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Transcriptome and metabolome profiling provide insights into hormone-mediated enhanced growth in autotetraploid seedlings of banana (*Musa* spp.)

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Introduction: Reconstructive breeding based on autotetraploids to generate triploid varieties is a promising breeding strategy in banana (*Musa* spp.). Therefore understanding the molecular mechanisms underlying the phenotypic differences between the original diploid and its autopolyploid derivatives is of significant importance in such breeding programs of banana.

Methods: In this study, a number of non-chimeric autotetraploid plants, confirmed by flow cytometry and chromosome counting were obtained using colchicine treatment of 'Pisang Berlin' (AA Group), a diploid banana cultivar highly resistant to Fusarium wilt Tropical Race 4 (*Foc* TR4) and widely cultivated in Asia.

Results and discussion: The autotetraploids showed significant increase in plant height, pseudostem diameter, root length, leaf thickness, leaf area, and leaf chlorophyll content. Transcriptomic analysis indicated that differentially expressed genes were mainly enriched in plant hormone signal transduction, mitogen-activated protein kinase (MAPK) signaling pathway, and carbon fixation in photosynthetic organelles. The genes related to the metabolism, transport or signaling of auxin, abscisic acid (ABA), cytokinin (CTK) and gibberellin (GA), as well as the genes encoding essential enzymes in photosynthetic CO_2 fixation were differentially expressed in leaves of autotetraploids and most of them were up-regulated. Metabolomic analysis revealed that the differentially accumulated metabolites were mainly involved in plant hormone signal transduction, porphyrin and chlorophyll metabolism, indole alkaloid biosynthesis, and carbon fixation in photosynthetic organelles. The results therefore, demonstrate that the hormones IAA, ABA, and photosynthetic regulation may play a vital role in the observed enhancement in the autotetraploids. These could be used as molecular and biochemical markers to facilitate the generation of triploid progenies as suitable new varieties for cultivation.

KEYWORDS

autotetraploid, diploid, hormonal regulation, transcriptome, metabolome, colchicine, banana, *Musa* spp.

Introduction

Banana (*Musa* spp.) is one of the major cash crops in the tropics and subtropics, as well as one of the major food crops in most developing countries (Heslop-Harrison and Schwarzacher, 2007). Banana production can be affected by a number of pests and diseases as well as climatic change (Dita et al., 2011). Development of resistant varieties is considered the most sustainable solution to banana productivity challenges. However, traditional banana breeding is very complicated due to most cultivated bananas being triploid (2n = 3x = 33) and very low fertility or complete sterility. The lack of pest and disease resistance genes has also been noted in most edible varieties (Poerba et al., 2019). Several of the desired characteristics are however, available in the diploid germplasm (AA and AB groups), including high hand number, parthenocarpy, slim fruit fingers, dwarf plant height, pest and disease resistance but with low yields, and generally non-parthenogenesis (Ortiz and Vuylsteke, 1995; Silva et al., 2001; Ssebuliba et al., 2006).

Polyploidization is a breeding technique that offers an opportunity to create novel genetically improved polyploid germplasm with increased interspecific fertility that can further be used to generate triploid varieties through conventional crossing programs (Sattler et al., 2016). Polyploid induction is thus put forward as an important step in banana breeding. Triploid bananas can be generated by crossing between diploid and autotetraploid plants and this has become one of the most important strategies in crossbreeding of bananas and has led to the successful commercialization of banana varieties in the Cavendish subgroup (Jenny et al., 2002; Perrier et al., 2019).

Autotetraploid plants can be obtained by crossing triploids with diploids, chromosome doubling of diploids, somatic hybridization, or genetic engineerin (Do Amaral et al., 2015; Borges et al., 2016; De Carvalho Santos et al., 2019). Induction of chromosome doubling using colchicine is widely used in polyploid mutagenesis of various plants. To date, polyploidy has been successfully induced in the crops like apple (Zhang F. et al., 2015), citrus (Pablo et al., 2011; Wu et al., 2012), and pear (Kadota and Niimi, 2002; Sun et al., 2009). However, rather than the desired euploids, the aneuploids and even hyperpolyploidy may occur in the chemical mutagenesis of chromosome doubling. Polyploids generally showed organ enlargement than the diploids in kiwifruits (Wu et al., 2012) and rice (Zhang J. et al., 2015). Allario et al. (2011) found that the autotetraploid leaves, roots, stems, and other organs of lemon became larger. Polyploids also tended to be better adapted to the environment. Polyploids of *Hordeum vulgare*, *Citrus limonia* as well as *Manihot esculenta* showed enhanced drought resistance compared to the corresponding diploid parents (Chen and Tang, 1945; Nassar, 2006; Allario et al., 2013). Apple autotetraploids showed higher drought and salt resistance (Xue et al., 2015; Zhang F. et al., 2015). Moreover, it was found that polyploidy can accumulate more quality related metabolites, like sugars, amino acids, and organic acids (Cohen et al., 2013). However, the growth of some autopolyploids was weaker than those of the diploids, exhibiting the characteristics of delayed development and dwarfing. For example, the autotetraploids of *Arabidopsis* spp. were weaker than the diploids (Fort et al., 2015), and the autotetraploid orange and apple plants were reported to be dwarfing (Syvertsen et al., 2000; Allario et al., 2011).

Efforts have been made to generate desired banana genotypes through polyploidy induction (Poerba et al., 2019). Banana plants generated by chromosome doubling mainly showed increased pseudostem size, stomatal length, fruit, and bunch weight (Hamill et al., 1992). The autotetraploid plants obtained from the wild diploids were taller and more robust, but with droopy leaves, fewer suckers, more sparse roots, changed fruit size and shape, and doubled anthocyanin content in the leaves (Vakili, 1967). Chromosome doubling of SH-3362 (AA) resulted in stronger autotetraploid plants with thicker pseudostems, thicker roots, and wider leaves (Hamill et al., 1992). In genotypes 'Kluai Sa' (AA) and 'Kluai Leb Mu Nang' (AA), leaf and fruit shape changed in autotetraploid plants relative to their diploid counterparts under the same growth conditions (Kanchanapoom and Koarapatchaikul, 2012). Chromosome doubling of 'Pisang Lilin' (AA) showed increased pseudostem diameter and plant height. Leaf number at flowering and harvest stages as well as bunch and fruit weights significantly increased in the autotetraploids (Do Amaral et al., 2015). However, apart from the phenotypic changes, little is known on molecular mechanisms underlying the phenotypic difference between diploid and its autopolyploid counterparts.

The diploid banana variety Pisang Berlin (AA) is highly resistant to Fusarium wilt Tropical Race 4 (*Foc* TR4), and is widely grown in southern China and other Southeast Asian countries (Hapsari and Lestari, 2016; Zuo et al., 2018). It has superior fruit quality, but the yields are low due to its small hands and fruits size. Therefore in our breeding program, we attempted to increase fruit size through polyploidization and therefore improve the yields of the diploids with good fruit quality characteristics. The present study focused on the growth of the autotetraploid seedlings of Pisang Berlin and explored the molecular mechanisms underlying phenotypic variation caused by ploidy changes, which would allow better understanding and utilizing the autotetraploids in the banana breeding program.

Materials and methods

Plant materials

The diploid banana variety Pisang Berlin (*Musa acuminata*, AA genome; ITC0611, doi: 10.18730/9K96E) was used in this study. The meristematic shoot tips of this variety were obtained from the China's National Banana Collection at Guangzhou, and

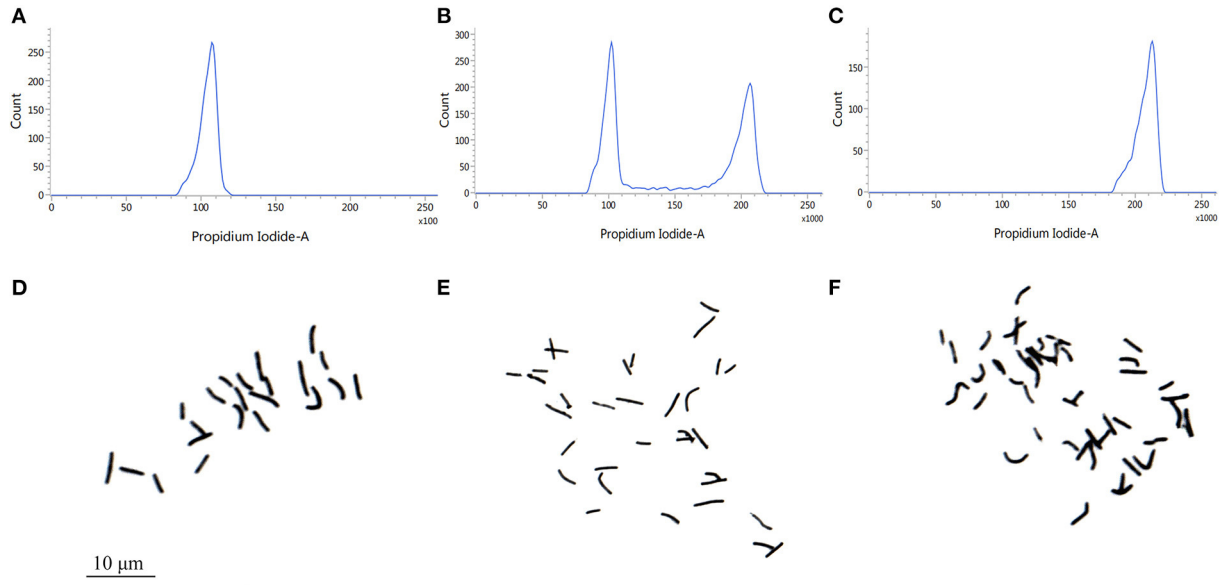

FIGURE 1
Ploidy analysis of of Pisang Berlin (AA). **(A)** DNA content of diploids (the main peak at channel 100). **(B)** DNA content of triploids (the main peak at channel 100–200). **(C)** DNA content of autotetraploid (the main peak at channel 200). **(D)** Chromosome number of diploids (2n = 2X = 22). **(E)** Chromosome number of triploids (2n = 3X = 33). **(F)** Chromosome number of autotetraploids (2n = 4X = 44).

propagated in the solid Murashige and Skoog (MS) medium under sterile conditions at 25°C (Swamy et al., 1983). The pH of the medium was adjusted between 5.7 and 5.8.

Chromosome doubling

In vitro induction of autotetraploids on shoot tips of Pisang Berlin (treated by colchicine) was carried out according to the method described by Bakry et al. (2007). Thereafter, the shoot tips were grown *in vitro* in the liquid MS medium (125 mL) consisting of adenine sulfate (651.5 μM) and 6-benzylaminopurine (22.2 μM, BAP). After that, they were kept at 27°C on a gyratory shaker at 80 rpm in liquid MS medium for 7 days under 16-h/8-h light/dark cycle. Selected shoot tips were treated with 1.25 mmol/L colchicine added to the 125 ml of liquid MS medium. The treated shoot tips were cultured in darkness for 48 h at 80 rpm. Thereafter, the shoots were rinsed in sterile water for 48 h at 80 rpm, followed by transfer into liquid MS medium for additional 7 days culture. Finally, the shoots were transferred into the solid MS medium for further sub-culture.

Rooting culture and plant regeneration

After 30-days of post-initial sub-culture, the surviving shoot tips were further sub-cultured twice or more at every 35–40 days, followed by transfer into the rooting medium (MS that contained 30 g/L sucrose and 7 g/L agar solidification). Thereafter, all shoots were maintained in the growing stage at 25 ± 2°C and 16-h/8-h light-dark cycle conditions. After 30-40 days, the rooted plantlets were transferred into liquid Hoagland nutrient solutions and retained for further 30 days (Hoagland and Arnon, 1950).

FCM-based ploidy degree determination

In order to determine the ploidy level, FCM was used using the method proposed by Roux et al. (2004). Meanwhile, sample preparation was conducted following the descriptions of Poerba et al. (2019). Leaf tissues (20–30 mg) were sampled using a sharp scalpel blade and placed in glass petri dishes.. 1 mL LB01 buffer (363 mg Tris, 148.9 mg Na_2EDTA, 20.2 mg spermine, 1.193 g KCl, 233.8 mg NaCl, 200 ul Triton X-100, 220 ul mercaptoethanol 200 ml deionized water with a pH of 7.5) (DoleŽel et al., 1989) was added to the plate. The nuclear DNA was stained with 2 μg/ml PI solution. Thereafter, the nuclear suspension was filtered using the 50-μm nylon mesh, followed by maintenance on ice till further experiments. FCM was performed to measure DNA concentrations (C units) within the samples, with 1C indicating the DNA concentration in the haploid chromosome set (n). Following FCM, the fluorescence intensity distribution (relative DNA concentration) was generally determined in arbitrary units (channel numbers). In this study, one

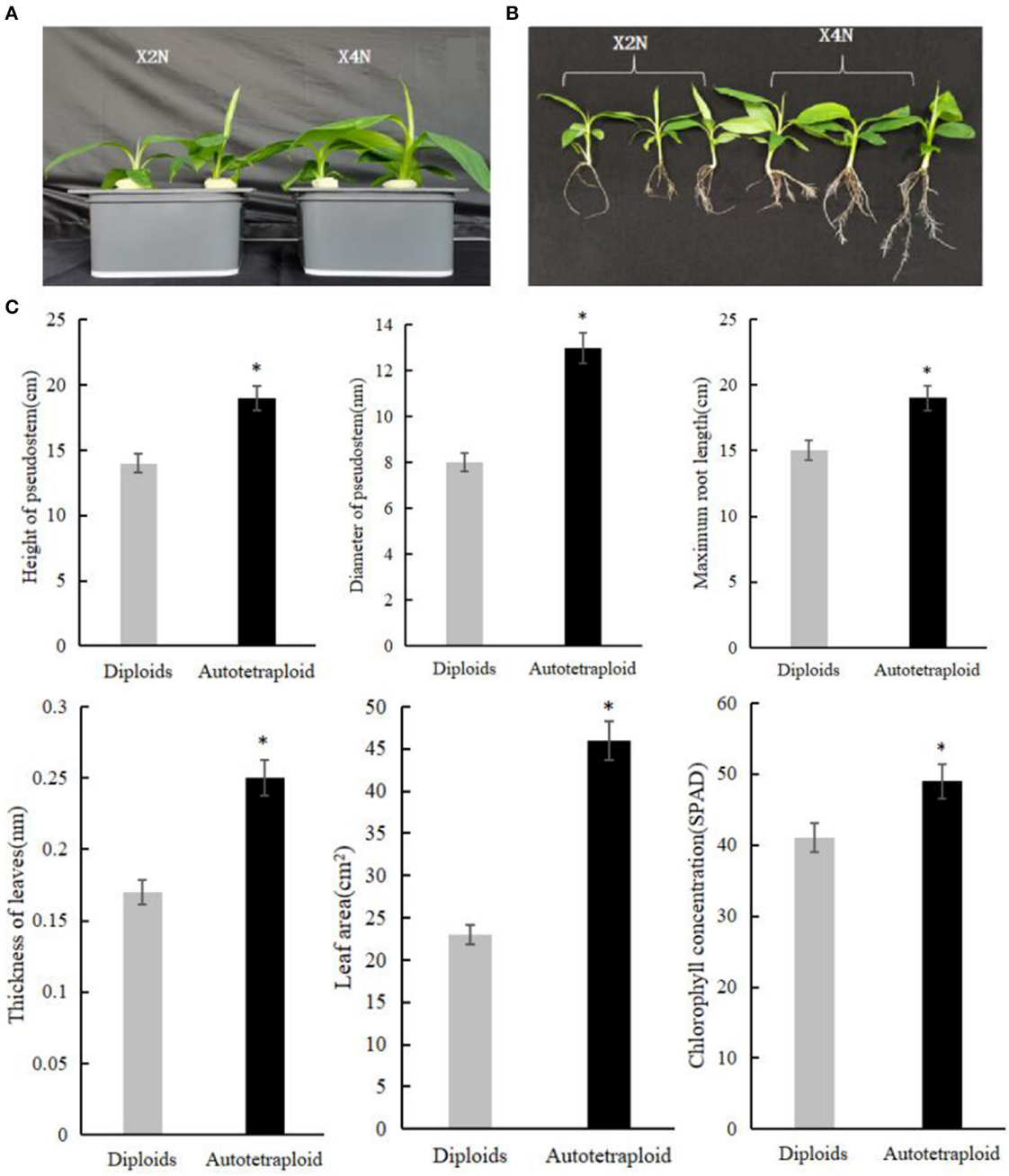

FIGURE 2
Phenotypic characterization of autotetraploid and diploid Pisang Berlin seedlings grown under the same conditions. **(A, B)** Phenotypic comparison of autotetraploid and diploid Pisang Berlin seedlings. **(C)** The pseudostem diameter, plant height, maximum root length, leaf thickness, leaf area, and chlorophyll content of autotetraploid and diploid Pisang Berlin seedlings. X2N represented diploids of Pisang Berlin. X4N represented autotetraploids of Pisang Berlin. * in the figure represent significant differences at $P < 0.05$ level.

Musa acuminata ssp. *malaccensis* (2n = 22) sample was utilized as the diploid reference (Poerba et al., 2019). The flow cytometer was adjusted to ensure that the peak represented the G1 nuclei appearing on channel 100. Later, additional samples were normalized to locations relative to the G1 peak.

Detection of chromosome number in the root tips

Young root tips of plants were selected and sliced by wall-removing low permeability and flame drying (Chen et al., 1979). Enzymolysis was carried out in a mixed enzyme solution

TABLE 1 RNA sequencing data and quality control.

Sample	Raw reads	Clean reads	Mapped reads	Clean bases	Q 30 (%)	GC content (%)
X1	49146166	47441494	44171524 (93.11%)	7.12G	94.81	50.96
X2	42717568	40921468	38203772 (93.36%)	6.14G	96.06	50.71
X3	46079646	44478910	41355175 (92.98%)	6.67G	95.95	50.29
X4	41287264	39209796	36417744 (92.88%)	5.88G	96.06	49.42
X5	46425252	43947988	40847833 (92.95%)	6.59G	95.48	49.95
X6	42116118	40230606	37307283 (92.73%)	6.03G	94.28	50.36

X1, X2, and X3 were diploids and X4, X4 and X4 were autotetraploid of Pisang Berlin.

(3% cellulase + 1% macerozyme) for 1.5 h. The smear was fixed after cleaning with water, dried by alcohol lamp flame, and then examined after staining with 5% Giemsa dye. A preliminary microscopic examination was performed under a 10-fold Olympus microscope. The splits were photographed under a 40x lens.

Phenotype analyses of diploid and autotetraploid

Diploid (control) and autotetraploid plantlets were grown in liquid Hoagland nutrient solutions for 30 days at 25 ± 2°C and 16-h/8-h light-dark cycle conditions, photographed, and the phenotypes were recorded as described by An et al. (2009). Plant height, length, and width of leaves were measured by measuring tape, stem diameter and leaf thickness were measured by an electronic micrometer, and chlorophyll value was measured by a portable chlorophyll meter. The leaf area was calculated as described by Liu et al. (2013).

cDNA preparation and illumina sequencing

Total RNA was isolated from the cultured leaves of diploids (X1, X2, and X3) and autotetraploids (X4, X5, and X6) of banana plants after 30 days with EASY spin Plus Plant RNA Kit (AidLab, Beijing). RNA concentration was detected by Nanodrop. Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was employed for detecting RIN and 28S/18S values. Then, RNA integrity and quality were measured by 1% AGE. Additionally, 18 cDNA libraries were prepared in line with specific protocols using NEB Next Ultra RNA Library Prep Kit. This was followed by the sequencing of cDNA libraries by Novogene (Beijing, China), adopting Illumina HiSeq TM 2000 platform. The quality of raw reads was checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) before data analysis. The impure reads were filtered, whereas the high-quality ones were

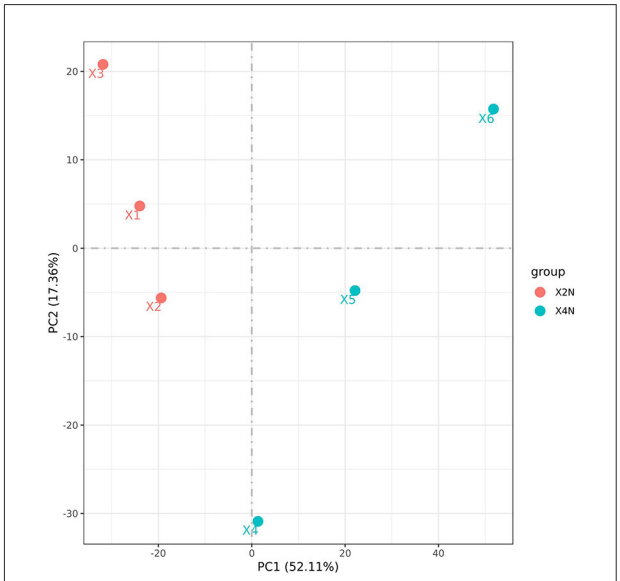

FIGURE 3
Principle component analysis (PCA) of diploids and autotetraploids of Pisang Berlin samples. X2N represented the diploids of Pisang Berlin (X1, X2, and X3 represented sample replicates). X4N represented the autotetraploids of Pisang Berlin (X4, X5, and X6 represented sample replicates).

acquired using Trinity by adopting default parameters, which were later utilized for constructing the distinct consensus sequences (Grabherr et al., 2011). Filtered reads were compared with the *Musa acuminata* subsp. *malaccensis* (ID: ensemblplants_musa_acuminata_subsp_malaccensis_asm31385 v1_gca_000313855_1) using TopHat (v2.0.10) (Trapnell et al., 2012).

Gene analysis and functional annotation

Using the RPKM (Reads Per kb per Million reads) approach, unigene expression was estimated by cuffdiff (Trapnell et al., 2012). DEGseq v1.14.0 software was used for identifying differentially expressed genes (DEGs). All

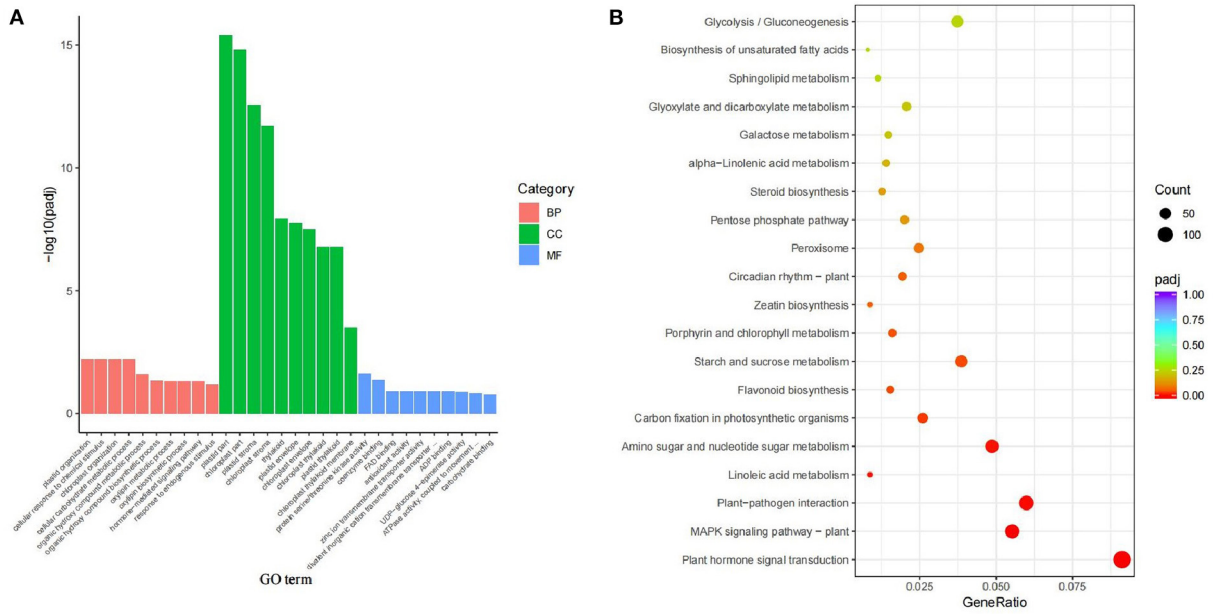

FIGURE 4
GO enrichment and KEGG enrichment of differentially expressed genes in diploids and autotetraploids of Pisang Berlin. **(A)** GO enrichment and different colors represented different functional categories, including BP biological processes, CC cellular components, and MF molecular functions. **(B)** KEGG enrichment and gene ratio represented the ration of the number of differentially expressed genes to the total gene number in the specific pathway, green indicated a low *Q*-value, while red indicated a high *Q*-value.

genes were assigned with corresponding *p*-values, and DEGs were selected upon the thresholds of $|\log2| \geq 1$ and $p \leq 0.05$. Later, acquired unigenes were mapped against Nr (Non-redundant Protein Sequence Database in GenBank), Swiss-Prot (Protein Sequence Database), GO (gene ontology; Conesa et al., 2005), COG (Cluster of Orthologous Groups of proteins; Tatusov et al., 2003), and KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa et al., 2006) databases.

Metabolic profiling

The leaf tissue samples of diploid and autotetraploid of banana plants were freeze dried in a lyophilizer (Scientz-100F) under vacuum, followed by grinding into fine powders. Later, 80% methanol (500 μL), added with 0.1% formic acid, to dissolve the powders (100 mg). After overnight preservation at 4 °C the samples were vortexed six times to enhance extraction efficiency. Later, the samples were centrifuged for 10 min at 15,000 g and the supernatants were then filtered using a 0.22 μm microporous filter, followed by processing for Ultra Performance Liquid Chromatography (UPLC)-mass spectrometry (MS/MS) (Want et al., 2010; Dunn et al., 2011). To characterize the secondary metabolites, around 0.1 g lyophilized leaf sample powders were subjected to methanol (80%) extraction. Then, the QTOF 6520 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was employed for profiling secondary metabolites by adopting the 1200 series Rapid Resolution HPLC system. Typical fragment ions were compared to the reference for identifying secondary metabolites as well as additional amino acids (Wang et al., 2016). Metabolites that shared close fragment ions were deemed as identical compounds. Statistical analysis of secondary metabolite data was performed using Analyst 1.6.1 software (AB SCIEX, Ontario, Canada). Variable importance in projection (VIP) values were determined through partial least squares discriminant analysis. The differentially changed metabolites (DCMs) were selected based on the thresholds of VIP $>$ 1, $p < 0.05$, and FC $>$ 1.5 or $<$0.667.

Reverse transcription of and qRT-PCR

M-MLV reverse transcriptase was adopted for synthesizing 2 μg RNA in line with Evo M-MLV RT Kit instructions (Accurate biotechnology, China) and further diluted to 1:6 for subsequent experiments. Ten genes were validated by qRT-PCR with thermal cycler apparatus (BIO-RAD iQ3 7700, Applied Biosystems). The 20 mm^3 qRT-PCR reaction system contained 2×SYBR Green Pro Taq HS Premix (10 mm^3), ROX Reference Dye (0.4 mm^3), respective primers (10 μmol ×

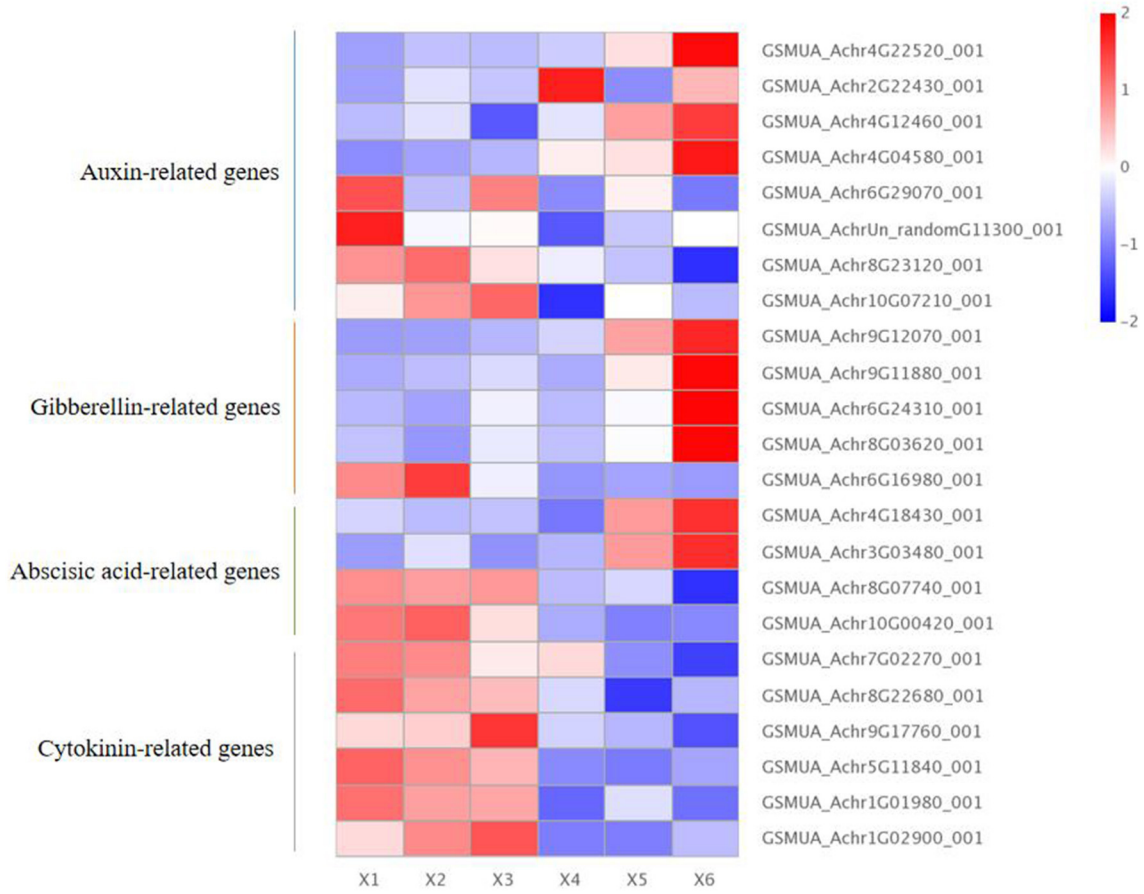

FIGURE 5
Endogenous hormone differentially expressed genes in diploid and autotetraploid of Pisang Berlin. X1, X2, and X3 represented the diploid banana plants, and X4, X5, and X6 represented the autotetraploid banana plants.

dm^{-3}, 0.4 mm^3 each), RNase Free ddH_2O (7.8 mm^3) and cDNA (1 mm^3). The thermal profile was comprised of two segments: 30-S at 95°C; 5-S denaturation at 95 °C, and 30-S annealing at 60 °C for altogether 40 cycles. Every assay was repeated thrice. Primer Express 2.0 Software (PE Applied Biosystems, USA) was applied in primer designing with default parameters. Supplementary Table S1 displayed the sequences of all the primers. Excel software and $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001) were used for data analysis with MaCAC-Q as the reference.

Statistical analysis

ANOVA was adopted using the SPSS 19.0 statistics software for comparing phenotypic data of different samples. The Fisher's least significance difference (LSD) test was applied for multiple comparisons of variables at 0.05 ($p < 0.05$).

Results

Autotetraploids generated by colchicine treatment

We carried out the chromosome doubling by treating shoot tips of Pisang Berlin (*Musa* spp. AA genome) with colchicine. Using flow cytometry, we checked the ploidy levels of the shoot tips in each sub-culturing step (without damaging their meristematic cells) in order to dissociate chimeras by repeated vegetative propagation. After three successive sub cultures, autotetraploid shoots were confirmed and then transferred into the rooting culture medium. Accurate chromosome counts in root tips were carried out to verify ploidy levels of the regenerated autotetraploid plantlets (Figure 1). Compared to the diploid plants, autotetraploid plants exhibited a better growth performance, exhibiting significant increase in pseudostem diameter, plant height, maximum root length, leaf thickness, leaf area, and chlorophyll content (Figure 2).

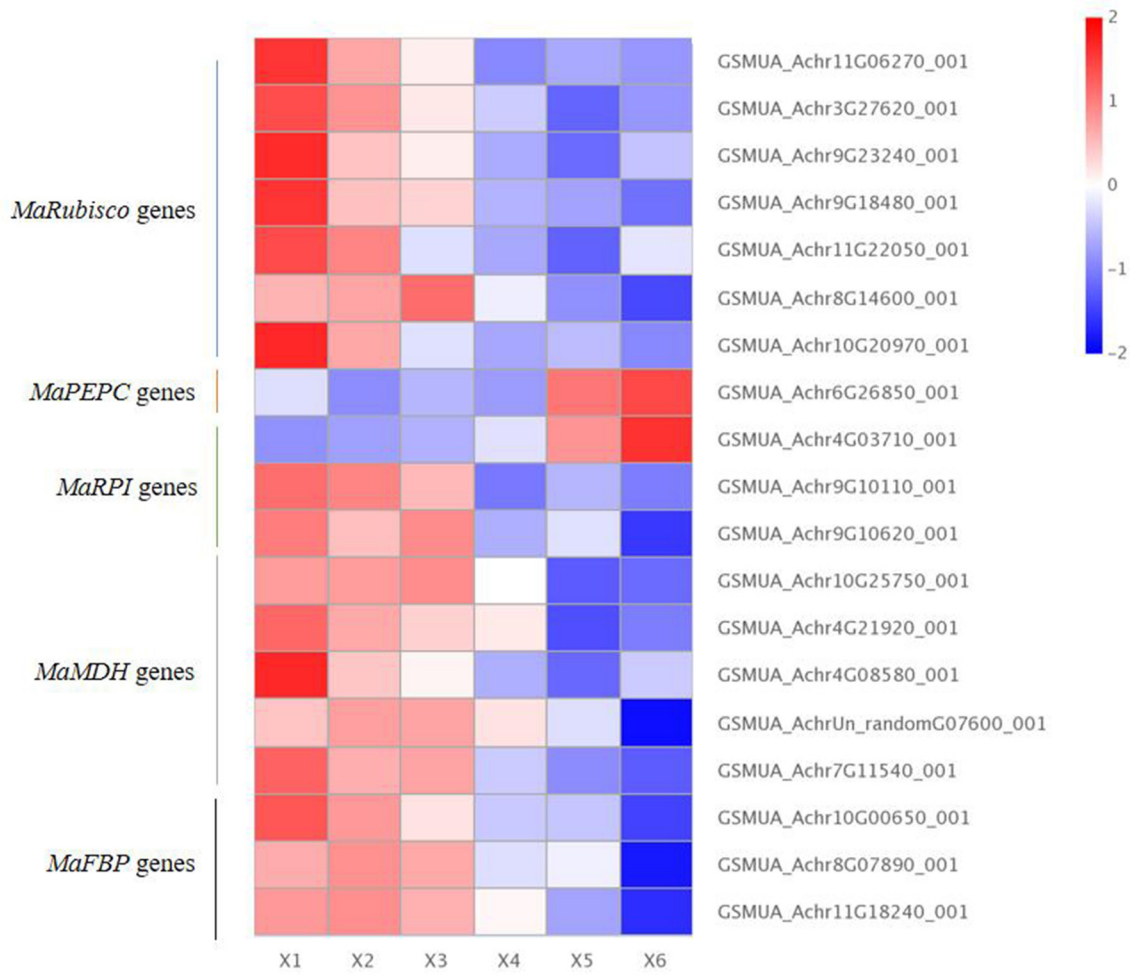

FIGURE 6
Photosynthesis differentially expressed genes in diploids and autotetraploids of Pisang Berlin. X1, X2, and X3 represented the diploid banana plants, and X4, X5, and X6 represented the autotetraploid banana plants.

Transcriptomic analysis and differentially expressed genes

Transcriptomic analysis was carried out on the diploid and autotetraploid plants to explore the genes responsible for the differential growth. The overall raw/clean reads within every sample ranged between 41,287,264 and 49,146,166. The sequence reads were aligned into the reference genome of banana, and results indicated that > 92% were map-able. This yielded altogether of 38.43 Gb of clean data. The > 94% Q30 and 50% GC concentrations suggested a high-quality transcriptomic results for the subsequent analyses (Table 1). The PCA analysis classified overall variation in two major components as 51.68 and 17.65%. These observations were validated through PCA, which suggested that diploids tissues fall away from autotetraploid tissues (Figure 3). These samples were also subject to hierarchical clustering on the basis of RPKM values. This approach classified six samples into two main groups: one (X2N) containing X1, X2, and X3, while the other (X4N) comprised X4, X5, and X6 (Figure 3).

There were 25334 DEGs in X4N vs. X2N. GO-function classification statistics were further performed on DEGs in autotetraploids and diploids after doubling. The DEGs were mainly found enriched in the plastid and chloroplast parts in the cellular composition of Pisang Berlin (X4N.vs.X2N) (Figure 4A). KEGG enrichment further indicated DEGs were mainly enriched in plant hormone signal transduction, MAPK signaling pathway, and carbon fixation in photosynthetic organisms (Figure 4B). The change in polyploid phenotype was tightly associated with plant endogenous hormones (Dai et al., 2015). Therefore, we focused on the DEGs related to plant hormone between autotetraploids and diploids because we found that the growth of autotetraploids at the seedling stage had a

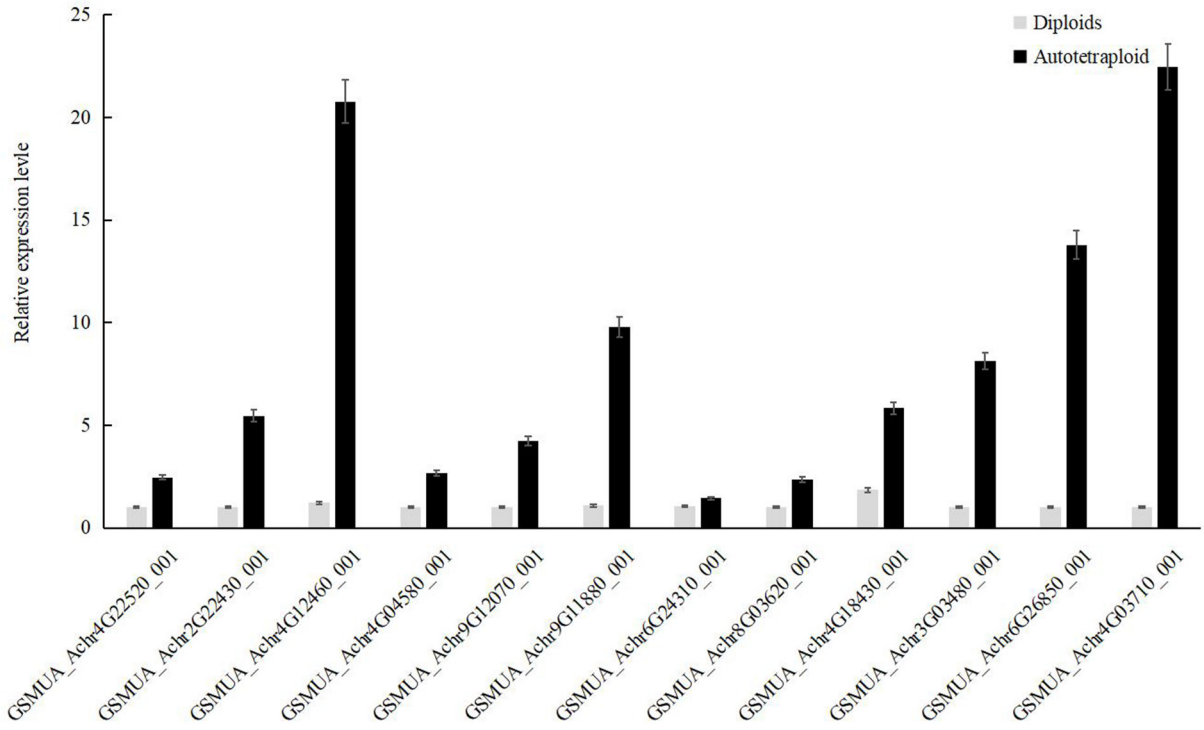

FIGURE 7
Quantitative RT-PCR results of up-regulated genes in autotetraploid of Pisang Berlin.

better plant performance compared with those of diploids (Figure 2).

There were eight auxin-related genes within the autotetraploids of Pisang Berlin, including four with up-regulation and four with down-regulation. The up-regulated DEGs included three *Ma5NG4* genes (GSMUA_Achr2G22430_001, GSMUA_Achr4G12460_001, and GSMUA_Achr4G04580_001 encoding auxin-induced 5NG4), which were auxin-induced protein with transport function and played a role in signaling of auxin (Kaur et al., 2017); *MaIAA30* (GSMUA_Achr4G22520_001) encoded an auxin-responsive protein (Sato and Yamamoto, 2008). The down-regulated DEGs included *MaARF18* gene (GSMUA_AchrUn_randomG11300_001 encoding auxin-response factor 18) which inhibited activity on downstream auxin genes (Chen et al., 2021); *MaARF7* gene (GSMUA_Achr6G29070_001 encoding auxin-response factor 7) negatively regulated auxin (Jong et al., 2009); Two *MaPIN* genes (GSMUA_Achr8G23120_001 and GSMUA_Achr10G07210_001) were auxin efflux carrier component and inhibited cell growth (Xu et al., 2005) (Figure 5; Supplementary Table S2).

Furthermore, we screened five gibberellin (GA)-related DEGs, including four DEGs up-regulated and one down-regulated in the autotetraploids. The four up-regulated DEGs included three *MaCIGR1* genes and one *MaGA2ox8* gene. The *MaCIGR1* genes (GSMUA_Achr9G12070_001, GSMUA_Achr6G24310_001, and GSMUA_Achr8G03620_001), are belonged to the *GRAS* family and encode chitin-inducible gibberellin-responsive protein 1, which could play an importance role in regulating plant height (Kovi et al., 2011). The *MaGA2ox8* gene (GSMUA_Achr9G11880_001) encodes gibberellin 2-beta-dioxygenase 8 and may regulate GA levels (Zhai et al., 2019). The down-regulated DEGs included *MaGID1* (GSMUA_Achr6G16980_001 encoding gibberellin receptor GID1) occluded gibberellin in a deep binding pocket covered by its N-terminal helical switch region (Murase et al., 2008) (Figure 5; Supplementary Table S2).

Moreover, there were four abscisic acid (ABA)-related DEGs within the autotetraploids, including two that were up-regulated and two that were down-regulated. The two up-regulated DEGs included one *ABA 8'-hydroxylase 1* gene (GSMUA_Achr4G18430_001) promoting the content of ABA (Kitahata et al., 2005), and one *MaPYL8* gene (GSMUA_Achr3G03480_001 encoding abscisic acid receptor PYL8) positively regulating ABA signaling during germination and abiotic stress responses (Saavedra et al., 2010). The two down-regulated DEGs were *MaABI5* genes (GSMUA_Achr8G07740_001 and GSMUA_Achr10G00420_001 encoding abscisic acid-insensitive 5) which would

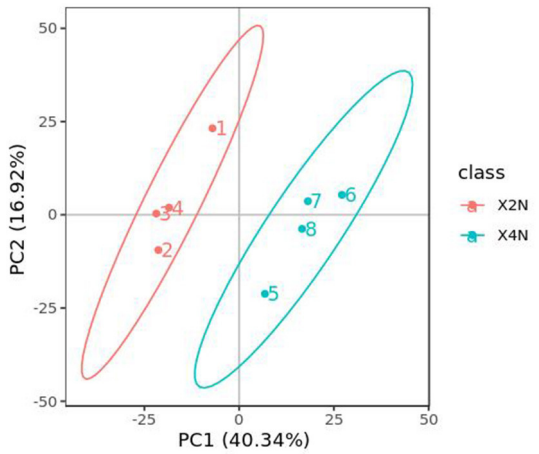

FIGURE 8
Principal component analysis (PCA) analysis of diploids and autotetraploids of Pisang Berlin samples.The ordinate represented the clustering of samples and the abscissa represented the clustering of metabolites. The diploids and autotetraploids of Pisang Berlin were represented as following, X2N represented diploids of Pisang Berlin (1, 2, 3, and 4 represented sample replicates), X4N represented autotetraploid of Pisang Berlin (5, 6, 7, and eight represented sample replicates).

negatively regulate ABA signaling (Brocard, 2002; Figure 5; Supplementary Table S2).

It was noted that six down-regulated DEGs related with cytokinin, and the six *MaCKX* genes (GSMUA_Achr7G02270_001,GSMUA_Achr8G22680_001, GSMUA_Achr9G17760_001,GSMUA_Achr5G11840_001, GSMUA_Achr1G01980_001, GSMUA_Achr1G02900_001 encoding cytokinin dehydrogenase) inactivated cytokinin (Chen et al., 2009; Figure 5; Supplementary Table S2).

Our results showed that there were seven differentially expressed *MaRubisco* genes encoding Ribulose 1,5-bisphosphate carboxylase, which were down-regulated in autotetraploids of Pisang Berlin. There is one *MaPEPC* (GSMUA_Achr6G26850_001 encoding phosphoenolpyruvate carboxylase), which was up-regulated in the autotetraploids of Pisang Berlin. PEPC catalyzed the irreversible carboxylation of phosphoenolpyruvate (PEP) to form oxaloacetate which played a key role in photosynthesis (Yasushi et al., 2003). There were three *MaRPI* DEGs encoding ribose-5-phosphate isomerase DEGs, including one with up-regulation (GSMUA_Achr4G03710_001) and the other two with down-regulation in the autotetraploids. There were five *MaMDH* DEGs encoding malate dehydrogenase and three *MaFBP* DEGs encoding fructose-1,6-bisphosphatase down-regulated in the autotetraploids of Pisang Berlin (Figure 6; Supplementary Table S3). The twelve DEGs that were up-regulated in the autotetraploids were further screened for qRT-PCR analysis, and the results were consistent with the transcriptome results (Figure 7).

Metabolome analysis and differential metabolites

PCA analysis classified overall variation as PC1 and PC2, contributing 16.92/18.17%, respectively (Figure 8). The correlations between samples with the same ploidy were high, indicating good repeatability of samples, as well as stability and reliability of the experimental data. The results of metabolome analysis revealed that metabolites of autotetraploid seedlings were significantly different from those of diploid banana seedlings (Figure 8).

Our results indicated that there were 276 differential metabolites in the autotetraploids and diploids of Pisang Berlin. We found 107 differential metabolites that were up-regulated in the autotetraploids. Furthermore, the KEGG pathway enrichment analysis showed that the significantly enriched pathways were biosynthesis of amino acids, porphyrin and chlorophyll metabolism, indole alkaloid biosynthesis, and carbon fixation in photosynthetic organisms (Figure 9; Supplementary Table S4).

We further analyzed metabolites associated with hormones and photosynthesis. We found that DL-tryptophan, tabersonine, trans-3-Indoleacrylic acid, Indole, and skatole related to auxin, as well as Abscisic acid, present at high levels in the autotetraploids. In contrast, zeatin-7-N-glucoside related to cytokinin, and biliverdin, D-ribulose 5-phosphate related to photosynthesis present at high levels in diploids of Pisang Berlin (Table 2).

Combined analysis of the metabolome and transcriptome revealed that, DEGs and differential metabolites in diploids and autotetraploids of Pisang Berlin, were found to be enriched in 30 metabolic pathways. DEGs and DCMs were mostly related to phenylpropanoid and amino acid biosynthesis, as well as plant hormone signaling in Pisang Berlin (Figure 10).

Discussion

Banana breeding for triploids is believed to be the optimum for cultivation in the banana industry, since the triploids exhibit better agronomic characteristics, good fruit quality and infertile or (seedless; Bakry et al., 2007). The reconstructive breeding to generate banana (*Musa* spp.) triploid varieties is a promising breeding strategy based on artificial autotetraploids as a breeding step. Induction of autotetraploid plants during mitosis with spindle fiber-inhibiting chemicals (such as colchicines and oryzalin) is a feasible approach to develop autotetraploids, but chimeras may occur during the polyploidy induction by colchicine, while some plants of ploidy variation could return to their original ploidy (De Carvalho Santos et al., 2019). In this study, regenerated plants of autotetraploids were obtained by colchicine induction (Figure 1). The mixoploidy phenomenon observed after the first and second generation of propagation was detected by the FCM approach (Pio et al., 2014), and

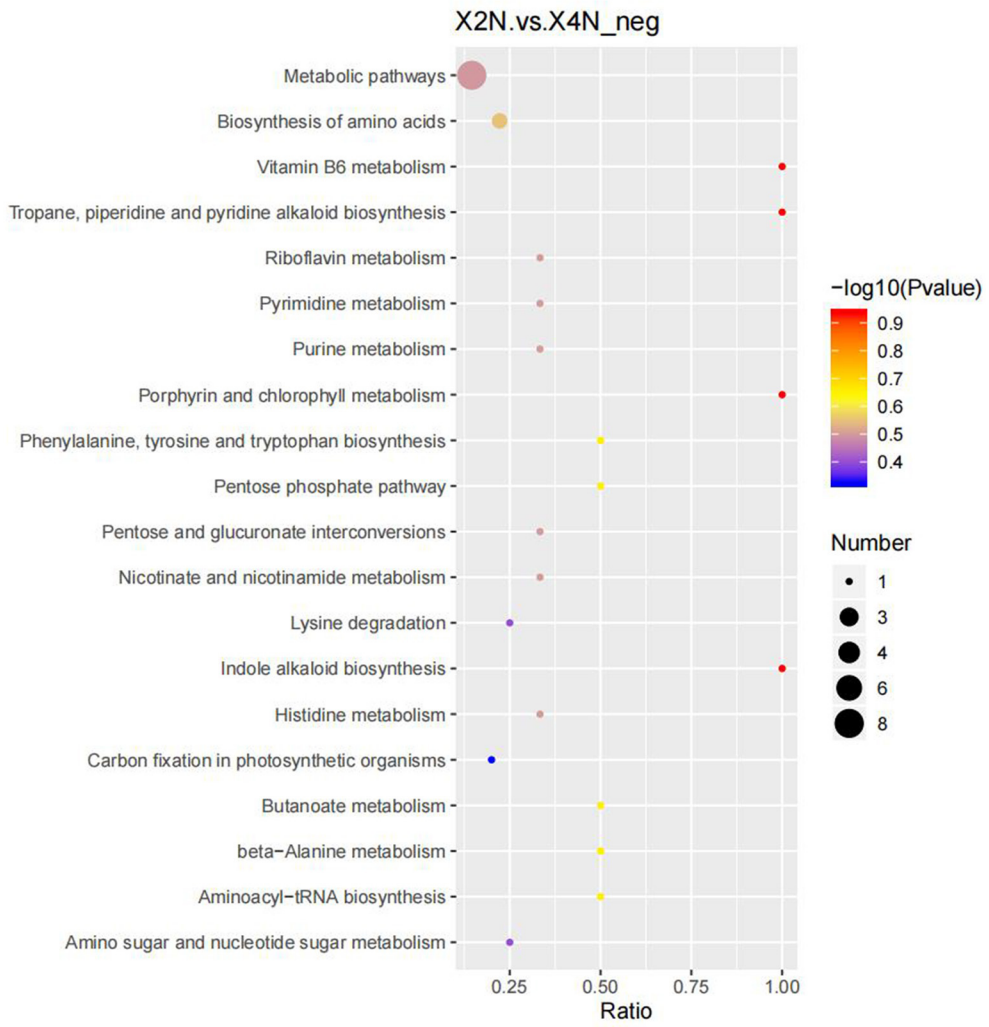

FIGURE 9
Scatter plot of 20 KEGG pathways between diploids and autotetraploids of Pisang Berlin of the differentially accumulated metabolites enrichment analysis. The degree of enrichment is shown by ratio, P-value, and the number of metabolites enriched in each pathway. X2N represented diploids of Pisang Berlin; X4N represented autotetraploid of Pisang Berlin.

this phenomenon, known as "retromutation" usually occurs in polyploid cells (Bakry et al., 2007). To avoid this phenomenon, we used the strategy of repeated vegetative propagation to dissociate chimeras (Roux et al., 2001), and obtained the non-chimeral autotetraploids (Figure 1).

Polyploids that were generated by chromosome doubling could have some superior agronomic features, including increased fruit size, disease tolerance, and seedless (Sanford, 1983; Predieri, 2001; Kanchanapoom and Koarapatchaikul, 2012). In this study, autotetraploids were obtained from treatment with colchicine on meristematic shoot tips of diploids Pisang Berlin (AA). The autotetraploid plantlets exhibited better plant performance, displaying significantly increases in height and diameter of pseudostem, root length, thickness and area of leaves, as well as leaf chlorophyll content, compared to the diploid control (Figure 2). This effect seems to be associated with the gene dosage effect as would be expected. Previous research based on colchicine derived polyploidy also obtained similar results (Vakili, 1967; Hamill et al., 1992). Polyploidy has the general effect of increasing gene expression levels, which would result in enhancing plant resistance and improving adaptation to adverse environmental conditions (Sattler et al., 2016).

Endogenous plant hormones are synthesized and transported to various parts that regulate growth and development by forming different concentration gradients and ratios (Arnao and Hernández-Ruiz, 2018). Polyploidy plants usually show organ hypertrophy and longer growth

TABLE 2 Differential metabolites associated with growth hormone.

Compound_ID	Name	log2FC	*P*-value	VIP	Up.Down
Com_622	DL-Tryptophan	2.688060296	0.000143895	2.142428434	up
Com_6035	Tabersonine	1.536882485	0.003161298	2.093947607	up
Com_21447	Abscisic Acid	2.66297236	0.027466353	2.305281688	up
Com_221	trans-3-Indoleacrylic acid	2.234765359	0.000545497	2.035622108	up
Com_1203	Indole	2.222727091	0.000570632	2.019590916	up
Com_5715	Skatole	1.572345839	0.001128781	1.83655634	up
Com_7656	Zeatin-7-N-glucoside	−0.736131109	0.004628143	1.345465678	down
Com_7524	Biliverdin	−0.635862077	0.040149073	1.13956098	down
Com_344	D-Ribulose 5-phosphate	−1.728866675	0.026882755	2.172300016	down

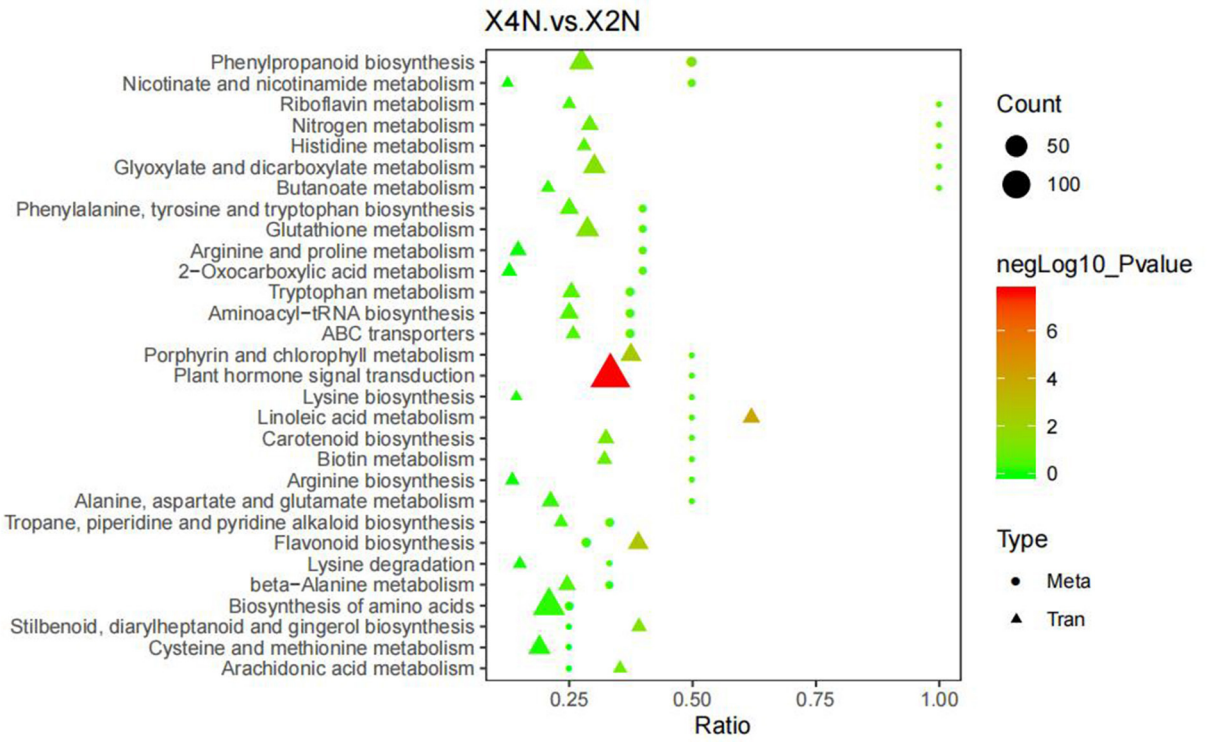

FIGURE 10
Association analysis of differential expression genes and differential metabolites. The abscissa represented the ratio of the metabolites or genes enriched in this pathway to the number of metabolites or genes annotated in this pathway, the ordinate represented the KEGG pathway enriched by metabolome and transcriptome. Count represented the number of metabolites or genes enriched in the pathway. X2N, diploids of Pisang Berlin; X4N, autotetraploid of Pisang Berlin.

periods, mainly due to the alterations of endogenous hormone levels. Previous reports showed that the DEGs related to cell development and carbohydrate metabolism, showed up-regulation within poplar polyploids (Cheng et al., 2015). In autotetraploid plants of *Atractylodes lancea*, their increasing stress resistance and phenotypic changes in leaves, may be related to the DEGs including *DnaK/Hsp70*, *ACO*, *ETR/ERS*, *FAD2*, *AOS*, *LOX*, *PHS1/PAS2* and *YWHAE* (Ul Haq et al., 2019). Another study found that in autotetraploids of birch (*Betula platyphylla*) and mulberry (*Morus alba*), the phenotypic changes would be attributed to the differentially expressed genes involved in signal transduction of plant hormones (including cytokinin, gibberellins, ethylene, and auxin; Mu et al., 2012; Dai et al., 2015).

The metabolic changes caused by polyploidy usually endue plants with stronger environmental adaptability. For example in willow plants, it was observed that the endogenous hormones (auxin and gibberellin) accumulated more in autotetraploids' root tips (Dudits et al., 2016), in comparison to the diploids. In our study, we analyzed genes associated with endogenous hormones. In autotetraploids of Pisang Berlin (AA), the genes *Ma5NG4* and *MaIAA30* were up-regulated, whereas the genes *MaARF18*, *MaARF7* and *MaPIN* were down-regulated, which may result in higher auxin content and afterwards lead to growth promotion (Figures 2, 5, Supplementary Table S2). The gibberellin-related genes, *MaCIGR1* and *MaGA2ox8* were up-regulated, *MaGID1* was down-regulated, indicating that these genes might play an importance role in increasing the plant height of autotetraploids (Supplementary Table S2).

Abscisic acid (ABA) has the effect on the slow growth when *in vitro* preservation of Mahesak (*Tectona grandis*) (Tongsad et al., 2020). In our study, the up-regulation of *ABA 8'-hydroxylase 1* and *MaPYL8*, as well as down-regulation of *MaABI5*, may lead to the high levels of abscisic acid found in the autotetraploids (Figure 5, Supplementary Table S2). Interestingly, we recently observed the slow plant growth occurred in autotetraploid plants of Pisang Berlin in the field, which also could be due to the inhibition effect caused by ABA (Humplík et al., 2017). Furthermore, we found that six genes of *MaCKX* cytokinins in the autotetraploids were down-regulated, and zeatin-7-N-glucoside related to cytokinin present at low levels (Figure 5, Supplementary Table S2). These evidences confirmed that *MaCKX* genes might play an important role in inactivation of cytokinins.

The photosynthetic process is considered as a series of enzymatic reactions, which involves genes related to Ribulose 1,5-bisphosphate carboxylase (Rubisco), malate dehydrogenase (MDH), ribose-5-phosphate isomerase (RPI), phosphoenolpyruvate carboxylase (PEP), fructose-1,6-bisphosphatase (FBP) (Whitney and Andrews, 2001; Liang et al., 2011; Wang et al., 2011; Ciou et al., 2015). Rubisco is responsible for the first step of the dark reaction in photosynthesis, but its catalytic efficiency is very low (Sharwood, 2017; Galmés et al., 2019). From our work, we identified the down-regulated Rubisco DEGs, as well as the up-regulated DEGs of PEP and RPI (Figure 6, Supplementary Table S3), which would possibly have critical positive effects on photosynthesis of the autotetraploids. Dai et al. (2015) also found that, the larger plants of autotetraploid mulberry trees were associated with the differentially expressed genes involved in photosynthesis biosynthesis.

Conclusion

Through *in vitro* chromosome doubling, we obtained the non-chimeral autotetraploids of *M. acuminata* (AA) "Pisang Berlin", a highly resistant banana diploid variety to *Foc* TR4. The autotetraploids showed significant increases in plant height, pseudostem diameter, main root length, and leaf thickness, area, and chlorophyll content. The evidences from transcriptome and metabolome profiling demonstrated that the hormones IAA, ABA, and photosynthetic regulation may play a vital role in the plant growth of autotetraploids in bananas. However, we contend that further investigations are needed to analyze the autotetraploids cultivated and hybridized with diploid elite cultivars for producing superior triploid hybrids that might have better values in sustainable breeding programs.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA900716.

Author contributions

OS, GY, NZ, LL, and LZ provided substantial contributions to the conception and design of the work. NZ, LL, and LZ performed the experiments, analyzed and interpreted the data, and wrote the manuscript. OS, WH, QY, FB, and GD revised the manuscript. OS, NZ, and AK reviewed and finalized manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1070108/full#supplementary-material

References

Allario, T., Brumos, J., Colmenero-Flores, J. M., Iglesias, D. J., Pina, J. A., Navarro, L., et al. (2013). Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. *Plant Cell Environ.* 36, 856–868. doi: 10.1111/pce.12021

Allario, T., Brumos, J., Colmenero-Flores, J. M., Tadeo, F., Froelicher, Y., Talon, M., et al. (2011). Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. *J. Exp. Bot.* 62, 2507–2519. doi: 10.1093/jxb/erq467

An, J. J., Li, M. F., Huang, M. J., and Li, S. P. (2009). Regression model building on leaf area of banana. *Guangxi Agr. Sci.* 40, 724–727

Arnao, M. B., and Hernández-Ruiz, J. (2018). Melatonin and its relationship to plant hormones. *Ann. Bot.* 121, 195–207. doi: 10.1093/aob/mcx114

Bakry, F., De La Reberdiere, N. P., Pichot, S., and Jenny, C. (2007). In liquid medium colchicine treatment induces non chimerical doubled-diploids in a wide range of mono-and interspecific diploid banana clones. *Fruits* 62, 3–12. doi: 10.1051/fruits:2006043

Borges, V. P., Marques, T. D. S., Reis, A. S. D., Oliveira, N. H. C., Jesus, J. A., Silveira, D. G., et al. (2016). Desenvolvimento *in vitro* de bananeira'Ouro'após poliploidização com antimitóticos. *Pesquisa Agro. Bra.* 51, 1789–1793. doi: 10.1590/s0100-204x2016001000011

Brocard, I. M. (2002). Regulation and role of the Arabidopsis abscisic acid-insensitive 5 gene in abscisic acid, sugar, and stress response. *Plant Physiol.* 129, 1533–1543. doi: 10.1104/pp.005793

Chen, J. W., Li, Y., Li, Y., Li, Y. Q., Wang, Y., Jiang, C. Y., et al. (2021). auxin response factor 18–histone deacetylase 6 module regulates floral organ identity in rose (*Rosa hybrida*). *Plant Physiol.* 186, 1074–1087. doi: 10.1093/plphys/kiab130

Chen, L., Zhao, J., Song, J., and Jameson, P. E. (2009). Cytokinin dehydrogenase: a genetic target for yield improvement in wheat. *Plant Biotechnol. J.* 18, 614–630. doi: 10.1111/pbi.13305

Chen, R. Y., Song, W. Q., and Li, X. L. (1979). A new method for preparing mitotic chromosomes from plant. *J. Integr. Plant Biol.* 21, 297–298.

Chen, S. L., and Tang, P. (1945). Studies on colchicine-induced autotetraploid barley. III. Physiological studies. *Am. J. Bot.* 32, 177–179. doi: 10.1002/j.1537-2197.1945.tb05104.x

Cheng, S., Huang, Z., Li, Y., Liao, T., Suo, Y. J., Zhang, P. D., et al. (2015). Differential transcriptome analysis between Populus and its synthesized allotriploids driven by second-division restitution. *J. Integr. Plant. Biol.* 57, 1031–1045. doi: 10.1111/jipb.12328

Ciou, S. C., Chou, Y. T., Liu, Y. L., Lu, J. W., Huang, S. F., Chou, Y. T., et al. (2015). Ribose-5-phosphate isomerase A regulates hepatocarcinogenesis via PP2A and ERK signaling. *Int. J. Cancer* 137, 104–115. doi: 10.1002/ijc.29361

Cohen, H., Fait, A., and Tel-Zur, N. (2013). Morphological, cytological and metabolic consequences of autopolyploidization in Hylocereus (Cactaceae) species. *BMC Plant Biol.* 13, 1–14. doi: 10.1186/1471-2229-13-173

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., and Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21, 3674–3676. doi: 10.1093/bioinformatics/bti610

Dai, F., Wang, Z., Luo, G., and Tang, C. (2015). Phenotypic and transcriptomic analyses of autotetraploid and diploid mulberry (*Morus alba* L.). *Int. J. Mol. Sci.* 16, 22938–22956. doi: 10.3390/ijms160922938

De Carvalho Santos, T. T., De Oliveira Amorim, V. B., Dos Santos-Serejo, J. A., Da Silva Ledo, C. A., Haddad, F., Ferreira, C. F., et al. (2019). Genetic variability among autotetraploid populations of banana plants derived from wild diploids through chromosome doubling using SSR and molecular markers based on retrotransposons. *Mol. Breed* 39, 1–13. doi: 10.1007/s11032-019-0996-1

Dita, M., Teixeira, L., Li, C., Zheng, S., O'Neill, W., and Daniels, J. (2011). Phenotyping Musa spp. for host reaction to Fusarium oxysporum f. sp. cubense, under greenhouse and field conditions In editor M Dita, *Practical Guidelines for Early Screening and Field Evaluation of Banana against Fusarium Wilt, Pseudocercospora Leaf Spots and Drought* (Montpellier France: Bioversity International), 5–18.

Do Amaral, C. M., De Almeida Dos Santos-Serejo, J., De Oliveira E Silva, S., Da Silva Ledo, C. A., and Amorim, E. P. (2015). Agronomic characterization of autotetraploid banana plants derived from 'Pisang Lilin'(AA) obtained through chromosome doubling. *Euphytica.* 202, 435–443. doi: 10.1007/s10681-014-1320-0

DoleŽel, J., Binarová, P., and Lcretti, S. (1989). Analysis of nuclear DNA content in plant cells by flow cytometry. *Biol. Plant* 31, 113–120. doi: 10.1007/BF02907241

Dudits, D., Török, K., Cseri, A., Paul, K., Nagy, A. V., Nagy, B., et al. (2016). Response of organ structure and physiology to autotetraploidization in early development of energy willow Salix viminalis. *Plant Physiol.* 170, 1504–1523. doi: 10.1104/pp.15.01679

Dunn, W. B., Broadhurst, D., Begley, P., Zelena, E., Francis-McIntyre, S., Anderson, N., et al. (2011). Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat. Protocols* 6, 1060–1083. doi: 10.1038/nprot.2011.335

Fort, A., Ryder, P., McKeown, P. C., Wijnen, C., Aartset, M. G., Sulpice, R., et al. (2015). Disaggregating polyploidy, parental genome dosage and hybridity contributions to heterosis in *Arabidopsis thaliana*. *New Phytol.* 209, 590–599. doi: 10.1111/nph.13650

Galmés, J., Capó-Bauçà, S., Niinemets, Ü., and Iñiguez, C. (2019). Potential improvement of photosynthetic CO_2 assimilation in crops by exploiting the natural variation in the temperature response of Rubisco catalytic traits. *Curr. Opin. Plant Biol.* 49, 60–67. doi: 10.1016/j.pbi.2019.05.002

Grabherr, M. G., Haas, B. J., Yassour, M., and Thompson, D. A., Amit, I. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol.* 29, 644–652. doi: 10.1038/nbt.1883

Hamill, S., Smith, M., and Dodd, W. (1992). *In vitro* induction of banana autotetraploids by colchicine treatment of micropropagated diploids. *Austral. J. Bot.* 40, 887–896. doi: 10.1071/BT9920887

Hapsari, L., and Lestari, D. A. (2016). Fruit characteristic and nutrient values of four Indonesian banana cultivars (*Musa* spp.) at different genomic groups. *J. Agri. Sci.* 38, 303–311. doi: 10.17503/agrivita.v38i3.696

Heslop-Harrison, J. S., and Schwarzacher, T. (2007). Domestication, genomics and the future for banana. *Ann. Bot.* 100, 1073–1084. doi: 10.1093/aob/mcm191

Hoagland, D. R., and Arnon, D. I. (1950). *The Water-Culture Method for Growing Plants Without Soil*. California: Agricultural Experiment Station, 1–32.

Humplík, J. F., Bergougnoux, V., and Van, V. E. (2017). To stimulate or inhibit? That is the question for the function of abscisic acid. *Trends Plant Sci.* 22, 830–841. doi: 10.1016/j.tplants.2017.07.009

Jenny, C., Tomekpe, K., Bakry, F., and Escalant, J. (2002). "Conventional breeding of bananas" in *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook*, ed L. Jacome (Rome: International Plant Genetic Resources Institute, Italy), 199.

Jong, M. D., Wolters-Arts, M., Feron, R., Mariani, C., and Vriezen, W. H. (2009). The Solanum lycopersicum auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. *Plant J.* 57, 160–170. doi: 10.1111/j.1365-313X.2008.03671.x

Kadota, M., and Niimi, Y. (2002). *In vitro* induction of tetraploid plants from a diploid Japanese pear cultivar (*Pyrus pyrifolia* N. cv. Hosui). *Plant Cell Rep.* 21, 282–286. doi: 10.1007/s00299-002-0509-1

Kanchanapoom, K., and Koarapatchaikul, K. (2012). In vitro induction of tetraploid plants from callus cultures of diploid bananas (Musa acuminata, AA group) 'Kluai Leb Mu Nang'and 'Kluai Sa'. *Euphytica* 183, 111–117. doi: 10.1007/s10681-011-0516-9

Kanehisa, M., Goto, S., Hattori, M., Aoki-Kinoshita, K. F., Itoh, M., Kawashima, S., et al. (2006). From genomics to chemical genomics: new

developments in KEGG. *Nucl Acids Res.* 34, D354–D357. doi: 10.1093/nar/gkj102

Kaur, S., Zhang, X., Mohan, A., Vikram, P., Singh, S., Zhang, Z. W., et al. (2017). Genome-wide association study reveals novel genes associated with culm cellulose content in bread wheat (*Triticum aestivum*, L.). *Front. Plant Sci.* 8, 1913. doi: 10.3389/fpls.2017.01913

Kitahata, N., Saito, S., Miyazawa, Y., Umezawa, T., Shimada, Y., Min, Y. K., et al. (2005). Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors. *Bioorgan. Med. Chem.* 13, 4491–4498. doi: 10.1016/j.bmc.2005.04.036

Kovi, M. R., Zhang, Y., Yu, S., Yang, G. Y., Yan, W. H., and Xing, Y. Z. (2011). Candidacy of a chitin-inducible gibberellin-responsive gene for a major locus affecting plant height in rice that is closely linked to Green Revolution gene sd1. *Theor. Appl. Genet.* 123, 705–714. doi: 10.1007/s00122-011-1620-x

Liang, W., Ouyang, S., Shaw, N., Joachimiak, A., Zhang, R., and Liu, Z. J. (2011). Conversion of D-ribulose 5-phosphate to D-xylulose 5-phosphate: new insights from structural and biochemical studies on human RPE. *FASEB J.* 25, 497–504. doi: 10.1096/fj.10-171207

Liu, Y. X., Zhou, Z. X., Tang, F. L., He, Y. D., Cao, H. X., Wang, B. Z., et al. (2013). Quantitative analysis of relationship between brazilian banana's leaf Area and its morphological characteristics. *Chin. J. Tropic Crops* 34, 1641–1645. doi: 10.3969/j.issn.1000-2561.2013.09.003

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\ \Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Mu, H. Z., Liu, Z. J., Lin, L., Li, H. Y., Jiang, J., and Liu, G. F. (2012). Transcriptomic analysis of phenotypic changes in birch (*Betula platyphylla*) autotetraploids. *Int. J. Mol. Sci.* 13, 13012–13029. doi: 10.3390/ijms131013012

Murase, K., Hlirano, Y., Sun, T. P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* 456, 459–463. doi: 10.1038/nature07519

Nassar, N. M. (2006). Chromosome doubling induces apomixis in a cassava×Manihot anomala hybrid. *Hereditas* 143, 246–248. doi: 10.1111/j.2006.0018-0661.01957.x

Ortiz, R., and Vuylsteke, D. (1995). Effect of the parthenocarpy gene P1 and ploidy on fruit and bunch traits of plantain-banana hybrids. *Heredity* 75, 460–465. doi: 10.1038/hdy.1995.162

Pablo, A., Yann, F., Sergio, S., Manuel, A., María, H., Jos,é, J., et al. (2011). Tetraploidization events by chromosome doubling of nucellar cells are frequent in apomictic citrus and are dependent on genotype and environment. *Ann. Bot.* 108, 37–50. doi: 10.1093/aob/mcr099

Perrier, X., Jenny, C., Bakry, F., Karamura, D., Kitavi, M., Dubois, C., et al. (2019). East African diploid and triploid bananas: a genetic complex transported from South-East Asia. *Ann. Bot.* 123, 19–36. doi: 10.1093/aob/mcy156

Pio, L. S., Pasqual, M., E., Silva, S. D. O., Rocha, H. S., Magalhaes, H. M., et al. (2014). Inducing and identifying artificially-induced polyploidy in bananas. *African J. Biotechnol.* 13, 3748–3758. doi: 10.5897/AJB2014.14009

Poerba, Y., Martanti, D., and Handayani, T. (2019). Morphology and reproductive function of induced autotetraploid banana by chromosome doubling. *SABRAO J Breed Genet.* 51, 175–190.

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. *Plant Cell Tissue Organ. Cult.* 64, 185–210. doi: 10.1023/A:1010623203554

Roux, N., Dolezel, J., Swennen, R., and Zapata-Arias, F. J. (2001). Effectiveness of three micropropagation techniques to dissociate cytochimeras in Musa spp. *Plant Cell Tissue Organ. Cult.* 66, 189–197. doi: 10.1023/A:1010624005192

Roux, N., Strosse, H., Toloza, A., and Swennen, R. (2004). *Detecting Ploidy Level Instability of Banana Embryogenic Cell Suspension Cultures by Flow Cytometry, in: Banana Improvement: Cellular Molecular Biology and Induced Mutations.* Enfield: Science Publishers Inc.

Saavedra, X., Modrego, A., Rodríguez, D., González-García, M. P., Sanz, L., and Lorenzo, G. N. (2010). The nuclear interactor PYL8/RCAR3 of fagus sylvatica FsPP2C1 Is a positive regulator of abscisic acid signaling in seeds and stress. *Plant Physiol.* 152, 133–150. doi: 10.1104/pp.109.146381

Sanford, J. (1983). *Ploidy Manipulations in Methods in Fruit Breeding.* West Lafayette, IN: Purdue University Press, 100–123.

Sato, A., and Yamamoto, K. T. (2008). What's the physiological role of domain II-less Aux/IAA proteins? *Plant Signal. Behav.* 3, 496–497. doi: 10.4161/psb.3.7.5994

Sattler, M. C., Carvalho, C. R., and Clarindo, W. R. (2016). The polyploidy and its key role in plant breeding. *Planta* 243, 281–296. doi: 10.1007/s00425-015-2450-x

Sharwood, R. E. (2017). Engineering chloroplasts to improve Rubisco catalysis: prospects for translating improvements into food and fiber crops. *New Phytol.* 213, 494–510. doi: 10.1111/nph.14351

Silva, S. D. O., Junior, M. T. S., Alves, É. J., Silveira, J. R. S., and Lima, M. B. (2001). Banana breeding program at Embrapa. *Crop Breed Appl Biotechnol.* 1, 399–436. doi: 10.13082/1984-7033.v01n04a09

Ssebuliba, R., Magambo, M., Talengera, D., Makumbi, D., Tenkouano, A., Rubaihayo, P., et al. (2006). Biological factors affecting seed production in East African Highland bananas. *J. Crop Improv.* 16, 67–79. doi: 10.1300/J411v16n01_05

Sun, Q., Sun, H., Li, L., and Bell, R. L. (2009). In vitro colchicine-induced polyploid plantlet production and regeneration from leaf explants of the diploid pear (Pyrus communis L.) cultivar,'Fertility'. *J. Horticult Sci. Biotechnol.* 84, 548–552. doi: 10.1080/14620316.2009.11512564

Swamy, R. D., Rao, N. S., and Chacko, E. K. (1983). Tissue-culture propagation of banana. *Sci Horticult.* 18, 247–252. doi: 10.1016/0304-4238(83)90028-6

Syvertsen, J., Lee, L., and Grosser, J. (2000). Limitations on growth and net gas exchange of diploid and tetraploid Citrus rootstock cultivars grown at elevated CO_2. *J Am Soc Horticult Sci.* 125: 228–234. doi: 10.21273/JASHS.125.2.228

Tatusov, R.L., Fedorova, N.D., Jackson, J.D., Jacobs, A., Kiryutin, B., Koonin, E., et al. (2003). The COG database: an updated version includes eukaryotes. *BMC Bioinform.* 4, 41. doi: 10.1186/1471-2105-4-41

Tongsad, P., Laipasu, P., Chareonsap, P. P., and Poeaim, A. (2020). The effects of abscisic acid and sorbitol on the slow growth in vitro preservation of Mahesak (*Tectona grandis* L.). *Int J. Agri. Technol.* 16, 1277–1286.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protocols* 7, 562–578. doi: 10.1038/nprot.2012.016

Ul Haq, S., Khan, A., Ali, M., Khattak, A. M., Gai, W. X., Zhang, H. X., et al. (2019). Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. *Int. J. Mol. Sci.* 20, E5321. doi: 10.3390/ijms20215321

Vakili, N. G. (1967). The experimental formation of polyploidy and its effect in the genus Musa. *Am. J. Bot.* 54, 24–36. doi: 10.1002/j.1537-2197.1967.tb06889.x

Wang, S., Tu, H., Wan, J., Chen, W., Liu, X., Luo, J., et al. (2016). Spatio-temporal distribution and natural variation of metabolites in citrus fruits. *Food Chem.* 199, 8–17. doi: 10.1016/j.foodchem.2015.11.113

Wang, Y. Q., He, F., Tan, X. F., Su, J. K., Xing, W. Y., and Bin-Linga, A. (2011). EST-based analysis of gene expression of photosynthesis in Ginkgo biloba mature leaves. *J. Central South Univ. Forest Technol.* 31, 54–59. doi: 10.14067/j.cnki.1673-923x.2011.03.024

Want, E. J., Wilson, I. D., Gika, H., Theodoridis, G., Plumb, R. S., Shockcor, J., et al. (2010). Global metabolic profiling procedures for urine using UPLC–MS. *Nat. Protocols* 5, 1005–1018. doi: 10.1038/nprot.2010.50

Whitney, S. M., and Andrews, T. J. (2001). The gene for the ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit relocated to the plastid genome of tobacco directs the synthesis of small subunits that assemble into Rubisco. *Plant Cell.* 13, 193–205. doi: 10.1105/tpc.13.1.193

Wu, R. M., Walton, E. F., Richardson, A. C., Wood, M., Hellens, R. P., and Varkonyi-Gasic, E. (2012). Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. *J. Exp. Bot.* 63, 797–807. doi: 10.1093/jxb/err304

Xu, M., Zhu, L., Shou, H., and Wu, P. (2005). A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant Cell Physiol.* 46, 1674–1681. doi: 10.1093/pcp/pci183

Xue, H., Zhang, F., Zhang, Z. H., Fu, J. F., Wang, F., Zhang, B., et al. (2015). Differences in salt tolerance between diploid and autotetraploid apple seedlings exposed to salt stress. *Sci. Horticult.* 190, 24–30. doi: 10.1016/j.scienta.2015.04.009

Yasushi, K., Hiroyoshi, M., and Katsura, I. (2003). Phosphoenolpyruvate carboxylase: three-dimensional structure and molecular mechanisms. *Arch. Biochem. Biophys.* 414, 170–179. doi: 10.1016/S0003-9861(03)00170-X

Zhai, R., Wang, Z. G., Yang, C. Q., Wang, K. L., Espley, R., Liu, J. L., et al. (2019). *PbGA2ox8* induces vascular-related anthocyanin accumulation and contributes to red stripe formation on pear fruit. *Hortic. Res.* 6, 137. doi: 10.1038/s41438-019-0220-9

Zhang, F., Xue, H., Lu, X., Zhang, B., Wang, F., Ma, Y., et al. (2015). Autotetraploidization enhances drought stress tolerance in two apple cultivars. *Trees* 29, 1773–1780. doi: 10.1007/s00468-015-1258-4

Zhang, J., Liu, Y., Xia, E. H., Yao, Q. Y., Liu, X. D., and Gao, L. Z. (2015). Autotetraploid rice methylome analysis reveals methylation variation of transposable elements and their effects on gene expression. *Proc. Natl. Acad. Sci.* 112, E7022–E7029. 10.1073/pnas.1515170112 doi: 10.1073/pnas.1515170112

Zuo, C. W., Deng, G. M., Li, B., Huo, H. Q., Li, C. Y., Hu, C. H., et al. (2018). Germplasm screening of *Musa* spp. for resistance to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4). *Eur. J. Plant Pathol.* 151, 723–734. doi: 10.1007/s10658-017-1406-3