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Identification of the GAox gene family in potato (*Solanum tuberosum* L.) and its expression analysis in response to drought stress

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Abstract

Background GAox is a key enzyme involved in GA biosynthesis pathway and plays an important role in regulating various processes in plant life cycle. However, it has not been systematic, studies have been conducted in potato, which is the world's fourth largest food crop.

Methods In this work, we systematically identified *GAox* gene family (*StGAox*) in potato by analyzing the potato genome sequence using a set of bioinformatics approaches, and analyze their physical, chemical properties, distribution on chromosomes, gene structure, conserved motifs, gene duplication events and expression patterns were analyzed.

Results The results showed that a total of 33 GAox proteins were identified and unevenly distributed on 10 chromosomes. Based on their protein structure and phylogenetic characteristics, these 33 *StGAoxes* were divided into 5 distinct subclasses. Collinearity analysis revealed that there were 5 pairs of duplicated genes in the *StGAox* gene family, and all of which evolved under purifying selection. Analysis of RNA-seq data of double haploid (DM) potatoes under different tissues, abiotic stresses and hormone treatments showed that *PG0002068*, *PG0024249* and *PG0027963* were higher expressed in leaves, *PG009427*, *PG0026762*, *PG0009021* and *PG0021095* were higher expressed in tubers, *PG2003479*, *PG0024249*, *PG0005698*, and *PG0009021*were higher expressed in shoots than those of other tissues. In addition, the expression of *PG0002068*, *PG2003479*, *PG0032156*, *PG0024249*, and *PG0021292* were up-regulated under mannitol and drought stress.

Conclusions Comparative genome-wide analysis of *StGAox* genes and their expression analyses revealed that members of this family may be involved in tissue-specific developmental and abiotic stress responses.

Keywords Potato, Gibberellin dioxygenase, Expression profiles, Drought stress

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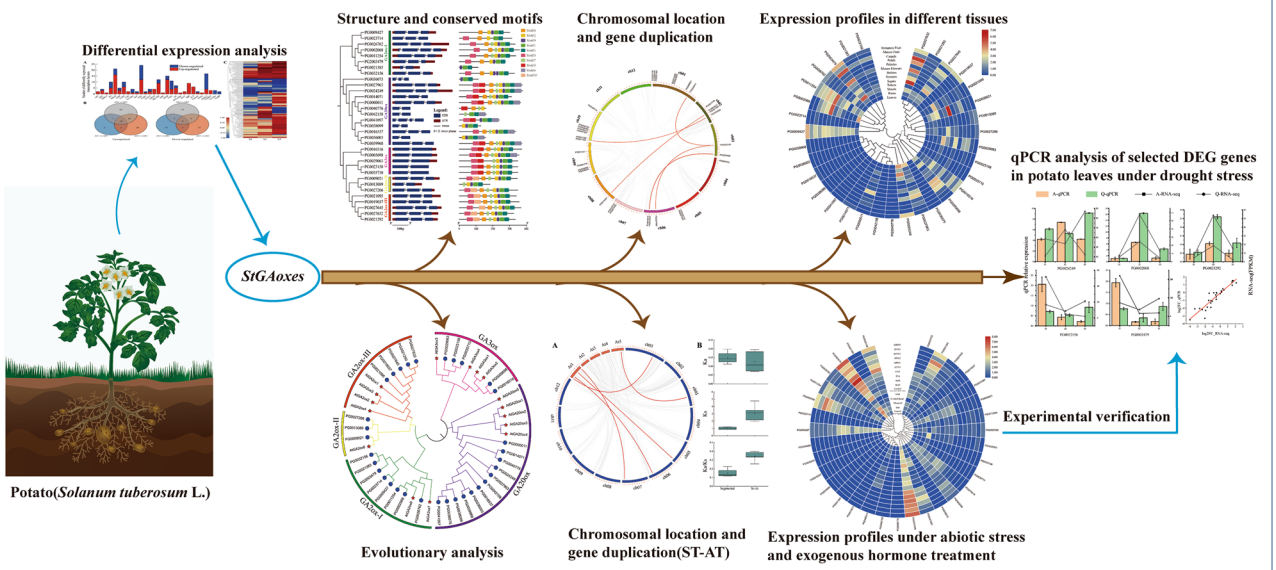
Graphical Abstract



Introduction

Gibberellin (GAs) is a kind of diterpenoid plant hormone, which is involved in physiological processes at various stages of plant growth cycle, such as stem elongation, root development, leaf extension, root hair growth and other life processes [1–4]. Numerous studies have certificated that dwarfism has been linked to deficiencies in gibberellin (GA) levels or signaling [5]. GA levels are widely manipulated in agriculture to stimulate fruit growth in seedless grapes, delay fruit senescence in oranges and lemons, increase fruit setting in mandarins, apples and pears, increase stem elongation in sugarcane, or decrease growth in cotton, canola and apple [6]. Furthermore, exogenous application of gibberellins or overexpression of gibberellin synthesis genes in fruits to increase endogenous gibberellin content can delay fruit ripening and senescence [7].

The biosynthesis pathways of GAs in higher plants have been clarified, and their essential elements have been found [8, 9]. The synthesis of GAs is catalyzed by a series of enzymes, among which there are three key GA oxidases, namely GA20ox, GA3ox and GA2ox, each GA oxidase is independently encoded by a small gene family, and their domains are highly homologous [10]. Overexpression of *GA20ox* not only can promotes internode elongation, but also contributes to leaf expansion in plant [11, 12]. However, unlike this, overexpression of *GA20ox* in zucchini resulted in dwarfing of the plant [13]. GA2ox can passivate the action of gibberellin and make gibberellin lose biological activity in plants. GA3ox can synthesize bioactive gibberellins [14]. Some research has also explored the expression of a regulatory gibberellin oxidation gene under exogenous hormone stimulation and abiotic stress, indicating that gibberellin oxidation gene not only regulates development and growth but also responds to various abiotic stresses [15, 16]. For instance, in *Camellia lipoensis*, transgenic plants which overexpressing *ClGA2ox1* or *ClGA2ox3* had poor growth and curled leaves [17]. Overexpression of *OsGA2ox5* increased the ability of rice seedlings to withstand salt stress, whereas the plant height was significantly reduced [18]. Overexpression of *AtGA2ox7* and *AtGA2ox8* in tobacco showing dwarf phenotype with decreased gibberellin content, whereas the flower and fruit phenotypes were normal [19]. In grapes, overexpression of GA3ox severely inhibited pollen germination and pollen tube growth, resulting in seedless phenomenon [20]. In spinach, overexpression of *SoGA2ox3* resulted in smaller plant height and delayed flowering [21]. Overexpression of *StGA2ox1* in potato plants improves plant tolerance to exogenous hormones and low-temperature stresses [22].

Potato (*Solanum tuberosum* L.) is one of the major cash crops in China, and has long been favoured by human beings. In recent years, the decline in potato yield and quality has attracted the attention of many researchers and genetic and molecular biological approaches have been used to improve potato yield and quality under drought stress [23, 24]. During potato tuber storage, spraying gibberellin can shorten the dormancy period of tubers, which promotes the growth of sprouts,

accelerates the accumulation of sugar, and improves the cold-resistance response [25]. Potato tuber formation may be associated with the phasiRNA siRD29 (−) mediated *StGA3ox3* gene, which has an important role in the transition from potato stolon to tuber [26].

Although gibberellin oxidase gene has been widely explored in numerous plant species [27–29]. However, there are fewer studies on the functional characterisation of the *GAox* gene in potato. Therefore, it is important to reveal the function, evolution and expression profile of *GAox* genes in potato. In the present study, gibberellin oxidase genes were identified and analyzed by bioinformatics method. Using RNA-seq data, *StGAox* genes in doubled monoploid (DM) potato that might be involved in different organ development, as well as in response to abiotic stresses were screened. Furthermore, candidate genes in tetraploid potato cultivar that might be involved in drought stress were explored. The results provide a theoretical basis for further study of the function of GAox gene families in potato.

Materials and methods

Plant materials and treatments

The experiment was carried out in the field under rainproof shed in Dingxi Academy of Agricultural Sciences, Gansu Province, China. The drought-sensitive cultivar 'Atlantic' (A) and drought-tolerant variety 'Qingshu No.9' (Q) [30–32] were used as experimental materials. The experiment was set up with two treatments: drought stress and a well-watered control (watering provided by a drip irrigation system). The experimental design was randomized block with three replications in each group. Before seedling emergence (the first 4 weeks), all plants in both treatments were watered optimally and equally, after seedling, the plants under drought stress treatment went unwatered for whole growth period, while the plants under control treatment were still irrigated optimally during whole growth period until the foliage began to die naturally. The foliage of three pooled plants for each replicate was collected at 25 days (early flowering stage), 50 days (full-blooming stage and 75 days (flower-falling stage), respectively, and then immediately frozen in liquid nitrogen and stored in a − 80 °C freezer for RNA extraction and gene expression analysis.

Identification of *GAox* gene members in potato

The potato's genome sequence, proteins, CDS and chromosomal locations are downloaded from the Potato Genome Sequencing Consortium (PGSC, http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml) [33]. In the process of identifying potato GAox members, two conditions were used as follows: (1) GAox conservative structural domain was downloaded from the Pfam database as template sequence [34], the Arabidopsis Information Resources (TAIR, http://www.arabidopsis.org/) were used to retrieve the amino acid sequences of members of the GAox family as well [35]. HMM models were created using the HMMER 3.1(http://hmmer.org/download.html) program with a cutoff value set to 0.01, which were subsequently used to search for GAox members in potato genome database. (2) The BLASTP algorithm (threshold $E < 1^{e-5}$) was used to find GAox members in the *Arabidopsis* GAox amino acid sequence [36]. Based on previous research, the TAIR was used to obtain the AtGAox protein sequences [35]. In the end, candidate members were manually verified in the SMART website (http://smart.embl-heidelberg.de) [37] and the NCBI-CDD (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [38].

Sequence analysis and structure characterization of *StGAox* genes in potato

The gene characteristics, such as AA (amino acids), MW (molecular weight), PI (theoretical isoelectric point), and other physical and chemical properties, were obtained from online software ExPASy Protparam Server [39]. Using the CELLO website to predict subcellular localization of potato GAox protein [40]. The locally created GAox protein database was imported into the online software MEME (maximum motif = 10, width = 6 ~ 50) to examine the conserved structural domains of GAox proteins [41]. Using the online Gene Structure Display Server (GSDS2.0, http://gsds.cbi.pku.edu.cn/) tool, the exon–intron of each GAox gene was rigorously studied, finally the *StGAox* gene structure was graphically presented [42].

Chromosome distribution and gene duplication analysis

According to the position information of *StGAox* family members on chromosomes in PGSC database, MapChart software [43] was used to plot the chromosomal position map and relative distance of *StGAox*. Duplication events of *StGAox* genes were analyzed by MCScanX (http://chibba.pgml.uga.edu/mcscan2/) [44] and visualizedby Circus v0.69 [45]. Tandem duplication events of the *StGAox* gene were determined according to the following two conditions: (1) the relatively constant short sequences that are more than 70% of the length of the long sequences; (2) the similarity of two aligned sequences is greater than 70%. The genes involved in tandem duplication are those that are closely spaced out along the same chromosome segment, separated by less than 100 kb, and have fewer than five genes in common. The synonymous (Ks) and non-synonymous (Ka) values of the *StGAox* genes were computed using the KaKs

Calculator 2.0(https://sourceforge.net/projects/kakscalculator2/) [46].

Evolutionary analysis and classification of *StGAox*

ClustalW software was used to perform multi-sequence alignment of 33 newly identified GAox amino acid sequences in potato and 16 known GAox amino acid sequences in Arabidopsis. An unrooted maximum likelihood phylogenetic tree was constructed using MEGA7.0 software with a bootstrap test with 1000 iterations using the Poisson method [47].

RNA isolation and quantitative real-time RT-PCR (qPCR)

The RNA extraction kit (DP419, TIANGEN, Beijing) was used to extract total RNA of leaves. Subsequently, RNA integrity and concentration were determined using agarose gel electrophoresis and a Nanodrop ND-2000 spectrophotometer (Nanodrop Technologies, USA). Then, the first strand of cDNA was synthesized after removing genomic DNA contamination using the FastKing RT kit with gDNase (KR116, TIANGEN, Beijing). The SuperReal PreMix Plus kit (SYBRGreen FP205, TIANGEN, Beijing) purchased from Tiangen Biochemical Technology Corporation was used for qRT-PCR on CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA), and three biological replicates were performed for each treatment.

The following reaction system for qPCR was used: cDNA (50 ng/μL), forward primer 0.6 μM, lower primers 0.6 μM, 2×SYBRGreen MasterMix 10 μL, ddH_2O 6.8 μL. The following qPCR settings were used: 30 s at 95 °C, 40 cycles of 5 s at 95 °C, 30 s at 60 °C, followed by detection of the melting curve at 65–95 °C. *StEF-1α* (AB061263) [48] was used as the internal reference gene. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of *GAox* genes [49]. The primers are shown in Additional file 1: Table S1.

Expression pattern analysis of *StGAox* in potato

Using Illumina RNA-seq data, *StGAox* gene expression in DM potatoes were analyzed in different tissues (immature fruit, ripe fruit, carpels, petals, petioles, flowers, stolons, stamens, sepals, tubers, buds, roots and leaves). The expression level of *StGAox* gene family under abiotic stress (salt stress: 150 mM NaCl, 24 h; Mannitol-induced drought stress treatment: 260 μM mannitol, 24 h; Heat stress: 35 ℃, 24 h) and hormone treatment (GA_3, IAA, ABA, BAP) were also detected. TBtools software [50] was used to generate the heat map.

To further understand the expression of *StGAox* gene family under drought stress. this study used RNA-seq data, the expression of *StGAox* gene in two varieties of potato 'Atlantic' (A, drought-sensitive variety) and 'Qingshu 9' (Q, drought-resistant variety) under drought stress was analyzed at three time points (S1:25 days, S2:50 days and S3:75 days). Bowtie v2.2.9 software and edge R package was used to RNA-Seq data analysis [33].

Results

Evolutionary analysis and classification of StGAox proteins

The amino acid sequences of 16 GAox members in Arabidopsis and 33 GAox members in potato were used to construct a phylogenetic tree (Fig. 1). They were divided into five subfamilies, namely GA3ox, GA20ox, GA2ox-I, GA2ox-II and GA2ox-III. The evolutionary relationship showed that 17 GAox genes (5 *AtGAoxes* and 12 *StGAoxes*) were classified in the GA20ox. 9 *GAox* genes (4 *AtGAoxes* and 5 *StGAoxes*) were classified in the GA3ox, 10 *GAox* genes (2 *AtGAoxes* and 8 *StGAoxes*) were classified in the GA2ox-I, 4 *GAox* genes (1 *AtGAox* and 3 *StGAoxes*) were classified in the GA2ox-II, 9 *GAox* genes (4 *AtGAoxes* and 5 *StGAoxes*) were classified in the GA2ox-III.

Physicochemical properties and subcellular localization of StGAox proteins

The amino acid length and physicochemical properties of potato GAox proteins are quite different (Additional file 2: Table S2). The amino acid length ranged from 113 (PG0036075) to 378 (PG0024249), the MWs were between 12,855.48 Da (PG0036075) and 43,143.76 Da (PG0024249) and the predicted PI ranged from 5.01

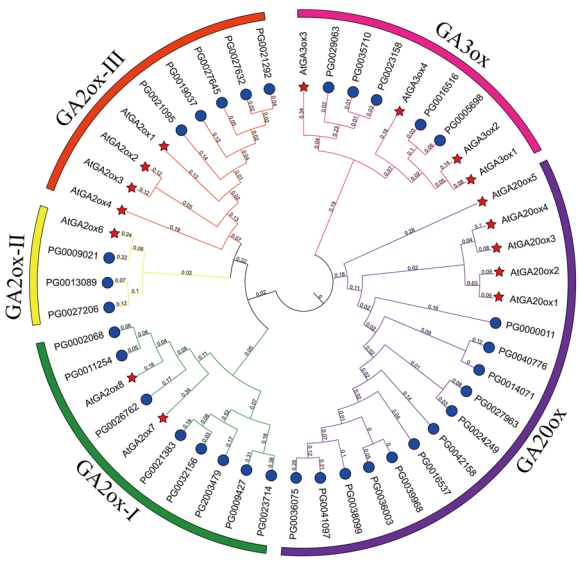


Fig. 1 Phylogenetic classification of GAoxes between Potato and Arabidopsis. The different subfamilies were marked with different colors. The blue circles represent *StGAox* genes, and the red circles represent *AtGAox* genes. Maximum likelihood method was adopted, and the bootstrap value was set to be equal to 1000

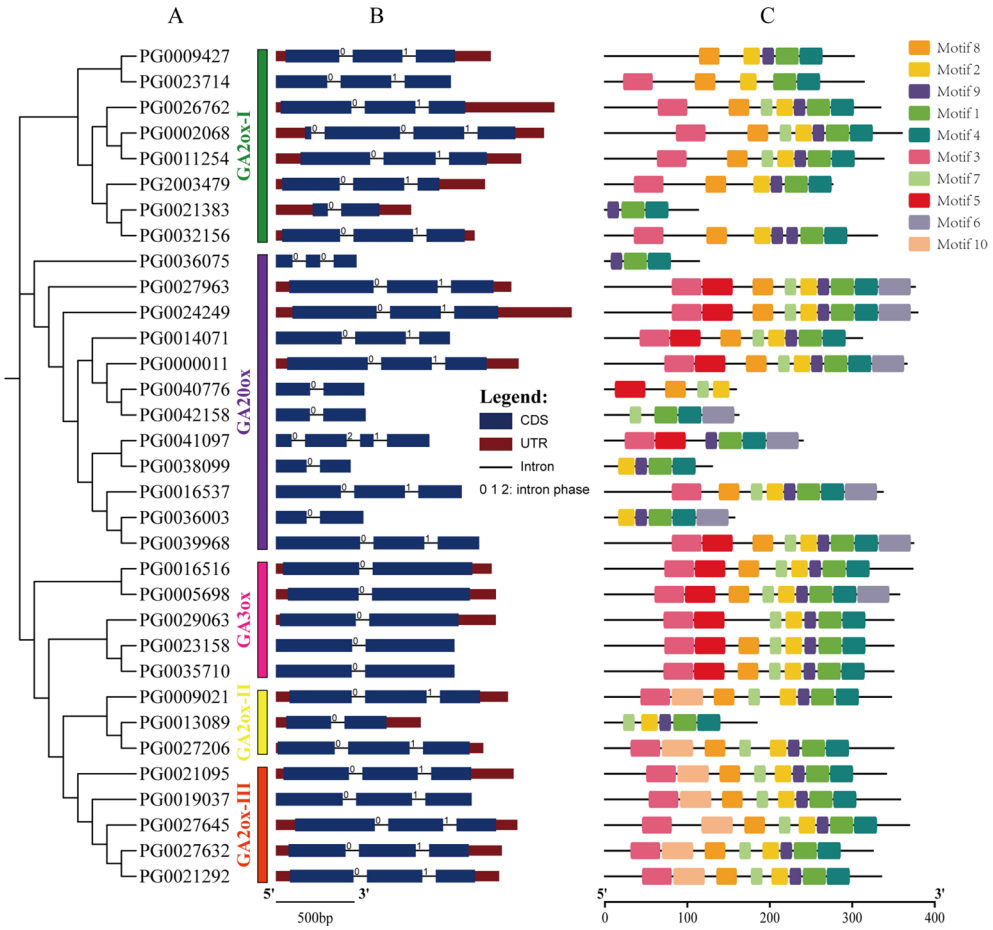


Fig. 2 Phylogenetic relationships, gene structure, and conserved motifs analysis of *StGAox*. **A** The evolutionary tree of *StGAox*. **B** Exon/intron structure of the *StGAox* gene. Blue boxes indicate exons, and black lines of the same length indicate introns. The red boxes indicate the upstream/downstream areas. The numbers 0, 1, and 2 indicate the splicing stage of the intron. **C** The distribution of conserved motifs in *StGAox*. The 10 different colored boxes represent 10 different presumed motifs

to 8.64. The full gene length ranged from 339 bp (PG0021383) to 1848 bp (PG0009427), most of the GAox members were within 1.1 kb, except 6 members longer than 1.1 kb, which were PG0027645, PG0027963, PG0024249, PG0039968, PG0009427, PG0016516, respectively. In addition, subcellular localization prediction showed that *StGAox* genes were mainly expressed in the nucleus, cytoplasm and cytoplasmic matrix, with an exception of PG0016537 being expressed in the cell membrane (Additional file 2: Table S2).

Structure and conserved motifs of *StGAox*

Same division as in Fig. 1, the unrooted phylogenetic tree was structured using amino acid sequences of 33 *GAox* in potato (Fig. 2A). The exon/intron structures of *GAox* genes were visualized by GSDS2.0, the number of exons in *StGAox* genes between 2 and 3, except for *PG0002068* and *PG0041097* with the most number of 4 exons. At the same time, the number of introns in *StGAox* gene family was relatively small with the number of introns less than 3 (Fig. 2B).

The conservative motifs of StGAox proteins were analyzed using the MEME program (v.5.0.4) (Fig. 2C). The details of the 10 motifs were referred in Additional file 3: Table S3. In the same subfamily, the conserved motifs of *StGAox* have similar structures, where, Motif 1, motif 2, and motif 4 were conservative being shared by all members. DIOX_N (PF14226) domain is common to the GA2ox-III and GA3ox subfamily and consists of motif 5 and motif 10. 20G-FeII_Oxy (PF03171) domain, contained in the GA20ox and GA3ox subfamilies, may consist of motifs 3 and 6–9. Notably, some motifs exist only in certain subfamily, such as motifs 5 and motif 6 were unique to the GA3ox and GA20ox subfamilies, and Motif 10 was unique to the GA2ox-II and GA2ox-III subfamilies. In general, the *StGAox* gene is relatively conserved in evolution.

Chromosomal location and gene duplication of *StGAox* genes

The chromosome localization analyses showed that 33 *StGAox* genes were unevenly distributed on 10 chromosomes (Fig. 3). 7 *StGAox* genes were on Chr01 and Chr10 showing the largest number of distribution, followed by those on Chr02 and Chr09 with 4 *StGAox* genes, only one *StGAox* gene was on Chr04 and Chr08, respectively. Chr11 and Chr12 had no *StGAox* gene distribution.

We analyzed the duplication events of the *StGAox* genes and found that 5 pairs of *StGAox* genes (9/33, 27.27%) were segmental duplication genes, of which, Chr03 and Chr06 encompassed 2 pairs of fragment duplication genes (*PG0024249* and *PG0027963*, *PG0005698* and *PG0016516*), the remaining fragment duplication genes were located on Chr01, Chr02, Chr08 and Chr10, respectively. Each pair of duplicated genes belonged to the same subfamily (Fig. 4, Additional file 4: Table S4). The results suggest that fragment duplication is essential in the evolution of the *StGAox* genes.

Non-synonymous substitution rate (*K*a) and synonymous substitution rate (*K*s) are a criterion for determining the positive selection pressure of repeated events. *K*a/*K*s = 1 stands for natural selection, *K*a/*K*s < 1 stands for purified selection, while *K*a/*K*s > 1 signifies positive selection [41]. The results showed that the *K*a/*K*s of the 5 pairs of duplicated genes ranged from 0.113 to 0.197, with an average of 0.149. Thus, all *GAox* duplicate genes have *K*a/*K*s < 1, which means that most of *GAox* genes had evolved under the effect of purifying selection. In addition, we analyzed the duplication events of *GAox* genes between potato and *Arabidopsis*, the results indicated 5 pairs of *GAox* genes were homologous between potato and *Arabidopsis*, and the Ka/Ks ranged from 0.071 to 0.196, with an average of 0.126 (Fig. 4, Additional file 5: Table S5).

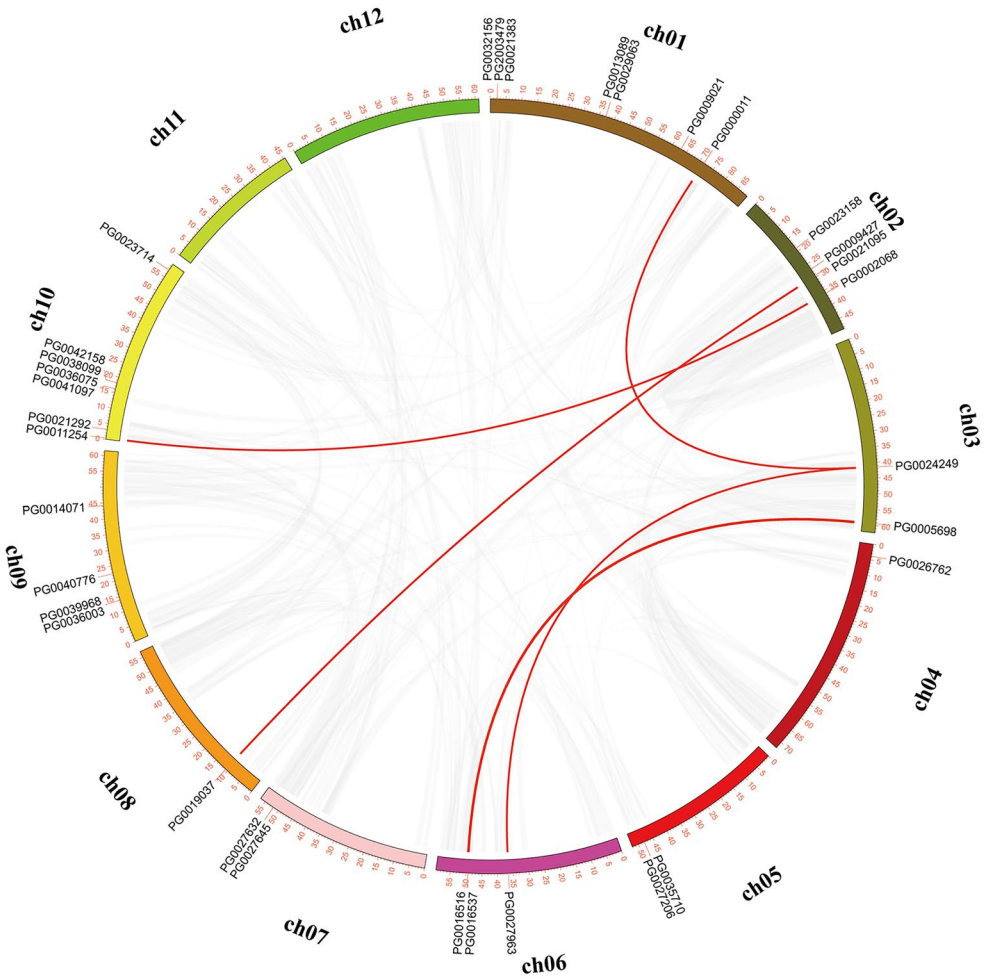


Fig. 3 The segmental replication events of *StGAox* genes in potato. Gray lines indicate all synteny blocks in the potato genome and red lines indicate segmental duplication of *StGAox* genes. The number of chromosomes is shown at the bottom of each chromosome

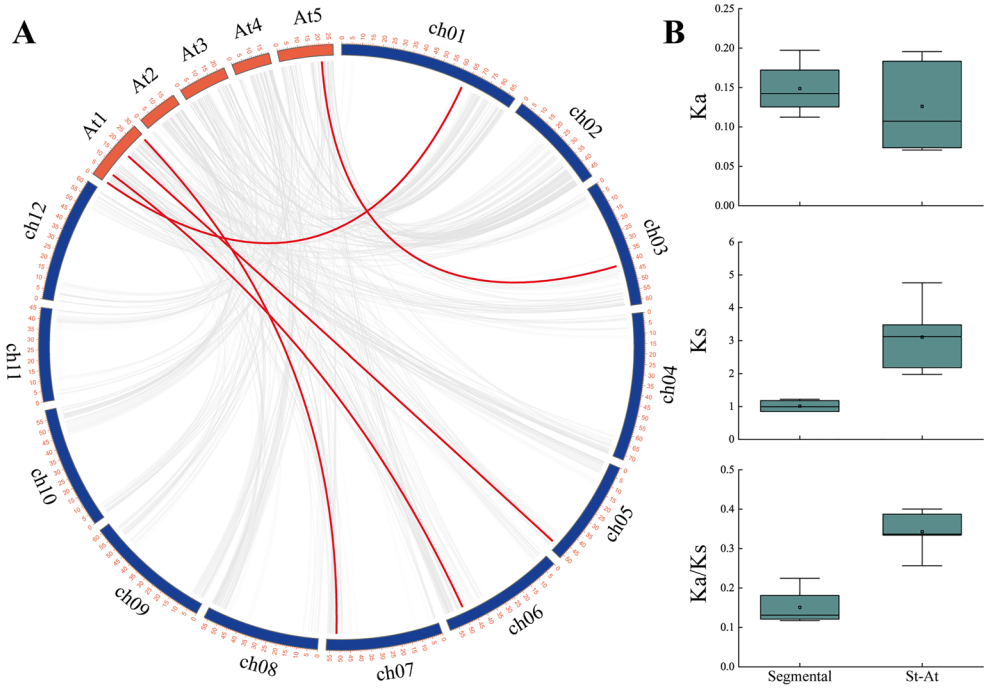


Fig. 4 Comparative physical mapping showing the homologous relationships of *StGAox* genes with Arabidopsis (**A**). *B* is the average values of Ka, Ks, and Ka/Ks. The horizontal axis represents segmental duplication, the duplication between potato and Arabidopsis (St-At) in **B**

Expression profiles of *StGAox* genes in different tissues of DM potato

Using RNA seq data from 13 tissues (immature fruit, mature fruit, carpels, petals, petioles, flowers, stolons, stamens, sepals, tubers, shoots, roots and leaves) of DM potato downloaded from PGSC, the expression patterns of *StGAox* in different tissues were analyzed. it is clear that 8 *StGAox* genes (*PG0009427*, *PG2003479*, *PG0032156*, *PG0027963*, *PG0016516*, *PG0027206*, *PG0027645*, *PG0027632*) were expressed in all tissues, 9 *StGAox* genes (*PG0036075*, *PG0042158*, *PG0041097*, *PG0038099*, *PG0039968*, *PG0036003*, *PG0016537*, *PG0040776*, *PG0014071*) were not expressed in all tissues. In addition, several *StGAox* genes showed tissue-specific expression patterns, such as *PG0009021* was only highly expressed in stolons, *PG0027632* was highly expressed in carpels and sepals, 4 genes (*PG0000011*, *PG0005698*, *PG0011254*, *PG0002068*) were specifically expressed in mature flowers, *PG0021095* was specifically expressed in tubers, 2 genes (*PG2003479*, *PG0024249*) were expressed specifically in carpels and shoots (Fig. 5).

Expression profiles of *StGAox* genes under abiotic stress and exogenous hormone treatment

To investigate the function of the *StGAox* gene, the expression pattern of *StGAox* was analyzed under abiotic stress and hormone treatment in this study. The results showed that 11, 10 and 9 genes were differentially expressed under salt, mannitol and heat treatment, respectively, compared to the control group (Fig. 2). Of which, 6 genes (*PG0009427*, *PG0024249*, *PG0016516*, *PG0005698*, *PG0009021*, *PG0027632*) were up-regulated, 2 genes (*PG0027645* and *PG0027963*) were differentially expressed only in response to heat stress. In addition, 10 genes (*PG0036075*, *PG0014071*, *PG0040776*, *PG0042158*, *PG0041097*, *PG0038099*, *PG0016537*, *PG0036003*, *PG0039968*, *PG0019037*) were not expressed under three stress treatments (FPKM = 0).

The expression patterns of *StGAox* genes under different hormone treatments were also examined (Fig. 6). The results showed that the expression profile of *StGAox* gene was complex under GA_3, ABA, BAP, and IAA treatments. Under BAP treatment, no genes were up-regulated, the expression levels of 7 *StGAox* genes (*PG0009427*, *PG2003479*, *PG0024249*, *PG0016516*, *PG0005698*, *PG0011254*, *PG0021383*) were down-regulated, among them, the expression level of *PG0021383* was down-regulated by 5 times compared to the control. Under ABA treatment, 3 genes (*PG0009021*, *PG0021292*, *PG0016516*) were up-regulated, among other, *PG0021292* has the highest expression level, which was 4.2 times than the control. 5 genes (*PG0021383*, *PG0032156*, *PG0024249*, *PG0027632*, *PG0005698*) were down-regulated, among other, *PG0005698* was

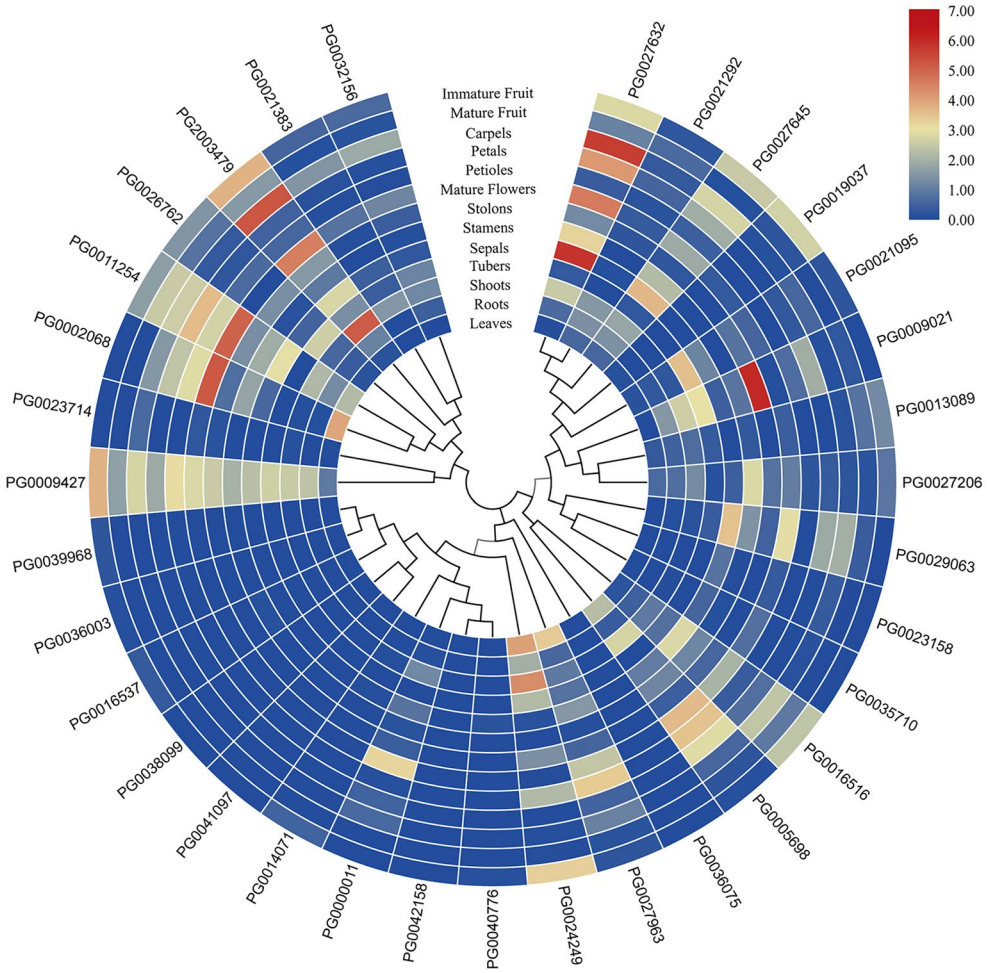


Fig. 5 Expression profile analysis of *StGAox* genes in different tissues (carpels, petals, flowers, immature fruits, mature fruits, stolon, tubers, leaves, petioles, roots, and buds) based on the transcriptome data. The color scale was plotted using the log2 mean of FPKM of each gene

down-regulated three times compared to the control. Under IAA treatment, 3 genes (*PG2003479*, *PG0009427, PG0016516*) were up-regulated, the expression levels were 1.23, 1.21, and 1.53 times higher than those of the control, respectively. Under GA_3 treatment, 5 genes (*PG2003479*, *PG0032156*, *PG0009021*, *PG0021292*, *PG0027632*) were up-regulated, among other, the most up-regulated was *PG0021292*, which was 2.7 times higher than the control. 5 genes (*PG0009427, PG0024249, PG0005698, PG0021383, PG0032156*) were down-regulated, the expression level of *PG0005698* was down-regulated by 3 times compared with the control.

This study conducted that drought stress on A and Q to further examine if the *StGAox* genes responded to drought stress. The results indicated that 9 *StGAox* genes were not expressed in A and Q (FPKM = 0), 12 *StGAox* genes were expressed less than 1 in A and Q. 11 *StGAox* genes were up- or down-regulated (FPKM ≥ 1, and $|\log_2 FC| \geq 1$) in Q under drought stress, of which, 4 genes were differentially expressed in three periods, 7 genes were differentially expressed at 25 d (S1), 8 genes were differentially expressed at 50d (S2), and 9 genes were differentially expressed at 75 d (S3). *PG0021292*, *PG0024249* and *PG0002068* were not significantly expressed in A and Q at S1 stage, but were highly expressed in Q at S2 and S3 stages. *PG0032156* and *PG2003479* were down-regulated in Q at S1 and S2 stages, but up-regulated in S3 by 90.2% and 80.6%, respectively. By analyzing the data, it was found that the differential expression profiles of 5 genes (*PG0024249*, *PG0002068*, *PG0021292*, *PG0032156* and *PG2003479*) genes increased at first and then decreased in Q.

Combined with the analysis of RNA-seq data under mannitol stress and drought stress, it was found that 10 *StGAox* genes were differentially expressed in DM under mannitol stress, of which 5 genes (*PG2003479*, *PG0002068*, *PG0024249*, *PG0032156*, *PG0021292*) were differentially expressed in A and Q under drought stress

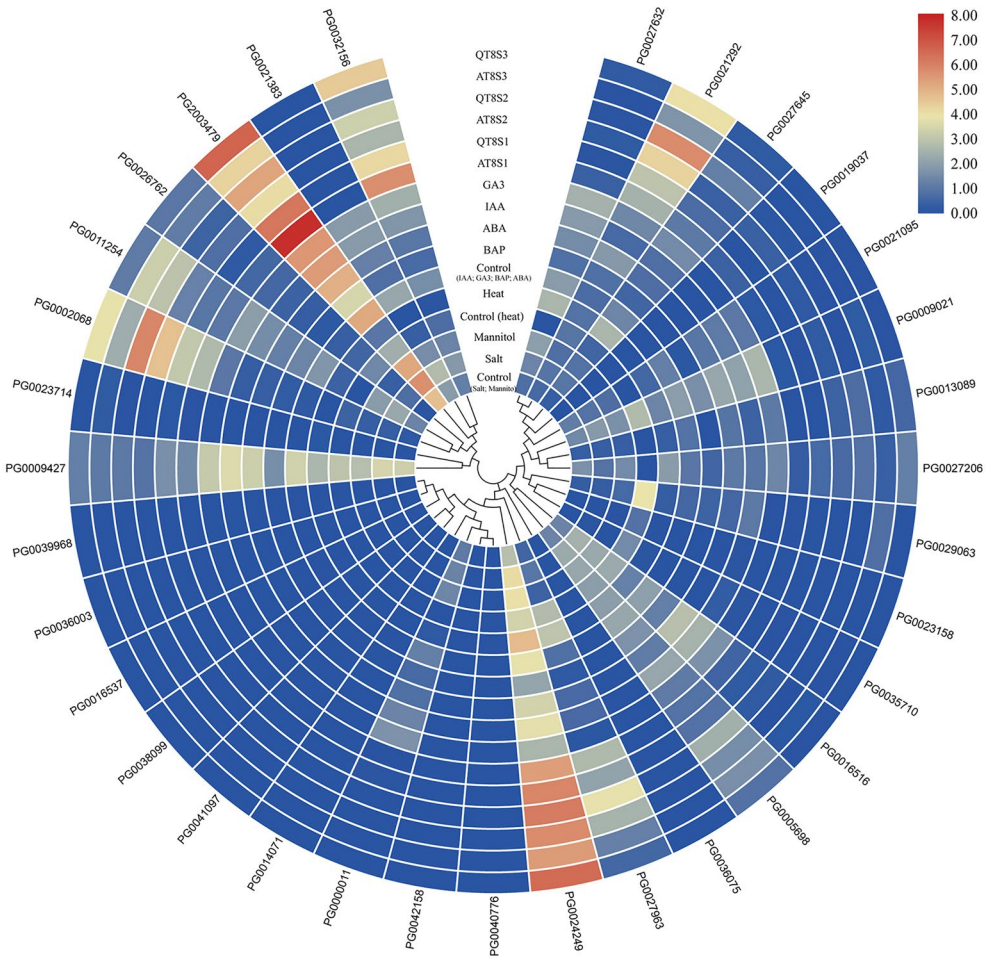


Fig. 6 The expression profiles of *StGAox* genes under hormone and abiotic stress. The expression profiles of *StGAox* genes under salt, mannitol, heat stress and hormone (GA_3, IAA, ABA, BAP) treatments in DM potato, and under drought stress for 25 days (S1), 50 days (S2), 50 days (S3) in the drought-sensitive cultivar Atlantic A and drought-tolerant cultivar Qingshu NO. 9 (Q). The color scale was plotted using log2 mean of FPKM of each gene

(Q compared with A), 3 genes (*PG2003479*, *PG0032156*, *PG0024249*) showed similar expression patterns under mannitol stress and drought stress. In addition, *PG0011254* displayed an opposing expression pattern under the stresses of mannitol and drought, with expression up-regulated under the former treatment and down-regulated under the latter treatment (Q compared with A).

To confirm the accuracy of the RNA-seq data, we chose 5 *StGAox* genes with considerably increased expression in Q under drought stress for quantitative real-time PCR (qPCR). The results showed that although there were some differences between qPCR expression patterns and RNA-seq data, the overall trend was basically consistent (Fig. 7). The linear relationship between the RNA-Seq data and qPCR is $y=0.853x-0.06325$, $R^2=0.853$. The result showed a high correlation between RNA-seq and qPCR, which further indicated that RNA-seq data were true and reliable.

Discussion

In *Arabidopsis*, rice, cucumber and other plants, some *GAox* genes play important roles in abiotic stress response [11, 16, 30, 51]. The unrooted evolutionary trees were constructed to fully understand the phylogenetic relationship between potato and *Arabidopsis* GAox family [52]. However, the response of *GAox* genes to hormonal and abiotic stresses has not been reported in potato. Therefore, we identified the potato GAox gene family and analyzed the expression of these genes in different tissue and under hormonal and abiotic stresses.

Analysis of the evolutionary characterisation of the gibberellin dioxygenase gene family showed that the GA2ox, GA3ox and GA20ox genes are distributed in different

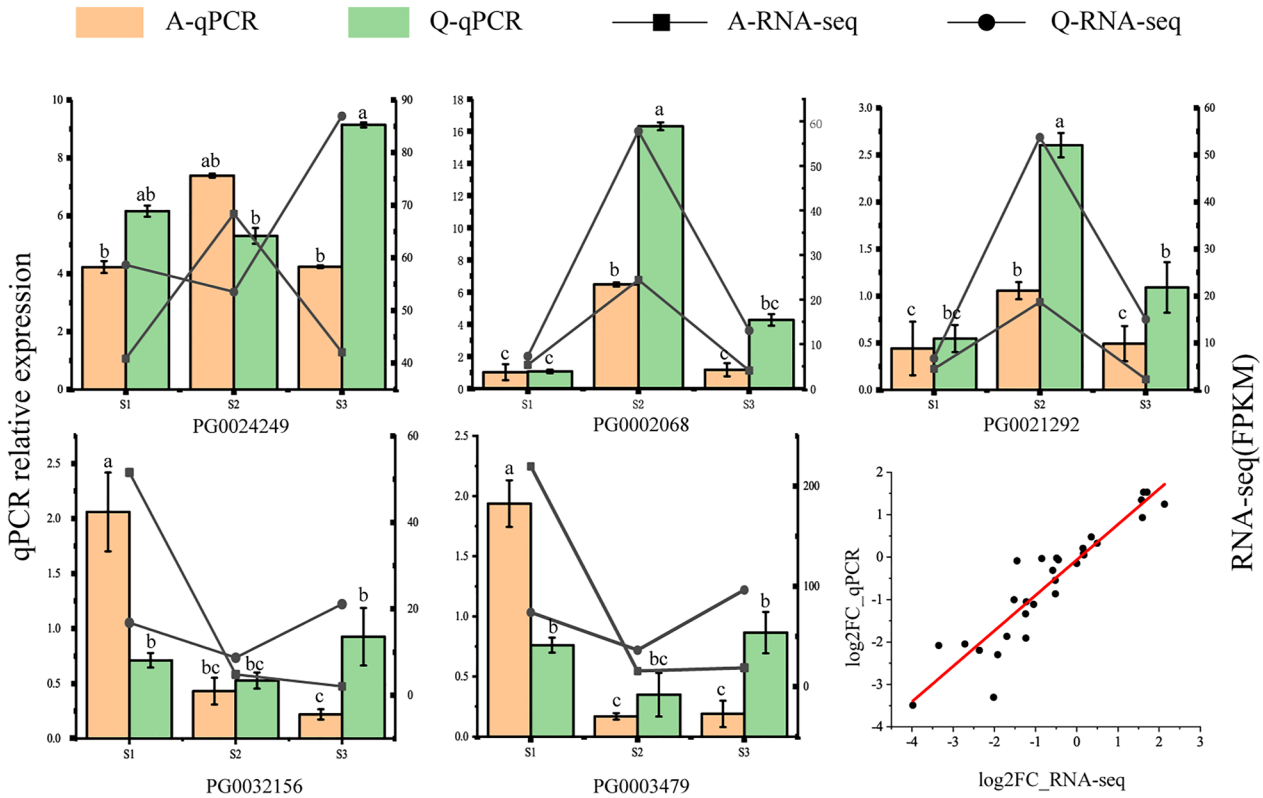


Fig. 7 The qPCR analysis of selected DEG genes in potato leaves under drought stress. The qPCR expression analysis of five *StGAox* genes in drought-sensitive varieties (Atlantic, A) and drought-tolerant varieties (Qingshu No. 9, Q) was performed on 25 days (S1), 50 days (S2), 75 days (S3) of drought treatment. Data represent the mean of three independent experiments ± standard error of the mean. Standard errors were shown as bars above the columns. Different letters above bars denote significant difference at $P < 0.05$

subfamilies, with *OsGA2ox1*, *OsGA2ox3*, *OsGA2ox4*, *OsGA2ox7*, *OsGA20ox1*, and *OsGA20ox4* were found in rice [53]. *AtGA2ox1*, 2 and 6, *AtGA3ox1*, 2 and 4 were found in Arabidopsis thaliana [29]. and *StGA2ox1* were found in potato, we classified the GAox gene family into five distinct subfamilies: GA2ox-I, GA2ox-II, GA2ox-III, GA20ox and GA3ox [26].

Repeat events in the gibberellin dioxygenase gene family suggest that 75% of *GAox* genes in the soybean genome undergo fragment replication events. In cucumber, the tandem or fragment repeat events are crucial in the expansion of the GA2ox and GA3ox gene families [54]. In this research, five pairs of fragment duplication genes were determined, it is suggested that fragment duplication is one of the main reasons for the evolution of the *GAox* gene family in potato. it is worth noting that replication events have been reported in other plants, but different amounts, such as eight segment duplicate pairs were found in soybean, three pairs of fragment replicating genes and two pairs of fragment replicating genes were found in rice and *Arabidopsis thaliana*, respectively [55]. We identified five pairs of segmental duplication genes, which suggests that segmental duplication is the main way in which plant gene families evolve [56].

In our work, we found that several segmental repeat genes displayed different expression patterns under abiotic stresses. For instance, *PG0024249* and *PG0027963* were a pair of duplication genes. *PG0024249* was up-regulated in drought-tolerant cultivar Q at flower-falling stage under drought stress, but *PG0027963* was not expressed under drought stress (FPKM = 0). Similar reports have also been reported in *Arabidopsis thaliana* and rice [57]. *AtGA3ox2* and *AtGA3ox4* are a pair of tandem repeating genes in *Arabidopsis Thaliana*, *AtGA2ox2* was mainly expressed in flowers, whereas *AtGA2ox4* was mainly expressed in roots [58, 59]. *OsGA20ox2* and *OsGA20ox4* in rice were a pair of fragment repeating genes, *OsGA20ox2* was up-regulated under salt stress, but *OsGA20ox4* was not expressed [60, 61]. In the case of gene duplication, these genes may lose their original functions and gain new ones to enhance plant fitness [62].

PG0002068 and *PG0011254* were a pair of fragment. Duplication genes located in the same cluster of GA2ox-I,

and they were specifically expressed in mature flowers and petioles. *PG0027963* and *PG0024249* are a pair of fragment duplication genes located in the same cluster of GA20ox, which were specifically expressed in petioles, the results suggest that these duplicated genes are associated with petiole growth and development. A similar expression pattern is found in segmental repeat genes in soybean and rice [63]. *GmGA2ox5* and *GmGA2ox8* were a pair of fragment repeating genes, both of which are expressed specifically in roots [64]. *OsGA20ox1 and OsGA20ox3* in rice were a pair of fragment repeating genes, *OsGA20ox1* is expressed specifically in flowers and *OsGA20ox3* is expressed specifically in carpels [65]. In conclusion, gene duplication provides a large number of functional genes for potato growth and development [66].

Genes in the same cluster have similar functions [67]. *AtGA2ox1* gene was a highly expression level in roots and stems in *Arabidopsis thaliana*, which was the key to the growth of *Arabidopsis thaliana* plants [68]. *PG0027645*, which was divided into the same subfamily as *AtGA2ox1*, was specifically expressed in mature flowers and immature fruits of potato. This indicates that *PG0027645* have to do with the growth and development of potatoes.

Under hormone (BAP, ABA, IAA and GA_3) treatments, there were 7, 6, 4, and 10 *StGAox* genes differentially expressed, respectively. among them, *PG0021383* of the GA2ox subfamily and *PG0024249* of the GA20ox subfamily were down-regulated under four hormone treatments. It is worth noting that under BAP stress, none of the *StGAox* genes were up-regulated. It is interesting that some genes exhibit different expression patterns under the action of different hormones, but the amplitude of changes was similar. Such as, *PG0009427* of the GA2ox subgroup and *PG0016516* of the GA3ox subgroup were up-regulated under the action of IAA, while down-regulated under the action of GA_3, which increased (decreased) by 1.2 and 1.5 times compared to the control, respectively. *CsGA2ox3* and *CsGA3ox2* are up- or down-regulated at least four times under the action of ABA and GA_3, which is consistent with the results of this study [69]. Similar conclusions have been reported in species such as *Arabidopsis* and tomato [70, 71].

The *GAox* gene plays a crucial role in response to various abiotic stresses [17, 20, 72]. In *Arabidopsis thaliana*, the double mutants of *AtGA3ox1* and *AtGA3ox2* enhanced plants tolerance to salt stress [14]. *PG0016516* and *PG0005698* are a pair of duplicated genes in GA3ox, which were also up-regulated in salt and mannitol stress, suggesting that two *StGAox* genes may have similar capabilities as *AtGA3ox1*. Mutants of *AtGA20ox1*, *AtGA20ox2* and *AtGA20ox3* in *Arabidopsis* showed significantly increased under drought tolerance [73]. Drought stress increases the expression of *OsGA20ox5* and *OsGA20ox6* genes, which further suppresses the activity level of GA in response to adversity stress [74]. Mutations in the promoter sequence of CsGy7G019320 cause the si-2 mutant phenotype by repressing the expression of *GA3ox*, which leads to a reduced GA content in cucumber [75]. It was shown that osmotic stress and drought stress lead to GA accumulation in Arabidopsis [71]. Overexpression of *StGA2ox1* in potato enhances tolerance to low-temperature stress [22]. *GAox* genes are known to regulate hormone biosynthesis, which in turn enhances plant tolerance to stress [76]. In our work, the expression level of *PG0024249* (*GA20ox* member) was up-regulated under mannitol and salt stress in DM potato, and also up-regulated under drought stress in drought-tolerant variety Q, indicating that *PG0024249* might be related to drought stress response. *PG2003479* and *PG0032156* in GA2ox-I clade was up-regulated in DM and Q under drought, while *PG0021292* and *PG0002068* in GA2ox-III clade was also up-regulated in Q under drought stress, declaring that these genes may be related to drought stress response. In conclusion, *StGAox* gene may play an important role in potato growth and development and resistance to abiotic stress, which is worth further investigation.

Conclusions

In this study, 33 *StGAox* genes were analyzed at the genome-wide level, unevenly distributed across 10 chromosomes. Based on highly conserved gene structure and motif, the *StGAox* gene is delimited five subgroups. Collinearity analysis suggested that the fragment duplication events play a pivotal role in the expansion of the *StGAox* genes. According to the Ka/Ks ratio, these gene pairs evolve under purification selection. In addition, 5 candidate genes related to potato drought resistance were identified. These findings provide important information for the study of the *GAox* gene family in potato.

Abbreviations

ABA	Abscisic acid
AA	Amino acid
BAP	6-Benzylaminopurine
DM	Doubled monoploid
GA_3	Gibberellin 3
GA20ox	GA20-oxidases
GA2ox	GA2-oxidases
GA3ox	GA3-oxidases
HMM	Hidden Markov model
IBA	3-Indolebutyric acid
Ks	Synonymous substitution rate
Ka	Non-synonymous substitution rate
MW	Molecular weight
NJ	Neighbor-Joining
PG	PGSC0003DMG40
PI	Isoelectric point
qRT-PCR	Quantitative real-time PCR
TF	Transcription factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00574-0.

Additional file 1: Table S1. Primers for qPCR used in this study.

Additional file 2: Table S2. Physicochemical properties and subcellular location of *GAox* proteins in *Solanum tuberosum* L.

Additional file 3: Table S3. Conserved motifs of *StGAox* proteins.

Additional file 4: Table S4. *StGAox* Family Gene Replication Event in potato.

Additional file 5: Table S5. Ka/Ks ratio of homologous genes between Arabidopsis and potato.

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Author contributions
Shujuan Jiao: Conceptualization, Software, Methodology, Writing-original draft preparation. Zhen Liu: Methodology, Data curation, Formal analysis. Yichen Kang: Investigation. Ruyan Zhang: Data curation. Yong Wang: Formal analysis. Junlian Zhang: Visualization. Yuhui Liu, funding acquisition, project administration. Shuhao Qin: Resources. All authors have read and agreed to the submitted version of the manuscript.

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Availability of data and materials
The raw data of the transcriptome analysis used in this study were submitted to the Sequence Read Archive (SRA) at NCBI under Project ID PRJNA541096 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA541096/), and the expression data was also available Potato Genome Sequencing Consortium (PGSC, http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). The accession number and the website listed above were publicly available. The databases used in this study were publicly accessible and no special permissions were required. Access to these databases or websites is open. No new sequence data were generated in this study request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests
The authors declare no competing interests.

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