Incomplete genome doubling enables to consistently enhance plant growth for maximum biomass production by altering multiple transcript co-expression networks in potato

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Abstract
Key message Cytochimera potato plants, which mixed with diploid and tetraploid cells, could cause the highest and significantly increased biomass yield than the polyploid and diploid potato plants.

Abstract Polyploidization is an important approach in crop breeding for agronomic trait improvement, especially for biomass production. Cytochimera contains two or more mixed cells with different levels of ploidy, which is considered a failure in whole genome duplication. Using colchicine treatment with diploid (Dip) potato (Solanum chacoense) plantlets, this study generated tetraploid (Tet) and cytochimera (Cyt) lines, which, respectively, contained complete and partial cells with genome duplication. Compared to the Dip potato, we observed remarkably enhanced plant growth and biomass yields in Tet and Cyt lines. Notably, the Cyt potato straw, which was generated from incomplete genome doubling, was of significantly higher biomass yield than that of the Tet with a distinctively altered cell wall composition. Meanwhile, we observed that one layer of the tetraploid cells (about 30%) in Cyt plants was sufficient to trigger a gene expression pattern similar to that of Tet, suggesting that the biomass dominance of Cyt may be related to the proportion of different ploidy cells. Further genome-wide analyses of co-expression networks indicated that down-regulation (against Dip) of spliceosomal-related transcripts might lead to differential alternative splicing for specifically improved agronomic traits such as plant growth, biomass yield, and lignocellulose composition in Tet and Cyt plants. In addition, this work examined that the genome of Cyt line was relatively stable after years of asexual reproduction. Hence, this study has demonstrated that incomplete genome doubling is a promising strategy to maximize biomass production in potatoes and beyond.

Introduction
Polyploidization is a basic feature of plant evolution and diversification via genome duplication (Wendel 2000), and polyploid plants often exhibit increment of organs, buffering of deleterious mutations, increased heterozygosity, and growth vigor (Cai et al. 2007; Corneillie et al. 2019; Shelley et al. 2018). Regarding such features, polyploidization thus provides an effective non-transgenic breeding approach to increase grain and biomass yields in crops (Cai et al. 2007; Corneillie et al. 2019; Shelley et al. 2018). For instance, the application of polyploidization has shown remarkably increased biomass in bioenergy Miscanthus (Chae et al. 2013) and willow crops (Serapiglia et al. 2015). Notably, the tetraploid Arabidopsis plants have almost doubled biomass yields than those of the diploid ones, but the hexaploid and octoploid Arabidopsis plants do not show any significantly higher biomass yields than those of the diploid ones,
raising a question if the maximum biomass yield could only be achieved in the tetraploid crops (Corneillie et al. 2019). In addition, the polyploidization causes a constant reduction of lignin content, leading to significantly improved lignocellulose recalcitrance, which is beneficial to bioethanol production (Corneillie et al. 2019; Madadi et al. 2021).

In principle, there are two different polyploid types in plants: allopolyploids derived from interspecific hybridization followed by genome doubling, and autopolyploids originated from a single species (Comai 2005; Otto 2007). Allopolyploidy can be confounded by the entanglement of both whole genome duplication (WGD) and hybridization (Zhang et al. 2015). For instance, investigations in maize indicated that gene expression is altered less by WGD than by hybridization (Auger et al. 2005). In contrast, synthesized autopolyploids represent ideal materials to investigate the consequences of genome duplication, especially to ascertain whether there are molecular and functional rules about increasing biomass following polyploidization events (Fasano et al. 2016). However, the consequences of chromosome doubling have been widely studied in allopolyploids, whereas more limited data are available for autopolyploid plants (Parisod et al. 2010). Extensive studies of genetic and epigenetic changes associated with polyploidization have been demonstrated that chromosomal rearrangements, sterility, aneuploidy, gene-expression aberrations, and DNA methylation changes are common in newly induced polyploids (neopolyploids) (Adams and Wendel 2005; Comai 2005; Ha et al. 2009; Leitch and Leitch 2008; Song et al. 1995; Xiong et al. 2011). However, few investigations are explored to examine the associations between growth vigor and gene expression in neopolyploids response to WGD.

A plant cytochimera is defined as an individual composed of two or more groups of cells that are genetically different for ploidy. In the case of cultured tissues, which are often induced spontaneously by somaclonal variations or artificially by mutagen treatment that lead to “cytochimera” (Frank and Chitwood 2016). For instance, polyploids can be induced by colchicine treatment of lateral buds, while cytochimeras may arise in the case as a result of incomplete penetration of colchicine (Hashimoto-Freitas and Nassar 2013). Sometimes cytochimera presence is more valuable than that of the normal type, especially in an ornamental plant, but in most cases, it is considered to negatively affect breeding performance (Fujishige et al. 1996). Since the characteristics of a mixture of different ploidy cells, unstable heredity, and usually without desirable traits, cytochimeras are deliberately avoided and abandoned during the plant polyploid breeding.

Because there is a variety of wild or domesticated diploid, tetraploid and hexaploid materials (Carputo et al. 2003), its special tolerance on ploidy manipulation (Ortiz and Ehlenfeldt 1992), and the strict tetraploid inheritance of cultivated potato (Carputo et al. 2003), the potato is a good model plant for autopolyploid research. Cultivated autopolyploid potatoes are often more vigorous and larger in size than their diploid relatives, indicating a fitness advantage associated with higher ploidy (Stupar et al. 2007). However, the diploid Solanum phureja is most vigorous and generates the greatest biomass than its derived monoploid and synthetic autopolyploid (Stupar et al. 2007). Synthetic autotetraploid of S. commersonii and S. bulbocastanum have no growth vigor compared with their diploid progenitors (Fasano et al. 2016). In this study, therefore, we generated tetraploid (Tet) and cytochimera (Cyt) potato lines and observed consistently enhanced plant growth in both polyploidy lines. We then determined distinctively altered lignocellulose composition in the Cyt straw. Finally, this study attempted to sort out why the Cyt potato line was of the highest biomass yield by performing a genome-wide analysis of transcript co-expression networks, providing a promising strategy to maximize biomass yields in crop breeding.

### Materials and methods

#### Plant growth and collection

Seeds of wild diploid potato (Solanum chacoense, PI 500,042) were obtained from the US potato gene bank. Sterilized seeds were germinated to form tissue culture seedlings on Murashige and Skoog (MS) basal medium containing 3% sucrose and 0.6% agar without exogenous growth regulators (pH 5.8). One tissue culture seedling was selected as the original diploid line for later experiments and was propagated to generate diploid plants by cutting nodes with an axillary bud and incubated in vitro under the fixed conditions (16-h light/8-h dark, 24 °C, 40 μmol m^-2s^-1). The tetraploid (Tet-1, 2) and cytochimera (Cyt-1, 2) lines were generated by treating the diploid (Dip) plants with 0.05% colchicine. The obtained tetraploid (Tet-1, 2) and cytochimera (Cyt-1, 2) lines were maintained and vegetatively propagated to generate multiple plantlets by cutting node and tissue culture. To produce mini-tubers, fifty plantlets from each line were shifted from tissue culture to greenhouse pots. Fifty minitubers from each line were grown in the field with a plant spacing of 50 cm and row spacing of 1 m for ploidy identification and phenotype determination. The harvested tubers were then grown in the field in the following year to collect stems and leaves for paraffin section, cell wall analysis, and RNA-seq.

#### Ploidy plant identification

A small section of node (approximately 1 cm length) with an axillary bud from propagated diploid was cultured onto...
the MS medium supplied with various concentrations of colchicine (0.025%, 0.05%, 0.075%, and 0.1%) for 10 days, and then transferred to the medium without colchicine. The 0.05% colchicine was finally selected for polyploidy induction experiments. Then, a small part of the leaf (2 mm²) of colchicine-treated plantlets was fixed in Carnoy’s fluid and digested with 1% pectolyase Y-23 and 2% cellulase R-10, and the ploidy was identified using fluorescence in situ hybridization (FISH) method with 5S rDNA as probes on the somatic nucleus. Because the diploid has one pair of 5S rDNA locus located on chromosome one, two signals of 5S rDNA on the somatic nucleus, while tetraploid by doubling the genome has four hybrid signals (Dong et al. 2000), but cytochimera cells contain two or four signals. To detect the percentage of tetraploid cells in the cytochimera plants from different lines, somatic nuclei from various organs of root tips, a section of tender young stem, a slice of young leaf, and small flower buds at the flowering stage were prepared for 5S rDNA FISH. To test the genetic stability of Cyt lines, the percentage of tetraploid cells was analyzed by 5S rDNA FISH using leaves of cytochimera (Cyt) potato plantlets from three generations (G1, G5, G30). Three biological replicates from each organ were conducted for identifying the ploidy level. Each replicate was tested by 5S rDNA FISH and about 200 cells from each replicate were photographed. Totally, about 600 cells from three replicates were accounted and the percentage of tetraploid cells was calculated. The significant difference in the percentage between different organs or generations was examined by the Chi-square test at 0.05 level using R-3.6.3. To check the ploidy level of the field-grown plants, small flower buds were collected as experimental samples, and multi-color FISH using 5S rDNA, 45S rDNA, and chromosomal-specific BAC clone BAC079E02 as probes were performed as previously described (Xiong et al. 2011, 2021).

**Phenotype measurement**

The field-grown plants of each line were randomly selected to determine the height (cm), number of main stems, leaf coverage area (cm²), pollen activity (%), yield of tubers (g), fresh and dry weights (g) at the young, flowering, and mature stages using the standard methods. The fresh straws were weighed and then dried at 50 °C for 3–5 days as dry matter. Three biological replications were carried out for each line, and statistical significance was calculated with one-way ANOVA analysis followed by LSD test at 0.05 level using R-3.6.3.

**Cell wall polymer extraction and assay**

Plant cell wall fractionation was performed as previously described (Peng et al. 2000). The well-mixed biomass powders were extracted with potassium phosphate buffer (pH 7.0), chloroform–methanol (1:1, v/v), DMSO–water (9:1, v/v) and ammonium oxalate 0.5% (w/v) to obtain lipid, starch, and pectin, respectively. The remaining crude cell walls were extracted with 4 M KOH containing 1.0 mg/mL sodium borohydride for 1 h at 25 °C, and the combined supernatants were used as the hemicelluloses fraction. The hexose of the remaining final pellet was determined as cellulose level by treatment with H₂SO₄ (67%, v/v) for 1 h at 25 °C using the anthrone/H₂SO₄ method (Fry et al. 1988). Total hemicellulose levels were calculated by measuring all hexoses and pentoses in the hemicellulose fraction and pentoses in the final pellet using the orcinol/HCl method (Dochse et al. 1962). Total pectin was calculated by measuring hexoses, pentoses, and uronic acids of the pectin fraction. Total lignin was measured according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al. 2008). All experimental analyses were performed in independent triplicates, and statistical analyses were completed with one-way ANOVA calculation followed by LSD test at 0.05 level using R-3.6.3.

**RNA sequencing**

The field-grown plants from Dip, Tet-1, and Cyt-1 lines were collected for RNA sequencing. Three independent plants propagated from each line (Dip, Tet-1, or Cyt-1) were used for biological replicates. The ploidy levels of Dip and Tet-1 and the proportion of tetraploid cells in Cyt-1 plants were double-checked by 5S rDNA FISH to ensure the homogeneity of replicates. The plant growth and tissue sampling were consistently conducted across ploidy genotypes and biological replicates. The samples of tender stems and young leaves were collected at the same growth stage and position for each plant, and a total of 18 samples were obtained. Total RNA was extracted from fresh stems and leaves using an RNeasy Plant Mini Kit (Qiagen). The individual cDNA was synthesized using the random hexamers as primers and mRNA templates. The resultant products were connected with adapters, followed by size selection and PCR amplification. Then, the constructed library was analyzed by an Illumina HiSeq X-Ten sequencing platform (Novogene, Beijing, China).

**Co-expression network analysis and functional enrichment**

The potato reference genome and annotation files were downloaded from Ensembl Plants, and BioMart converted its different ID (http://plants.ensembl.org/index.html). Adapter sequences and low-quality reads were initially filtered by fastp with default settings (Chen et al. 2018), and the remaining ones were called clean reads. Then, FPKM (fragments...
per kilobase of exon model per million mapped fragments) expression matrix was obtained through the transcript-level analysis with HISAT, StringTie, and Ballgown (Pertea et al. 2016).

For transcripts with average FPKM value $\geq 2$ of all samples, logarithms-transformed values ($\log_2$(FPKM + 1)) were used to generate the co-expression networks by the WGCNA package in R-3.6.3 (Langfelder and Horvath 2008). The samples were clustered by the hclust function with the “average” parameter in R-3.6.3 to detect whether there were abnormal samples. The modules were then obtained using the automatic network construction function blockwiseModules with default settings, except that TOMType was signed, the power, minModuleSize, and mergeCutHeight were 27, 30, and 0.25, respectively. To visualize the expression profiles of the modules, the eigengene (first principal component) for each module was plotted using ggplot2 in R-3.6.3. To relate the trait measurements with the network, the module eigengenes (MEs) were correlated with the trait data. To identify hub transcripts within the modules, the module membership (MM) for each transcript was calculated based on the Pearson correlation between the expression level and the ME. Transcripts within the module with the highest MM are highly connected within that module. To associate individual transcripts with the traits, we calculated gene significance (GS) as described in the WGCNA package. Then, networks were visualized using Cytoscape 3.7.1 (https://cytoscape.org/).

Both annotations of GO (http://geneontology.org/) and KEGG (https://www.kegg.jp/) were carried out to identify functional transcripts. Subsequently, functional enrichment of GO and KEGG pathway were analyzed using clusterProfiler package in R-3.6.3, respectively (Yu et al. 2012). The terms were considered to be significantly enriched if FDR (false discovery rate) $\leq 0.05$.

Results

Distinctively increased biomass yields in polyploid potato straws

Based on a colchicine treatment with the diploid (Dip) potato plantlets, this study established efficient methods to screen and identify polyploid plants by counting the fluorescent signals from 5S rDNA probes on the somatic nucleus. Because two characteristic signals of 5S rDNA on the somatic nucleus accountable for Dip plants, we observed four hybrid signals in root, stem, leaf, and flower organs of the tetraploid (Tet) plant at the flowering stage (Fig. S1), indicating that the Tet plants were of doubled genomes. Notably, this study also identified the cytochimera (Cyt) potato plants that mixed diploid and tetraploid cells (Fig. S1). In detail, two Cyt lines (Cyt-1, Cyt-2) were examined with 24.2–41.8% tetraploid cells (% total cells) in four major organs of potato plants (Table S1), indicating that the Cyt plants were partial duplicated and contained mostly diploid cells. In addition, the ploidy level and the chromosome number of Dip, Tet-1, and Cyt-1 from field-grown plants were further verified by FISH using three different chromosomal specific markers located separately on chromosomes 1, 2, and 3 (Fig. 1a). We found that the Tet-1 plant had a complete 48 chromosomes including two pairs of chromosomes 1, 2, and 3 each, while the Cyt-1 plant contained both diploid cells with parental Dip karyotype and tetraploid cells with Tet-1 like karyotype (Fig. 1a).

Among the polyploid potato lines generated in this study, we selected two elite lines of each polyploid type (Tet-1, 2; Cyt-1, 2) to compare their distinct plant growth and biomass yields with the Dip plants (Fig. 1b). In the two-year field experiments, two Tet lines exhibited much-enhanced plant growth and development than those of the Dip plants including main stems (Table S2) and leaf coverage area (Table S3), consistent with the previous findings that genome duplication could cause a remarkable increase of plant growth in tetraploid plants (Corneillie et al. 2019). Hence, compared to the Dip plants, the Tet plants were of consistently higher biomass yields in three major growth stages, with the biomass up to 6.6-fold for fresh weight (Fig. 1c; Table S4) and 4.4-fold for dry matter (Fig. 1d; Table S4) at the mature stage. Notably, this study examined that the Cyt plants could consistently show more enhanced plant growth and development than those of the Dip plants, with the significantly increased fresh (Fig. 1c; Table S4) and dry biomass yields (Fig. 1d; Table S4) by 9.1-fold and 5.4-fold at the mature stage. Thus, despite the tetraploid plants were of the highest biomass yields among the polyploid plants examined in the previous studies (Corneillie et al. 2019; Sattler et al. 2016), this study indicates that the cytochimera plants should be optimal to produce maximum biomass in potato plants. We also examined significantly reduced tuber yields in the Cyt and Tet plants relative to the Dip plants (Table S4). The data thus indicated that the mixture of diploid and tetraploid cells in Cyt potato crop may be specific for increasing biomass rather than for tuber production.

Enlarged stem and leaf organs but reduced pollen activity in polyploid potato plants

Concerning the biomass yields increased in the Tet and Cyt plants, we observed stem and leaf dissections. Compared to the Dip plants, both Tet and Cyt lines exhibited a significant enlargement in different types of stem cells including the sizes of the epidermis, cortex, and xylem cells (Fig. 2a). Furthermore, the Cyt lines showed either significantly increased stem radius and cortex sizes or...
reduced xylem sizes than those of the Tet plants, but Tet and Cyt plants had a similar epidermis dimension (Fig. 2c). Meanwhile, the Tet and Cyt plants also exhibited distinctively enlarged leaf thickness than the Dip plants, in particular on the spongy tissues (Fig. 2b, d). The data thus revealed that the Cyt plants were of the most enhanced stem and leaf growth, which was accountable for its highest biomass yield examined above. However, this study found significantly reduced pollen activity in the Cyt and Tet plants, compared to the Dip plants (Table S4).

**Relatively stable genotype and phenotype of Cyt by asexual reproduction**

Since the cytochimera plant containing two different ploidy cells, to understand whether the cytochimera potato is stable, the genotype status and phenotype stability of Cyt plants were analyzed by using materials from different generations and two different asexual propagations. Percentages of tetraploid cells in the different organs including root, stem, leaf, and flower of Cyt plants at the
flowering stage were calculated using 5S rDNA FISH. We found that there is no significant difference in the percentages of tetraploid cells among the four organs at 0.05 level (Table S1). Meanwhile, this study undertook five years to consistently generate more than 30 generations of nodal culture propagations in vitro using the nodal stem segments from original Cyt plants and examined that two Cyt lines of the 30th generation could maintain 30.2–33.5% tetraploid cells (Table S5). Therefore, there was no significant difference in the proportion of tetraploid cells among generations by Chi-square test, suggesting that the stage of incomplete duplication of Dip genomes could steadily maintain in the Cyt potato plant (Table S5). In addition, Cyt plants propagated by tubers in the field from three continuous generations also showed that the frequencies of tetraploid cells were relatively stable, which kept around one-third of the total cells (Table S1).

The phenotypes of Cyt plants from different generations and two different asexual propagations were also surveyed. We found that the phenotype including plant growth and biomass yields of the two elite lines of cytochimera line (Cyt-1, 2) was also very stable. For example, in the two-year field experiments, the two Cyt lines exhibited the most enhanced plant growth and biomass yields than those of the Dip and Tet plants (Fig. 1b, c, d).

![Cross-section observation of stem and leaf in a Dip, Tet-1, and Cyt-1 potato samples at the flowering stage. Images of the stem (a) and leaf (b) dissections under microscopy. ep, epidermis; co, cortex; ph, phloem; xy, xylem; pt, palisade tissue; st, spongy tissue.](image)

![Measurements of stem (c) and leaf (d) dissections. Multiple comparisons were performed by LSD test at 0.05 significant level and the differences among samples were marked with different letters. Data as means ± SD (n = 3).](image)
Altered cell wall composition in polyploid potato straws

Because plant cell walls are the major components of straw biomass, this study determined wall polymers levels in the polyploid potato straws (Table 1). We calculated wall polymers levels against dry matter (% DM), and both cellulose and lignin were significantly reduced in the Tet and Cyt plants relative to the Dip plants, whereas the hemicellulose and pectin were increased (Table 1). In addition, the Cyt plants showed significantly different contents of four wall polymers from the Tet plants, suggesting that Tet and Cyt plants have distinct cell wall compositions. Hence, we assumed that the raised non-cellulosic polysaccharides (hemicellulose, pectin) may compensate for the decreased cellulose and lignin to maintain plant mechanical strength and biomass yield in the Cyt straw (Madadi et al. 2021).

Characteristic co-expression networks among ploidy potato plants

To analyze why Cyt plants have the highest biomass and significantly altered cell wall polymers, RNA sequencing was performed on three lines with different ploidy. In general, the obtained sequencing data were of good quality at 150 G in total, and the mapping rates with the reference genome were varied around 80% (Table S6). From the 18 samples examined, the transcripts with a mean FPKM < 2 were filtered and a total of 14,584 transcripts were thus retained for the following co-expression network analysis. Because the samples exhibited a similar curve of FPKM distribution (Fig. 3a), we justified that they were of perfect homogeneity. Meanwhile, this study generated the cluster tree with two classic branches corresponding for stem and leaf tissues, whereas both Tet and Cyt plants showed significantly different contents of four cell wall polymers from the Tet plants, suggesting that Tet and Cyt and Cyt had outliers (sam08 and sam13) in the branches of Cyt and Tet, respectively, suggesting that the genome doubling should cause a similar expression pattern between the Tet and Cyt samples. Based on the means of traits (Table S4) described above and in previous research (Madadi et al. 2021), this study generated a heatmap (Fig. 3b) and the Cyt samples were superior to other samples in the most traits examined, consistent with the enhanced plant growth and biomass yield in Cyt plants. Then, the co-expression network was constructed to obtain nine distinct functional modules, except the grey module with unclassified transcripts (Fig. 3c; Supplementary file1).

By correlating the above nine modules with the traits, it was found that the green module was highly correlated with major agricultural traits about overall plant growth and development (Fig. 4a). Using the green module as an example (Fig. 4b), we examined that transcripts of the module were of the high significance measures with the traits and the high module membership with the module eigengenes (MEs). In addition, the line diagrams of MEs showed an expression trend of overall transcripts for each module (Fig. 4c). Although stem and leaf tissues were of different expression as the fluctuation range of lines, the transcripts within the green module did not show tissue-specific expression, and thus they may mainly respond for polyploidization.

Furthermore, GO and KEGG enrichment analyses were conducted for transcripts of the nine modules (Table 2; Supplementary file1). The turquoise module exhibited a reducing expression trend in the Tet and Cyt plants (Fig. 4c), which may mainly affect plant cell wall formation and assembly, as well as xylan biosynthesis, consistent with the altered cell wall composition (Table 1). In particular, the green module may associate with the spliceosome by participating in pre-mRNA processing. More importantly, this module was highly correlated with many agricultural traits, suggesting that genome doubling can downregulate the expression of spliceosomal associated transcripts and affect transcriptional regulation by changing alternative splicing, thus specifically improving some traits in Tet and Cyt plants (Fig. 4a, c; Table 2). In summary, although the expression trends of Cyt and Tet were similar, such as the turquoise module containing the most transcripts and the green module highly correlated with traits (Fig. 4a). However, the differential expression of transcripts for lipid transport (pink module), brassinosteroid mediated signaling pathway (yellow module), fatty acid biosynthesis (brown module), and steroid biosynthesis (black module) may be related to the higher biomass of Cyt than that of Tet (Fig. 4c; Table 2).

Finally, this study identified a total of 267 transcripts with an average degree of 65 in the co-expression network of the green module (Fig. 4d). Among them, the PGSC0003DMT400023450 (Splicing factor 3a) belongs to the spliceosomal pathway sot03040 (Table 2) and the

Table 1  Plant cell wall compositions (% dry matter) of mature straw in the diploid (Dip), tetraploid (Tet), and cytochimera (Cyt) potato samples

| Samples | Cellulose   | Hemicellulose | Lignin   | Pectin   |
|---------|-------------|---------------|----------|----------|
| Dip     | 19.02 ± 0.54<sup>a</sup> | 9.77 ± 0.21<sup>c</sup> | 19.09 ± 0.87<sup>a</sup> | 6.5 ± 0.13<sup>c</sup> |
| Tet-1   | 16.34 ± 0.42<sup>b</sup> | 11.85 ± 0.18<sup>b</sup> | 15.61 ± 0.65<sup>b</sup> | 7.66 ± 0.21<sup>b</sup> |
| Tet-2   | 16.01 ± 0.20<sup>b</sup> | 11.52 ± 0.43<sup>b</sup> | 16.02 ± 0.30<sup>b</sup> | 7.59 ± 0.25<sup>b</sup> |
| Cyt-1   | 12.20 ± 0.40<sup>c</sup> | 14.30 ± 0.30<sup>c</sup> | 12.90 ± 0.32<sup>c</sup> | 8.87 ± 0.43<sup>c</sup> |
| Cyt-2   | 11.80 ± 0.63<sup>c</sup> | 13.99 ± 0.45<sup>c</sup> | 12.65 ± 0.49<sup>c</sup> | 8.82 ± 0.40<sup>c</sup> |

Multiple comparisons were performed by LSD test at 0.05 significant level and the differences among samples were marked with different letters (a, b, and c). Data as means ± SD (n = 3)
**PGSC0003DMT400023679** is an RNA binding protein with multiple splicing, confirming that the green module is highly associated with the spliceosome. On the other hand, the related transcripts have been examined in other plant species. For instance, *Arabidopsis* homologous gene AT5G06160 (ATO) of PGSC0003DMT400023450 has been examined to implicate pre-spliceosome formation as a novel regulator of gametic cell fate (Moll et al. 2008). In addition, this study also identified a total of 131 transcripts with an average degree of four in the co-expression network of the turquoise module (Fig. 4e). Using the *Arabidopsis* homologous gene AT2G38080 (LAC4) of PGSC0003DMT400028629 (Lac-case-4) as an example, which plays a major role in catalyzing lignin monomer polymerization (Berthet et al. 2011; Zhao et al. 2013), confirming that the turquoise module is highly associated with the cell wall.

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**Discussion**

Polyploidization has been widely implemented in plant breeding for improving grain yield or quality and stress resistance in food crops (Bamakhramah et al. 1984). It is also applied to increase biomass yield and to alter cell wall composition in bioenergy crops (Chae et al. 2013). Recently, it has been examined that the tetraploid *Arabidopsis* plants are of almost double dry biomass yields than those of the diploid ones, but the hexaploid and octoploid plants show significantly reduced biomass yields, compared to the tetraploid plants (Corneillie et al. 2019). As an exception, some polyploid plants could not show any higher grain and biomass yields, as compared to their diploid parents. Hence, the characteristic of polyploidization depends on genomic structure, reproduction patterns, ancestral ploidy level, and the purpose for which the crop has been cultivated (Manzoor et al. 2019). Notably, this study has demonstrated a novel strategy to maximize biomass yield in the potato crop by performing incomplete genome doubling (Fig. 1).

Based on the co-expression network analysis, this study has examined that some important transcripts in genome doubled potatoes (Tet, Cyt) are differently expressed compared to the diploid ones (Dip), which may play a dominating role in enhanced biomass yield. Among the nine modules, the green module associated with the plant growth and biomass yield shows a similar expression pattern between the Tet and Cyt plants (Fig. 4c). Alternative splicing is an
important mechanism to regulate gene expression and produce proteome diversity, which plays an important role in plant growth and development (Huang et al. 2020). Because the green module was highly associated with the spliceosome (Table 2), this study suggests that alternative splicing may play an important role in the novel traits observed in the Tet and Cyt plants, consistent with the previous report (Fasano et al. 2016). On the other hand, despite the tetraploid cell with a low level (~ 30%) in Cyt plants (Table S1; Table S5), these cells are enough to trigger a similar gene expression pattern to the Tet plants. A possible explanation is that sRNA transfers between different ploidy cells of Cyt to change the expression of their target genes (Li et al. 2013). Interestingly, another cytochimera line with 52% tetraploid cells shows the fresh biomass up to 4.1-fold against the Dip (Cyt-1, 2 up to 9.1-fold), probably due to different proportions of tetraploid cells resulting in distinct epigenetic impacts on the cytochimeras (Hashimoto-Freitas and Nasar 2013). Therefore, crop breeding for proportionally differentiated cytochimeras should be a powerful approach for improved agronomic traits.

In this study, we examined significantly reduced pollen activity in the Cyt plants (Fig. 3b; Table S4), but this disadvantage can be avoided by the vegetative propagation of potato tubers and nodal stem segments, indicating that incomplete genome doubling should be still applicable for the most vegetatively propagated plants. However, trait changes by chimerism may be dependent on crop species.
such as a significant increase in root tuber yield rather than the biomass of cytochimera cassava (Hashimoto-Freitas and Nassar 2013). In addition, recent studies have achieved the maximum bioethanol yield of Cyt potato straws compared to other genetically modified and mutant plants (Madadi et al. 2021), suggesting that it may be applicable for perennial bioenergy crops such as Miscanthus and switchgrass.

Although cytochimeras are of desirable traits, the genetic stability should be considered as the key factor for the purpose of commercial variety. The apical meristem of typical

| Table 2 | Analysis of GO and KEGG enrichment |
|---------|------------------------------------|
| Modules | ID | Description | P value | FDR |
| Pink    | GO:0,006,869 | Lipid transport | 0.000195 | 0.008606 |
|         | sot00052     | Galactose metabolism | 0.002788 | 0.013942 |
|         | sot00040     | Pentose and glucurionate interconversions | 0.014177 | 0.035442 |
| Magenta | GO:0,006,098 | Pentose-phosphate shunt | 0.00111 | 0.042948 |
|         | sot00030     | Pentose phosphate pathway | 0.003803 | 0.04306 |
|         | sot00710     | Carbon fixation in photosynthetic organisms | 0.006625 | 0.04306 |
| Turquoise | GO:0,042,254 | Ribosome biogenesis | 1.69E-06 | 0.000328 |
|         | GO:0,034,660 | ncRNA metabolic process | 1.79E-06 | 0.000328 |
|         | GO:0,015,031 | Protein transport | 2.41E-06 | 0.000328 |
|         | GO:0,006,412 | Translation | 4.00E-06 | 0.000328 |
|         | GO:0,009,832 | Plant-type cell wall biogenesis | 1.64E-05 | 0.000921 |
|         | GO:0,045,492 | Xylan biosynthetic process | 3.19E-04 | 0.006981 |
|         | GO:0,009,664 | Plant-type cell wall organization | 3.69E-04 | 0.007507 |
|         | GO:0,048,193 | Golgi vesicle transport | 0.001523 | 0.035748 |
|         | sot03010     | Ribosome | 4.88E-10 | 5.61E-08 |
|         | sot03050     | Proteasome | 4.40E-05 | 0.002532 |
| Yellow  | GO:0,009,742 | Brassinosteroid mediated signaling pathway | 0.000262 | 0.020615 |
|         | GO:0,010,605 | Negative regulation of macromolecule metabolic process | 0.000342 | 0.024274 |
| Red     | GO:0,006,260 | DNA replication | 6.32E-10 | 7.11E-08 |
|         | GO:0,006,412 | Translation | 6.86E-10 | 7.11E-08 |
|         | GO:0,000,819 | Sister chromatid segregation | 0.000287 | 0.016242 |
|         | GO:0,033,044 | Regulation of chromosome organization | 0.000287 | 0.016242 |
|         | sot03010     | Ribosome | 8.79E-13 | 5.62E-11 |
|         | sot03030     | DNA replication | 9.15E-07 | 2.93E-05 |
|         | sot00190     | Oxidative phosphorylation | 6.04E-06 | 0.000129 |
| Blue    | GO:0,019,684 | Photosynthesis, light reaction | 1.17E-08 | 3.21E-06 |
|         | GO:0,009,658 | Chloroplast organization | 2.16E-07 | 4.45E-05 |
|         | GO:0,006,662 | Glycerol ether metabolic process | 3.87E-05 | 0.003981 |
|         | GO:0,051,186 | Cofactor metabolic process | 0.000107 | 0.009727 |
|         | GO:0,046,148 | Pigment biosynthetic process | 0.000132 | 0.010858 |
|         | sot00195     | Photosynthesis | 2.16E-15 | 2.23E-13 |
|         | sot00710     | Carbon fixation in photosynthetic organisms | 1.07E-05 | 0.000372 |
|         | sot00630     | Glyoxylate and dicarboxylate metabolism | 1.29E-05 | 0.000372 |
|         | sot03010     | Ribosome | 1.45E-05 | 0.000372 |
|         | sot01200     | Carbon metabolism | 8.85E-05 | 0.001822 |
|         | sot00860     | Porphyrin and chlorophyll metabolism | 0.000108 | 0.001847 |
|         | sot0906     | Carotenoid biosynthesis | 0.002171 | 0.031951 |
| Brown   | GO:0,006,633 | Fatty acid biosynthetic process | 1.20E-10 | 7.92E-08 |
|         | sot00061     | Fatty acid biosynthesis | 7.74E-05 | 0.007435 |
|         | sot01212     | Fatty acid metabolism | 0.000715 | 0.02746 |
|         | sot00602     | Fatty acid elongation | 0.000858 | 0.02746 |
| Black   | sot00100     | Steroid biosynthesis | 1.02E-08 | 3.96E-07 |
| Green   | sot03040     | Spliceosome | 0.000142 | 0.01195 |
plants has three layers: the outermost layer (L1), the second outer layer (L2), and the inner layer (L3) (Aida et al. 2020; Filippis et al. 2013; Satina et al. 1940; Tilney-Bassett 1986). Structures of the cell layers are maintained during development and have specific patterns of distribution. For example, the L1 forms epidermis of the most organs, the L2 forms the sub-epidermal tissues including the gametes, and the L3 forms the innermost tissues including the pith and endodermis (Aida et al. 2020; Geier 2012). A chimeric plant could change its structure of cell layers in types of chimera depended manner (Aida et al. 2020). Periclinal chimera plants, which have genetically distinct cell layers, but the genotype of the same layer is consistent, which can stably maintain their chimerism by vegetative growth and/or vegetative propagation (Geier 2012; Tilney-Bassett 1986).

It has also suggested that the Cyt potato lines should be the periclinal cyto-chimera containing only one cell layer of genome duplication due to the relative genomic stability of the frequency of tetraploid cells during vegetative propagation. In general, the stability depends on how precisely the patterns of cell divisions in each meristem layer are maintained. Satina and coworkers treated seeds of Datura with colchicine to induce polyploidy and found that the outer two layers, L1 and L2, were maintained as separate lineages since their cells underwent only anticlinal divisions within the meristem, which allows the existence of stable periclinal chimeras (Satina et al. 1940; Szymkowski and Sussex 1992). Further studies might sort out the genome duplication of which layer induces the increase of biomass and how Cyt potato maintains its ploidy stability.

In conclusion, the periclinal cytochimera with incomplete genome doubling is a promising new strategy to increase biomass for food and bioenergy crops.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03976-y.

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**Author contribution statement**
KZ completed the major experiments of potato polyploidy identification and bioinformatic analysis, and wrote the manuscript; NJ, LW, Zhijun X, JW, and JD participated in the potato polyploidy selection and characterization; MM completed the most potato biomass process experiments; Youmei W, ST, and Yanting W participated in the tissue culture, biomass chemical analysis, and experiment discussion; LP supervised biomass process project and finalized the manuscript, and Zhiyong X designed and supervised the potato polyploidy project.

**Data availability**
The RNA sequence is available from the National Centre for Biotechnology Information (NCBI) as BioProject ID PRJNA591474.

**Declarations**

**Conflict of interest**
The authors declare no conflict of interest.

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