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Perspectives on Elevated Ploidy in Regenerants from Plant Tissue Culture and Transformation

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Short Communication

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ABSTRACT

Elevated ploidy is a common phenomenon among plants regenerated from tissue culture. Contributors to this effect include cells of elevated ploidy present within normally diploid or polyploidy explant tissues, endoreduplication by individual cultured cells, and effects of *in vitro* environmental factors on cell division. Because polyploidization can result in morphological changes and thus, different adaptabilities, the determination of ploidy in transgenic regenerants plants is recommended when evaluating phenotypes and conducting mutant screens subsequent to genetic transformation.

INTRODUCTION

Plant tissue culture and regeneration systems have been used in diverse agriculture and life science applications, including commercial micropropagation, viral and other pathogen elimination, germplasm preservation, gene function studies, genetic manipulation, and many others. In most plant species, the availability of an effective *in vitro* regeneration system is a prerequisite for *Agrobacterium*-mediated and other forms of stable plant transformation as applied for gene introduction, genome editing, and other purposes of genetic manipulation.

Plant somatic cells, including fully differentiated cell types in various tissues used as explant sources, have the capacity to regenerate into whole plants *in vitro*, a capacity known as totipotency [1]. In the past decades, whole plants have been regenerated from explants derived from many different plant parts, including but not limited to roots, hypocotyls, cotyledons, leaves, stems, and flowers. Regeneration is a process by which mature cells revert to an undifferentiated or embryogenic cell status, and then are stimulated to re-enter the division cycle, setting the stage for redifferentiation. Within each tissue and organ type, the differentiated cells have specific sizes, shapes, functions, and patterns of gene expression during plant growth and development in nature. During somatic development, genetically and genomically variant cells and cell lines may also arise due to mutation, errors in cell division, limitations affecting adaptability and other causes. Thus, a given explant tissue cannot be assumed to be genetically or genomically homogeneous when put on artificial culture medium [2].

Because the environmental conditions, including biochemical composition of the culture medium and especially the plant hormone levels and ratios, exposure to plant tissue culture provides the opportunity for both normal as well as genetically/genomically variant plant cells to express their totipotency. Thus, while the aim of culture may be to recover regenerants that faithfully maintain the explant genotype, or to depart from the original genotype only in an intended manner, the unintended occurrence of genetically/genomically variant plants with populations of regenerants is to be expected, and indeed somatic variation and ploidy changes in regenerants have been widely reported in plants such as *Arabidopsis thaliana*, broccoli, cucumber, maize, tomato, apple and many others [3-8].

In our study of 16 expressed sequences of unknown function in the diploid strawberry species, *Fragaria vesca*, we used tissue culture and an Agrobacterium-mediated transformation system to introduce RNAi gene silencing constructs into leaf explants from the model variety 'Hawaii 4' [9]. While various construct-specific phenotypes were observed among regenerants,

an initially puzzling finding was that a certain type of phenotypic variant characterized by atypically widened leaflets occurred with low frequency among regenerant populations derived from each of the 16 constructs. This phenotype was reminiscent of that associated with colchicine-induced tetraploids in *F. vesca*, as had been observed in a previous study [10]. The hypothesis that the distinctive regenerants were tetraploid was confirmed by performing chromosome counts and flow cytometry on a subset of variants. Based on the confirmed relationship between elevated ploidy and variant leaflet morphology, we developed and employed a reliable phenotypic metric, reconfirmed by flow cytometry, to examine 992 transgenic plants resulting from transformations with 16 different constructs, and found 173 or about 17% of the transformed plants were tetraploid [9].

Previous to our study, the only report of elevated ploidy regenerants in strawberry was that of Nyman and Wallin, who used flow cytometry to detect 15 plants of elevated ploidy among 51 protoplast-derived regenerants of an octoploid *Fragaria x ananassa* breeding line [11]. Our finding concerning the occurrence and phenotypic characteristics of elevated ploidy regenerants of *F. vesca* 'Hawaii 4' was the first reported in a diploid strawberry, and the first reported in any transgenic strawberry. Thus, our report contributed an important new insight into the properties of the diploid strawberry model plant, *F. vesca* 'Hawaii 4' [9]. Notably, 'Hawaii 4' had been selected from among 14 F. vesca varieties as the best performer in a comparative study of regenerative capacities, on which basis it was adopted by the strawberry research community as a model plant leading to the sequencing of its genome [12,13]. Thus, enhanced knowledge of the 'Hawaii 4' model system is of particular importance in the field of strawberry genetics/genomics.

Based upon our study of transgenic regenerants in the 'Hawaii 4' model system, we suggested the tetraploid regenerants may have come from the original tetraploid cells in explants, or alternately may have been caused by the tissue culture and transformation procedures. A flow cytometric analysis has since shown that about 13% of nuclei in 'Hawaii 4' leaves from diploid plants grown in greenhouse exhibit tetraploidy. However, nuclei sampling under a broader range of plant physiological conditions will be required to definitively assess the extent of endopolyploidy in the leaves of diploid 'Hawaii 4' plants.

To study the cause of ploidy level changes in transgenic tomato, Ellul et al. used flow cytometry to measure nuclear DNA contents in cells of donor cotyledonary explants from five diploid cultivars and the transformants [7]. It was found that cotyledonary tissues of diploid cultivars were polysomatic, being composed of a mix of diploid and polyploid cells. The relative percentages of 2C, 4C, and 8C cotyledonary nuclei varied among plant genotypes. The transformation procedures also affected the percentage of elevated ploidy regenerants, as the percentages of transformed plants with elevated ploidy levels were related to explant source genotype and transformation/selection procedures but not to the proportions of 4C and 8C nuclei in the respective explant sources.

Somatic polyploidy or endopolyploidy is a widespread occurrence in plants, generally occupying conspicuous portions of the plant body, comprising cells and tissues with the most varied degrees of differentiation and types of function, including flowering time and number, mainly generated by endoreduplication [2,14-16]. To use cell culture techniques for plant breeding and to further elucidate the degree of endopolyploidy in the explant tissue source, Kudo and Kimura studied the patterns of endopolyploidy during seedling development in diploid cabbage [17]. It was found there were only 2C cells in un-germinated embryos, but seedlings, including their root, cotyledons, hypocotyls, shoot tips and leaves contained cells from 2C to 16C. It was shown that the patterns of endopolyploidy are specific to each developmental stage. In general, the older tissues exhibited higher levels of multiploidy than younger tissue did within the same plants. Because endopolyploidy is a common feature or a factor in plant tissue development, it is not surprising that tetraploid plants could be regenerated from differentiated diploid explants in tissue culture and transformation.

Endoreduplication is a process where cells replicate their genome without mitosis, accompanied with rapid cells growth, development and differentiations. It can also be an adaptive response to environmental changes and various abiotic and biotic stressors [18]. Tissue culture and transformation systems put cells and tissue under potentially stressful artificial conditions by controlling light, temperature, medium composition and plant hormones. These factors are varied for the respective needs of different genotype plant regeneration. Polyploid cells generally tolerate stressors better than diploid cells can in strongly fluctuating environments [19]. Thus, polyploidy can be advantageous for cell and plant survival, which may explain why the frequency of tetraploid regenerants may exceed the frequency of tetraploid cells within the respective explant, as we have observed in 'Hawaii 4' [20]. Accordingly, an even higher percentage of tetraploid plants might be expected among regenerants from protoplast culture because of the longer culture times required for protoplasts as compared with general tissue culture [21]. In fact, the study reported that 29% of regenerants from protoplasts of an octoploid strawberry breeding line exhibited elevated ploidy [11].

In summary, the possibility of elevated ploidy among regenerants should be routinely factored into the phenotypic and genetic evaluation of genetically manipulated plants derived via *in vitro* procedures. A characteristic phenotype is likely to be associated with tetraploid regenerants, as seen in the diploid strawberry model plant 'Hawaii 4', although the precise nature of the tetraploidy-related phenotype may vary among species.

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REFERENCES

1. Altaf H, et al. Plant tissue culture: current status and opportunities. INTECH. 2012.
2. Martin SL and Husband BC. Whole genome duplication affects evolvability of flowering time in an autotetraploid plant. Plos One. 2012;7:e44784.
3. Fras A and Mausznska J. Regeneration of diploid and tetraploid plants of Arabidopsis thaliana via callus. Acta Biol Cracov Bot. 2003;45:145-152.
4. Metz TD, et al. Agrobacterium tumefaciens-mediated transformation of broccoli (Brassica oleracea var. italica) and cabbage (B. oleracea var. capitata). Plant Cell Rep.1995;15:287-292.
5. Filipecki M, et al. Tissue-culture-responsive and autotetraploidy-responsive changes in metabolic profiles of cucumber (Cucumis sativus L.). J Appl Genet. 2006;47:17-21.
6. Wan Y, et al. Polyploid level of plant regeneration from mixed ploidy Maize callus culture. In Vitro Dev Biol. 1992;28:87-89.
7. Ellul P, et al. The ploidy level of transgenic plants in Agrobacterium-mediated transformation of tomato cotyledons (Lycopersicon esculentum L. Mill.) is genotype and procedure dependent. Theor Appl Genet. 2003;106:231-238.
8. Velasco R, et al. The genome of the domesticated apple (Malus × domestica Borkh.) Nat Genet. 2010;42:833-839.
9. Zhang Q, et al. Somatic embryogenesis, tetraploidy, and variant leaf morphology in transgenic diploid strawberry (Fragaria vesca subspecies vesca 'Hawaii 4'). BMC Plant Biol. 2014;14:14-23.
10. Williamson SC. Germplasm development and assessment in the diploid strawberry species Fragaria vesca L. Master's Thesis. Durham, NH, University of New Hampshire. 1993.
11. Nyman M and Wallin A. Improved culture technique for strawberry (Fragaria x ananassa Duch.) protoplasts and the determination of DNA content in protoplast derived plants. Plant Cell Tissue Organ Cult. 1992;30:127-133.
12. Oosumi T, et al. High-efficiency transformation of the diploid strawberry (Fragaria vesca) for functional genomics. Planta. 2006;223:1219-1230.
13. Shulaev V, et al. The genome of woodland strawberry (Fragaria vesca). Nat Genet. 2011;43:109-116.
14. Damato F. Endopolyploidy as a factor in plant tissue development. Caryologia. 1964;17:41-52.
15. Joubes J and Chevalier C. Endoreduplication in higher plants. Plant Mol Biol. 2000;43:735-745.
16. Barow M. Endopolyploidy in seed plants. Bio Essays. 2006;28:271-281.
17. Kudo N and Kimura Y. Patterns of endopolyploidy during seedling development in cabbage (Brassica oleracea L.). Ann Bot. 2001;87:275-281.
18. Scholes DR and Paige KN. Plasticity in ploidy: a generalized response to stress. Trends in Plant Science. 2015;20:165-175.
19. Beest M, et al. The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot. 2012;109:19-45.
20. Comai L. The advantages and disadvantages of being polyploid. Nature. 2005;6:836-846.
21. Newell CA, et al. Cytogenetic analysis of plants regenerated from tissue explants and mesophyll protoplasts of winter rape, Brassica napus L. Can J Genet Cytol. 1984;26:752-76