Video Article

Measuring Endoreduplication by Flow Cytometry of Isolated Tuber Protoplasts

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Abstract

Endoreduplication, the replication of a cell's nuclear genome without subsequent cytokinesis, yields cells with increased DNA content and is associated with specialization, development and increase in cellular size. In plants, endoreduplication seems to facilitate the growth and expansion of certain tissues and organs. Among them is the tuber of potato (*Solanum tuberosum*), which undergoes considerable cellular expansion in fulfilling its function of carbohydrate storage. Thus, endoreduplication may play an important role in how tubers are able to accommodate this abundance of carbon. However, the cellular debris resulting from crude nuclear isolation methods of tubers, methods that can be used effectively with leaves, precludes the estimation of the tuber endoreduplication index (EI). This article presents a technique for assessing tuber endoreduplication through the isolation of protoplasts while demonstrating representative results obtained from different genotypes and compartmentalized tuber tissues. The major limitations of the protocol are the time and reagent costs required for sample preparation as well as relatively short lifespan of samples after lysis of protoplasts. While the protocol is sensitive to technical variation, it represents an improvement over traditional methods of nuclear isolation from these large specialized cells. Possibilities for improvements to the protocol such as recycling enzyme, the use of fixatives, and other alterations are proposed.

Video Link

The video component of this article can be found at https://www.jove.com/video/57134/

Introduction

Endoreduplication is the process by which a cell forgoes the typical cell cycle and instead undergoes an alternate course of development consisting of repeated rounds of DNA replication without cellular division. The resulting cell will have increased DNA content and nuclear size which is thought to play a role in cellular regulation, expansion, and specialization. Generally, a round of endoreduplication (termed an endocycle) and the corresponding increase in DNA content are associated with larger cell volume, an observation that precipitated the "karyoplasmic theory" that increased DNA content is required to properly regulate a larger, perhaps more complex, cell[1]. This phenomenon is common in higher plants, having been observed in a range of tissues including those with structural/defensive (trichomes)[2,3], nutritive (maize endosperm)[4,5], and sink/storage (tomato pericarp; potato tuber)[6,7,8] functions. In fruit, it has been suggested that endoreduplication plays a role in facilitating the rapid expansion of the pericarp as evidenced by the negative relationship between endoreduplication and fruit developmental period[9]. For instance cells with DNA content up to 512C (512 times the haploid genome) have been observed in the tomato pericarp[8]. Furthermore Chevalier et al. (2014) demonstrated that alterations in expression of cell cycle genes can lead to increases in endoreduplication levels within the pericarp which then results in larger fruit[10]. Thus, alteration of genes promoting endoreduplication provides a potential target for improvement of biomass or yield through plant breeding or genetic manipulation. However, such improvement is contingent upon greater understanding of the causes and consequences of endoreduplication.

Endoreduplication is most often measured via flow cytometry whereby nuclei, released in crude tissue preparations[11], are incubated with a DNA-binding fluorophore, such as propidium iodide[12] (PI). The filtered samples are then passed by the laser of a flow cytometer where emission wavelengths specific to the fluorophore can be observed. The intensity of fluorescence in each event (*i.e.*, nucleus) is directly correlated with the DNA-content of the particle. Thus, by comparing to a known standard, the relative and absolute DNA content of cells in a given sample may be calculated. Endoreduplication indices (EI) are determined by establishing the average number of endocycles per cell within a sample by observing cellular DNA content (C-value) where 1C is the DNA content of a haploid cell (formula presented in step 6.7 of the protocol). For instance, in a diploid organism, the base DNA content of somatic cells is 2C. If a sample has few cells with 4C, corresponding to a single round of endoreduplication, or greater it would have an EI near 0; however if nearly all the cells are 4C the EI would be approximately 1. However, as multiple rounds of endoreduplication are common in higher plants observed EI values may be much greater. While calculating endoreduplication indices may be relatively straightforward, it requires that relative abundances of nuclei C-values be reliably ascertained, which is precluded in certain species and tissues (including potato tubers). It is likely that differences in cellular anatomy, chemistry or cell wall composition[13] cause these samples to be recalcitrant to the typical preparations using a razor blade to release the nuclei directly into appropriate buffers that are commonly used with tissues such as tomato pericarp, a model for endoreduplication studies[8].

In potato, the examination of endoreduplication within the tuber remains limited to just a couple studies, perhaps due in part to a lack of a reliable protocol as the aforementioned crude preparations yield inconsistent results. While such approaches work well for leaves the tuber samples experience severe degradation and an abundance of noise in the form of debris, making differentiation of peaks comprised of nuclei with differing C-values nearly impossible. This is perhaps due to differences in cellular composition and a profusion of cellular debris within tuber cells (*e.g.* starch-storing amyloplasts). To overcome this hurdle, we recently developed a more reliable protocol for acquiring distinguishable peaks in flow cytometry of potato tubers. The frequency of cells within each peak can then be used for calculating accurate endoreduplication levels for different genotypes or different tissues that comprise a tuber. The protocol, which employs a modified protoplast extraction method[14,15] antecedent previously described flow cytometry[11], showed considerable variation within potato relatives, cultivars, and tissues[16]. Here we present the protocol in detail by evaluating EI in the contexts of ploidy, tissue, and tuber size.

Protocol

NOTE: It is important to include appropriate controls, usually in the form of leaf tissue of the same ploidy as experimental samples. This is because the 2C peak of tuber samples may be small and difficult to identify.

1. Preparations

NOTE: The solutions listed here may be prepared ahead of time and stored at 4 °C but those presented in subsequent steps must be made fresh on the day of use.

1. Make an appropriate volume of Plasmolysis Incubation Solution (PIS) (15 mL/sample): 0.55 M mannitol, 2 mM $CaCl_2$, 1 mM KH_2PO_4, 1 mM $MgCl_2$, 50 mM Tris buffer pH 7.5 (adjusted with HCl).
2. Make an appropriate volume of Plasmolysis Wash Solution (PWS) (15 mL/sample) which is identical to PIS except for addition of 0.71 M mannitol.
3. Make 250 mL of modified Galbraith's Flow Cytometry Buffer[11] (FCB): 13.6 mM sodium citrate (trisodium), 8 mM MOPS, 18 mM $MgCl_2$, 0.4% v/v Triton X-100. Stir for at least 30 min to distribute the Triton X-100.
4. Filter sterilize PIS and PWS solutions with a 0.22 μm asymmetric polyethersulfone (aPES) filter. Use vacuum driven filtration units.
5. **Prepare 106 μm mesh filters for lysed protoplasts. Prepare 1.5 mL microcentrifuge tubes which may be directly nested into collection tubes, however any method of passing suspensions through a 106 μm filter may be used.**
   NOTE: These do not need to be sterile.
   1. If using 1.5 mL microcentrifuge tubes, cut 1 cm off the tip each microcentrifuge tube. Using a hot plate or other heat source heat a 1 cm^2 piece 106 μm steel mesh on a piece of aluminum foil. Press the cut end of the tube into the mesh until they have fused.

6. Wash potatoes to be sampled, removing all soil and debris.
   NOTE: Tuber size and age should be appropriate for the goals of the experiment but do not seem to affect quality of results. We have successfully applied this protocol to tubers which have been in cold storage (4 °C, 95% RH) up to 8 months. While tubers with defects (*e.g.* tuber end rot, hollow heart, brown necrotic areas) may be responsive, they should be avoided, if possible.

2. Day 1: Plasmolyze Tuber Tissue

NOTE: Control leaf samples can be included here to produce protoplasts or at step 5.2 if crude preparations are preferred. This must be performed with sterile tools in an aseptic environment such as a laminar flow hood.

1. Surface sterilize potato tubers via immersion in 75% ethanol for at least 5 min.
2. Remove tubers from ethanol and air dry. This usually takes ~5 min, longer if the tuber is larger than 100 g.
3. Prepare and label sterile 50 mL conical tubes for each sample and aliquot 15 mL of filter sterilized PIS into each.
4. **Sample approximately 1 cm^3 of the desired tissue from sterilized tubers using either sterilized scalpel or cork borer.**
   NOTE: Depending on the tissue being sampled one may use either a sterilized cork borer or knife/scalpel. For instance, if the pith is desired the cork borer may be used to take a core from the apical to distal end of the tuber and the central fraction of the core may be sampled. However, due to the asymmetric internal morphology of potato tubers, it may be more precise to sample parenchyma or cortex by halving the tuber and excising the desired tissue. See **Figure 1** for a depiction of internal tuber morphology.
   1. Coarsely chop tissue using a sterilized scalpel into approx. 3 mm^3 pieces and transfer tissue to conical tube containing PIS. Incubate overnight at 4 °C.
      NOTE: This is to maximize surface area so that the PIS, and later the enzyme solution, may fully permeate the tissue resulting in more complete digestion.
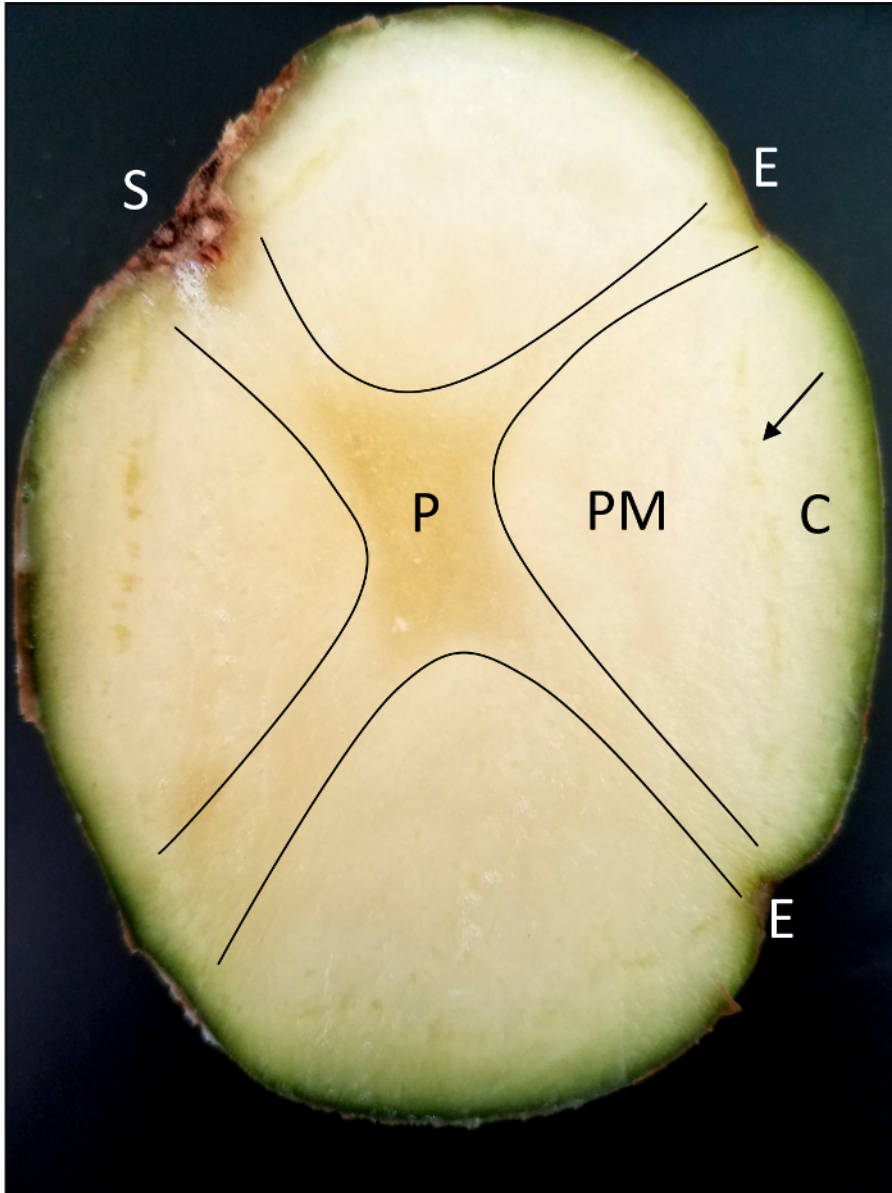
Figure 1: Internal morphology of a potato tuber. The separation between pith (P) and perimedullary parenchyma (PM) is illustrated with black lines. The vascular ring, which separates parenchyma from the cortex (C) is denoted with a black arrow. The stolon end (S) and tuber eyes (E) are labeled as well. Please click here to view a larger version of this figure.

3. Day 2: Generate Potato Protoplasts

NOTE: This must be performed with sterile tools in an aseptic environment such as a laminar flow hood.

1. Prepare and filter (0.45 µm aPES filter) an appropriate amount of Enzyme Solution (ES) (10 mL/sample): 0.71 M mannitol, 3 mM $CaCl_2$, 1 mM KH_2PO_4, 1 mM $MgCl_2$, 4% Onozuka R-10 cellulase, 0.8% macerozyme R-10, 1% hemicululase, 10 mM MES buffer, pH 5.8. Ensure that appropriate filters are used in this step; filters with pore size smaller than 0.45 µm are prone to clogging whereas a low protein binding membrane, such as aPES, minimizes loss of enzyme during filtration.
2. Aspirate off PIS from samples in conical tubes using a sterile serological pipette.
3. Add 10 mL of ES to each sample and invert 2–3 times.
4. Incubate samples overnight at 29 °C with 180 rpm horizontal shaking. Incubate samples for at least 16 h.

4. Day 3: Harvest Protoplasts and Wash Protoplasts

NOTE: Aseptic conditions are no long necessary at this point.

1. Remove samples from shaker and allow them to settle for ~10 min.
2. **Aspirate off as much of the ES solution as possible.**

1. Remove as much of the ES solution as feasible with a serological pipette, taking care to avoid removing the digested tissue.
2. Using a micropipette remove any remaining ES solution, again avoiding the digested tissue. This is most easily done by holding the tube at an angle so that the ES solution flows toward the lid while the digested tissue remains at the bottom.

3. Add 15 mL of PWS to each sample and invert gently 2–3 times. Allow protoplasts to settle for 10 min.
4. Remove PWS with a serological pipette. Use a micropipette to remove any remaining liquid.
   NOTE: This step is absolutely critical to ensure the integrity of the sample nuclei during flow cytometry. In troubleshooting, this step was found to be the most likely source of failure in a sample.

5. Day 3: Prepare Samples for Flow Cytometry

NOTE: The samples should be kept on ice from here on unless otherwise noted.

1. Prepare propidium iodide (0.4 mg propidium iodide/mL FCB) and RNase solutions (0.8 mg RNase A/mL FCB) by dissolving each in FCB.
   CAUTION: Propidium iodide is highly toxic. Avoid contact with skin or eyes. Wear appropriate PPE.
2. **Add 1.5 mL of ice cold FCB to each sample. Briefly shake or vortex each sample to break up aggregated tissue. It is important to break up the clumps of tuber tissue so that the FCB may fully permeate the cells and release the nuclei. Different samples may require more or less agitation depending on tissue and genotype.**
   NOTE: The FCB lyses the protoplasts, releasing the nuclei. Hence the necessity of keeping samples on ice to prevent degradation.
   1. If control flow cytometry samples were not included in the protoplast generation step they may be included here. If a control is to be added, finely chop a small leaf (~3 cm^2) in 1.5 mL of ice cold FCB using a razor blade. Control samples should be the same ploidy as the experimental samples, preferably the same genotype. *In vitro* plantlets tend to give cleaner peaks than greenhouse or field-grown plants.

3. **Pass 1 mL of the FCB/tissue suspension through a 106 μm mesh filter. Use a 1.5 mL microcentrifuge tube with the tip cut off and metal mesh melted to the bottom as described in step 1.5.1. This microcentrifuge tube may be nested directly into a 2 mL microcentrifuge tube and the sample passed directly through. If using another method of filtration, the filtrate may be collected in an ice-cold Petri plate and then transferred to a 2 mL tube.**
   NOTE: sometimes the remaining aggregates and cellular debris may clog the mesh filter. In this case, simply tap the two nested microcentrifuge tubes a few times to dislodge the debris.
4. Add 250 μL of the RNase solution to each sample. Invert and incubate for 30 min at room temperature (RT).
   NOTE: This step is intended to remove RNA from the samples, leading to less noise during flow cytometry. However, as the nuclei are short-lived, researchers may decide to decrease the incubation time or perform it on ice if they are experiencing severe degradation of their samples.
5. Add 125 μL of the propidium iodide solution to each sample. Invert and incubate on ice for 30 min.
   NOTE: Samples should be used for flow cytometry as soon as possible as degradation is apparent within 2 h, even on ice.

6. Day 3: Flow Cytometry of Potato Tuber Nuclei

NOTE: Flow cytometer operation and software will vary depending on the instrument and manufacturer. Detailed instructions on the specific operation of the flow cytometer will be provided in the instrument's User Guide.

1. Create two dot plots using logarithmic scale of forward scatter vs. side scatter and propidium iodide (PI) vs side scatter. Also create a histogram with PI on the x-axis. Logarithmic scale is required to ensure all events are within scale as nuclei may differ vastly in fluorescence.
2. **Load a known control sample tube and adjust the voltage so that all events are on scale. We use in vitro leaf tissue from a sample genotype. If samples of different ploidies are to be run, a control sample for each should be included.**
   1. Make note of the channel of the 2C peak in the control sample. The 2C peaks of the experimental samples should fall in the same location.

3. Load an experimental (tuber) sample and again ensure that all events are on scale. If adjustments are required, repeat step 6.2 to identify the channel of 2C peaks.
4. Manually gate the protoplast nuclei using the side scatter vs PI plot.
5. Set the PI histogram to only show the gated protoplast nuclei region.
6. Collect the desired number of events from each sample. Frequently researchers use 10,000 gated events for flow cytometry; however we use 2,000 events for tuber protoplast samples to accommodate more samples and samples with low concentrations.
7. Calculate each sample's EI from the PI histograms using the following formula:

$$EI = \frac{4C + (2 * 8C) + (3 * 16C) + (4 * 32C) + (5 * 64C) + (6 * 128C)...}{100}$$

where 4C is the percentage of nuclei which are 4C (representing a single round of endoreduplication), 8C is the percentage which are 8C, and so on. See **Figure 4** for examples of histograms and C-values of peaks.

Representative Results

Production of protoplasts

The generation of protoplasts is necessary to achieve repeatable flow cytometry results from potato tubers, the general morphology of which is displayed in **Figure 1**. Researchers may wish to ensure high quality protoplasts have been produced prior to the addition of the FCP, especially in the event that troubleshooting is required. The majority of the protoplasts should be spherical with minimal protrusions (**Figure 2**) and may differ greatly in size, which is perhaps reflective of differences in EI.
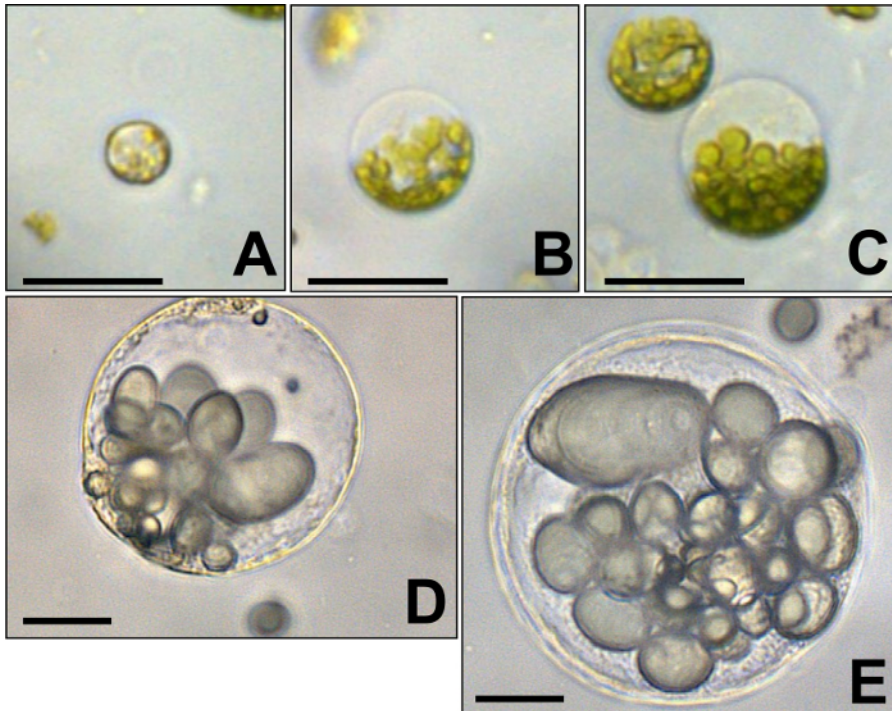

Figure 2: Representative leaf and tuber protoplasts acquired at step 4.4 of the protocol. Isolated protoplasts should be spherical and symmetrical with plasma membrane intact. Note the size difference between leaf (**A-C**) and tuber (**D, E**) protoplasts as well as the differences in size within a tissue which may indicate different levels of endoreduplication. Leaf protoplasts contain chloroplasts whereas amyloplasts are visible within the tuber protoplasts. Scale bars = 1,000 µm. Please click here to view a larger version of this figure.

Evaluation of flow cytometry results

The success or failure of an experiment may be gauged from the width of peaks and their separation in the flow cytometry histograms. As measuring endoreduplication requires calculating relative abundance of nuclei in each peak, mere presence or absence of peaks is insufficient if there is too much noise to draw meaningful boundaries between them. **Figure 3** displays the variation in results researchers may encounter in both the flow cytometry scatter plots and histograms. Potential causes for failed samples are presented within the discussion.
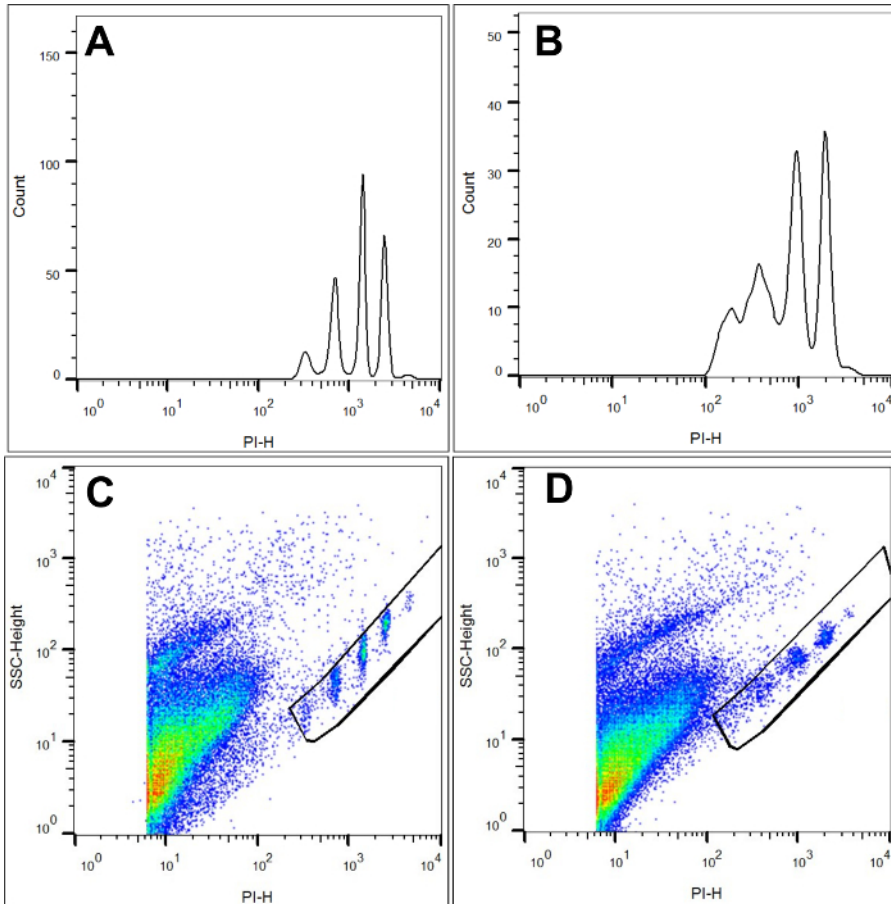
Figure 3: Representative results obtained from flow cytometry of protoplast nuclei of intact and degraded pith samples. Intact nuclei (**A**) show clean separation between peaks to quantify relative abundance of cells in each using the appropriate software whereas peaks in degraded samples (**B**) show shifted, wide and overlapping bases. In the scatterplots of intact (**C**) and degraded (**D**) samples, black boxes indicate nuclear event gating for histograms and the clustering of events is reflected in the width of the histogram peaks. Events outside the gates indicate debris, severely degraded nuclei, or other aggregates. PI-H corresponds to intensity of fluorescence which is measured in arbitrary units. Troubleshooting methods to prevent sample degradation are discussed within the text. Please click here to view a larger version of this figure.

Endoreduplication differences between tissues

Previously we reported that tuber pith tissue has significantly greater levels of endoreduplication than cortex tissue[16]. To confirm and expand on this we looked at endoreduplication levels of three different tissues in cv. Superior: pith, perimedullary parenchyma, and cortex, replicated in triplicate with 2,000 gated events per sample. Our results confirmed our earlier observation that pith tissue has significantly greater EI than cortical tissue with means of 1.79 and 1.12 endocycles per cell, respectively ($p = 0.018$; Student's t-test). Somewhat surprisingly, the parenchyma tissue demonstrated a profile similar to cortex and was also significantly different from pith tissue (mean = 1.14; $p = 0.013$; Student's t-test). These results are summarized in **Figure 4**.
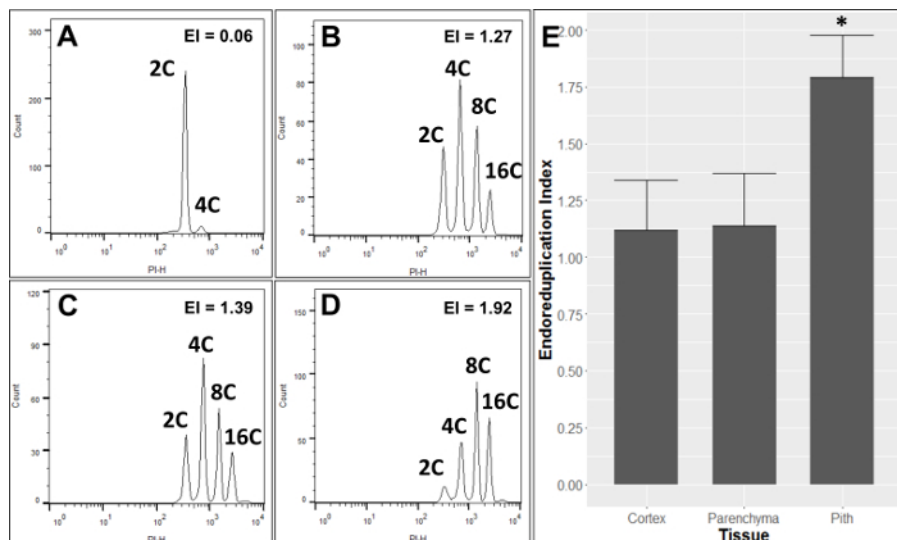
Figure 4: Endoreduplication in three tissues of cv. Superior. Histograms of leaf (**A**), tuber cortex (**B**), parenchyma (**C**), and pith (**D**) tissues are shown along with the EI value calculated from those histograms. Differences in relative abundance of nuclei comprising each peak are readily apparent, especially between leaf and tuber tissues. C-values for each peak are also displayed. PI-H corresponds to intensity of fluorescence which is measured in arbitrary units. Mean comparisons of EI of tuber tissues are displayed in E where asterisk indicates significant difference (p <0.05) and error bars show standard deviation. Please click here to view a larger version of this figure.

Influence of tuber size and ploidy

We previously reported that, for a given genotype, tubers of different size but similar maturity did not display a corresponding difference in EI[16]. We aimed to confirm this result as well as evaluate the relationship between ploidy and endoreduplication. For this experiment, we used three replicates of parenchyma tissue from three different genotypes: cv. Superior (4x), VT_SUP_19 (2x) which is a dihaploid extracted from cv. Superior by prickle pollination[17], and VT_SUP_19 4x which is a doubled dihaploid[18] isogenic to VT_SUP_19. We included a set of replicates for large (90–130 g) and small (<35 g) tubers for cv. Superior while tubers for the other two genotypes were 90–130 g. The tubers were all harvested from greenhouse grown plants at full maturity, *i.e.* the tops of the plants had senesced. We observed a significant difference between VT_SUP_19 and its progenitor Superior (p = 0.04); however, there was no significant difference between VT_SUP_19 and VT_SUP_19 4x (p = 0.69). This indicates that while there is a likely genetic component to endoreduplication, as unmasked by the genomic reduction, it is not dictated by ploidy, at least in this background. Lastly, we once again observed no significant differences between large and small cv. Superior tubers as demonstrated in **Figure 5**.
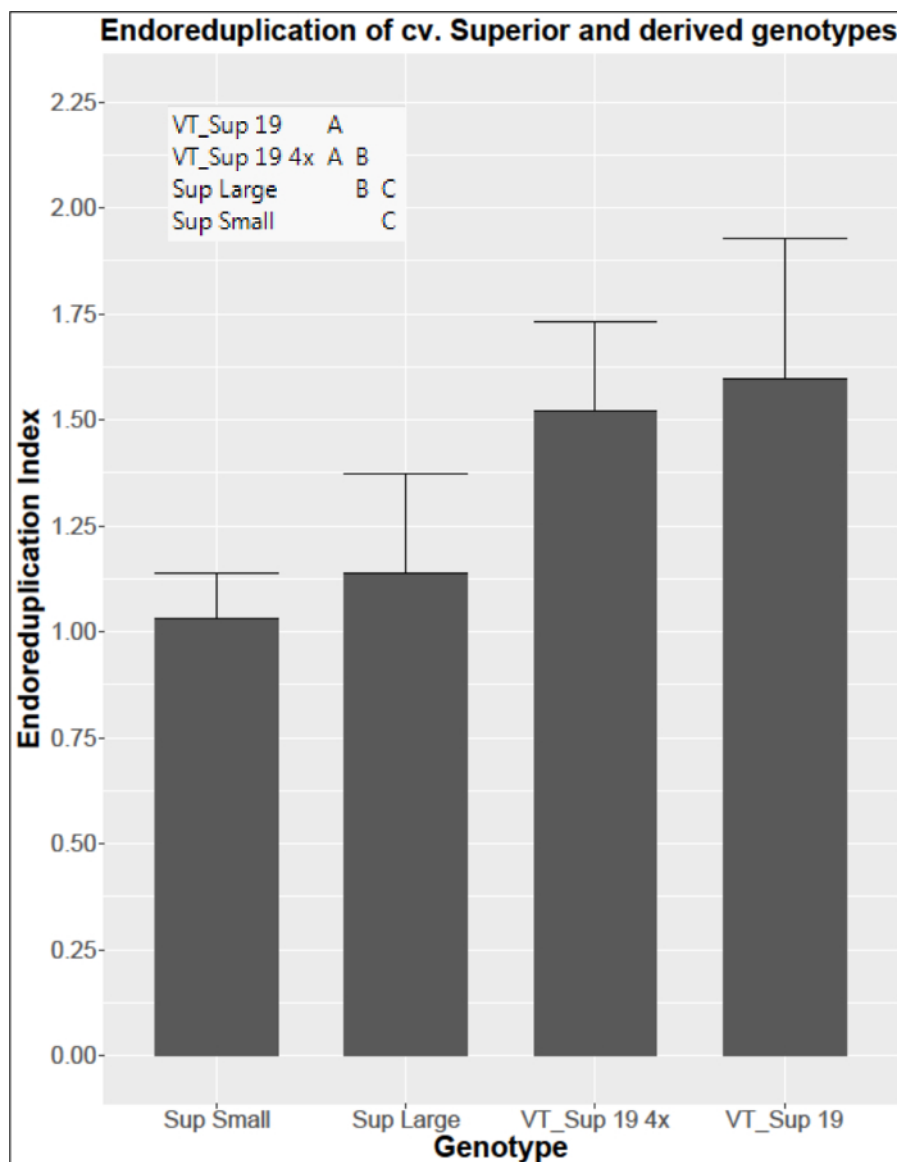
Figure 5: Endoreduplication in two sizes of tubers of cv. Superior and its diploid (VT_Sup_19) and tetraploid (VT_Sup_19 4x) derivatives. The bar chart shows the mean for small (<35 g) and large (90–130 g) cv. Superior tubers as well as large (90–130 g) tubers of the dihaploid VT_SUP_19 and the isogenic doubled dihaploid VT_SUP_19 4x. Significant differences (p <0.05) are indicated by the connecting letter report. Error bars depict standard deviation. Please click here to view a larger version of this figure.

Discussion

The protocol presented herein provides researchers with a means to assess endoreduplication within potato tubers, whose modified cellular content and increased cell size seemingly preclude other flow cytometry preparations. The protocol relies upon protoplast generation as a means to reduce noise and debris while maintaining nuclear integrity. Previously, researchers have described similar preparations for particularly recalcitrant flow cytometry samples as well as utilized tuber protoplasts to study a variety of topics such as pathogenesis[19,20]. However, to our knowledge, none have combined the use of such tuber protoplasts with flow cytometry for the purpose of studying endoreduplication. Furthermore, we found the use of protoplasts to be more reliable than typical crude preparations as well as the technique utilized in the two only other studies to assess tuber endoreduplication[6,7]. Here we discuss the shortcomings of the protocol, potential pitfalls in its execution and sample preparation, and results of a typical experiment which employs it.

Despite the utility and repeatability of the tuber flow cytometry protocol, it does have a few weaknesses which should be discussed. To begin, the protocol is time intensive, requiring two overnight incubations. Furthermore, the protocol requires some expensive reagents, particularly the cellulase and macerozyme. Additionally, the preparations are highly time-sensitive, degrading within a few hours, which may limit throughput within a single day, especially if many events are desired. Lastly, the protocol, while reliable, is sensitive to errors within sample preparation all of which seem to result in damage to the nuclei and lower quality results. For example, microbial contamination may occur during the plasmolysis and protoplast generation steps (1–3.4) due to improper aseptic technique. Sample contamination, while not always precluding the success of a sample, seems to dramatically decrease quality of obtained histograms, again likely due to damage to the nuclei.

As previously stated, the major drawbacks of the protocol are costs, time, and sensitivity to errors. Researchers may wish to take steps to mitigate each of these. As for cost, researchers intending to apply the protocol to many samples may consider modifying the enzyme concentrations and/or duration of the digestion step as different tissues and genotypes may vary in responsiveness. This includes the possibility of filtering and recycling the ES which has previously been demonstrated but not employed here[21]. As for time, in our observation, it is possible to dispense with the first incubation (the plasmolysis step) and still extract protoplasts; however, their integrity and abundance will suffer, which negatively impacts results. To reduce the deterioration of samples researchers may consider that some flow cytometry approaches involve the use of fixatives (*e.g.,* formaldehyde) to preserve sample integrity[22]. This may be a useful means to both prevent deterioration and allow for sample storage rather than protoplast exaction and flow cytometry occurring on the same day as presented. Another means of preventing the attrition of nuclei may be to include a nuclease inhibitor to the ES and/or FBC; while 2-mercaptoethanol effected no noticeable changes during development, other inhibitors have not been tested herein.

In our observation, the single most critical step is step 4.4, the removal of all plasmolysis wash solution before the addition of the flow cytometry buffer (FCB). If even a small volume (<10 µL) remains, the samples are much more likely to be degraded which can lead to a poor or even failed sample. This is likely due to impurities within the enzyme solution (*e.g.* nucleases, proteases)[23,24] which quickly degrade the nuclei during the incubation periods and time between sample runs, even at low concentrations. Another important consideration is the duration of the PI and RNase incubation steps. As noted in the protocol, the samples do not remain stable for more than a few hours after addition of the FCB so researchers may decide to reduce the duration of these steps to accommodate more samples or lengthy flow cytometry runs resulting from low nuclei concentrations. This also requires the researcher to consider the tradeoff between number of events per sample and total number of samples to be run or consider the use of fixatives as previously mentioned.

Differences between Tissues and Genotypes

To provide results representative of the protocol we designed two simple experiments to confirm previously reported variation by tissue and examine the influence of ploidy and genotype. The results of the tissue experiment demonstrate that the protocol yields reproducible results, as pith tissue was once again found to have the highest EI. Somewhat surprisingly parenchyma tissue, which had not previously been evaluated, had an EI value similar to cortical tissue and significantly lower than pith. This was unanticipated as parenchyma cells are typically larger than either pith or cortical cells, at least at maturity. One possible explanation is that the tubers (90–130 g) were too immature for the parenchyma cells, which comprise the majority of tuber volume at maturity, to have fully expanded and reached their maximum C-values[25]. This may also explain why the dihaploid (VT_SUP_19) and the doubled dihaploid (VT_SUP_19 4x) demonstrated significantly greater EI than cv. Superior tubers; while tubers of approximately equal size were sampled from each genotype, the maximum size of tubers from either VT_SUP_19 or the isogenic tetraploid is much smaller than that of the progenitor. Thus, it may be that they displayed greater EI simply because they were larger relative to their maximum attainable size. Alternatively, the difference may be a consequence of the genetic complement VT_SUP_19 received from its tetraploid progenitor. Another possibility is that the genomic reduction that occurred on extraction of the dihaploid from the tetraploid may have unmasked deleterious alleles resulting in plant-wide stress, which has also been shown to contribute to elevated EI[26]. Nevertheless, this demonstrates the careful consideration researchers must employ when selecting tubers and tissues to be used for comparison of EI values, especially between genotypes.

Future applications

The protocol described in this article provides researchers with an important tool for understanding endoreduplication in potato. It may allow for studies into the genetic and environmental components of endoreduplication, the time-course of development across tuber tissues, and assessment of natural variation. Ultimately, endoreduplication may make a promising target for potato improvement, an undertaking which will require a reliable means of assessment.

Disclosures

The authors declare that they have no competing financial interests.

Acknowledgements

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March 2018 | 133 | e57134 | Page 9 of 10

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