SORTING OF DIFFERENT *ARABIDOPSIS* SEED TYPES

OBJECTIVE

The purpose of this experiment was to test the feasibility of using the COPAS™ PLUS instrument to analyze and distinguish between mixed populations of different types of *Arabidopsis thaliana* seeds. In order to verify the experiment results, seeds were sorted for microscopic inspection. Wild type seeds were used as negative control.

INTRODUCTION

The COPAS PLUS instrument is able to analyze and sort large objects (200-600 microns) on the basis of the physical characteristics of size, density, and fluorescence signals. In this experiment using the COPAS PLUS instrument, we analyzed and sorted mixed populations of different types of *Arabidopsis* seeds and then dispensed the seeds into 96-well plates. Wild type seeds were used as negative control. The seeds were visually inspected for accuracy.

MATERIALS AND METHODS

The following seed samples were mixed, analyzed, and sorted:

- Tetraploid seeds were mixed with wild type.
- GFP labeled seeds were mixed with wild type seeds.
- Transparent testa mutant seeds were added to the mixture of GFP positive and wild type seeds.

Data was acquired using the COPAS PLUS and analyzed using the WinMDI software.

RESULTS

Sample 1:

Tetraploid seeds are known to be larger than wild type seeds. It was therefore expected that the COPAS instrument would be able to distinguish these seeds from wild type seeds using the length measurement, which is called Time Of Flight (TOF). After initial set up of the instrument, the sample was analyzed and it was determined that the two populations are not completely distinct. We then analyzed the sample using the TOF measurement and a measurement of the optical density of the seeds, which is called Extinction (EXT). The dot plot in Figure 2 shows a mixed population on the basis of TOF and EXT. Using the multi-parametric approach, tetraploid seeds could be distinguished from the wild type seeds. A region (R2) representing the largest objects in the sample was selected for sorting. These were dispensed into wells of a 96-well plate. Visual inspection confirmed that all of the seeds sorted from Region 2 were tetraploid.

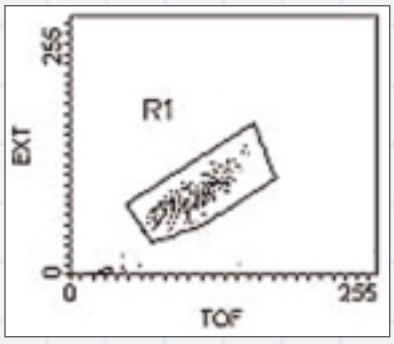

Figure 1. TOF/EXT for wild type seeds.

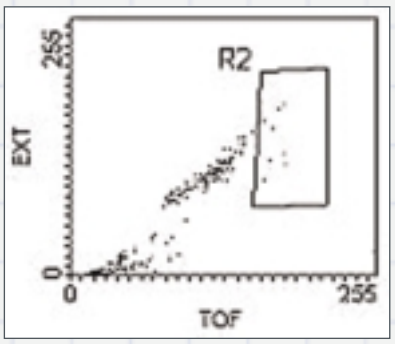

Figure 2. TOF/EXT for a mixed population of wild type and tetraploid seeds.

WORK PERFORMED SEPTEMBER 2003 BY:

Bergervoet, J.H.W.[1], Bongaarts, R.[2]
1 Plant Research International, Wageningen, Netherlands
2 Union Biometrica, Geel, Belgium

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Sample 2:

Wild type seeds were mixed with GFP positive seeds and run on the COPAS PLUS to verify that the instrument can distinguish between fluorescent and non-fluorescent seed populations. Two fluorescence parameters were used, FLU1 and FLU2, where FLU1 represents the green emission (515 nm) and FLU2 represents the red emission (585 nm). A region (R1) representing the brightest objects in the sample was selected for sorting (Figure 4). These were dispensed into wells of a 96-well plate. Visual inspection confirmed that all seeds sorted from Region 1 were GFP positive.

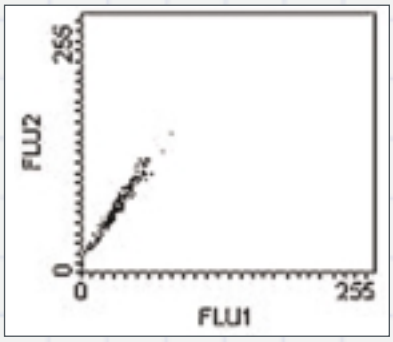

Figure 3. FLU1/FLU2 for wild type seeds.

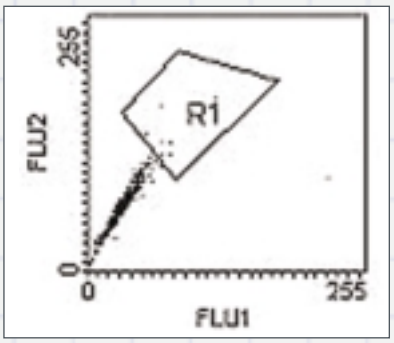

Figure 4. FLU1/FLU2 for a mixed population of wild type and GFP labeled seeds

Sample 3:

Transparent testa seeds were mixed with the wild type and GFP positive seeds and run on the COPAS PLUS to determine if the instrument can identify a difference between the seeds with a transparent seed coat from the other two populations on the basis of fluorescence. Seeds from this *Arabidopsis thaliana* mutant line appear yellow, due to the lack of condensed tannin pigments in the seed coat. The autofluorescence of the transparent testa seeds as viewed by microscopy is very high and more intense than the GFP signal and therefore was anticipated to be distinguishable using the measurements of FLU1 and FLU2. The instrument settings were adjusted (lower sensitivity) to visualize the transparent testa seeds in the mixed population. Once this was done, transparent testa seeds could be easily identified and sorted. A region (R1) representing the brightest objects (FLU1, green emission vs. FLU2, red emission) in the sample was selected on a dot plot for sorting (Figure 6). These were dispensed into wells of a 96-well plate. Visual inspection confirmed that all sorted seeds from Region 1 were transparent testa mutant seeds.

Figure 5. Microscopic image of transparent testa (tt16 on left) seeds and wild type (WT on right) seeds.

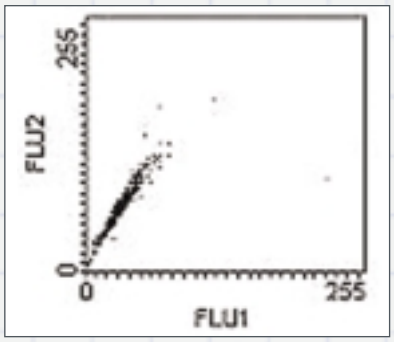

Figure 6. FLU1/FLU2 for mixed seeds.

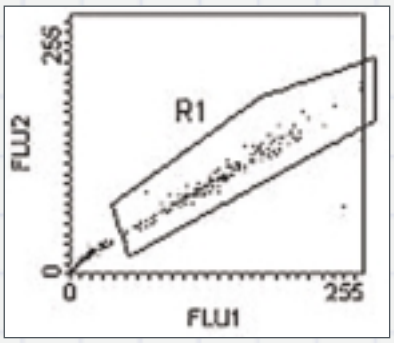

Figure 7. FLU1/FLU2 for mixed seeds after adjusting the instrument to separate (or distinguish) the transparent testa seeds in the mixed population.

CONCLUSION

These three experiments demonstrate that the COPAS PLUS may be used to analyze, sort, and dispense the different types of *Arabidopsis* seeds and also distinguish between them. All experiments resulted in 100% purity of the selected seed type.

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