Production and characterization of a novel interspecific somatic hybrid combining drought tolerance and high quality of sweetpotato and *Ipomoea triloba* L.

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Research Article

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Abstract

Key message **A novel interspecific somatic hybrid combining drought tolerance and high quality of sweetpotato and *Ipomoea triloba* L. was obtained and its genetic and epigenetic variations were clarified.**

Somatic hybridization can be used to overcome the cross-incompatibility between sweetpotato (*Ipomoea batatas* (L.) Lam.) and its wild relatives and transfer useful and desirable genes from wild relatives to cultivated plants. However, most of the interspecific somatic hybrids obtained to date can't produce storage roots and show no good agronomic characters. In the present study, a novel interspecific somatic hybrid, named XT1, was obtained through protoplast fusions between sweetpotato cv. Xushu 18 and its wild relative *I. triloba* L.. This somatic hybrid was found to produce storage roots and to exhibit significantly higher drought tolerance and quality compared with its cultivated parent Xushu 18. Transcriptome and real-time quantitative PCR (qRT-PCR) analyses showed that the well-known drought stress-responsive genes in XT1 and *I. triloba* were significantly up-regulated under drought stress. Evidence of genome structural reconstructions between the two genomes of the fusion parents in XT1 was confirmed by genomic in situ hybridization (GISH) and specific nuclear and cytoplasmic DNA markers. Its DNA methylation variations were characterized by methylation-sensitive amplified polymorphism (MSAP). This study not only reveals the significance of somatic hybridization techniques in the genetic improvement of sweetpotato but also provides valuable materials and knowledge for investigating the mechanism of storage root formation in sweetpotato.

Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam., is an important food and industrial material crops worldwide. The narrow genetic background limits further improvement of this crop (Li et al. 2008; Liu 2017). Many wild *Ipomoea* species possess useful and desirable agronomic traits lacking in the cultivated sweetpotato, such as high starch content, disease and insect resistance and drought tolerance (Huang and Sun 2000; Cao et al. 2009). These favorable wild germplasm resources, however, cannot be used directly by conventional hybridization due to their cross-incompatibility with sweetpotato (Guo et al. 2006). Somatic hybridization technique represents a successful and effective approach overcoming the sexual barriers in transferring various desirable genes from wild species to cultivated species or creating new beneficial characters in several crops such as potato (Chen et al. 2013; Tu et al. 2021), citrus (Xiao et al. 2014; Ruiz et al. 2018), cotton (Sun et al. 2004; Yu et al. 2012), *Brassica* species (Wang et al. 2003; Kumari et al. 2020) and wheat (Xia et al. 2003; Liu et al. 2009).

Somatic hybridization can combine the nuclear, mitochondrial and plastid genomes of two desirable parents and generate a novel variability through bypassing sexual crosses (Iovene et al. 2007). Newly synthetical hybrids require an adaptation of different genomes within one cell, they may possess extensive genetic and epigenetic variations. These variations can lead to chromosome rearrangement, global gene expression changes such as novel expression and gene silence (Xia 2009; Sahu et al. 2013), dormant transposon activation (Sarilar et al. 2013), DNA methylation alteration (Shen et al. 2012) and

histone modification (He et al. 2010). Evidence from many studies suggests that the genomics and epigenetics changes caused by somatic hybridization can explain the phenotypic variations of the hybrid derivatives (Liu and Xia 2014).

Identification of the genome composition is a prerequisite for a better use of somatic hybrids (Iovene et al. 2007). Genomic in situ hybridization (GISH) is usually used to identify the chromosomal constitutions of hybrids (Parokonny et al. 1992). For cytoplasmic composition analysis, at present, several universal primers homologous to conserved sequences of mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) have been used (Scotti et al. 2003; Yu et al. 2012; Chen et al. 2013). Besides the genetic variations, epigenetic variations play a significant role in the growth and development of plants. One of the key epigenetic modifications is changes in cytosine methylation pattern, and it can be reflected using the technique of methylation-sensitive amplified polymorphisms (MSAP). In recent years, amplified fragment length polymorphism (AFLP) and MSAP markers are still a relatively cheap and credible alternative to identify the genetic and epigenetic variations in many plants including potato (Smyda-Dajmund et al. 2021), *Brassica* (Sheng et al. 2013), citrus (Xu et al. 2014), wheat (Liu et al. 2015) and marine macroalgae (Gupta et al. 2015).

To date, many interspecific somatic hybrids between sweetpotato and its wild relatives have been produced through protoplast fusions (Liu 2011). However, most of these hybrids can't produce storage roots and not show good agronomic characters, which limit their use in sweetpotato genetic improvement. In addition, little information is available about the performance and genomic components of the somatic hybrids. The mechanism of phenotypic inheritance associated with genome structure and DNA methylation in somatic hybrids are also unknown.

I. triloba L., a wild relative of sweetpotato, has been shown to have the tolerance to drought and may be a potential source of agriculturally desirable traits (Martin and Jones 1973; Yang et al. 2009; Jia et al. 2017). In the present study, a novel interspecific somatic hybrid, named XT1, was obtained through protoplast fusions between sweetpotato cv. Xushu 18 and *I. triloba*. This somatic hybrid produced storage roots and exhibited significantly higher drought tolerance and quality compared with Xushu 18. Its genomic components were identified by GISH and specific nuclear and cytoplasmic DNA markers and the DNA methylation variations were characterized by MSAP.

Materials And Methods

Plant materials and growth conditions

Sweetpotato cv. Xushu 18 (2*n*=6*x*=90), a commercial variety widely planted in China, and its wild relative *I. triloba* L. (K121, 2*n*=2*x*=30) were used for protoplast isolation and fusion. The *in vitro*-grown plants of *I. triloba* were cultured on Murashige and Skoog (MS) basal medium at 27±1 °C under 13 h of cool-white fluorescent light at 54 μmol·m^{-1}·s^{-1}. Embryogenic suspension cultures of Xushu 18 were prepared as described previously (Liu et al. 2001).

Protoplast isolation, fusion and culture

Protoplasts were isolated from young petioles of *I. triloba* and embryogenic suspension cultures of Xushu 18, respectively, as described previously (Liu et al. 1991; Guo et al. 2006). After purification, the protoplasts of *I. triloba* and Xushu 18 were suspended in W5 salt solution respectively (Negrutiu et al. 1986), and then completely mixed in an 1:2 ratio. The mixed protoplasts were quickly fused with polyethylene glycol (PEG) method (Liu et al. 1998). The culture of the fusion products was performed as described by Yang et al. (2009). The regenerated plantlets were cultured continuously on fresh MS medium for developing into whole plants.

RAPD analysis and chromosome counts

Total genomic DNA was extracted from fresh leaves of 4-weeks-old *in vitro*-grown regenerated plants and both fusion parents with the EasyPure Plant Genomic DNA Kit (Transgen, Beijing, China). For random amplified polymorphic DNA (RAPD) analysis, 20 RAPD primers (Supplementary Table S1) were used according to the method of Guo et al. (2006). The root tips of somatic hybrids were used to determine the chromosome counts (Guo et al. 2006).

Morphological characterization

Somatic hybrid plants and their fusion parents were grown in 7 cm × 7 cm pots with soil and then transferred to a field for observing their morphological characteristics according to 'Descriptors for sweetpotato' (CIP 1991).

GISH and cytoplasmic genome analyses

For the chromosomal composition analysis, GISH was used to study the genomic dosage of the parental genomes in a storage root-bearing somatic hybrid followed the procedures of Yang et al. (2009). Six pairs of cpDNA and 6 pairs of mtDNA universal primers (Supplementary Table S1) were used to detect the cytoplasmic components of the storage root-bearing somatic hybrid (Jia et al. 2017).

AFLP and MSAP analyses

In order to detect the genetic variations among the storage root-bearing somatic hybrid and its parents, 36 AFLP primer pairs (Supplementary Table S1) were used. The AFLP procedure was performed from the report of Zhao et al. (2013). For detecting the methylation changes on specific sites, MSAP analysis was carried out using 64 selective primer pairs (Supplementary Table S1) following the general procedure of Gupta et al. (2012). AFLP and MSAP data originated from the electrophoresis of PCR products were converted into binary matrix of 1 (present) and 0 (absent). Due to the differential cleavage of the restriction site (5'-CCGG) recognized by the two isoschizomers *Hpa*II and *Msp*I, the hemi-methylation of the external cytosine (^{5m}CCGG) or full-methylation of the internal cytosine (C^{5m}CGG) can be obviously distinguished (Kanchanaketu et al. 2012). Therefore, the methylation status and level at the site can be

reflect by the different band pattern from PCR amplification, and the methylation types were assigned to the corresponding patterns described by Xu et al. (2014).

Assay for drought tolerance

In order to evaluate the drought tolerance, *in vitro*-grown plants of the storage root-bearing somatic hybrid and its parents were cultured on MS medium containing 0 and 20 % polyethylene glycol (PEG) 6000, respectively. After four weeks, the growth and rooting of each plant were observed, the fresh weight (FW), proline and malonaldehyde (MDA) contents and superoxide dismutase (SOD) activity of each plant were analyzed (Gao et al. 2011).

The 25-cm-long cuttings from the storage root-bearing somatic hybrid and its parents grown in a field for 6 weeks were planted in a transplanting box. After irrigation with water for 2 weeks, they were subjected to drought stress without water for 6 weeks. Growth and rooting ability were continuously investigated, the FW and dry weight (DW) of each plant were measured (Zhai et al. 2016).

For further identification, the storage root-bearing somatic hybrid and its parents were planted in a drought stress facility. After 30 days of planting, the plants were subjected to drought stress for 70 days (Yang et al. 2009). After 100 days of planting, the photosynthetic rate, stomatal conductance, transpiration rate and relative chlorophyll content were measured (Liu et al. 2014). The plants were harvested and FW of the storage roots was measured.

Transcriptome analysis

Total RNA was extracted from storage roots of the storage root-bearing somatic hybrid and its parents, which treated with normal well-watered irrigation and drought stress, respectively, using the RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China) for Illumina RNA-seq and real-time quantitative PCR (qRT-PCR) analyses. Transcriptome sequencing and analysis were conducted by Novogene Bioinformatics Institute (Beijing, China). Two biological replicates were performed using independent tissue samples for normal irrigation and drought stress. The differentially expressed transcripts (≥200 bp) with more than 2-fold changes in each drought sample compared with the control sample were selected for homology and annotation analyses (Zhai et al. 2016).

Expression analysis of the related genes

The expression levels of drought stress-responsive genes encoding xanthoxin dehydrogenase (ABA2), 9-cis-epoxycarotenoid dioxygenase (NCED), phosphatidylinositol-4-phosphate 5-kinase (PIP5K) , phospholipase C (PLC), phospholipase D (PLD), pyrroline-5-carboxylate synthase (P5CS), pyrroline-5-carboxylate reductase (P5CR), SOD, glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), late embryogenesis abundant protein (LEA), trehalose-6-phosphate synthase (TPS), Nifu-like domain-containing protein (NFU), dehydration responsive element binding protein (DREB), phosphoribulokinase (PRK) and D1 polypeptide of photosystem II (psbA) were verified by qRT-PCR (Liu et al. 2014). The specific primers used were listed in Supplementary Table S1.

Quality analysis

Storage roots of the storage root-bearing somatic hybrid and its parent Xushu 18 were employed to analyze their dry matter, soluble sugar, starch, protein, fiber matter and total carotenoid contents. The dry matter and soluble sugar contents were determined as described by Yang et al. (2009). The starch, protein and fiber matter contents were measured following the method of Ji et al. (2015). The total carotenoid content was determined as described by Cervantes-Flores et al. (2011).

Statistical analysis

The experiments were repeated three times and the data presented as the mean ± standard error (SE) were analyzed by Duncan's multiple range test. A *P* value of < 0.05 was considered to be statistically significant.

Results

Production of somatic hybrids

Petiole protoplasts of *I. triloba* were easily fused with embryogenic suspension culture protoplasts of sweetpotato cv. Xushu 18 using the PEG-mediated method (Supplementary Fig. S1 A,B). After 10-12 weeks of culture in liquid MS medium with 0.5 mg L^{-1} kinetin (KT) and 0.05 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), the fused products formed small calluses with diameter of 1-2 mm (Supplementary Fig. S1 C). The small calluses grew into calluses of 5-10 mm in diameter when they were transferred to solid MS medium with 0.5 mg L^{-1} KT and 0.05 mg L^{-1} 2,4-D for 3-4 weeks (Supplementary Fig. S1 D). These calluses were transferred to MS medium supplemented with 2 mg L^{-1} benzylaminopurine (BAP) and began to regenerate plantlets after about 2 weeks (Supplementary Fig. S1 E). After further transfer to MS basal medium for 4 weeks, these plantlets developed into the whole plants (Supplementary Fig. S1 F). A total of 375 regenerated plants were obtained from 48 of the transferred 169 calluses.

Identification of somatic hybrids

RAPD analysis was performed on all of the 375 regenerated plants, and 341 (90.93%) of them showed the specific bands of both parents or new bands which both parents did not have, indicating that all the 341 plants were somatic hybrids (Supplementary Fig. S1 G). The number of chromosomes of these hybrids ranged from 46 to 90, much less than the sum (90+30=120) of those of both parents. These results indicated that the chromosome elimination phenomenon occurred in these somatic hybrids. Interestingly, one of them, named XT1, had 90 chromosomes, which was consistent with the chromosome number of sweetpotato ($2n = 6x = 90$).

After transferred to soil, all the somatic hybrids survived (Supplementary Fig. S1 H). They were further grown in a field and found that most of them displayed the intermediate morphology of both parents. XT1 showed similar growth habit of vines to Xushu 18 and produced storage roots (Fig. 1 A-F), while other somatic hybrids had similar growth habit to *I. triloba* and did not form obvious storage roots. The color of abaxial leaf veins and petioles of XT1 was the same as that of *I. triloba* and obviously different from that of Xushu 18 (Fig. 1 D), but its stem diameter and internode length were the middle type of both parents

(Fig. 1 E). XT1 had light-yellow root skin and light-orange root flesh, which were different from the red root skin and white root flesh of Xushu 18 (Fig. 1 F,G).

GISH karyotype of the somatic hybrid XT1

The chromosome composition of XT1 was characterized using the GISH method. Chromsomes of XT1 were stained by 4,6-diamidino-2-phenylindole (DAPI) (Green), while chromosomes of *I. triloba* were colored red by digoxigenin-labeled genomic DNA probes. The results showed that XT1 had 2 chromosomes from *I. triloba*, 55 chromosomes from Xushu 18 and 33 recombinant chromosomes of both parents (Fig. 2 A).

Cytoplasmic genome compositions of the somatic hybrid XT1

To determine the cytoplasmic genome compositions of XT1, the polymorphisms between the parental cpDNA and mtDNA were detected using the universal primer pairs (Table S1). All the cpDNA primers and 2 mtDNA primers (*rpS*14/*cob* and *nad*1-*exon*B/*nad*1-*exon*C) generated polymorphic and clear bands, showing that XT1 had the same bands as Xushu 18 (Fig. 2 B,C). These results demonstrated that the cpDNA and mtDNA of XT1 were originated from Xushu 18.

The nuclear genome components of the somatic hybrid XT1

The nuclear genome components of XT1 were clarified by AFLP analysis. Thirty-six AFLP primer pairs yielded a total of 2026 bands, including 1062 (52.42%) inherited bands, 823 (40.62%) altered bands and 141 (6.96%) novel bands in XT1 (Fig. 2 D, Table 1). Of the 52.42% inherited bands, 18.41% came from both parents, 2.96% from *I. triloba* and 31.05% from Xushu 18. The 40.62% altered bands, including both parent bands (1.48%), *I. triloba* specific bands (30.80%) and Xushu 18 specific bands (8.34%), were absent in XT1 (Table 1).

DNA methylation variations of the somatic hybrid XT1

MSAP analysis was used to explore the DNA methylation variations at the 5’- CCGG sites. By using 64 *EcoR*I + *Hpa*II/*Msp*I primer combinations (Table S1), 787, 1035 and 987 clear and reproducible sites were generated in *I. triloba*, XT1 and Xushu 18, respectively (Fig. 2 E, Table 2). The ratios of unmethylated (pattern *Hpa*II = 1/*Msp*I = 1), full-methylated (pattern *Hpa*II = 0/*Msp*I = 1) and hemi-methylated (pattern *Hpa*II = 1/*Msp*I = 0) CCGG sites were 53.24%, 34.56% and 12.20% in *I. triloba*, 37.78%, 31.50% and 30.72% in XT1 and 41.95%, 29.69% and 28.37% in Xushu 18, respectively (Table 2). These results showed that the unmethylation ratio was lower in XT1 than in both parents.

DNA methylation alterations of XT1 in connection with its parents were further analyzed (Table 3). A total of 1473 sites were observed in XT1, which included 837 (56.82%) inherited sites, 427 (28.99%) altered sites and 209 (14.19%) novel sites. Of the 56.82% inherited sites, 14.46% were originated from both parents, 4.88% from *I. triloba* and 37.47% from Xushu 18. The 28.99% altered sites, including both parent sites (2.85%), *I. triloba* specific sites (21.86%) and Xushu 18 specific sites (4.28%), were absent in XT1 (Table 3).

Drought tolerance evaluation of the somatic hybrid XT1

By *in vitro* assays, XT1 and its parents exhibited normal growth and rooting on MS medium without PEG6000 (Fig. 3 A). When subjected to PEG stress, in contrast to the poor growth of Xushu 18, XT1 and

I. triloba showed vigorous growth and rooting (Fig. 3 A, Supplementary Table S2). Under PEG stress, the proline content and SOD activity were increased and the MDA content was decreased in XT1 and its parents, but these physiological indexes of XT1 were between the two parents (Supplementary Table S2).

The cuttings of field-grown plants were planted in transplanting boxes under greenhouse conditions for evaluating their drought tolerance. After 6 weeks of drought treatment, XT1 and *I. triloba* produced obvious new leaves and roots, but Xushu 18 gradually turned brown to death (Fig. 3 B), the number and length of roots, FW and DW of XT1 plants were observably higher than those of Xushu18 (Supplementary Table S3).

In the drought stress facility, XT1 and its parents showed vigorous growth and the yield of XT1 was significantly lower than that of Xushu 18 under the well-watered irrigation (Fig. 3 C); under drought stress, XT1 exhibited the similar outward appearance to *I. triloba* which showed only a little weaker growth, but Xushu 18 grew slowly with yellowing and necrosis of some leaves, and the yield of XT1 was significantly higher than that of Xushu 18 (Fig. 3 C). Under drought stress, the stomatal conductance, transpiration rate and relative chlorophyll content were obviously higher in XT1 and *I. triloba* than in Xushu 18, though *I. triloba* and Xushu 18 exhibited significantly different levels (Fig. 4). Our results demonstrated that XT1 had higher drought tolerance than Xushu 18.

Expression analysis of drought-responsive genes

Under drought stress, the transcriptome of XT1 and both parents was analyzed by RNA-Seq. Totally, 2940 (54.14%), 2107 (65.33%) and 501 (41.30%) differentially expressed genes (DEGs) were up-regulated, and 2490 (45.86%), 1148 (34.67%) and 712 (58.70%) DEGs were down-regulated in *I. triloba*, XT1 and Xushu 18, respectively (Fig. 5 A). To display the number of up-regulated DEGs expressed commonly and specifically in XT1 and its parents, venn diagrams were generated (Fig. 5 B). The results indicated that XT1 and its parents shared 213 DEGs. Except for these genes detected across all accessions, the diagram showed that 729 DEGs were commonly expressed in XT1 and *I. triloba*, 328 were coexpressed in XT1 and Xushu 18, and 1985 were specifically detected in XT1. Further analysis indicated that 986 (36.33%) of the 729 and 1985 DEGs expressed in XT1 were known genes, of which the genes involved in phosphatidylinositol (PI) and abscisic acid (ABA) signalling pathways, reactive oxygen species (ROS) scavenging system and stress responses were up-regulated under drought stress. The qRT-PCR analysis further demonstrated that the well-known drought-responsive genes related to ABA biosynthesis (*ABA*2, *NCED*), PI signalling pathways (*PIP5K*, *PLC*, *PLD*), proline biosynthesis (*P5CS*, *P5CR*), ROS-scavenging system (*SOD*, *GPX*, *CAT*, *APX*, *POD*), drought-responsive proteins (*LEA*, *TPS*, *NFU*), dehydration-responsive elements (*DREB*) and photosynthesis (*PRK*, *psbA*) were significantly up-regulated in XT1 and *I. triloba* compared with those of Xushu 18 under drought stress though XT1 and *I. triloba* showed different expression levels (Fig. 6).

Quality analysis of the somatic hybrid XT1

Dry matter, soluble sugar, starch, protein, fiber matter and carotenoids were the main nutritional compositions in sweetpotato. They were compared between XT1 and its cultivated parent Xushu 18. The results indicated that the dry matter and fiber matter contents between XT1 and Xushu 18 had no significant differences (Table 4). The soluble sugar, protein and carotenoid contents were significantly higher in XT1 than in Xushu 18, but the starch content of XT1 was significantly lower than that of Xushu 18 (Table 4). Our results provide evidence that *I. triloba* not only has drought tolerance related genes, but also has good quality genes.

Discussion

Production of a novel somatic hybrid

Somatic hybridization technique is an efficient and feasible way to produce somatic hybrids between sexually cross-incompatible cultivated sweetpotato and its wild species (Liu et al. 1998; Zhang et al. 2002; Guo et al. 2006; Yang et al. 2009; Jia et al. 2017). In the present research, a novel somatic hybrid, XT1, was successfully produced between sweetpotato cv. Xushu 18 and *I. triloba*. This somatic hybrid was biased to Xushu 18 in growth habit of vines and formation of storage roots (Fig. 1) and to *I. triloba* in drought tolerance (Fig. 3). It also showed higher soluble sugar, protein and carotenoid contents compared with Xushu 18 (Table 4). Evidence of genome structural reconstructions between the two genomes of its fusion parents in XT1 was determined by GISH and specific nuclear and cytoplasmic DNA markers (Fig. 2). In addition, this study provides important insights into epigenetic modifications in the somatic hybrid of sweetpotato and its wild relatives (Fig. 2).

Effect of somatic hybridization on genetic variations

Evidence from studies involving potato (Smyda-Dajmund et al. 2021), *Brassica* (Sheng et al. 2013), citrus (Xu et al. 2014), wheat (Shaked et al. 2001; Liu et al. 2015) and marine macroalgae (Gupta et al. 2015) has demonstrated that somatic hybridization among distantly related species may bring about drastic changes in genome DNA, such as chromosomal rearrangements and directional sequence elimination. In our research, GISH analysis displayed that the number of chromosomes of XT1 was 90, which included 2 chromosomes from *I. triloba*, 55 chromosomes from Xushu 18 and 33 recombinant chromosomes of both parents (Fig. 2 A). These results showed that large-scale elimination and more recombination or introgression of chromosomes occurred in XT1. The previous studies on protoplast fusion between sweetpotato and its wild relatives resulted in somatic hybrids with huge changes in chromosome number, all of which had no more than 88 chromosomes (Liu et al. 1998; Zhang et al. 2002; Guo et al. 2006; Yang et al. 2009; Jia et al. 2017). The happening of a non-additive chromosome count is a general phenomenon as reported in many plants (Kasha and Kao 1970; Chen et al. 2004; Tu et al. 2009; Wang et al. 2011).

In this study, the nuclear genome components of XT1 were investigated by AFLP markers (Table 1). The proportion of parental bands loss in XT1 was about 47.58%, and the parent-specific bands loss was non-random, it was likely to lose most of the genome DNA sequences from *I. triloba*, which was coincident with the result of GISH. We propose that the more distant relationship between Xushu 18 and *I. triloba* may cause diversified forms of incompatibility in genomes, and hence lead to drastic changes in genome structure. A similar trend was also noted by Sun et al. (2014), who discovered that the frequency of chromosome fragment loss was lower in the *japonica* + *indica* somatic hybrids than in the *japonica* rice + wheat somatic hybrids and proposed that chromosomes were easily eliminated when the two fusion parents had more distant relationship.

Effect of somatic hybridization on DNA methylation variations

Cytosine methylation within DNA is a common form of DNA covalent modification that antagonizes transcription in most eukaryotes, which provides a ubiquitous epigenetic control (Zilberman et al. 2006). It has been reported that distant hybridization can also trigger numerous epigenetic variations. For example, in a somatic hybrid no. 38 (2n=34, the sum of two parents) between *Brassica nigra* (L.) Koch and *Brassica oleracea* L. var. *italica*, the DNA methylation alteration ratio was 4.07% (Sheng et al. 2013). Xu et al. (2009) found that the proportion of the methylation variations was 7.29% in leaves from juvenile plants of the newly synthesized *B. napus* allopolyploid and 7.04% in mature plants. Our study displayed that the ratio of total changed methylation sites in XT1 was 43.18% (Table 3), much higher than those of other somatic hybrids. This could be due to the prominent chromosome recombination and elimination in XT1. Our results suggest that somatic hybridization-induced changes in the methylation of cytosine might represent a common response (Sheng et al. 2013; Xu et al. 2014).

Further analysis revealed that XT1 retained more methylation sites from Xushu 18 genome specific sites (37.47%) and both parental genome sites (14.46%), and tended to lose the methylation sites from *I.triloba* (4.88%) (Table 3). These non-random or biased alterations of methylation sites were coincident with changes in the genome structure. We also found that the novel sites induced by somatic hybridization in the cytosine DNA methylation (14.19%, Table 3) were much more frequent than the incidence in genome DNA sequences (6.96%, Table 1). These results suggest that changes of DNA cytosine methylation induced by somatic hybridization might not depend on the DNA sequence changes.

Effect of parent-biased changes in genome structure on cytoplasmic genome compositions

Because of the novel variability in the nuclear and cytoplasmic DNA induced by somatic hybridization, studies on the inheritance of mitochondrial and chloroplast DNA of somatic hybrids are very important (Iovene et al. 2007). Previous studies have revealed that recombination and (or) coexistence of mitochondrial DNA from both parents is an universal phenomenon; on the contrary, chloroplast DNA often has random and biased segregation, recombination and mixed populations of chloroplast DNA have scarcely ever been detected (Li and Sink 1992; Cardi et al. 1999; Zhou et al. 2001; Xiang et al. 2004). To date, the mechanism of the cytoplasmic genome compositions of somatic hybrids is not still determined. Collonnier et al. (2001) proposed that the difference in the rate of organelle replication might lead to the biased chloroplast segregations. Chen et al. (2013) showed that lower frequency of cpDNA recombination than mtDNA in somatic hybrids between *Solanum tuberosum* and *S. chacoense* might be due to the mitochondria structure which contains some master circles and subgenomic circles, or a large of organelle replication.

In our study, electrophoresis of PCR products amplified by cpDNA and mtDNA universal primers revealed that XT1 had the cpDNA and mtDNA banding patterns completely identical to the cultivated parent Xushu 18, which possibly indicated that only Xushu 18 contributed to the cytoplasmic components (Fig. 2). Similar findings have been documented by Xu et al. (2004), who found that all the regenerated plants of *Microcitrus papuana* Swingle and sour orange (*Citrus aurantium*) had banding patterns completely identical to the embryogenic parent *M. papuana* for cpDNA and mtDNA, and no polymorphic

bands from the leaf parent could be detected. In this study, GISH karyotype and AFLP analyses showed that XT1 and Xushu 18 had nearer genetic relationship, and enormous DNA sequences of *I. triloba* were eliminated. We speculate that parent-biased changes in genome structure might increase the frequencies of nuclear-cytoplasmic interactions in the somatic hybrid, and hence could make the cytoplasmic genome of *I. triloba* gradually disappear in subsequent cell divisions.

Effect of parent-biased changes in genome structure and DNA methylation on phenotypes

One of the primary targets of somatic hybridization between sweetpotato and its wild relatives is gaining the somatic hybrids with storage roots and good agronomic traits. In the present study, XT1 in the morphology was similar to Xushu 18, and it not simply could produce storage roots but also had better drought tolerance, and also displayed several new traits. We think that the beneficial traits of XT1 presented here are possibly due to the genetic and epigenetic changes caused by protoplast fusion. XT1 had 90 chromosomes, consistent with those of sweetpotato ($2n=6x=90$). Furthermore, in XT1 the proportions of Xushu 18-specific genome compositions and DNA methylation sites were much larger than those of *I. triloba*. This might result in the morphological similarity of XT1 to Xushu 18.

Drought tolerance assay showed that XT1 had better growth, more developed root system and higher photosynthetic activity when suffered from severe drought, compared with the cultivated parent Xushu 18 (Figs. 3,4). Meanwhile, the transcriptome analysis of XT1 and its parents indicated that 729 DEGs were commonly expressed in XT1 and *I. triloba* (Fig. 5 B). Further analysis revealed that the genes of PI and ABA signalling pathways, proline biosynthesis, ROS-scavenging and stress responses were up-regulated under drought stress (Fig. 6). It is thought that these modifications of morphology and physiology result from genetic recombination in XT1, resulting in its better drought adaptation.

In addition, epigenetic regulation, as a change in cytosine methylation, plays a central role in genome organization, gene expression and plant growth and development (Joyce and Cassells 2002; Rangwala and Richards 2004; Cai et al. 2007). Our results suggest that the storage root nature, increased drought tolerance, high quality and several new traits of XT1 may be partly due to genetic and epigenetic variations.

In conclusion, a novel interspecific somatic hybrid, XT1, which produced storage roots and exhibited high drought tolerance and quality, was obtained through protoplast fusions between sweetpotato cv. Xushu 18 and its wild relative *I. triloba*. Its genetic and epigenetic variations were clarified. Our study not only shows the significance of somatic hybridization in the genetic improvement of sweetpotato but also provides valuable materials and knowledge for investigating the mechanism of storage root formation in sweetpotato.

Declarations

Author contribution statements QL, LJ and HZhai conceived and designed the experiments. LJ and YY performed the experiments. LJ, YY , SH, GX, NZ, HZhang and SG analyzed the data. QL, LJ and YY

wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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Tables

Table 1 The nuclear genome components of XT1

Type	Inherited bands		Changed bands			
	No.	%	Altered bands		Novel bands	
			No.	%	No.	%
Both parental genome sharing bands	373	18.41	30	1.48		
K121 genome specific bands	60	2.96	624	30.80		
X18 genome specific bands	629	31.05	169	8.34		
Total	1062	52.42	823	40.62	141	6.96

Table 2 Comparison of DNA methylation levels between XT1 and its parents *I.triloba* (K121) and Xushu 18 (X18) based on MASP analysis

Individuals	Total sites	Unmethylated CCGG sites		Methylated CCGG sites (%)				
				Total sites	Full-methylated sites of internal cytosine		Hemi-methylated sites of external cytosine	
		No.	%	No. %	No.	%	No.	%
K121	787	419	53.24	368 46.76	272	34.56	96	12.20
XT1	1035	391	37.78	644 62.22	326	31.50	318	30.72
X18	987	414	41.95	573 58.05	293	29.69	280	28.37

Table 3 DNA methylation variations of XT1

Type	Inherited methylation sites		Changed methylation sites			
	No.	%	Altered sites		Novel sites	
			No.	%	No.	%
Both parental genome sites	213	14.46	42	2.85		
K121 genome specific sites	72	4.88	322	21.86		
X18 genome specific sites	552	37.47	63	4.28		
Total	837	56.82	427	28.99	209	14.19

Table 4 Comparison of the main nutritional compositions between XT1 and its cultivated parent Xushu 18 (X18)

ndividuals	Dry matter content (% FW)	Soluble sugar content (% DW)	Starch content (% DW)	Protein content (% DW)	Fiber matter content (% DW)	Carotenoid content (mg $100g^{-1}$ FW)
ζT1	30.57 ± 1.33a[1)]	11.26 ± 2.15a	57.62 ± 4.37b	8.33 ± 1.69a	0.86 ± 0.02a	0.22 ± 0.01a
ζ18	34.13 ± 1.99a	7.92 ± 1.31b	69.42 ± 4.37a	5.21 ± 0.08b	0.89 ± 0.06a	0.04 ± 0.00b

[1)] Data are presented as means ± SE (n=3). Different letters represent significant difference at $P<0.05$.

Figures

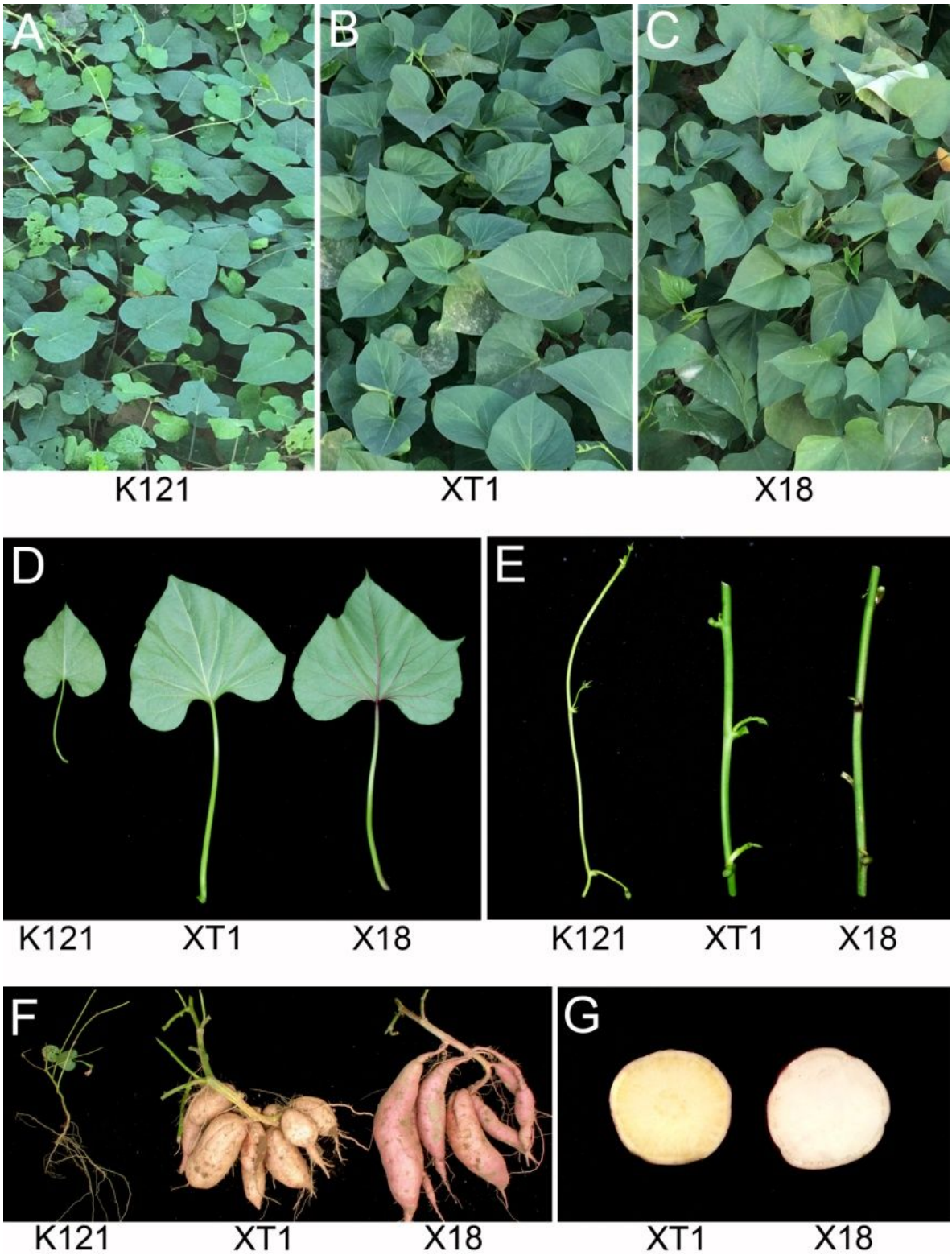

Figure 1

Morphological characteristics of the somatic hybrid XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18) grown in a field. **A-C** Plants. **D** Leaves. **E** Stems. **F, G** Storage roots

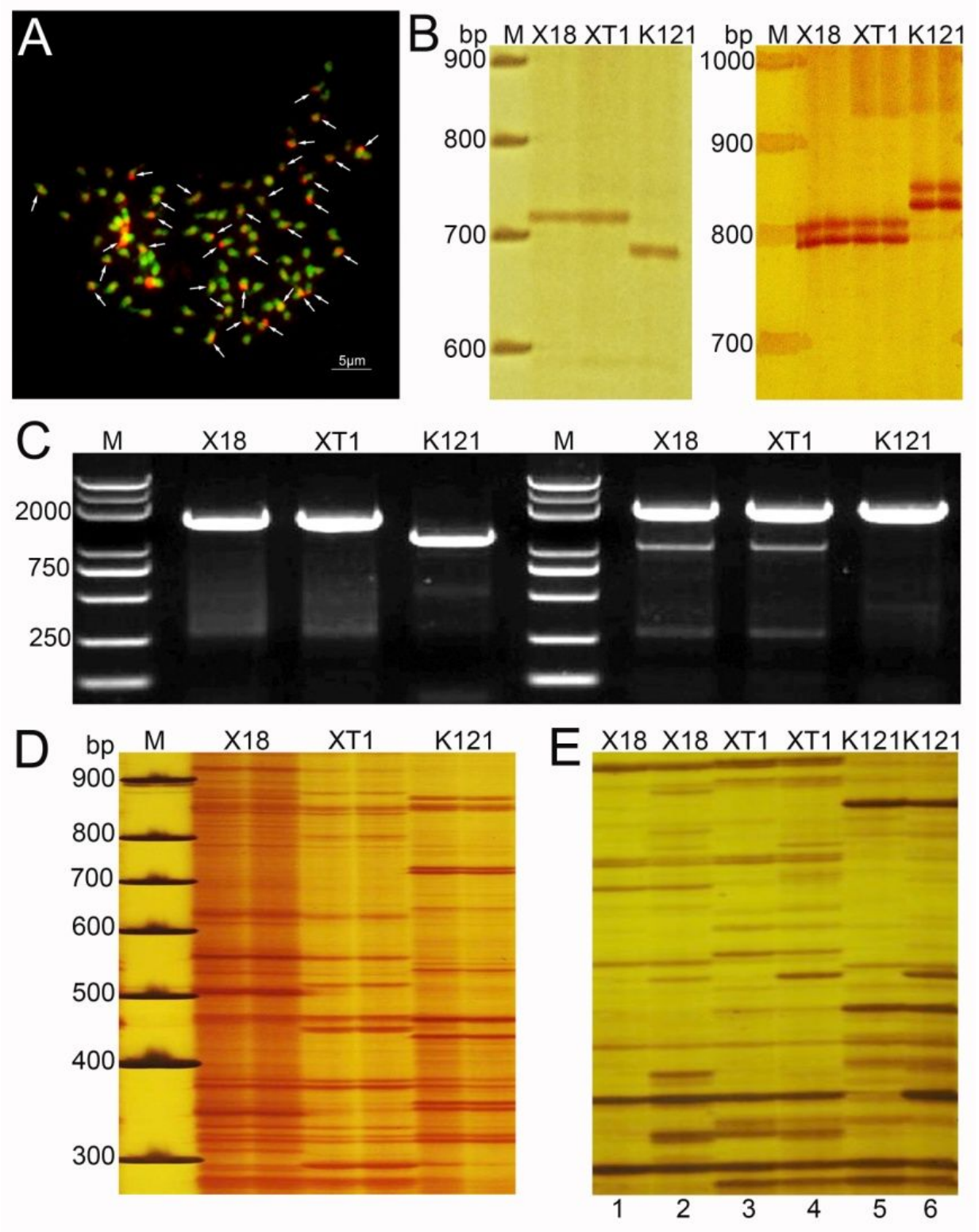

Figure 2

Genome analysis of the somatic hybrid XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18). **A** GISH karyotype of XT1. Red color indicates chromosomes or their fragments of *I. triloba* and green color indicates chromosomes or their fragments of Xushu18. Arrows indicate recombinant chromosomes. **B** Chloroplast genome analysis by primers NTCP3 (*left*) and ccSSR-13 (*right*), respectively. *M* 100 bp DNA ladder. **C** Mitochondrial genome analysis by primer sets nad1-exonB/nad1-exonC (*left*) and rpS14/cob

(*right*), respectively. *M Trans*® 2K plus DNA marker. **D** AFLP analysis by primer combination *EcoR*I-CTA/*Mse*I-GCT. *M* 100 bp DNA ladder. **E** MSAP by primer combination *EcoR*I-ACG/*Hpa*II (*Msp*I)-TCG showing different types of locus specific DNA methylation in XT1 and both parents. *Lanes 1, 3* and *5* are amplification results of *Hpa*II-digested genomic DNA. *Lanes 2, 4* and *6* represent *Msp*I-digested results

Figure 3

Drought tolerance evaluation of the somatic hybrid XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18). **A** Responses of XT1, K121 and X18 cultured on MS medium with no stress (Normal) and 20% PEG 6000 for 4 weeks, respectively. **B** Responses of XT1, K121 and X18 grown in transplanting boxes for 8 weeks under normal condition (Normal) and drought stress (Drought), respectively. **C** Phenotypes and yield of XT1, K121 and X18 planted in a drought stress facility for 100 days under the normal well-watered irrigation (Normal) and drought stress, respectively. Data are presented as means ± SE (n=3), and different letters represent significant difference at $P<0.05$

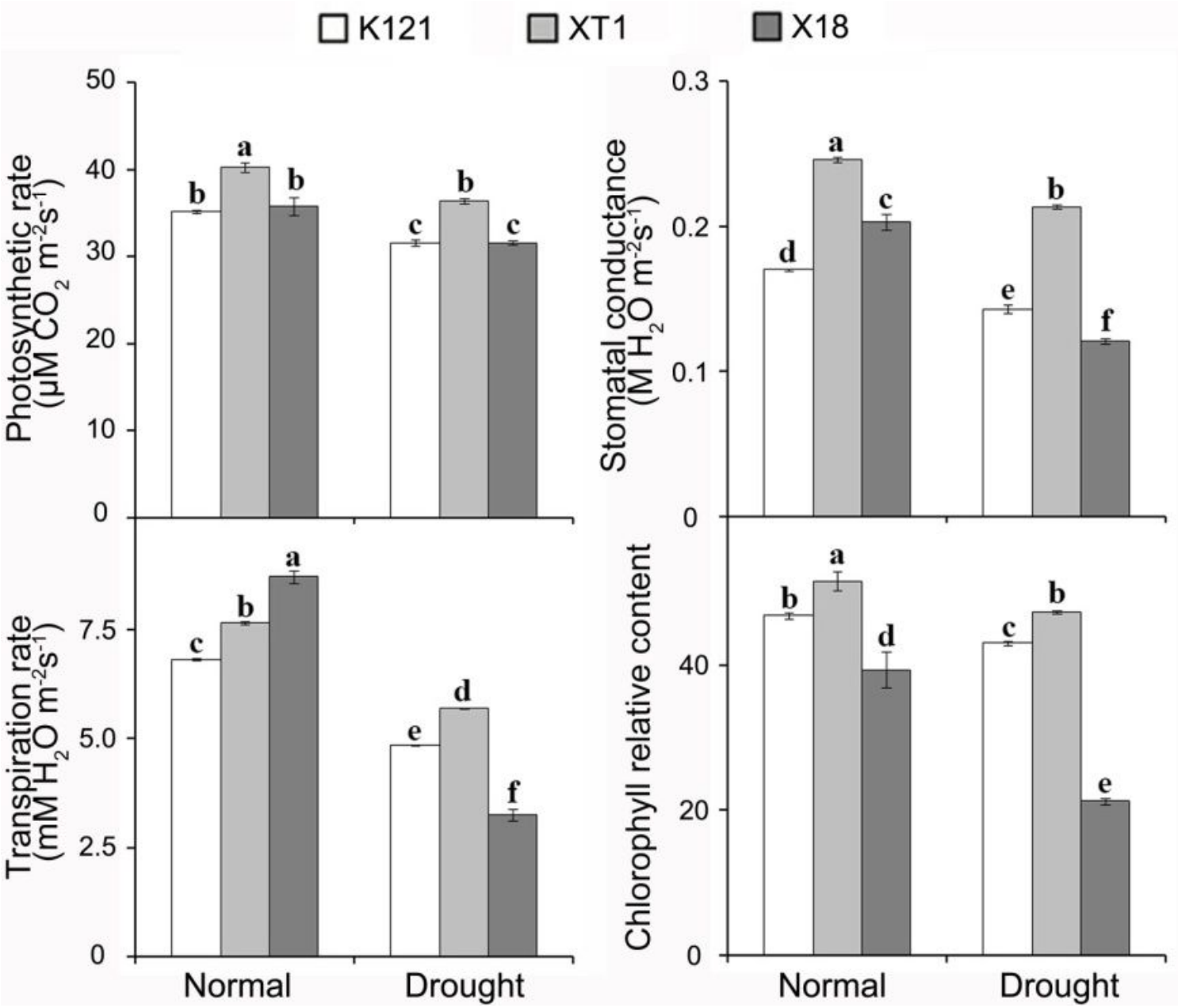

Figure 4

Photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll relative content in the leaves of XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18) planted in a drought stress facility under the normal well-watered irrigation (Normal) and drought stress (Drought), respectively. Data are presented as means ± SE (n=3) and different letters represent significant difference at $P<0.05$

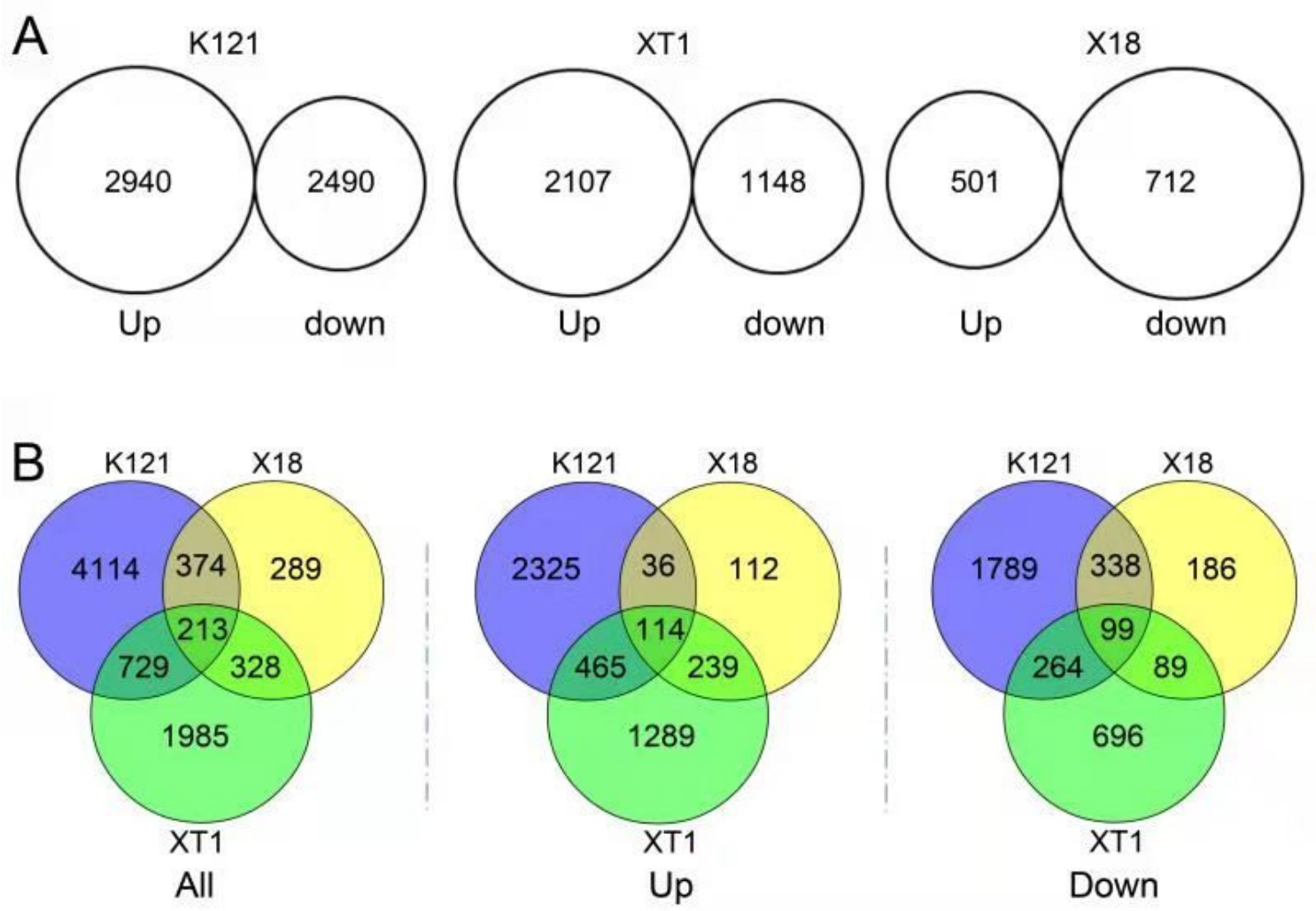

Figure 5

Analysis of global differential expressed genes (DEGs) in XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18) under drought stress. **A** DEGs in XT1 and its parents. **B** Venn diagrams showing the number and overlap of DEGs in XT1 and its parents. All, all of the DEGs; Up, up-regulated DEGs；Down, down-regulated DEGs

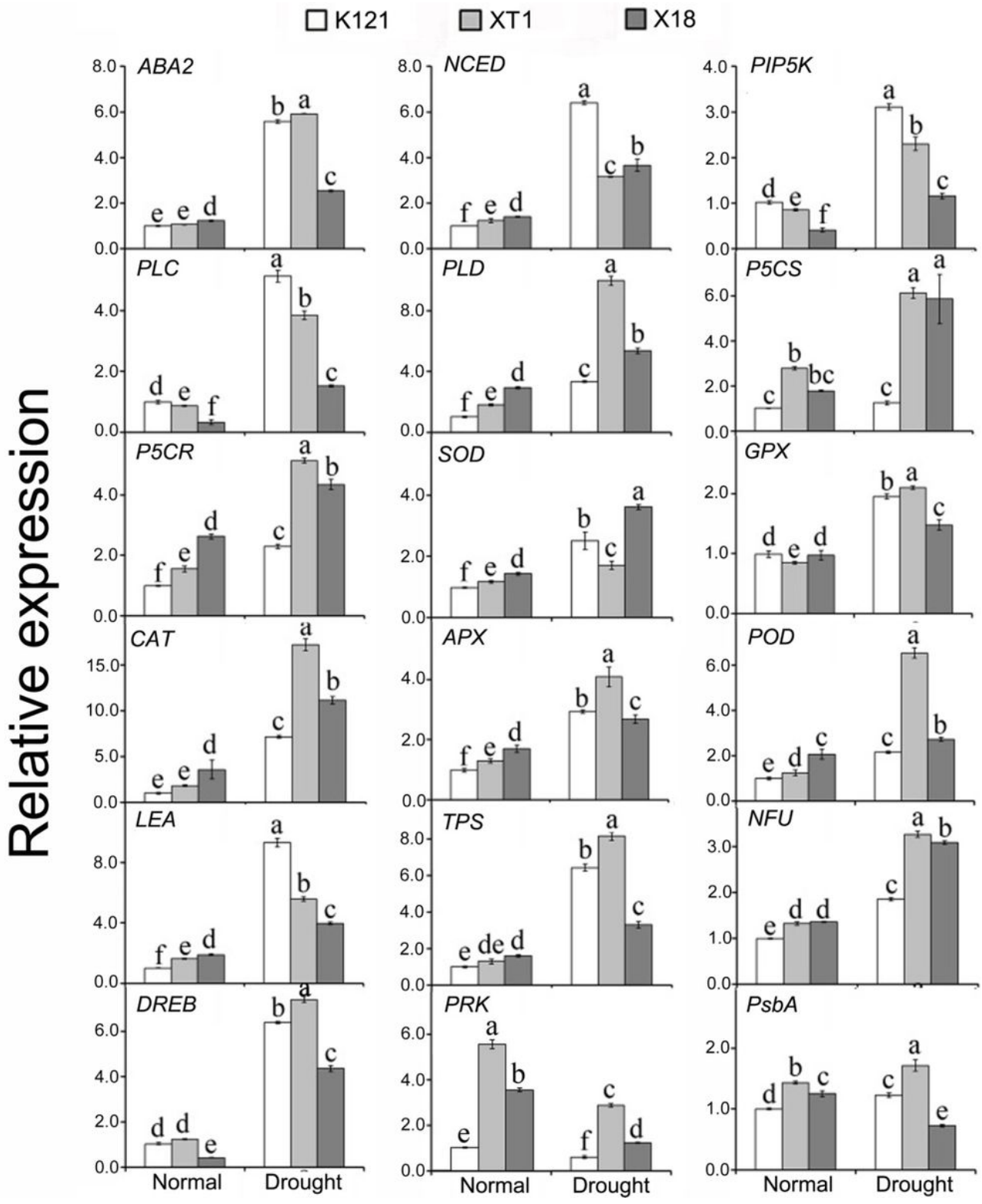

Figure 6

Relative expression level of the genes related to drought tolerance in XT1 and its parents *I. triloba* (K121) and Xushu 18 (X18). The results are expressed as relative values based on *I. triloba* without drought stress as reference sample set to 1.0. Data are presented as means ± SE (n=3) and different letters represent significant difference at $P<0.05$

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