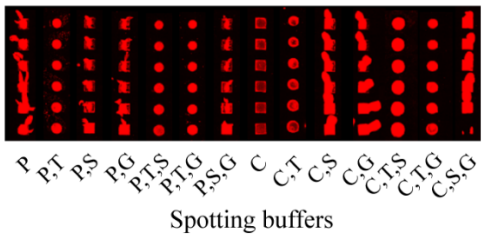




C



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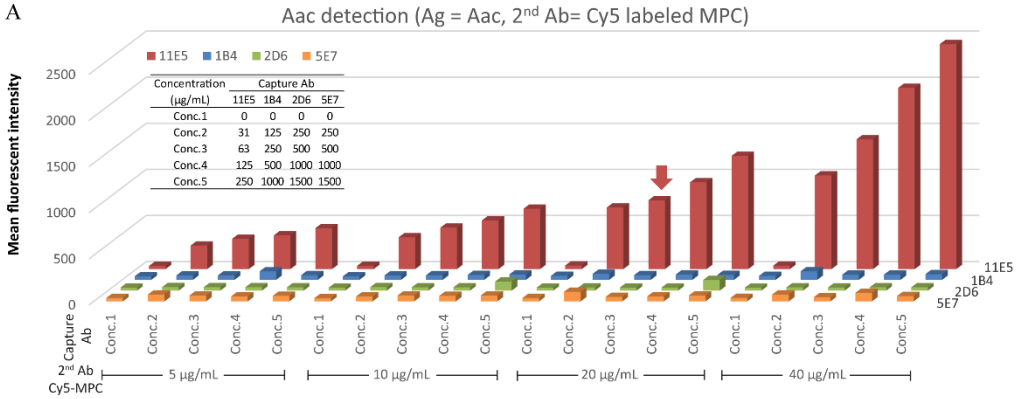
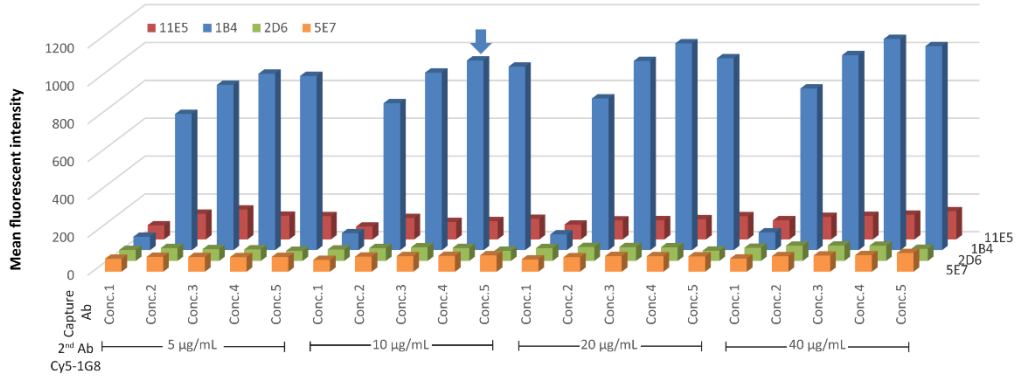
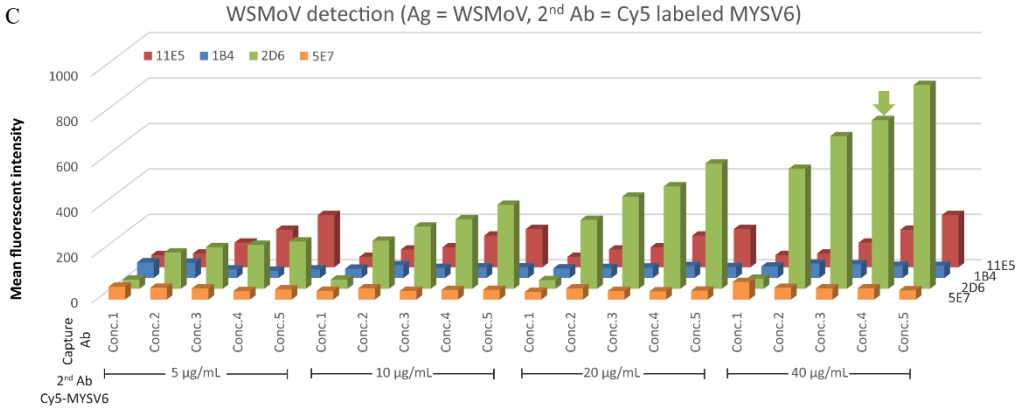
A


Aac detection (Ag = Aac, 2nd Ab= Cy5 labeled MPC)
11E5 1B4 2D6 5E7
Mean fluorescent intensity
2500
2000
1500
1000
500
0
Concentration (μg/mL) | Capture Ab 11E5 1B4 2D6 5E7
Conc.1 0 0 0 0
Conc.2 31 125 250 250
Conc.3 63 250 500 500
Conc.4 125 500 1000 1000
Conc.5 250 1000 1500 1500
Capture Ab
Conc.1 Conc.2 Conc.3 Conc.4 Conc.5
2nd Ab Cy5-MPC
5 μg/mL
10 μg/mL
20 μg/mL
40 μg/mL
11E5
1B4
2D6
5E7

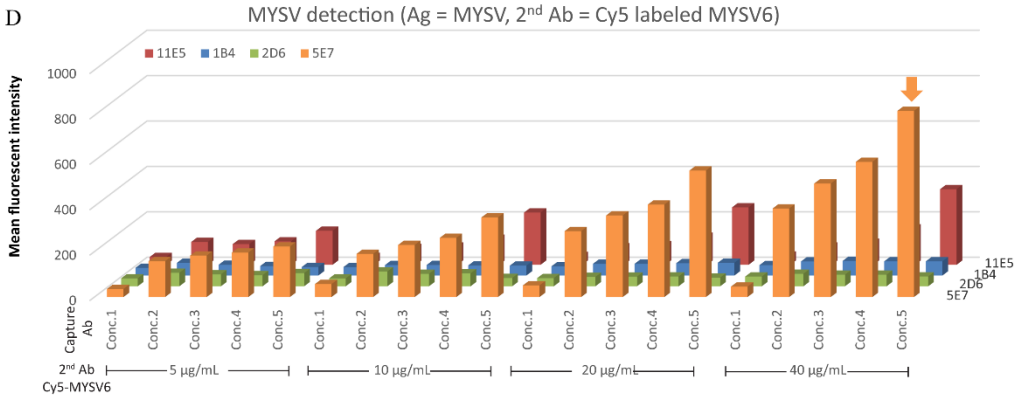
B


ChiVMV detection (Ag = ChiVMV, 2nd Ab = Cy5 labeled 1G8)
11E5 1B4 2D6 5E7
Mean fluorescent intensity
1200
1000
800
600
400
200
0
Capture Ab
Conc.1 Conc.2 Conc.3 Conc.4 Conc.5
2nd Ab Cy5-1G8
5 μg/mL
10 μg/mL
20 μg/mL
40 μg/mL
11E5
1B4
2D6
5E7

C


WSMoV detection (Ag = WSMoV, 2nd Ab = Cy5 labeled MYSV6)
11E5 1B4 2D6 5E7
Mean fluorescent intensity
1000
800
600
400
200
0
Capture Ab
Conc.1 Conc.2 Conc.3 Conc.4 Conc.5
2nd Ab Cy5-MYSV6
5 μg/mL
10 μg/mL
20 μg/mL
40 μg/mL
11E5
1B4
2D6
5E7

D


MYSV detection (Ag = MYSV, 2nd Ab = Cy5 labeled MYSV6)
11E5 1B4 2D6 5E7
Mean fluorescent intensity
1000
800
600
400
200
0
Capture Ab
Conc.1 Conc.2 Conc.3 Conc.4 Conc.5
2nd Ab Cy5-MYSV6
5 μg/mL
10 μg/mL
20 μg/mL
40 μg/mL
11E5
1B4
2D6
5E7

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