PostDoc Journal
Vol. 3, No. 4, April 2015

Journal of Postdoctoral Research
www.postdocjournal.com

Is a plant's ploidy status reflected in its metabolome?

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Abstract

With the enrichment of metabolomic information in a diverse number of plants, the obtained datasets reflect on important aspects of plant growth, function, physiology, productivity, and adaptation to changing biotic and abiotic environments. Many of these plant species are natural polyploids that are either model plants, crops, or are of commercial interest. Decades of efforts have resulted in artificially induced polyploids from *in vitro* micropropagation practices as well. Recent efforts using next generation, high throughput genome sequencing approaches have contributed to our growing understanding of their genome ploidy status and the inherent biosynthetic potentials of encoded metabolomes. However, the ever perplexing questions galore regarding the metabolic status of these polyploids across plant genomes remained. Thus we collated information on metabolomes of polyploids and asked whether they reflect the ploidy status of these plants. We conclude that polyploids, either natural or those induced artificially, demonstrate (i) enhanced primary metabolism, (ii) enhanced secondary metabolism, i.e., terpenoids, phenylpropanoids, flavonoids, and alkaloids content, and (iii) increased bioactive constituents to enable polyploids to counteract against environmental challenges in a more efficient manner in the plant species included in this review.

Keywords: colchicine, genome, metabolism, micropropagation, ploidy, specialized

Polyploidy is rampant in all plants and important for metabolic diversity

Ploidy represents the number of sets of chromosomes (or DNA amount) in the nucleus of a cell. Plant genome sizes vary more or less depending on the species (Michael, 2014). Polyploidy (defined as having more than two genomic copies) is an important mechanism of plant speciation and genome evolution (Arrigo and Barker, 2012) as well as for numerous other eukaryotes (Ainouche and Jenczewski, 2010). Almost all plant species have doubled their genomes at least once during their course of evolution, followed by diploidization and then repeated polyploidization (Adams and Wendel, 2005). Moreover, polyploids form at a relatively high frequency (1 in 100,000) in flowering plants (Ramsey, and Schemske, 1998). Because of this high formation rate and the polyploidy tolerance, stable polyploidy is prevalent in plants. Thus, polyploidy is an important evolutionary factor for the genesis of new plant species as well as the creation of commercially cultivated plant species such as crops. For example, in wheat, which is the progenitor of contemporary hexaploid bread wheat, three genetically distinct types that are economically important include: tetraploid durum wheat, hexaploid hard bread wheat, and hexaploid soft bread wheat, where this classification is based on starch fractionation patterns during milling, a trait heritable via chromosome 5D (Gooding et al., 2009).

Genes involved in specialized metabolism are preferentially retained following whole genome duplication (WGD) in *Arabidopsis* (Maere et al., 2005). For example, over-retention of metabolic genes was observed in protozoan *Paramecium* following WGDs, which follows the tenets of metabolic control theory and selective pressure on the metabolic pathway rather than on

individual genes in the pathway (Gout et al., 2009). However, recently it was postulated that the expansion of specialized metabolic pathway genes appear due to local gene duplication events in plants (Chae et al., 2014). On the other hand, artificially induced polyploids obtained during *in vitro* micropropagation (cell, tissue, and organ culture approaches) or during field experiments that induce genomic multiplication are known to enhance production and/or qualitative improvement in the biochemical profile of secondary metabolites (Dhawan and Lavania, 1996). In comparison to diploids, genome doubling possibly has multi-level effects on the morphology, viability, and physiology of polyploids (Cohen et al., 2013). The increased cell size in polyploids affects enzyme activity and changes morphology in differential manner (Lavania et al., 2012). Polyploidization is often accompanied by increased cell size and conspicuous changes in secondary metabolism (Lavania, 2005). In addition, allopolyploidisation (defined as joining of two different parental genomes in a polyploid organism with diploid meiosis) possibly introduces natural complementation of biosynthetic pathways to harness the useful metabolites to yield pharmaceuticals, aroma chemicals, and commercially important and industrially relevant plant metabolites. Regulation of microRNAs confers natural variation in allopolyploids of *Arabidopsis thaliana* (Ng et al., 2011). Recently, the role of polyploidy and tandem-derived gene duplication events in chemical defense and pathogen recognition was probed using bioinformatics approaches where the collinear blocks (ohnologs) across nine high-quality genomes, including six major crops, revealed 14 pathways with above-average retention-score across all genomes and 37 pathway categories differentially enriched in species-specific sets of retained genes (Schranz, 2015).

In addition to such allo- and auto-polyploidiation events, another important phenomenon is endopolyploidization, i.e., repeated cycles of DNA replication occurring without M-phase and cell ploidy getting doubled with each endoreduplication cycle (e.g. 4C, 8C, 16C, etc.) in plant cells (Wildermuth, 2010). Cell- and tissue-specific patterns of plant endoploidy is species-specific and usually occurs in terminally differentiated, larger, single cells (e.g. trichomes) and organs with enhanced metabolic capacity (i.e., maize endosperm and fruits). In fact, endoduplications has been implicated as a common mechanism required to support the enhanced metabolic demands associated with plant-biotroph interactions (Wildermuth, 2010). Yeasts, which are susceptible to ethanol stress, displayed increases in protective metabolites such as polyols, amino acids, phospholipids, and unsaturated fatty acids in haploid strains but not in diploid strains (Ding et al., 2010), highlighting the clear-cut advantage of increased ploidy to cellular adaptation and survival. Thus polyploidization has provided plants enormous opportunity to accomplish metabolic flexibility and enrichment in addition to obtaining genomic diversity through heterosis or hybrid vigor and fixation of heterozygosity (Ng et al., 2012).

Polyploidy induced enhancement in specialized metabolism

Polyploidization can result in greater specialized (also known as secondary) metabolite production and yield (Predieri, 2001 and Urwin et al., 2007) and can induce changes in the quality and quantity of secondary metabolites (te Beest et al., 2011) such as phenolics, flavonoids, terpenoids, anthocyanins, and polyketides, among others. For instance, it was observed that autopolyploids (defined as doubling of a single genome) in many drug plants including species such
as *Atropa*, *Camellia*, *Hyocyamus* and *Solanum*,
have sharply increased quantities of useful secondary metabolites per unit dry weight, whereas the species such as *Datura* and *Mentha* have a decreased production of these compound (Dhawan and Lavania, 1996). In *Hylocereus* species (Cactaceae), species changes associated with autopolyploidization in two systems of induced somatic polyploids, diploid-autotetraploid and triploid-autohexaploid, were studied which indicated a higher abundance of

sugars in duplicated lines compared to their donor lines (Cohen et a., 2013). Increased contents of amino acids, tricarboxylic acid (TCA) cycle intermediates, organic acids and flavonoids, while decreased betacyanins content in fruits upon genome doubling was confirmed following combined metabolomics approaches using gas chromatography-mass spectrometry (GC-MS) and ultra-performance liquid chromatography (UPLC) Q-TOF (time of flight). Moreover, breeding strategies have taken advantage of correlating the ploidy levels with secondary metabolite profiles, and morphological traits analyses to define a breeding strategy for trifoliate yam (*Dioscorea dumetorum* (Kunth) Pax) (Adaramola et al., 2014). Traditionally, colchicine and oryzalin have been used to induce polyploidy in diverse plant species. In the following sub-sections, several important studies that have relied upon such approaches in recent years, with or without involving micropropagation practices, are listed.

Terpenoids

A clear-cut, metabolite-dependent link to polyploidy-mediated change in body size was evident in a study consisting of unique diploid-autotetraploid paired sets of eight diverse clones of six species of the lemon grass, *Cymbopogon,* that accumulate qualitatively different monoterpene essential oils (terpenoids are an abundant group of specialized metabolites made up of isoprene C5- units) in their vegetative biomass (Lavania et al., 2012). Metabolites originating from plastids, such as flavonoids, terpenoids, and indole glucosinolates, show significant quantitative changes with increased ploidy, with the specific class of affected metabolite being dependent on the species under consideration (Wildermuth, 2010). Colchicine- induced stable tetraploid clones of *Artemisia annua* L. produced six times more artemisinin, a sesquiterpenoid phytochemical, as compared to the diploid clones of hairy roots (Jesus-Gonzalez and Weathers, 2003). Induced autotetraploids generated by colchicine treatment in essential oil bearing plant basil (*Ocimum basilicum* L.) revealed that major constituents of the essential oil, i.e., linalool and 1,8-cineole were increased by 13 % and 77 %, respectively in the tetraploids compared to the diploid plants (Omidbaigi et al., 2010). In fruits of triploid watermelons (*Citrullus lanatus*), the levels of lycopene were higher than their diploid progenitors, whereas the triploids tended to contain more lycopene than tetraploids (King et al., 2009). Adventitious roots of *Panax ginseng* C.A. Meyer (a natural tetraploid) treated with colchicine for octoploid roots revealed that the total ginsenoside and Rb-group ginsenoside contents were less in the octoploid roots than in the tetraploids (Kim et al., 2004). In contrast, a study in ginger (*Zingiber officinale* Roscoe) did not find significant differences in gingerol concentrations between the tetraploid clones and their parent diploid cultivar (Wohlmuth et al., 2005). Above discussed examples suggest that with higher ploidy content the terpenoid content increased generally, although depending on the species and plant part under study, the level of metabolites may show different trends.

Phenylpropanoids

Induction of tetraploidy (2*n*=4*x*=48) in *Solanum commersonii*, a wild potato species, by oryzalin treatment revealed from the HPLC-UV and LC-MS analyses that the metabolite profiles between the diploid *S. commersonii* and its tetraploids showed comparable qualitative profiles, except that phenylpropanoid content was significantly higher in the tetraploids than in the diploids (Caruso et al., 2011). In colchicine induced *Echinacea purpurea* (L.), Moench tetraploid plants obtained from diploid explants revealed higher caffeic acid derivatives and alkamides and cichoric acid content, alongside elevated phenylpropanoid biosynthesis (Xu et al., 2014). Triploid progeny population obtained from diploid and tetraploid of *Camellia* tea species indicated that the catechin and caffeine levels of the triploid progenies were higher than their diploid parent and showed heterosis for caffeine and EGCG, thus allowing quantitative enhancement of some of the quality-related

parameters in tea (Das et al., 2013). The ploidy level in licorice plantlets obtained from treatment with 0.08% (to induce mixoploidy [defined as presence of both diploids and polyploids in a population]) and 0.1% (to induce tetraploidy) of colchicine demonstrated that the anthocyanin level was significantly increased in callus obtained from mixoploid plantlets whereas the amount of glycyrrhizic acid increased upon induction of polyploidization, proving an increased production of metabolites in polyploid licorice tissues (Bernard et al., 2012). In copper-exposed diploid and tetraploid chamomile (*Matricaria chamomilla*) roots, it was demonstrated that flavonoids and phenolic acids did not differ with respect to ploidy (Kováčik et al., 2010). In general it is evident that with increases in ploidy levels, the phenylpropanoid contents increased in all these above discussed instances.

Flavonoids

Several instances are available in literature where flavonoid levels were correlated with ploidy status, possibly due to ease of qualitative and quantitative evaluation of flavonoids from plant tissues. While the amount of the coumarin herniarin or methoxylated flavonoids was significant higher in tetraploid cv. 'Lutea' of *Matricaria chamomilla* (Repcˇa´k et al., 1999), the flavonoid apigenin was found to be more abundant in diploid cv. 'Novbona' (Sˇvehlı´kova´ and Repcˇa´k, 2000). Colchicine induced tetraploids from diploid yellow-flowered cyclamen *in vitro* yielded two tetraploid plants which demonstrated greater ability to accumulate chalcone than their diploid relatives (Takamura and Miyajima, 1996). Similarly, upon colchicine treatment for induction of polyploidy, the flavonol biosynthetic pathway of *Petunia* 'Mitchell' demonstrated differential effect with increased relative concentration of the major metabolite quercetin-3-sophoroside and decreased relative concentration of the minor metabolite quercetin-3 7-diglucoside without affecting the total concentration or specific concentration of the other four minor flavonols (Griesbach and Kamo, 1996). Although it is difficult to infer if the induction is just a stress-response upon treatment with colchicine, the question remains as to why the flavonoid biosynthetic pathways would be induced upon increased ploidy level? However, it can be safely concluded that higher ploidy levels are clearly reflected in higher accumulation of flavonoids in plants.

Alkaloids

The alkaloid profiles of hairy roots of *Datura stramonium* obtained from diploid and tetraploid plants were found to be comparable in terms of the major alkaloids, but they differed significantly in case of minor species, whereas maximal yield of hyoscyamine was recorded for hairy roots from tetraploid plants that were cultivated on a specific tissue culture medium (Pavlov et al., 2009). In Egyptian henbane (*Hyoscyamus muticus* L.), it was revealed that in spite of their lower biomass production, tetraploid clones could produce more scopolamine than their diploid counterparts under similar growth conditions (Dehghan et al., 2012). In colchicine treated polyploids of *Cannabis sativa* L., the reducing sugars, soluble sugars, total protein, and total flavonoids increased significantly in mixoploid plants compared with tetraploid and diploid plants, although polyploidization increased the contents of tetrahydrocannabinol in mixoploid plants only, while tetraploid plants had lower amounts of this substance in comparison with diploids (Bagheri and Mansouri, 2014). Thus, the alkaloid contents showed ploidy-dependent increases in plants that are alkaloid biosynthesizing species.

Other bioactive constituents

Evaluation of diploid and mixoploid *Trigonella foenum-graecum* L. aqueous extracts, against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Fusarium oxysporum* f. sp. *lycopersici* revealed that aqueous extracts of diploid plants were less toxic than mixoploid

ones, indicating the presence of effective bioactive phytochemicals in the mixoploids (Omezzine et al., 2014). Similar studies conducted in tetraploid *Mitracarpus hirtus* L. correlated antibacterial properties with ploidy status (Pansuksan et al., 2014). Among other advantages conferred by ploidy is the possible amplification in detoxification machinery. Tetraploid soybean (*Glycine max*) plants were relatively tolerant while diploid plants were highly susceptible to a toxin metribuzin [4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one] applied both in field grown plants as well as in suspension cell cultures (Abusteit et al., 1985). Thus, it can be generally concluded that plants with higher ploidy levels biosynthesized more bioactive constituents, demonstrated more efficient detoxification machinery, or were more resistant to toxins-- all indicating towards increased adaptive mechanisms for surviving challenges from the environment.

Polyploids exhibit enhanced primary metabolism Large-scale transcriptional changes related to primary metabolism upon induction of ploidy is well known in yeast (Li et al., 2010) and numerous plants (Albuzio et al., 1978; Mishra et al., 2010), even at the level of smallRNAs (Guan et al., 2014). However, the changes in metabolic responses in response to ploidy status are interesting. Polar lipids in wheat can help distinguish between ploidy levels (Armanino et al. 2002). In experiments involving the genus *Cenchrus* under water-stress conditions, the relative reduction in photosynthetic characteristics was more pronounced in annual diploid than perennial diploid and tetraploid, while water-stress tolerance in terms of plant height, rolling, and wilting of leaves implied better adaptation of tetra- and hexaploid species over diploids (Chandra and Dubey, 2009). The inheritance patterns of sugar and organic acid contents of ripe berries in a tetraploid × diploid table grape cross population were investigated and revealed that the sugar contents in tetraploid progeny was significantly higher than that in the diploid progeny and that the sugar contents appeared to increase with ploidy level (Liang et al., 2011).

Genes involved in glycolysis, respiration (TCA cycle and mitochondrial electron transport chain), and fermentation exhibit preferentially increased ploidy-associated expression and are over-retained following a WGD (Wildermuth, 2010). For instance, the transcriptomic expression levels also indicated an up-regulation in primary metabolism of haploid marine alga *Emiliania huxleyi* cells in terms of carbon metabolism, tricarboxylic acid cycle and general energy metabolism (Rokitta et al., 2011), although diploid cells showed 20% richer (that is, the total number of mRNA species) transcriptome (von Dassow et al., 2009). On the basis of the resource acquisition theory, it was conceived that any environmental stress that can lower plant resource availability would favor survival in a slow-growing polyploid as compared with that of a fast-growing diploid, as shown in tobacco (Deng et al., 2012). Tetraploid *Spathiphyllum* plants are more resistant to drought stress compared to their diploid counter parts (Van Laere et al., 2010). In tetraploid cucumbers (*Cucumis sativus* L.), the quantitative changes in 14 metabolites, as compared to regenerated diploids, indicated increases in serine, glucose-6P, fructose-6P, oleic acid, and shikimic acid levels as revealed from GC-MS analysis of a total 48 metabolites, although the variation in metabolic profiles did not correlate directly with the genomic changes in tetraploids (Filipecki et al., 2006). Overall, primary metabolism seems upregulated in polyploids.

Current understanding of the links between ploidy and plant metabolism

The gene-balance hypothesis proposes that genes coding for products that are highly connected- within protein complexes or biochemical pathways- are “dosage-sensitive”, i.e., they must be present in the same number of genomic copies as the genes with whose products they interact (Buggs et al., 2012). This

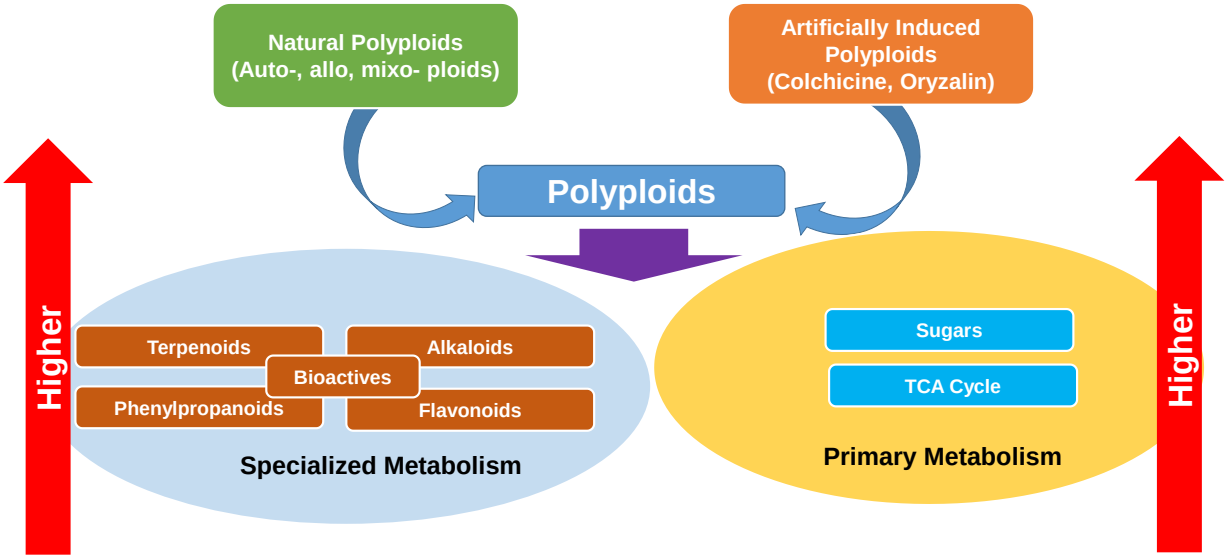

Figure 1. Thematic diagram summarizing the effects of ploidy status (either natural or artificial) on increasing the primary and specialized metabolism in plants.

'dosage balance hypothesis' predicts that, for instance, transcription factors or signal transductors will be retained after polyploidy such that gene networks maintain proper levels of upstream regulators to control the multiplied downstream pathways (Arrigo and Barker, 2012). However, an opposite pattern predicted by the "dosage balance" hypothesis indicated that signaling genes and transcription factors were significantly lost across whole genome duplications in multiple species, as compared to metabolic and structural genes that were strongly retained in duplicate copies (Barker et al., 2008). The metabolic pathways that are sensitive to net gene dosage were, among others, fermentative capacity, transporters, and plastidial production of specialized metabolites (Wildermuth, 2010). For instance, soybean that has undergone two separate polyploidy events revealed that transcription factors and ribosomal genes were differentially expressed in many tissues (i.e., apical meristem, flower, green pods, leaves, nodules, root, and root tip), suggesting that the main consequence of polyploidy in soybean may be at the regulatory level (Roulin et al., 2013). Transcriptome analyses support the idea that induced endoreduplication is a mechanism to increase metabolic capacity and identify specific processes and genes of established or predicted importance (Wildermuth, 2010). In addition, the dynamic nature of polyploid genomes- with alterations in gene content, number, arrangement, expression and transposon activity-may generate sufficient novelty that every individual in a polyploid population or species may be unique (Soltis et al., 2014), thus enabling higher survival rates andfitness as well as subsequent adaptive capabilities. In Figure 1, we summarize the findings highlighting the effects of ploidy status on plant metabolism in general.

Conclusions and future prospects

Both primary and specialized metabolism are directly responsive to increments in ploidy levels of plants, as evidenced from above. Nonetheless, there are exceptions and species-specific anomalies in this over-simplified generalization. Furthermore, artificially induced polyploidization and polyploidization induced over natural course of plant evolution might act differently on the genome-induced metabolomic changes in plants. As more plant genomes get sequenced in this post-genomic era, and more plant metabolomes are characterized, there would be an incremental

focus on correlating the genome ploidy with metabolomic enrichment.

In fact, recently ploidy level was proposed as a general normalization factor, providing an advanced method for quantifying transcripts, proteins, and also metabolites in *A. thaliana* cells (Shimada et al., 2010), and thus indicating the importance of correlating ploidy with metabolome. Although it is a major challenge for the complete depiction of a comprehensive map of plant central metabolism (Sulpice and McKeown, 2015), efforts are under way to understand the metabolic models operating in these sequenced polyploidy plant genomes, such as PlantSEED (Seaver et al., 2014). The objective of PlantSEED (http://plantseed.theseed.org) is that by producing consistent annotations for about 10 reference genomes, a functioning metabolic model for each genome, gap filling to identify missing annotations, and proposing gene candidates for missing annotations would be achieved. These advancements and resources would provide the information content on ploidy levels of numerous plants, and the metabolomics data from these species could be correlated to their ploidy status.

Competing interests

The authors declare that there are no competing interests.

Acknowledgments

The first author acknowledges a PhD studentship carried at Biotechnology Department, FM University, Balasore, India. The corresponding author acknowledges a Postdoctoral Research Associate Fellowship availed at the University of Florida, Gainesville, USA.

References

Abusteit, E. O., Corbin, F. T., Schmitt, D. P., Burton, J. W., Worsham, A. D., & Thompson Jr, L. (1985). Absorption, translocation, and metabolism of metribuzin in diploid and tetraploid soybean (*Glycine max*) plants and cell cultures. Weed Science, 618-628.

Adams, K. L., & Wendel, J. F. (2005). Polyploidy and genome evolution in plants. Current opinion in plant biology, 8, 135-141. http://dx.doi.org/10.1016/j.pbi.2005.01.001

Adaramola, T. F., Sonibare, M. A., Sartie, A., Lopez-Montes, A., Franco, J., & Albach, D. C. Integration of ploidy level, secondary metabolite profile and morphological traits analyses to define a breeding strategy for trifoliate yam (*Dioscorea dumetorum* (Kunth) Pax). Plant Genetic Resources, 1-10.

Ainouche, M. L., & Jenczewski, E. (2010). Focus on polyploidy. New Phytologist, 186, 1-4. http://dx.doi.org/10.1111/j.1469-8137.2010.03215.x

Albuzio, A., Spettoli, P., & Cacco, G. (1978). Changes in gene expression from diploid to autotetraploid status of *Lycopersicon esculentum.* Physiologia plantarum, 44, 77-80. http://dx.doi.org/10.1111/j.1399-3054.1978.tb01617.x

Armanino, C., De Acutis, R., & Festa, M. R. (2002). Wheat lipids to discriminate species, varieties, geographical origins and crop years. Analytica Chimica Acta, 454, 315-326. http://dx.doi.org/10.1016/S0003-2670(01)01548-3

Arrigo, N., & Barker, M. S. (2012). Rarely successful polyploids and their legacy in plant genomes. Current opinion in plant biology, 15, 140-146. http://dx.doi.org/10.1016/j.pbi.2012.03.010

Bagheri, M., & Mansouri, H. (2014). Effect of Induced Polyploidy on Some Biochemical Parameters in *Cannabis sativa* L. Applied biochemistry and biotechnology, 1-10. http://dx.doi.org/10.1007/s12010-014-1435-8

Barker, M. S., Kane, N. C., Matvienko, M., Kozik, A., Michelmore, R. W., Knapp, S. J., & Rieseberg, L. H. (2008). Multiple paleopolyploidizations during the evolution of the Compositae reveal parallel patterns of duplicate gene retention after millions of years. Molecular Biology and Evolution, 25, 2445-2455. http://dx.doi.org/10.1093/molbev/msn187

Bernard, F., Moghbel, N., & Hassannejad, S. (2012). Treatment of licorice seeds with colchicine: changes in seedling DNA levels and anthocyanin and glycyrrhizic acid contents of derived callus cultures. Natural product communications, 7, 1457-1460.

Buggs, R. J., Chamala, S., Wu, W., Tate, J. A., Schnable, P. S., Soltis, D. E., et al. (2012). Rapid, repeated, and clustered loss of duplicate genes in allopolyploid plant populations of independent origin. Current Biology, 22, 248-252. http://dx.doi.org/10.1016/j.cub.2011.12.027

Caruso, I., Lepore, L., De Tommasi, N., Dal Piaz, F., Frusciante, L., Aversano, R., et al. (2011). Secondary metabolite profile in induced tetraploids of wild *Solanum commersonii* Dun. Chemistry & biodiversity, 8, 2226-2237. http://dx.doi.org/10.1002/cbdv.201100038

Chae, L., Kim, T., Nilo-Poyanco, R., & Rhee, S. Y. (2014). Genomic signatures of specialized metabolism in plants. Science, 344, 510-513.

Chandra, A., & Dubey, A. (2009). Assessment of ploidy level on stress tolerance of *Cenchrus species* based on leaf photosynthetic characteristics. Acta physiologiae plantarum, 31, 1003-1013. http://dx.doi.org/10.1007/s11738-009-0317-0

Cohen, H., Fait, A., & Tel-Zur, N. (2013). Morphological, cytological and metabolic consequences of autopolyploidization in *Hylocereus* (Cactaceae) species. BMC plant biology, 13, 173. http://dx.doi.org/10.1186/1471-2229-13-173

Das, S. K., Sabhapondit, S., Ahmed, G., & Das, S. (2013). Biochemical evaluation of triploidprogenies of diploid× tetraploid breeding populations of *Camellia* for genotypes rich in catechin and caffeine. Biochemical genetics, 51, 358-376. http://dx.doi.org/10.1007/s10528-013-9569-x

De Jesus-Gonzalez, L., & Weathers, P. J. (2003). Tetraploid *Artemisia annua* hairy roots produce more artemisinin than diploids. Plant cell reports, 21, 809-813.

Dehghan, E., Häkkinen, S. T., Oksman-Caldentey, K. M., & Ahmadi, F. S. (2012). Production of tropane alkaloids in diploid and tetraploid plants and *in vitro* hairy root cultures of Egyptian henbane (*Hyoscyamus muticus* L.). Plant Cell, Tissue and Organ Culture, 110, 35-44. http://dx.doi.org/10.1007/s11240-012-0127-8

Deng, B., Du, W., Liu, C., Sun, W., Tian, S., & Dong, H. (2012). Antioxidant response to drought, cold and nutrient stress in two ploidy levels of tobacco plants: low resource requirement confers polytolerance in polyploids? Plant Growth Regulation, 66, 37-47. http://dx.doi.org/10.1007/s10725-011-9626-6

Dhawan, O. P., & Lavania, U. C. (1996). Enhancing the productivity of secondary metabolites *via* induced polyploidy: a review. Euphytica, 87, 81-89. http://dx.doi.org/10.1007/BF00021879

Filipecki, M., Yin, Z., Wiśniewska, A., Śmiech, M., Malinowski, R., & Malepszy, S. (2006). Tissue-culture-responsive and autotetraploidy-responsive changes in metabolic profiles of cucumber (*Cucumis sativus* L.). Journal of applied genetics, 47, 17-21. http://dx.doi.org/10.1007/BF03194594

Gooding, M. J., Khan, K., & Shewry, P. R. (2009). The wheat crop. Wheat: chemistry and technology, (Ed. 4). 19-49.

Gout, J. F., Duret, L., & Kahn, D. (2009). Differential retention of metabolic genes following whole-genome duplication. Molecular biology and evolution, 26, 1067-1072. http://dx.doi.org/10.1093/molbev/msp026

Griesbach, R. J., & Kamo, K. K. (1996). The effect of induced polyploidy on the flavonols of *Petunia* 'Mitchell'. Phytochemistry, 42, 361-363. http://dx.doi.org/10.1016/0031-9422(95)00893-4

Guan, X., Song, Q., & Chen, Z. J. (2014). Polyploidy and small RNA regulation of cotton fiber development. Trends in plant science, 19, 516-528. http://dx.doi.org/10.1016/j.tplants.2014.04.007

Kim, Y. S., Hahn, E. J., Murthy, H. N., & Paek, K. Y. (2004). Effect of polyploidy induction on

biomass and ginsenoside accumulations in adventitious roots of ginseng. Journal of Plant Biology, 47, 356-360. http://dx.doi.org/10.1007/BF03030551

King, S. R. (2009). Lycopene and citrulline contents in watermelon (*Citrullus lanatus*) fruit with different ploidy and changes during fruit development. In IV International Symposium on Cucurbits 871 (pp. 543-550).

Kováčik, J., Klejdus, B., Hedbavny, J., & Zoń, J. (2010). Copper uptake is differentially modulated by phenylalanine ammonia-lyase inhibition in diploid and tetraploid chamomile. Journal of agricultural and food chemistry, 58, 10270-10276. http://dx.doi.org/10.1021/jf101977v

Lavania, U. C. (2005). Genomic and ploidy manipulation for enhanced production of phyto-pharmaceuticals. Plant Genetic Resources: Characterization and Utilization, 3, 170-177. http://dx.doi.org/10.1079/PGR200576

Lavania, U. C., Srivastava, S., Lavania, S., Basu, S., Misra, N. K., & Mukai, Y. (2012). Autopolyploidy differentially influences body size in plants, but facilitates enhanced accumulation of secondary metabolites, causing increased cytosine methylation. The Plant Journal, 71, 539-549. http://dx.doi.org/10.1111/j.1365-313X.2012.05006.x

Li, B. Z., Cheng, J. S., Ding, M. Z., & Yuan, Y. J. (2010). Transcriptome analysis of differential responses of diploid and haploid yeast to ethanol stress. Journal of biotechnology, 148, 194-203. http://dx.doi.org/10.1016/j.jbiotec.2010.06.013

Liang, Z., Sang, M., Ma, A., Zhao, S., Zhong, G. Y., & Li, S. (2011). Inheritance of sugar and acid contents in the ripe berries of a tetraploid × diploid grape cross population. Euphytica, 182, 251-259. http://dx.doi.org/10.1007/s10681-011-0487-x

Maere, S., De Bodt, S., Raes, J., Casneuf, T., Van Montagu, M., Kuiper, M., & Van de Peer, Y. (2005). Modeling gene and genome duplicationsin eukaryotes. Proceedings of the National Academy of Sciences of the United States of America, 102, 5454-5459. http://dx.doi.org/10.1073/pnas.0501102102

Michael, T. P. (2014). Plant genome size variation: bloating and purging DNA. Briefings in functional genomics, 13, 308-317. http://dx.doi.org/10.1093/bfgp/elu005

Ming-Zhu Ding, Bing-Zhi Li, Jing-Sheng Cheng, and Ying-Jin Yuan. (2010). Metabolome analysis of differential responses of diploid and haploid yeast to ethanol stress. OMICS: A Journal of Integrative Biology, 14: 553-561. http://dx.doi.org/10.1089/omi.2010.0015

Mishra, B. K., Pathak, S., Sharma, A., Trivedi, P. K., & Shukla, S. (2010). Modulated gene expression in newly synthesized auto-tetraploidof *Papaver somniferum* L. South African Journal of Botany, 76, 447-452. http://dx.doi.org/10.1016/j.sajb.2010.02.090

Ng, D. W., Lu, J., & Chen, Z. J. (2012). Big roles for small RNAs in polyploidy, hybrid vigor, and hybrid incompatibility. Current opinion in plant biology, 15, 154-161. http://dx.doi.org/10.1016/j.pbi.2012.01.007

Ng, D. W., Zhang, C., Miller, M., Palmer, G., Whiteley, M., Tholl, D., & Chen, Z. J. (2011). cis-and trans-Regulation of miR163 and target genes confers natural variation of secondary metabolites in two *Arabidopsis* species and their allopolyploids. The Plant Cell Online, 23, 1729-1740. http://dx.doi.org/10.1105/tpc.111.083915

Omezzine, F., Daami-Remadi, M., Ladhari, A., & Haouala, R. (2014). Variation in phytochemical content and antifungal activity of *Trigonella foenum-graecum* L. with plant developmental stage and ploidy level. South African Journal of Botany, 92, 120-125. http://dx.doi.org/10.1016/j.sajb.2014.02.014

Omidbaigi, R., Mirzaei, M., & Moghadam, M. S. (2010). Difference of growth traits, essential oil content and compositions between diploid and induced tetraploid plants of basil (*Ocimum basilicum* L.). Journal of Essential Oil Bearing Plants, 13, 579-587. http://dx.doi.org/10.1080/0972060X.2010.10643866

Pansuksan, K., Sangthong, R., Nakamura, I., Mii, M., & Supaibulwatana, K. (2014). Tetraploid induction of Mitracarpus hirtus L. by colchicine and its characterization including antibacterial activity. Plant Cell, Tissue and Organ Culture, 117, 381-391. http://dx.doi.org/10.1007/s11240-014-0447-y

Pavlov, A., Berkov, S., Weber, J., & Bley, T. (2009). Hyoscyamine biosynthesis in *Datura stramonium* hairy root in vitro systems with different ploidy levels. Applied biochemistry and biotechnology, 157, 210-225. http://dx.doi.org/10.1007/s12010-008-8264-6

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. Plant cell, tissue and organ culture, 64, 185-210. http://dx.doi.org/10.1023/A:1010623203554

Ramsey, J., & Schemske, D. W. (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual Review of Ecology and Systematics, 467-501. http://dx.doi.org/10.1146/annurev.ecolsys.29.1.467

Repcák M., Švehlıková V., Imrich J., and Pihlaja K. Pathogens and the role of glycosidic precursors In (1999). Jaceidin and chrysosplenetin chemotypes of roots. In: Handbook of Phytoalexin Metabolism and

Soltis, P. S., Liu, X., Marchant, D. B., Visger, C. J., & Soltis, D. E. (2014). Polyploidy and novelty: Gottlieb's legacy. Philosophical Transactions of the Royal Society B: Biological Sciences, 369, 20130351. http://dx.doi.org/10.1098/rstb.2013.0351

Sulpice, R., & McKeown, P. C. (2015). Moving Towards a Comprehensive Map of Central Plant Metabolism. Annual Review of Plant Biology, 66. http://dx.doi.org/10.1146/annurev-arplant-043014-114720

Švehlíková, V., & Repčák, M. (2000). Variation of apigenin quantity in diploid and tetraploid *Chamomilla recutita* (L.) Rauschert. Plant Biology, 2, 403-407. http://dx.doi.org/10.1055/s-2000-5957

Takamura, T., & Miyajima, I. (1996). Colchicine induced tetraploids in yellow-flowered *Chamomilla recutita* (L.) Rauschert. Biochem. Syst. Ecol. 27, 727-732.

Rokitta, S. D., de Nooijer, L. J., Trimborn, S., de Vargas, C., Rost, B., & John, U. (2011). Transcriptome analyses reveal differential gene expression patterns between the life-cycle stages of *Emiliania huxleyi* (Haptophyta) and reflect specialization to different ecological niches. Journal of phycology, 47, 829-838. http://dx.doi.org/10.1111/j.1529-8817.2011.01014.x

Roulin, A., Auer, P. L., Libault, M., Schlueter, J., Farmer, A., May, G., et al. (2013). The fate of duplicated genes in a polyploid plant genome. The Plant Journal, 73, 143-153. http://dx.doi.org/10.1111/tpj.12026

Schranz, M. E. (2015). Interplay of polyploid-and tandem-derived gene duplicates in chemical defense and pathogen recognition. In Plant and Animal Genome XXIII Conference. Plant and Animal Genome.

Shimada, H., Obayashi, T., Takahashi, N., Matsui, M., & Sakamoto, A. (2010). Normalization using ploidy and genomic DNA copy number allows absolute quantification of transcripts, proteins and metabolites in cells. Plant methods, 6, 29. http://dx.doi.org/10.1186/1746-4811-6-29.

cyclamens and their characteristics. Scientia Horticulturae, 65, 305-312. http://dx.doi.org/10.1016/0304-4238(96)00896-5

te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubešová, M., & Pyšek, P. (2011). The more the better? The role of polyploidy in facilitating plant invasions. Annals of Botany, mcr277. http://dx.doi.org/10.1093/aob/mcr277

Urwin, N. A., Horsnell, J., & Moon, T. (2007). Generation and characterization of colchicine-induced autotetraploid *Lavandula angustifolia*. Euphytica, 156, 257-266. http://dx.doi.org/10.1007/s10681-007-9373-y

Van Laere, K., França, S. C., Vansteenkiste, H., Van Huylenbroeck, J., Steppe, K., & Van Labeke, M. C. (2011). Influence of ploidy level on

morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. Acta Physiologiae Plantarum, 33, 1149-1156. http://dx.doi.org/10.1007/s11738-010-0643-2

von Dassow, P., Ogata, H., Probert, I., Wincker, P., Da Silva, C., Audic, S., et al. (2009). Transcriptome analysis of functional differentiation between haploid and diploid cells of *Emiliania huxleyi*, a globally significant photosynthetic calcifying cell. Genome Biol, 10, R114. http://dx.doi.org/10.1186/gb-2009-10-10-r114

Wildermuth, M. C. (2010). Modulation of host nuclear ploidy: a common plant biotroph mechanism. Current opinion in plant biology, 13, 449-458. http://dx.doi.org/10.1016/j.pbi.2010.05.005

Wohlmuth, H., Leach, D. N., Smith, M. K., & Myers, S. P. (2005). Gingerol content of diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). Journal of agricultural and food chemistry, 53, 5772-5778. http://dx.doi.org/10.1021/jf050435b

Xu, C. G., Tang, T. X., Chen, R., Liang, C. H., Liu, X. Y., Wu, C. L., et al. (2014). A comparative study of bioactive secondary metabolite production in diploid and tetraploid *Echinacea purpurea* (L.) Moench. Plant Cell, Tissue and Organ Culture, 116, 323-332. http://dx.doi.org/10.1007/s11240-013-0406-z