Sorting of Different Arabidopsis Seed Types

Objective

The purpose of this experiment was to test the feasibility of using the COPAS™ PLUS instrument (1000µm flow channel) to analyze and distinguish between mixed population 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 a negative control.

Introduction

The COPAS family of instrumentation comprises a collection of large particle cytometers utilizing at least 1 excitation laser and up to 8 channels of fluorescence collection. Large objects can be analyzed for size, density, and fluorescence characteristics before being gently dispensed into a multi-well plate or other collection container for further investigation or reuse. In this experiment, the COPAS PLUS instrument with a 1000µm flow channel was used to analyze and sort mixed population of different types of *Arabidopsis* seeds into 96-well plates. Wild type seeds were used as negative control and plates were inspected for sort accuracy.

Results

Sample 1: Tetraploid seeds mixed with wild type.

Tetraploid seeds are known to be larger than wild type seeds. It was therefore expected that the COPAS PLUS instrument would be able to distinguish these seeds from wild type seeds using the length measurement, (Time of Flight or 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 (Extinction or EXT). The dot plot in figure 2 shows a mixed population on the basis of TOF and EXT. Using the multiparametric approach, tetraploid seeds could be distinguished from the wild type seeds. A region (R2) representing the larges objects in the sample was selected for sorting into the wells of a 96-well plate. Visual inspection confirmed that all for the seeds sorted from region 2 were tetraploid.

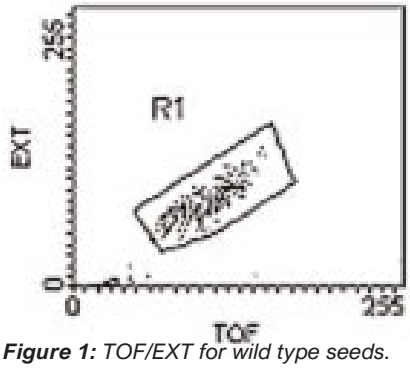

Figure 1: *TOF/EXT for wild type seeds.*

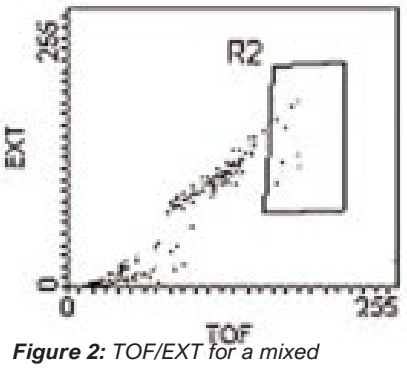

Figure 2: *TOF/EXT for a mixed population of wild type and tetraploid seeds.*

Sample 2: GFP labeled seeds mixed with wild type seeds.

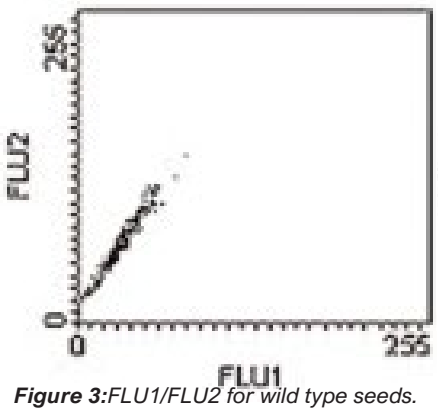

Figure 3:*FLU1/FLU2 for wild type seeds.*

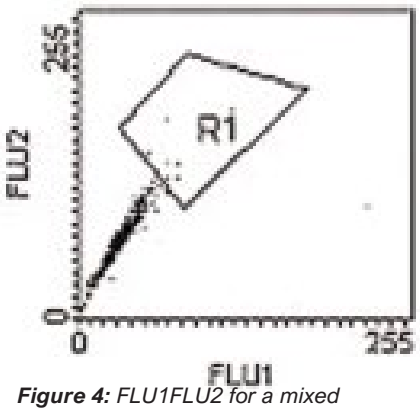

Figure 4: *FLU1FLU2 for a mixed population of wild type and GFP labeled seeds.*

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 (representing green emission at 512nm) and FLU2 (representing red emission at 585nm). A region (R1) representing the brightest objects in the sample was selected for sorting (figure 4) into wells of a 96 well plate. Visual inspection confirmed that all seeds sorted from Region 1 were GFP positive.

Sample 3: Transparent testa seeds mixed with wild type and GFP positive seeds.

The COPAS PLUS was used to 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 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 2 were transparent mutant seeds.

Figure 5: *Microscopic image of transparent testa (tt16 on left) seeds and wildtype (WT on right) seeds.*

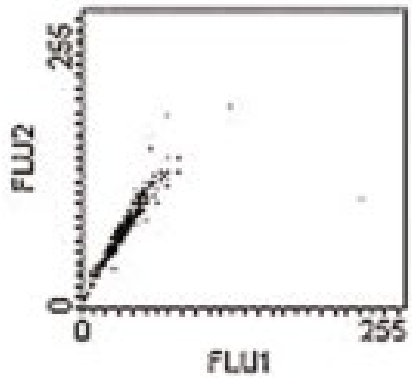

Figure 6: *FLU1/FLU2 for mixed seeds.*

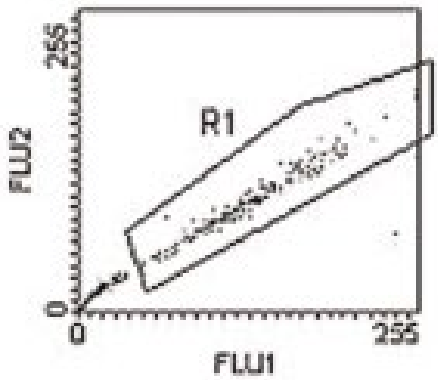

Figure 7: *FLU1/FLU2 for mixed seeds after adjusting the instrument to separate (or distinguish the transparent testa seeds in the mixed population*

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.

Work Performed 2003, reissued 2024 by:
BergerVoet, J.H.W.[1], Bongaarts, R[2]
1 Plant Research International, Wnageningen, Netherlands
2 Union Biometrica, Geel, Belgium