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Modified lignocellulose and rich starch for complete saccharification to maximize bioethanol in distinct polyploidy potato straw

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ABSTRACT

Potato is a major food crop with enormous biomass straw, but lignocellulose recalcitrance causes a costly bioethanol conversion. Here, we selected the cytochimera (Cyt) potato samples showing significantly-modified lignocellulose and much increased soluble sugars and starch by 2–4 folds in mature straws. Under two pretreatments (8 min liquid hot water; 5% CaO) at minimized conditions, the potato Cyt straw showed complete enzymatic saccharification. Further performing yeast fermentation with all hexoses released from soluble sugars, starch and lignocellulose in the Cyt straw, this study achieved a maximum bioethanol yield of 24 % (% dry matter), being higher than those of other bioenergy crops as previously reported. Hence, this study has proposed a novel mechanism model on the reduction of major lignocellulose recalcitrance and regulation of carbon assimilation to achieve cost-effective bioethanol production under optimal pretreatments. This work also provides a sustainable strategy for utilization of potato straws with minimum waste release.

1. Introduction

Bioethanol has been evaluated as an excellent additive to gasoline along with the benefit of reduction in net carbon release (Ragauskas et al., 2006; Sun et al., 2016). Despite the implementation of sugar- and starch-derived bioethanol for commercial purposes, its large-scale production conflicts with food security around the world (Ajanovic, 2011). Alternatively, cellulosic ethanol is being considered as a promising solution. However, due to lignocellulose recalcitrance, cellulosic ethanol conversion currently requires an extremely strong biomass pretreatment and costly enzymatic saccharification for final yeast fermentation along with potential formation of secondary wastes (Liu et al., 2018; Raud et al., 2019). In addition, a sustainable supply of biomass resources becomes another important factor for large-scale bioethanol production (Liu et al., 2019). Lignocellulose recalcitrance is basically determined by plant cell wall composition, wall polymer feature, and wall network style (Chaturvedi et al., 2015). Thus, genetic modification of plant cell walls has been attempted to reduce lignocellulose recalcitrance in several bioenergy crops (Mohapatra et al., 2019). Since plant cell walls are of complex structures and diverse biological functions, specific manipulation of one or multiple genes associated with cell wall biosynthesis and assembly could have the limitation for effective improvement of lignocellulose recalcitrance with a potential effect on plant growth and biomass yield (Bhatia et al., 2017). Moreover,

Abbreviations: Dip, diploid; Tet, tetraploid; Cyt, cytochimera; LHW, liquid hot water; CaO, calcium oxide; DP, degree of polymerization; MS, Murashige and Skoog; H, *p*-coumaryl alcohol; G, coniferyl alcohol; S, sinapyl alcohol.

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although various physical and chemical pretreatments can effectively break down lignocellulose recalcitrance by partially removing and disrupting non-cellulosic polymers (Ho et al., 2019; Mesa et al., 2017), it is critical to find out the lignocellulose-modified biomass resource sustainable for cost-effective and mild pretreatments. As a basic characterization of plant evolution *via* genome duplication, polyploidization provides an effective non-transgenic breeding approach for sustainable lignocellulose and grain resources in crops (Cai et al., 2007; Comai, 2005; Corneillie et al., 2019; Jansky & Spooner, 2017; Wendel, 2000). For instance, the application of polyploidization has shown remarkably increased biomass yields in bioenergy *Miscanthus* (Chae et al., 2013) and willow (Serapiglia et al., 2015). Notably, the tetraploid *Arabidopsis* plants (4n) have almost doubled biomass yields than those of the diploid ones (2n), but the hexaploid (6n) and octaploid (8n) *Arabidopsis* plants do not show any significantly higher biomass yields than those of the tetraploid ones. In addition, the polyploidization causes a constant reduction of plant cell wall thickness from 2n to 8n *Arabidopsis* plants, leading to significantly improved lignocellulose recalcitrance (Corneillie et al., 2019). Nevertheless, it remains elusive about how the lignocellulose of ploidy plants can be modified for efficient enzymatic saccharification towards high bioethanol production under mild biomass process technology. Potato is one of the most important food crops for starch-rich tuber production around the world (Fao, 2017). To date, although most potato cultivars are tetraploid plants as food supply, little is yet reported about its lignocellulose utilization for biofuel production. In this study, we selected three different ploidy levels of potato plants and determined the contents of soluble sugars, starch, and lignocellulose in the cytochimera (Cyt) potato straw. Notably, this study examined much improved lignocellulose recalcitrance in the Cyt straw, leading to a complete biomass saccharification under two environment-friendly pretreatments. Furthermore, this study achieved maximum bioethanol yields in the Cyt straw by performing a classic yeast fermentation with all hexoses sources derived from soluble sugars, starch and lignocellulose. Hence, this study proposed a novel hypothetic model to explain why the lignocellulose was completely digested and how the maximum bioethanol production was achieved under minimized pretreatments (Zahoor et al., 2017; Hu et al., 2018; Wu et al., 2019). This provides a sustainable strategy for cost-effective biomass utilization in potato straw and other bioenergy crops.

2. Materials and methods

2.1. Plant growth and biomass collection

Seeds of wild diploid potato (*Solanum chacoense*, PI 500042) were obtained from the US potato gene bank. Sterilized seeds were germinated to form tissue culture seedling on Murashige and Skoog (MS) basal medium containing 3% sucrose (w/v) and 0.6 % agar (w/v) without exogenous growth regulators (pH 5.8). For all the experiments, seedlings were propagated from one original tissue culture plant through cutting node with an axillary bud and incubated *in vitro* under fixed conditions (16-h light/8-h dark, 24 °C, 40 mmol m-2s-1). The tetraploid (Tet-1, 2) and cytochimera (Cyt-1, 2) lines were generated by treating the diploid (Dip) plants with colchicine. The harvested tubers were grown in the field to collect stems and leaves for cell wall characterization of fully senescent plants. For cell wall characterization, the mature stem tissues were first inactivated at 105 °C for 10 min and dried at 60 °C until constant weight. The dried tissues were further ground through a 40-mesh screen and stored in plastic bags until in use. Plants for cell wall staining were cultured in the MS medium, as a small section of stems (2−3 cm) with an axillary bud were transferred into the MS medium under a light-grown condition (16-h light/8-h dark) for 12 weeks at 24 °C.

2.2. Ploidy potato selection and identification

A small section of the node (approximately 1 cm length) with an axillary bud from propagated diploid was cultured onto the MS medium supplied with various concentration 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 to screen and determine the somatic ploidy level. A small part of the leaf (2 mm^2) was fixed in Carnoy's fluid and digested with 1% pectolyase Y-23 and 2% cellulase R-10, and the ploidy was then identified using fluorescence *in situ* hybridization (FISH) method with 45S rDNA and 5S rDNA as probes on the somatic nucleus. Further, the somatic ploidy levels of the seedlings including original diploid/Dip, tetraploid/Tet, and cytochimera/Cyt samples were confirmed by flow cytometry (MoFlo XDP high-speed cell sorter, Beckman). The diploid has one pair of 5S rDNA locus located on chromosome one, and 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 both two or four signals. To determine the chromosome integrality of field-grown plants, multi-color FISH using 5S rDNA, 45S rDNA, and chromosomal specific BAC clone BAC 079E02 as probes was performed as previously described (Xiong et al., 2011).

2.3. Biomass yield measurement

The well-grown potato plants were randomly selected to determine the fresh and dry weights (g) of mature straws, number and yield (g) of tubers. The total 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 significance analysis was performed by the LSD test at $P < 0.05$.

2.4. Immunofluorescence detection of xylan and pectin epitopes

Using a glycan-directed immunolabeling approach, the immune histochemical observations of xylan and pectin were carried out as previously described (Cao et al., 2014). The stems of 12-weeks-old samples (2 cm above basal) were cut into 0.2–1.5 cm pieces and fixed with 4% (w/v) paraformaldehyde, and anhydrous through an ethanol-water gradient (30 %, 50 %, 70 %, 90 %, and 100 %, each for 30 min) and inserted in paraplast plus. Further, the slices were cut using a microtome (RM2265, Leica, Leica Microsystems, Nussloch, Germany) with 8 μm in thickness and placed on lysine-treated slides which were dried at 37 °C for 48 h, de-waxed with xylene, and hydrated through a series of ethanol (100 %-0%). Monoclonal antibodies CCRC-M148 and CCRC-M38 (de-esterified homogalacturonan) were used to detect xylan and pectin, relatively. The sections (8 μm thickness) were incubated with 1% PBS (phosphate buffer saline) contained 3% SMP (skim milk powder, w/v) for 60 min, and then treated with 1% PBS buffer containing 10 μg/mL antibody (CCRC-M148 or CCRC-M38) for another 60 min. The immunolabelled sections were washed three times (5 min each) with 1% PBS and incubated with a 100-fold dilution of anti-mouse-IgG in dark for 2 h. The anti-mouse-IgG antibody was labeled by fluorescein-isothiocyanate (FITC). Immunofluorescence sections were imaged under an Olympus BX-61 microscope.

2.5. Starch and cell wall polymer extraction and assay

Plant cell wall fractionation was performed as previously described by 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 H_2SO_4 (67 %, v/v) for 1 h at 25 °C using the anthrone/H_2SO_4 method (Fry, 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 (Dische et al., 1962). Total pectin was calculated by measuring hexoses, pentoses, and uronic acids of the pectin fraction. Starch was calculated by determining the hexose of the starch fraction. Finally, a UV/VIS Spectrometer (Shanghai MAPADA Instruments Co., Ltd. V-1100D) was used for the absorbance measurement of hexoses (at 660 nm) and pentoses (at 620 nm). All experimental analyses were performed in independent triplicates.

2.6. Soluble sugars analysis

The biomass powder (0.300 g) was transferred into the plastic tube with 6 mL distilled water and shaken at 150 rpm for 2 h at 50 °C. After centrifugation at 3000×*g* for 5 min, the supernatant was collected for analysis of total hexoses and pentose in independent triplicates.

2.7. Total lignin and monomers assay

Total lignin was measured according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008). Three lignin monomers (H, *p*-coumaryl alcohol; G, coniferyl alcohol; S, sinapyl alcohol) were detected by HPLC (1525, Waters Corp., MA, USA) using the nitrobenzene oxidation method as previously described (Alam et al., 2019).

2.8. Monosaccharide determination of hemicellulose

The monosaccharide composition of hemicellulose was detected by GC–MS (Shimadzu GCMS-QP2010 Plus) as previously described (Xu et al., 2012). Trifluoroacetic acid (TFA) and *myo*-inositol were obtained from Aladdin Reagent Inc. *1*-Methylimidazole was purchased from Sigma-Aldrich Co. LLC. Acetic anhydride and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd.

2.9. Detection of degree of polymerization (DP) of cellulose

The relative DP values of β-1,4-glucans were measured by the viscometry method using the crude cellulose samples extracted with the 4 M KOH solution containing sodium borohydride at 1.0 mg/ML followed with the 8% (w/v) $NaClO_2$ as previously described (Deng et al., 2020). All experiments were conducted in independent triplicate.

2.10. Cellulose accessibility detection

Congo Red (CR) stain was performed to evaluate cellulose surface areas as described by Alam et al. (2019) with minor modification by Deng et al. (2020). All measurements were conducted in independent triplicate.

2.11. Fourier transforms infrared (FTIR) spectroscopy scanning

FTIR spectroscopy was carried out to observe chemical linkages in biomass samples as previously described (Alam et al., 2019).

2.12. Biomass pretreatments and enzymatic hydrolysis

The biomass pretreatments and sequential enzymatic hydrolysis were carried out as previously described (Jin et al., 2016; Zahoor et al., 2017) with minor modification on the incubation time and chemical concentration at the lab scale. In the liquid hot water (LHW) pretreatment, the well-mixed biomass powder (0.300 g) was suspended in 2.4 mL distilled water into a Teflon gasket well-sealed in stainless steel bombs at 5% solid loading and heated at 200 °C at 15 rpm for 4, 8, 12, 16, and 32 min. The samples were then shaken under 150 rpm for 2 h at 50 °C. In the Lime (CaO) pretreatment, the well-dried biomass powder (0.300 g) was added into 6 mL CaO, at various concentrations (1%, 2.5 %, 5%, 7.5 %, and 10 %, w/w) with 5% solid loading, and shaken at 150 rpm for 48 h at 50 °C. Biomass sample (0.300 g) with 6 mL distilled water only was shaken at 150 rpm for 2 h at 50 °C as a control.

The pretreated biomass residues were washed with distilled water at least 5 times to reach pH 7.0, and once with 10 mL of enzymatic hydrolysis buffer. The washed residues were incubated with 6 mL (2 g L^{-1}, w/v) of mixed-cellulase (containing cellulases at 10.60 FPU g^{-1} biomass and xylanase at 6.72 U g^{-1} biomass from Imperial Jade Biotechnology Co, Ltd) and shaken at 150 rpm for 48 h at 50 °C. Then, the samples were centrifuged at 3000×*g* for 5 min, and the supernatants were collected to determine the yields of total hexoses and pentoses released from enzymatic hydrolysis. All experiments were carried out in independent triplicates.

2.13. Yeast fermentation and ethanol assay

The yeast fermentation and ethanol measurement were performed at the lab scale as described previously (Deng et al., 2020; Huang et al., 2019) using total hexoses released from enzymatic hydrolysis described above. In brief, the engineered commercial *S. cerevisiae* strain (purchased from Angel yeast Co., Ltd., Yichang, China) was used in all fermentation reactions. The dry yeast powder at 0.5 g L^{-1} was dissolved in 0.2 mM phosphate buffer (pH 4.8) for 30 min. The fermentation was performed in plastic centrifuge tubes at 37 °C and incubated for 48 h. The ethanol yield was estimated using $K_2Cr_2O_7$ method. The distillation of fermentation liquid was performed at 100 °C for 10 min, then 1 mL of produced ethanol sample and 2 mL 5% $K_2Cr_2O_7$ into glass tubes were heated for 10 min in a boiling water bath. After cooling at room temperature, the distilled water was added to make the volume up to 10 mL the absorbance was measured at 600 nm by a UV/VIS Spectrometer. Absolute ethanol (99.9 %) was used as the standard. All analyses were performed in independent triplicates.

3. Results and discussion

3.1. Remarkably raised biomass yields in polyploidy potato straws

Based on a classic chemical (colchicine) treatment with the diploidy (Dip) potato seedlings, this study generated two polyploidy potato samples, the tetraploidy (Tet) plants of doubled genomes and the cytochimera (Cyt) plants that mixed diploid and tetraploid cells. Among the polyploidy potato lines generated in this study, we selected two elite lines of each polyploidy type (Tet-1,2; Cyt-1,2) and then compared their distinct plant growth and biomass yields with the Dip plants (Fig. 1). In the two-year field experiments, two Tet potato lines exhibited much-enhanced plant growth and development than those of the Dip plants (Fig. 1A and B). This was consistent with the previous findings that genome duplications could cause a remarkably increased plant growth in the tetraploidy plants examined (Corneillie et al., 2019). Hence, compared to the Dip plants, the Tet potato plants showed constantly higher biomass yields in mature straws, with the increased rates of dry biomass up to 4-fold (Fig. 1D). Meanwhile, we examined the tuber yields including tuber sizes and numbers, and did not find any significant difference at $P < 0.05$ level between the Cyt and Tet plants in the two-year field experiments (Table 1 and Fig. 1C). Hence, the data suggested that the mixture of diploid and tetraploid cells in Cyt potato crop may be specific for improving lignocellulose quantity and quality rather than for tuber production, which should be an interesting issue to further explore in the future. Furthermore, the Cyt plants could show even more enhanced plant growth with significantly increased dry biomass yield by 29 % at $P < 0.01$ levels (n = 3), compared to the Tet plants. Despite the highest biomass yields in the Tet plants among the polyploidy plants examined in the previous studies (Corneillie et al.,

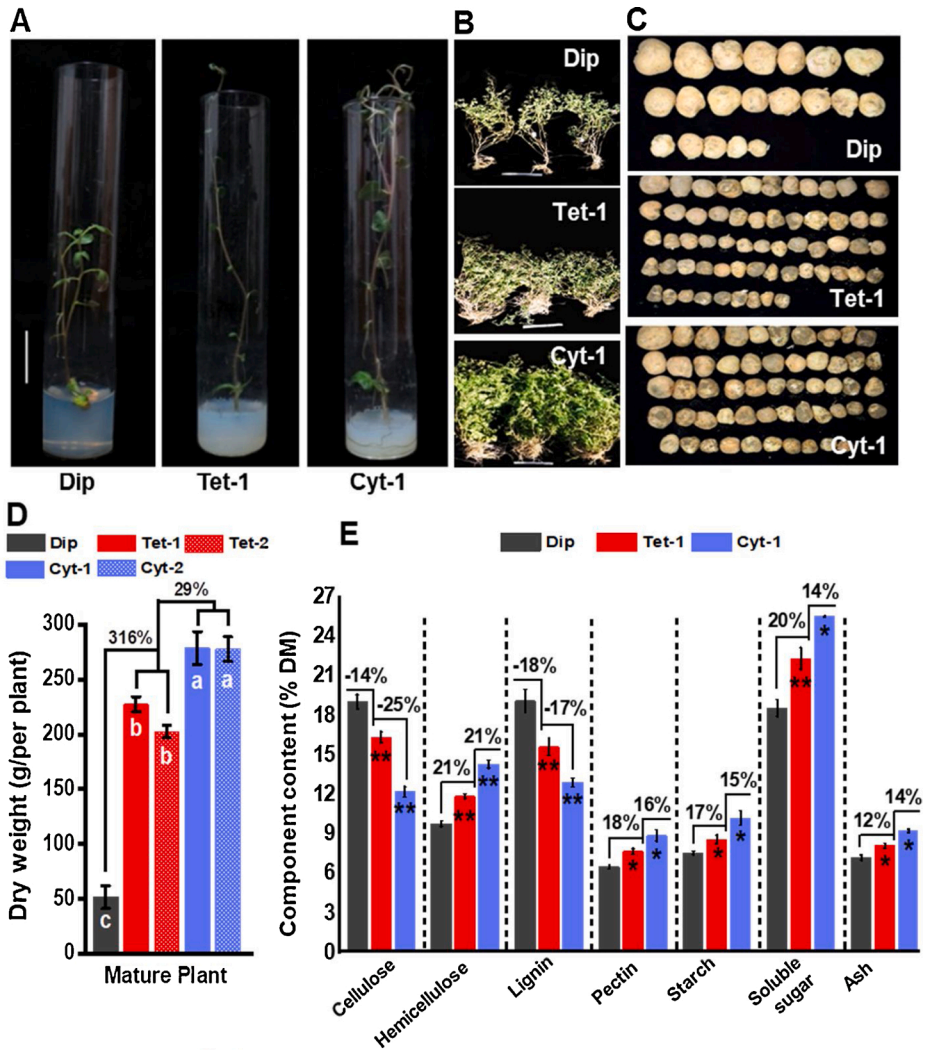

Fig. 1. Characterization of three types of ploidy (Dip, Tet, Cyt) potato plants. (A) Potato seedlings (12-weeks-old) cultured in the MS medium and grown under optimized condition. (B) Mature potato straws collected as biomass samples in the field. (C) Potato tubers from five plants in the two-year field experiments. (D) Dry biomass weight of the potato straws at the mature stage. (E) Biomass component content (% dry matter/DM). Bars indicated means ± SD (n = 3 biological replicates). Student's *t*-test between the two types of potato plants at * $P < 0.05$ and ** $P < 0.01$ with the increased/decreased (-) percentage and data as means ± SD (n = 3).

Table 1
Number and yield (g) of tubers per plant in the Dip, Tet-1, and Cyt-1 potato samples.

Samples	Number of tubers/ plant	Tuber's yield/ plant (g)
Dip	4 ± 0.57^{b}	3.47 ± 0.16^{a}
Tet-1	11 ± 0.81^{a}	2.12 ± 0.14^{b}
Cyt-1	10 ± 1.21^{a}	2.00 ± 0.06^{b}

Significance levels are indicated by different letters according to analysis of variance (LSD test, $P < 0.05$). The data as means ± SD (n = 30).

2019; Sattler et al., 2016), this study suggested that the Cyt plants should be optimal to produce the maximum biomass in potato straw and beyond.

3.2. Distinct increased starch and soluble sugars and altered lignocellulose

Since lignocellulose is the major biomass component of crop straw, this study determined cell wall composition, starch, and soluble sugars levels in the polyploidy potato plants. As a comparison with the Dip, the Tet straw showed remarkably raised wall polymers (cellulose, hemicellulose, lignin, and pectin), starch, soluble sugars, and ash contents (g/per plant) by 2–4 folds (Fig. S1), accounting for its much-increased biomass yield (Fig. 1D). Importantly, the cell wall compositions were distinctively altered among the Dip, Tet, and Cyt lines. To understand this finding, we calculated proportions of wall polymers, starch, and soluble sugars against the dry matter of straw (% dry matter/DM). By comparison, both cellulose and lignin were significantly reduced in the Tet and Cyt relative to the Dip, whereas the non-cellulosic polymers (hemicellulose, pectin), starch, and soluble sugars remained increased at $P < 0.01$ level (Fig. 1E). In terms of those components examined, the Cyt straw remained significantly different from the Tet at $P < 0.05$ or 0.01 level. The results thus indicated that the Cyt straw had a significantly altered cell wall composition and increased soluble sugars and starch accumulation among three types of potato straws.

Furthermore, this study examined that the soluble sugars consist of more than 80 % hexoses in mature potato straws, and the Cyt straw thus had much more hexoses levels than those of the Dip and Tet straws with increased rates of 34 % and 14 %, respectively (Fig. S2A). In terms of the increased hemicellulose and pectin levels in the Cyt straw, we observed two major types of hemicellulose and pectin *in situ* using the immunolabeling probes specific for targeting xylan and de-esterified homogalacturonan (Fig. S2B and C). The Cyt straw exhibited either relatively more fluorescence cells or stronger fluorescence intensity relative to the Dip and Tet straws, consistent with its most increased hemicellulose and pectin contents. Monosaccharide analysis of hemicellulose confirmed a major increase of xylose proportion in the Cyt plant (Table S1). With respect to the reduced lignin level, the Cyt straw showed three monolignols (H, G, S) proportions to be similar to the Dip plant (Table S2). Taken together, it is assumed that the reduced cellulose production of Cyt straw may be the major cause for both carbohydrates (soluble sugars, starch) accumulation and non-cellulosic polysaccharides

(hemicellulose, pectin) deposition, suggesting that the Cyt plant might have a dynamic regulation of carbon partitioning among all polysaccharides and carbohydrates. In other words, it is hypothesized that the raised non-cellulosic polysaccharides may compensate for the decreased cellulose and lignin to maintain plant mechanical strength and biomass yield in the Cyt straw.

3.3. Complete biomass hydrolysis under mild pretreatments

Biomass saccharification has been defined by measuring either hexose yields (% cellulose) released from enzymatic hydrolysis after pretreatment with the biomass residues or sugars (hexoses and pentoses) yields (% dry matter) from both enzymatic hydrolysis and pretreatments (Li et al., 2013, 2015). Using our previously established approaches (Zahoor et al., 2017; Alam et al., 2019; Wu et al., 2019), this study performed liquid hot water/LHW and CaO pretreatments with mature potato straws (Fig. 2), which are mild pretreatments. Under the LHW pretreatment at a time course or the CaO pretreatment at series of concentrations, the Cyt straw required either less incubation time of LHW (8 min) or lower concentration of CaO (5% w/w) to obtain the highest hexoses yields (% cellulose) and total sugars (hexoses and pentoses) yields (% dry matter), whereas the optimal LHW incubation time was 12 min for the Tet and Dip straws and the optimal CaO concentration was 10 % for the Dip straw (Fig. 2A–D).

Furthermore, under two minimized pretreatments (8 min LHW, 5% w/w CaO), the Cyt straw exhibited complete biomass saccharification with hexoses yield of 100 % (% cellulose), but both Dip and Tet straws showed significantly less enzymatic saccharification in particular for total sugars yields at $P < 0.01$ level (Fig. S3A-D). Further, GC/MS assay clarified more than 97 % of hexose yields to be derived from glucose in the Cyt straw, other than in the Tet straw (Fig. S3E), confirming that almost complete enzymatic hydrolysis of cellulose microfibrils occurred in the Cyt straw. Hence, under the two mild pretreatments, the Cyt straw demonstrated higher biomass enzymatic saccharification among the Dip, Tet, and Cyt potato samples, which could be due to much-reduced lignin deposition and specifically modified lignocellulose features.

3.4. Maximum bioethanol yields under optimal pretreatments

Yeast fermentation was conducted for bioethanol production using the hexoses released from enzymatic hydrolysis of both starch and pretreated lignocellulose residues. Without any pretreatment, this study detected much increased bioethanol yields in the Tet and Cyt straws at $P < 0.01$ level, compared to the Dip, but the Cyt straw showed significantly higher bioethanol yield than that of the Tet (Fig. 3A–E), mainly due to its higher content of soluble sugars and starch as described above. Furthermore, under two optimal pretreatments (8 min LHW, 5% CaO), the highest ethanol yields were achieved in the Cyt straw, consistent with its highest hexose yields obtained from soluble sugars and enzymatic hydrolyses of starch and lignocellulose. Although the Cyt straw showed bioethanol yields of 23 % and 24 % (% dry matter) under two mild pretreatments, it could theoretically obtain bioethanol yields of 28

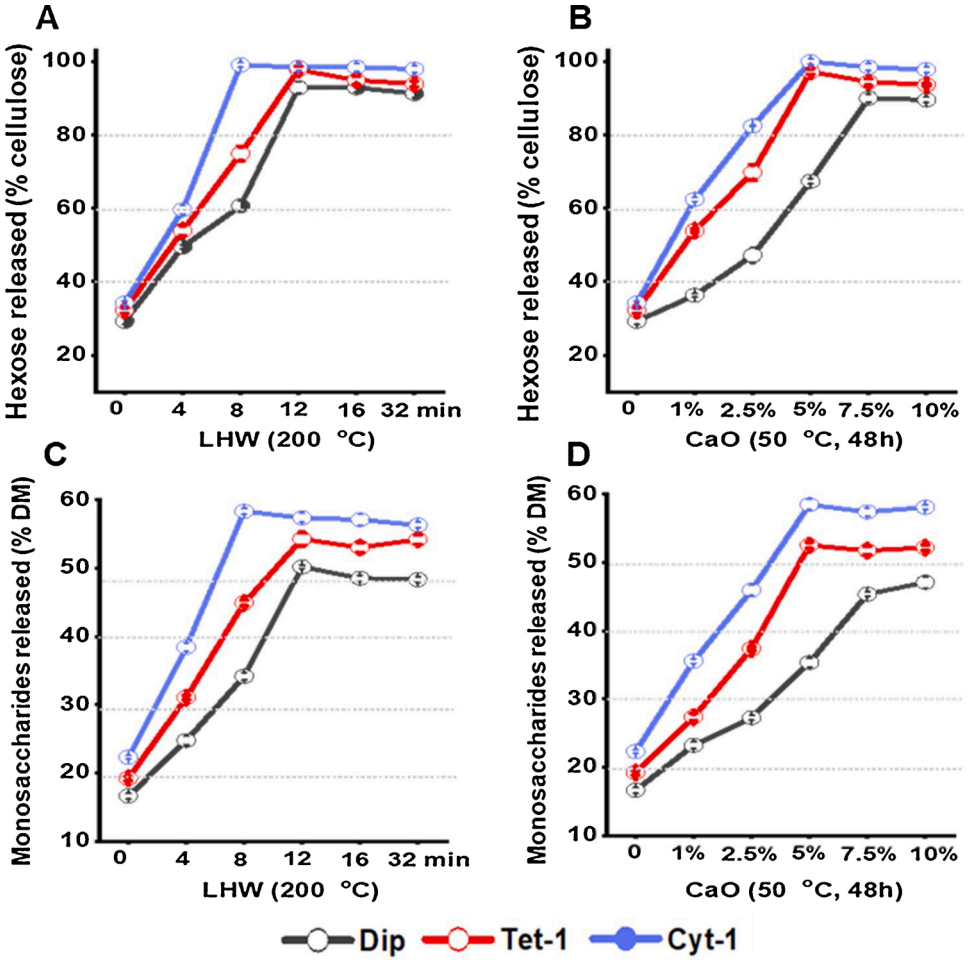

Fig. 2. Biomass enzymatic saccharification under two mild pretreatments with mature straws in Dip, Tet, and Cyt samples. (A) Hexose yield (% cellulose) released from enzymatic hydrolysis after LHW pretreatments under a time course. (B) Hexose yield after CaO pretreatment at various concentrations. (C, D) Total sugar yields (% dry matter) released from both pretreatment and sequential enzymatic hydrolysis under two pretreatments. Data as means ± SD (n = 3).

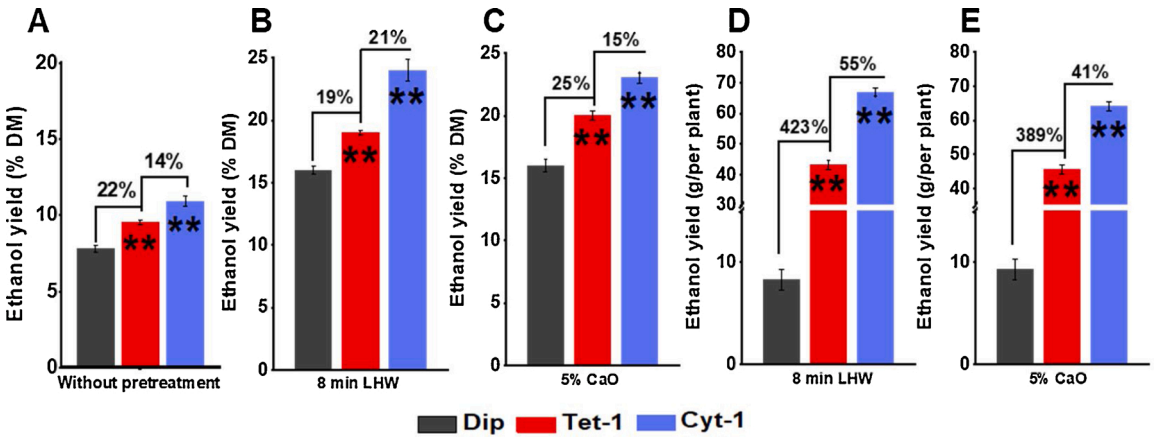

Fig. 3. Bioethanol production under two optimal (8 min LHW, 5% w/w CaO) pretreatments in Dip, Tet, and Cyt samples. (A, B, C) Bioethanol yields against dry matter (%DM). (D, E) Bioethanol yield against plant (g/per plant). Student's *t*-test among the Dip, Tet-1, and Cyt-1 samples at ** $P < 0.01$ with the increased/decreased (-) percentage and data as means ± SD (n = 3).

% and 29 % from the co-fermentation of hexoses and pentose (xylose), which were higher than those of the previously-reported bioenergy crops (Table 2). More importantly, a recent report has shown bioethanol yield of 24 % (% dry matter) achieved in the corn mutant, but it required stronger pretreatment (20 min LHW, 15 % CaO) conditions (Wu et al., 2019). Further analyses of mass balance confirmed that the highest bioethanol yields achieved in the Cyt straw was mainly due to its much soluble sugars and starch accumulation and complete lignocellulose enzymatic hydrolysis after two optimal pretreatments performed in this study (Figs. 4 and S4).

3.5. *Effective extraction of hemicellulose-lignin complexes*

To understand a complete biomass enzymatic saccharification in the Cyt straw, we examined extraction of cellular polymers from the optimal pretreatments. Under optimal LHW and CaO pretreatments, the Dip, Tet, and Cyt straws all showed much higher extraction of hemicellulose and lignin (Fig. 5A and B), consistent with the previous findings examined in other biomass residues (Alam et al., 2019; Wu et al., 2019). These consequently led to a relatively increased cellulose content in the pretreated biomass residues, compared to the raw materials (Fig. 5C). However, although the Dip and Tet straws required longer incubation times and higher chemical concentrations for their optimal LHW and CaO pretreatments, the Cyt straw showed constantly lower lignin levels in their pretreated biomass residues. Since lignin has been well characterized as a major barrier against cellulases accession and enzymatic digestion (Chen & Dixon, 2007; Oyarce et al., 2019; Xu et al., 2018), less lignin deposition in the pretreated residues could be one of the major causes for enhanced enzymatic saccharification in the Cyt straw. Meanwhile, the raw materials of Cyt straw contained higher hemicellulose content than those of the Dip and Tet straws, but its pretreated biomass resides showed the lowest hemicellulose levels. To detect any potential alterations of wall polymer interlinkages in the pretreated biomass residues, we used Fourier transforms infrared (FTIR) spectroscopy scanning to observe the shifted characteristic peaks (C—H; C—O—C; C═C; C═O) corresponding for inter-linkage styles among major wall polymers in the pretreated residues (Fig. 5D and Table S4). By comparison, the Cyt straw showed more shifted peaks or reduced peak intensity in its pretreated residues than those of the Dip and Tet straws, suggesting a more effective extraction of hemicellulose-lignin complexes from the two optimal pretreatments conducted in the Cyt straw.

3.6. *Considerably reduced lignocellulose recalcitrance*

With regard to the effective wall polymer extraction under two optimal pretreatments, this study attempted to sort out the mechanism of reduced lignocellulose recalcitrance for enhanced biomass enzymatic saccharification. First, we detected the cellulose DP and its surface areas (accessibility) in potato straws (Fig. 5E and F), which have been characterized as the major factors accounting for lignocellulose recalcitrance (Alam et al., 2019; Hu et al., 2018; Wu et al., 2019). In the raw materials (without any pretreatment), the Cyt sample showed a significantly lower cellulose DP value than those of the Dip and Tet samples. Under the two optimal pretreatments, all Dip, Tet, and Cyt straws had much-lower cellulose DP, but the Cyt showed the lowest DP value. By contrast, the two optimal pretreatments could lead to much more raised cellulose accessibility among three Dip, Tet, and Cyt samples. Significantly, the cellulose DP values were negatively correlated with the hexose yields released from enzymatic hydrolysis with an extremely high r value of

Table 2
Comparison of bioethanol yields (% dry matter) obtained in this study and other genetically modified and mutant plants in the previous studies.

Plant species	Pretreatment	Hexoses fermentation	Hexoses & pentoses co-fermentation[#]	Solid Loading	References
Potato (Cyt-1)	**8 min LHW (200 °C) 5% w/w CaO (50 °C, 48 h)**	**24 % 23 %**	**29 % 28 %**	**5%**	**This study**
Corn mutant	20 min LHW (200 °C) or 15 % w/w CaO (50 °C, 48 h)	19 %	24 %	5%	(Wu et al., 2019)
Wheat	Dilute H_2SO_4 (120 °C, 40 min)	10 %	12 %	2.5 %	(Li et al., 2011)
Barely	Dilute H_2SO_4 (120 °C, 40 min)	15 %	18 %	2.5 %	(Li et al., 2011)
Sugarcane bagasse	Dilute H_2SO_4 (170 °C, 10 min)	13 %	23 %	7%	(Ko et al., 2018)
Switchgrass	Ammonia fiber expansion (50 °C, 30 min)	17 %	24 %	19 %	(Serate et al., 2015)
Sorghum	Dilute H_2SO_4 (121 °C, 120 min)	13 %	22 %	10 %	(Serate et al., 2015)
Poplar	LHW (180 °C, 44 min)	19 %	25 %	5%	(Bhagia et al., 2016)
Eucalyptus	Hydrothermal (180 °C, 40 min)	20 %	24 %	7.5 %	(Sykes et al., 2015)

[#] Based on the average conversion rate (0.35 g ethanol/g xylose) as previously described by Rodrussamee et al (2018) and Valinhas et al (2018).

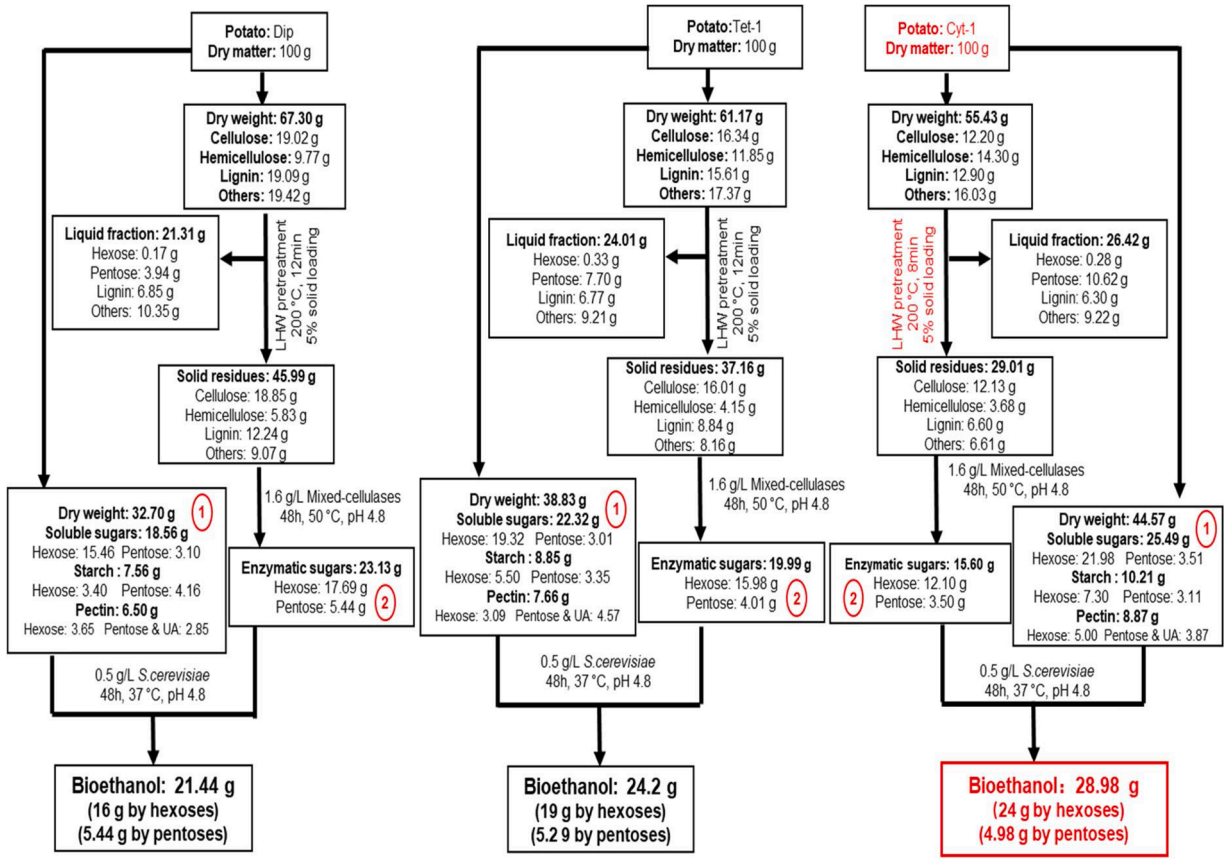

Fig. 4. Mass balance analysis for bioethanol production under optimal LHW pretreatment in the Dip, Tet-1, and Cyt-1 samples. The mass amounts of hexoses and pentoses are presented in streams 1 and 2.

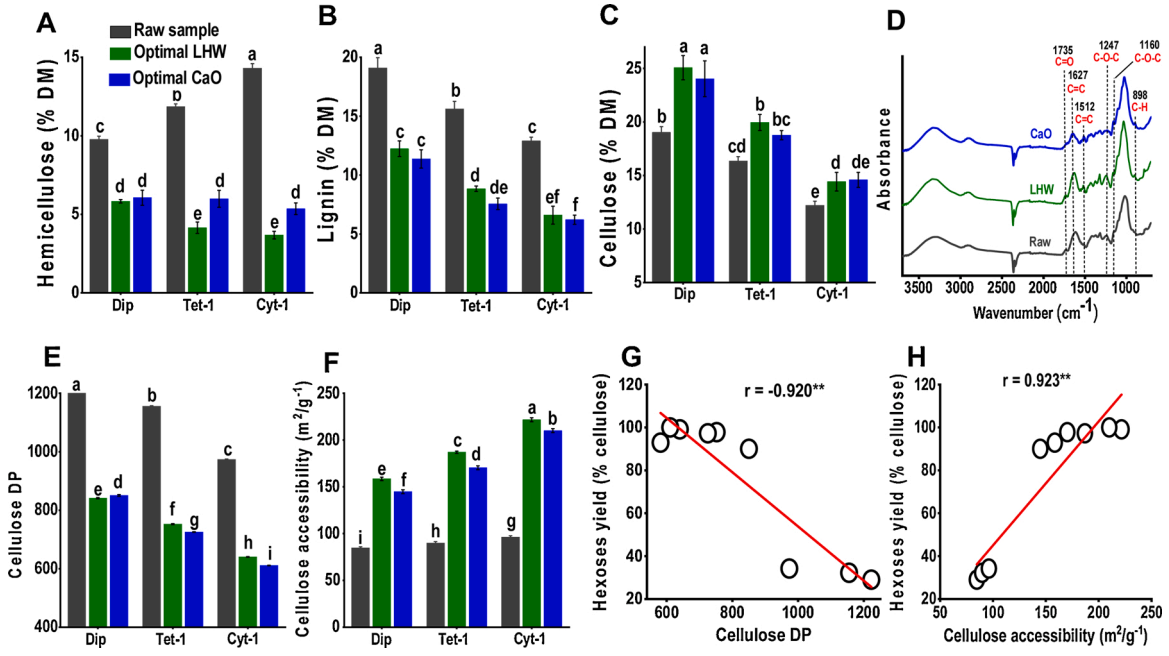

Fig. 5. Characterization of cell wall polymers levels, cellulose DP and accessibility from the optimal LHW and CaO pretreatments in the Dip, Tet, and Cyt potato samples. (A, B, C) Three major cell wall polymer compositions (% dry matter) in raw material and pretreated biomass residues. (D) Comparison of FTIR profiling in the raw and pretreated residues of the Cyt sample. Dot lines indicated the majorly altered bonds as indicated in Table S3. (E, F) Cellulose DP and cellulose accessibility in raw material and pretreated biomass residues. Cellulose accessibility is defined by Congo red stain area (m^2/g^{-1}). Different letters above bars indicate that the means differ according to the analysis of variance and LSD test ($P < 0.05$). (G, H) Correlation analysis between cellulose DP (n = 9) or accessibility (n = 9) and hexose yields released from enzymatic hydrolysis under two optimal pretreatments and without pretreatment (raw material). ** As significant correlation at $P < 0.01$.

0.92, whereas the cellulose accessibility showed a positive correlation (Fig. 5G and H). It thus confirmed that cellulose DP and accessibility were two crucial contrast factors of lignocellulose recalcitrance (Alam et al., 2019; Wu et al., 2019). Hence, the complete biomass saccharification of the Cyt straw could be due to its most reduced lignocellulose recalcitrance. It has also demonstrated that the Cyt plant generated from incomplete genome doubling could not only produce the most increased lignocellulose and carbohydrates (soluble sugars, starch) in mature straw, but it could also have remarkably modified lignocellulose for maximum bioethanol production.

3.7. Mechanism of maximum bioethanol from mild biomass processing

Based on the data obtained in this work and the findings achieved in the previous studies (Deng et al., 2020; Alam et al., 2019; Wu et al., 2019), this study proposed a model to explain why high bioethanol yield could be achieved in the Cyt potato straw (Fig. 6). Although polyploidization has been implemented to increase biomass yield (Chae et al., 2013; Sattler et al., 2016), this study demonstrated a novel approach to maximize biomass yield by performing incomplete genome doubling in plants. The incomplete genome doubling could also remarkably improve lignocellulose recalcitrance including reduced lignin level and cellulose DP value and increased cellulose accessibility. These should cause an integrated enhancement for complete biomass enzymatic saccharification, even though under minimized biomass pretreatments. Due to significantly reduced cellulose production, the Cyt straw was increased starch and soluble sugars, which could be used for direct yeast fermentation. In addition, the Cyt straw showed increased pectin and hemicellulose to maintain plant mechanical strength and biomass yield, and these non-cellulosic polysaccharides could be used as additional pentoses source for co-fermentation by the engineered yeast strain (Wu et al., 2019). Therefore, the incomplete genome doubling of the Cyt plant could dynamically regulate carbon partitioning among carbohydrates and wall polysaccharides, providing an applicable technology for cost-effective biomass processing to maximize bioethanol production in potato straw and beyond.

4. Conclusions

By selecting polyploidy potato plant (Cyt) with incomplete genome doubling, this study examined the increased biomass yield in mature Cyt straw including soluble sugars and starch. Due to reduced lignocellulose recalcitrance, the Cyt straw showed complete enzymatic saccharification under two optimal pretreatments performed. Further using all hexoses sources obtained from soluble sugars, starch, and lignocellulose in the Cyt straw, this study conducted a classic yeast fermentation to achieve the highest bioethanol production in history. Therefore, this work has proposed a novel mechanism model to highlight a powerful strategy for cost-effective bioethanol production under mild manner in potato straw.

CRediT authorship contribution statement

Meysam Madadi: Investigation, Methodology, Formal analysis, Writing - original draft. **Kanglu Zhao**: Investigation, Methodology, Formal analysis. **Youmei Wang**: Validation, Data curation. **Yanting Wang**: Validation, Project administration. **Nengzhou Jin**: Formal analysis, Methodology. **Zhijun Xu**: Software, Formal analysis. **Guanhua Li**: Software, Methodology. **Shang-wen Tang**: Editing, Project administration. **Tao Xia**: Editing, Methodology. **Zhi Qi**: Editing, Resources.

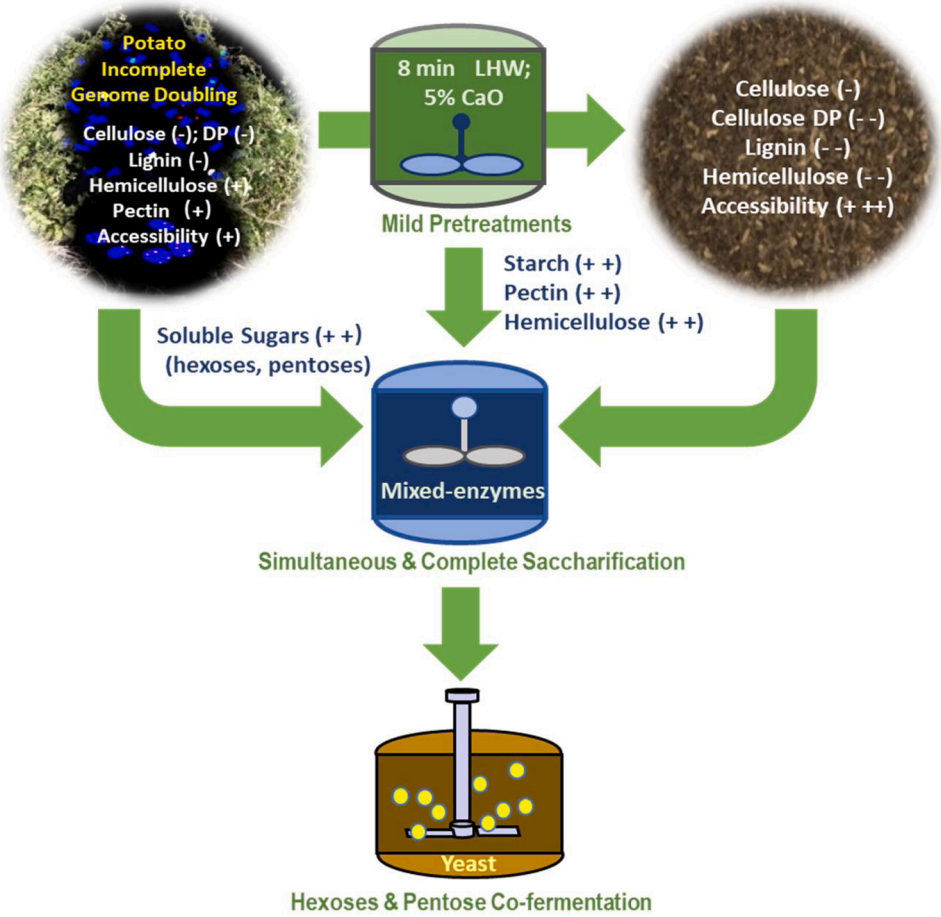

Fig. 6. A model to elucidate how the maximum bioethanol yields could be achieved under two optimal pretreatments by simultaneous saccharification of the most-modified lignocellulose and the most-increased starch and soluble sugars for co-fermentation of all hexoses and pentoses by engineered yeast strain. (-) and (+) respectively indicate reducing and enhancing cell wall polymers levels, cellulose DP and accessibility, and starch levels during biomass processing and bioethanol production in the model.

Liangcai Peng: Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Zhiyong Xiong**: Conceptualization, Writing - original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2021.118070.

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