Effects of *StAAP1* and *StAAP8* in Potato ATFs Transcription Factor Family on Plant Development

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Abstract

Background: Amino acid transporters mediated the transport of various amino acids in plants, which were essential for plant growth and development. ATFs (the amino acid transporter family) was one of amino acid transporters in plants and AAPs (amino acid permease) subfamily belongs to the ATFs family.

Results: In this research, eight *AAP* genes were identified in the potato and divided into two subgroups. The first subgroup is the *StAAP* gene containing six microporous transport channels (Pore-lining), and the second subgroup are the *StAAP* gene containing seven microporous transport channels. The subcellular location and overexpression vectors were built by *StAAP1* and *StAAP8*. The results indicated these two genes were localized on the cell membrane. Observing the phenotype of the transgenic plants, *StAAP1* and *StAAP8* promoted the growth of leaves, *StAAP1* changed the shape of tubers and enhanced their weight and *StAAP8* was no significant influence in tubers but decreased the weight a little.

Conclusions: In this study, the expression pattern showed *StAAP8* might regulate the transport of amino acids in potato roots and affect the osmotic potential of potato root cells. Thereby, the tolerance of plants to abiotic stress and *StAAP1* and *StAAP8* promote the amino acid transport into leaves and *StAAP1* had effected on tuber development and *StAAP8* might resist the weight of tubers.

Background

Nitrogen as one of most important nutrient for plants growth required in amount of compounds with different forms and it mainly existed in the form of amino acids in plants [1]. Amino acids were important ingredients of enzymes and proteins and important for metabolism and cell structure in plant [2]. In addition, they were also precursors of compounds necessary for plant growth and development, which including secondary metabolites and it played an irreplaceable function in some pathways in plants [3, 4]. Someone indicated amino acids had important function in some abiotic and biotic stress and regulated growth and development in plant [5-7].

Amino acids in plants assimilate within roots and leaves and transported in the phloem to some other organs and APC and ATF transcription factor families participated plant amino acid transport [2]. Amino acid permease (*AAPs*) was one of subfamilies in ATF transcription factor family and the others could be clustered into 5 subfamilies, lysine-histidine-like transporters (*LHTs*), proline transporters (*ProTs*), γ -aminobutyric acid transporters (*GATs*), auxin transporters (*AUXs*) and aromatic and neutral amino acid transporters (*ANTs*) [8-11]. Some researchers had been identified the *AAP* genes in *A. thaliana*, *G. max*, *O. sativa*, *P. trichocarpa* and *S.tubersum* [12-16], and these genes were all located in cell plasmalemma and played a role in H^+ movement during amino acid transport.

The *AAPs* family had been participated a variety of activity pathways, such as absorbing amino acids, loading phloem amino acids, transferring amino acids, storing seeds and other aspects [17]. *AtAAP1* was expressed in root tip, root epidermis, and root hair cells and it had important for uptake of acidic and

neutral amino acids during the soil solution concentration exceeds 50 mM [18]. On the contrary, *AtAAP5* played an important role in the intake of basic amino acids during the low soil solution concentration [19]. *OsAAP3* and *OsAAP6* also could promote the absorption of amino acids in the roots [20, 21].

Tan showed the loading of phloem amino acids was very important for the entire physiological process of plants [22]. Although the amino acid transporters involved in this process had not been identified, but related research suggests that it might be AAPs [23]. The tracer experiments indicated that *AtAAP2* was key for xylem to phloem transfer and sink N and C supply which according to expression studies of genes of N/C transport and metabolism in source and sink, and by phenotypic and metabolite analysis of *AtAAP2* mutant [24]. And the decreased amino acid levels in phloem in *AtAAP6* mutant also explained the important role of AAP genes in the transfer of amino acids from xylem to phloem.

Moreover, the growth and development of *AtAAP8* mutant was affected due to reduced loading of amino acids in the phloem and content in the pool. And Hammers showed embryos of *AtAAP1* mutant seeds had low levels of amino acids and protein levels in seeds after ingesting amino acids, and *AtCAT6* remedied the lost function of *AtAAP1* to avoid seed development [25]. Overexpression of *VfAAP1* could be enhancing the protein levels and increasing the weight of seeds in cotyledons of broad beans and wild peas [26]. Overexpression *OsAAP3* could increase some amino acid concentration but the total amino acids has not changed. And knock out *OsAAP3* could enhance grain number per spike, yield per plant and protein content in rice seeds [20].

In recent years, some researchers indicated the expression of AAP was affected by plant growth and environmental factors [27-29]. The expression of *AtAAP4* and *AtAAP6* were decreased during the salt stress [30], and the expression of AAP genes could be affected when the plant attacked by pathogens. Similarly, the expression levels of some genes were significantly up-regulated or down-regulated during some abiotic stresses in rice [16].

As an important crop, there were few related researches on potato amino acid absorption and transport. The growth of potato needed a plenty of nitrogen and these were influenced the tubers formation and yields. Zahir indicated that tryptophan could significantly promote the absorption of nitrogen by potatoes, and could also increase the concentration of potassium, nitrogen and phosphorus in tubers [31]. To further clarify the AAP genes, we provided support to the function of *StAAP1* and *StAAP8* in potato development.

Results

Identification of *StAAPs* and analysis of their physical and chemical parameters

The result of blast showed 8 genes were belonged to AAPs, and these proteins had uniquely transcript and gene sequences. In addition, each annotation report for each protein were amino acid transporter on the PGSC website. these 8 AAP transcript factors were *StAAP1* (PGSC0003DMP400008841), *StAAP2* (PGSC0003DMP400006005), *StAAP3* (PGSC0003DMP400029354), *StAAP4*

(PGSC0003DMP400020862), *StAAP5* (PGSC0003DMP400038556), *StAAP6* (PGSC0003DMP400038554), *StAAP7* (PGSC0003DMP400010690) and *StAAP8* (PGSC0003DMP400020470).

The basic physical and chemical parameters of *StAAPs* could be acquired from ProtParam website and these information which included Number of amino acids, theoretical pI, aliphatic index, instability index, grand average of hydropathicity (GRAVY) were showed in Table 1. The amino acid composition and some other information were not shown in this table. But we could clearly observe that 3 kinds of amino acid, *alanine*, *isoleucine* and *valine*, had higher frequency in *StAAPs*. The theoretical pI were greater than 8 which meant they were all basic proteins. Otherwise, the aliphatic index more than 95 and GRAVY more than 0.40 indicated they were hydrophobic proteins and instability index showed they were stable proteins. Plant-mPloc website could predict the proteins function sites and almost every *StAAPs* were located on cell membrane except *StAAP5*, it was consistent with the characteristics of AAP proteins were a hydrophobic protein. Only *StAAP5* might be located in Golgi apparatus to play biological functions.

Analysis of *StAAPs* transmembrane structures

The results from NCBI-CDD showed that *StAAPs* all belonged to *SdaC* (cl26160) superfamily which was amino acid permease and the function of amino acid transport and metabolism. Through PSIPRED Workbench website to predict the transmembrane structures in each proteins and 8 *StAAPs* all had 9 to 10 transmembrane domains (Fig 1). Analysis of gene structure showed that the N-terminus of 3 genes, *StAAP3*, *StAAP5* and *StAAP6*, was extracellular and C-terminus of all AAP genes was intracellular. According to the transmembrane domain prediction results, we could divide *StAAPs* into two categories. one was *StAAPs* with 6 micro Pore-linings, including *StAAP1*, *StAAP2*, *StAAP3*, *StAAP4*, *StAAP7* and *StAAP8*. And the other was 7 micro Pore-linings, including *StAAP5* and *StAAP6*. The prediction of membrane protein topology and signal peptides showed that no signal peptide sequence existed in the polypeptide chain of 8 *StAAPs*. These results further explanation that *StAAP* proteins were related to transport and amino acid channel proteins which located on the cell membrane.

Analysis of *StAAP* Protein Structure

The predication result displayed that 8 *StAAPs* had similar secondary structures (Fig 2A). we analyzed the protein secondary structures showed α -helix was the highest proportion in all structures in each protein. Among them, the polypeptide chain with the highest α -helix was *StAAP1*, which was ratio reached 46.99%. And the lowest was *StAAP6*, which was also reached 43.01%. The number of involved in the α -helix composition was greater than 200 amino acids in 8 polypeptide chains. on the contrary, β -turn was lowest proportion in secondary structures, and it was existed less than 4% in each protein. The ratio of other secondary structures were random coil and extended strand from high to low.

We also predicted tertiary structure of *StAAPs* by Phyre2.0 website (Fig 2B). The result showed that accurately predicted ratio of amino acid residues greater than 90%, which meant the result of Phyre 2.0 website were credible and the proportion of accurately predicted amino acid sites is greater than 95% in 8

StAAPs. So that, the prediction of protein tertiary structure was high reliable. We analysis of protein secondary structure and tertiary structure indicated, α -helix played an important role in the spatial structure of AAP and it might be a core element of the transmembrane domain, which formed the amino acid transport channel on cell membrane. The predictions and simulations illustrated that 8 *StAAPs* were amino acid permease enzymes which located on the cell membrane, and they were mainly responsible for absorption and efflux of amino acids between cells.

Collinear analysis and Phylogenetic tree construction about *StAAPs*

The collinear analysis showed that *StAAP1*, *StAAP2*, *StAAP3* and *StAAP8* had colinearity genes and some genes were not belonged to *StAAPs* (Fig 3). Besides, *StAAP1/ StAAP8* and *StAAP2/ StAAP4* were colinearity genes and these 2 pair genes were also segmental duplication gene pair, respectively. This phenomenal might indicate these genes were relatively conservation and had important functions to maintain plant adaptation in hole evolution. Interestingly, *StAAP2* had 6 colinearity genes in hole potato genome, and these genes were annotated Proline transporter, Isoform 2 of Lysine histidine transporter, Amino acid transporter and some other annotation. That could be identified *StAAP2* was a transporter from the other way and its family members also were transporter.

We used 5 plant species' AAP genes to construct phylogenetic tree, which including *S. tuberosum*, *N. tabacum*, *S. lycopersicum*, *Z. mays* and model plant *A. thaliana* (Fig 3). According to the results of phylogenetic tree, the AAP genes could be divided into two subgroups, namely Subgroup I and Subgroup II. For the *StAAP* gene family, subgroup II contained only *StAAP5* and *StAAP6*, which was consistent with the predicted results of protein transmembrane structure. At the same time, the AAP family genes in 5 plants were distributed in both subgroups, which further indicates that the AAP genes can be divided into two types according to the structural and functional characteristics of the genes that guided the production of proteins. These two types of AAP genes might perform different functions in plants.

The expression analysis of *StAAPs*

The potato RNA-seq expression database was acquired from Massa [32] which contained expression changes not only between different plant tissues but also under biotics, abiotics, and hormone treatments. We selected *StAAPs* to analyzed their expression patterns in various tissues and treatments and processed the RNA-seq database and generated a heatmap (Fig 4). Twelve different tissues, leaves, tubers, sepals, stamens, stolons, flowers, petioles, petals, roots, shoots, callus and carpels were used to analyze the expression sites in different genes(Fig 4). the results showed that *StAAP1*, *StAAP4*, *StAAP5*, *StAAP6* and *StAAP8* had same expression pattern, they basically kept a high expression level in each tissues. Only *StAAP1* and *StAAP4* also had a higher expression in tuber and stolon, *StAAP8* had lower expression in tuber. The expression pattern of *StAAP2*, *StAAP3* and *StAAP7* indicated they had low expression in all tissues, and *StAAP7* was only expressed in flower-related tissues. The results indicated that each genes was a certain difference in the expression of each gene in potato tissue and that is to say, *StAAPs* had spatial expression specificity.

The expression pattern of phytohormone treatment (BAP, IAA, ABA and GA3), biotic stress (*P. infestans*, BABA and BTH) and abiotic stress (salt, mannitol and heat) showed the same tendency compared with tissues'. *StAAP1*, *StAAP4*, *StAAP5*, *StAAP6* and *StAAP8* had same pattern which were up-regulated expression in each treatment and *StAAP1* and *StAAP4* were down-regulated expression in each treatment (Fig 4). *StAAP8* was obviously up-regulated expression under ABA treatment, and the expression changed was highest under heat treatment, it was almost enhanced about 37 times. Moreover, *StAAP4*, *StAAP5*, *StAAP6* and *StAAP8* were all up-regulated expression under salt and mannitol.

This indicated that *StAAP8* gene, as an amino acid transport channel protein, was also involved in the response of potato to abiotic stress. Combining with potato tissue-specific gene expression data, it was known that *StAAP8* gene might affect the osmotic potential of potato root cells by regulating amino acid transport in potato roots, thereby increasing the plant's tolerance to abiotic stress such as drought. Based on the above analysis results, it could be seen that *StAAP8* was more important in the other *StAAP* genes, and because the *StAAP1* gene was a colinear relationship with the *StAAP8* gene. Thus, we chosen *StAAP1* and *StAAP8* for gene function research.

Subcellular location of *StAAP1* and *StAAP8* proteins

In order to determine the subcellular localization of *StAAP1* and *StAAP8* genes, *pEGFP-StAAP1* and *pEGFP-StAAP8* subcellular localization expression vectors were constructed, and agrobacterium injection was used to infect tobacco leaves after 36 hours to observe the *StAAP1* and *StAAP8* genes expression localization with pCEGFP empty vector as control (Fig 5). The distribution of GFP signals in the cells of the control group was not specific, while *StAAP1* and *StAAP8* were localized on the cell membrane.

Subcellular localization analysis found that *StAAP1* and *StAAP8* genes were localized on the cell membrane, indicating that these two genes mainly played a role in the cell membrane, and it was speculated that they might participate in the transmembrane transport of amino acids. For the follow-up studied of the biological functions of *StAAP1* and *StAAP8* provided the foundation.

Overexpression of *StAAP1* and *StAAP8*

We selected pCambia1300 vector which contained CAMV35S promoter to rebuild the overexpression vectors. And using these vectors could study the function of *StAAP1* and *StAAP8* genes through agrobacterium-mediated potato genetic transformation method. Figure 6A showed the callus formation, differentiation and rooting, and this method had high conversion efficiency. We selected some plants which had normally rooting and good growth potential for identification from a molecular way.

In order to obtain the real transgenic plants, the PCR tested the DNA could distinguish the positive plants, primarily (Fig 6B). Due to pCambia1300 vector contained hygromycin resistance gene sequence which the plants could not have these gene in nature. We used HYG primer to amplify the transgenic plants and wild Desiree as negative control and pCambia1300 vector or rebuild vector as positive control to confirm

the transgenic plants. The results of PCR showed the positive and transgenic plants had same strip, about 467 bp and the negative had nothing. We finally identified 12 transgenic plants in each genes.

The expression of transgenic plants detection

In order to determine the expression changing of transgenic plants, we used qRT-PCR to identify the expression of 12 transgenic plants and analyzed their change to ensure the higher one was selected to observe their phenotypes (Fig 7). *StAAP1* and *StAAP8* transgenic plants had up-regulated expression compared to wild Desiree. Figure 7A indicated the highest enhance was *OXStAAP1-5* which was increased about 22 times. Similarly, *OXStAAP8-10* was increased about 28 times from figure 7B. Thus, we could confirm these transgenic plants were positive and some plants have good expression enhancement.

Analysis of the phenotypic changes in transgenic plants

We respectively selected three *StAAP1* and *StAAP8* transgenic plants planted into soil in the greenhouse. According to our observation showed no significant difference in plant height, but the leaves of two kinds of genes' transgenic plants were obviously bigger than wild plant. The numbers of leaves were existed some difference, the overexpression *StAAP8* transgenic plants were less than wild and the *StAAP1* plants were not different (Fig 8A).

Moreover, we acquired the tubers and collected them to analyze the size and shape (Fig 8B). The results showed the number of wild and transgenic potato tubers were 10 to 12, which were not obviously difference. But *StAAP1* tubers were bigger than wild tubers and *StAAP1* tubers were longer than wild, but the total weight was no significant change. Unlike *StAAP1* tubers, *StAAP8* tubers were smaller and lighter than wild and the shape of tubers were not changed. Their homologous gene, *AtAAP6*, had opposite phenotype. The *AtAAP6* mutant had larger rosette width than the wild type and seeds from the AAP6 mutant were also significantly larger than those from the wild-type plants [33]. Eventhough they had different results but they all influenced the leaves and *StAAP1* and *StAAP8* were related to potato tubers formation.

Discussion

Bioinformatics analysis of StAAP

In this study, the StAAP gene family transmembrane domain analysis, protein spatial structure prediction, and phylogenetic tree analysis were used to preliminary classify the StAAP gene family members into two groups. This classification was consistent across tobacco, tomato, Arabidopsis and corn.

Combining the location of StAAP genes and their colinearity genes on chromosomes showed that the members of the StAAP genes and their corresponding collinear genes were not evenly distributed on the chromosomes. this phenomenon might be because they were some segmental duplications and the functional of each gene duplication pairs might existed some difference. Among the members of the

StAAP gene family, there was a collinear relationship between the two pairs of genes *StAAP1* / *StAAP8* and *StAAP2* / *StAAP4*, which indicated that these genes might have gene duplication in the genome during the evolution process and have important physiological functions in the life process of potatoes.

Expression pattern analysis of StAAP

Expression pattern analysis showed that the expression of *StAAP8* gene was significantly increased under ABA treatment, salt stress and mannitol treatment which indicated that *StAAP8* not only as an amino acid transport channel protein but also involved in the response of potatoes to abiotic stress. On the other hand, the tissue-specific gene expression showed that *StAAP8* gene might affect the osmotic potential of potato root cells by regulating the transfer of amino acids in potato roots, thereby the tolerance of plants to osmotic stress-related abiotic stresses were increased.

The expression and phenotypic observations of transgenic plants

Because *StAAP8* changed more significantly in expression patterns, and might be played an important role under related stress in StAAPs. Thus, the basic gene function was performed on *StAAP8* and its colinearity gene, *StAAP1*.

The qRT-PCR results of *StAAP1* and *StAAP8* overexpressing transgenic plants showed that the gene expression levels in the overexpressing plants were all up-regulated, which confirmed that the transgenic plant construction was credible. The phenotypic observations of transgenic plants revealed that the leaf of transgenic plants was larger than wild type, which might be beneficial to photosynthesis of plants and promote plant development.

Conclusion

In this study, *StAAP* gene families were analyzed based on bioinformatics methods. A total of 8 *StAAPs* were identified and divided into two subgroups. Subgroup I had 6 *StAAPs* which contained 6 micro-porous transport channels (Pore-lining), including *StAAP1*, *StAAP2*, *StAAP3*, *StAAP4*, *StAAP7* and *StAAP8*. The others formed subgroup II which had 7 micro-porous transport channels. The expression pattern showed *StAAP8* might regulate the transport of amino acids in potato roots and affect the osmotic potential of potato root cells. Thereby, the tolerance of plants to abiotic stress such as drought is improved.

On the other hand, we used Desiree as model potato to clone and rebuild the overexpression and subcellular location vectors of *StAAP1* and *StAAP8*. Under the laser confocal microscope, it was observed that *StAAP1* and *StAAP8* were localized on the cell membrane. And the leaves of overexpression transgenic plants were bigger than wilds and the tubers of *OXStAAP1* transgenic plants had different type and more weight. Interestingly, the weight of *OXStAAP8* transgenic tubers less than wild type. Thus, we speculated that *StAAP1* and *StAAP8* promote the amino acid transport into leaves and *StAAP1* had effected on tuber development and *StAAP8* might resist the weight of tubers.

Methods

Identification of *StAAPs* and analysis of their physicochemical properties

Download potato proteins sequences to blast the StAAP proteins which using *AtAAPs* as a query and review the descriptions to each protein in order to preliminarily predict the transcription factor family members in potato¹. some other information were predicted from ProtParam² and Plant-mPloc³.

Predictive analysis of transmembrane and protein structures

We can use the MEMSAT-SVM model in PSIPRED Workbench website⁴ to predicted the protein transmembrane domain. TOPCPNS website⁵ was predicted membrane protein topology and signal peptides. SOPMA website⁶ and Phyre 2.0 website⁷ are using to predict protein secondary structure and tertiary structure of protein.

Collinear analysis and Phylogenetic tree construction about *StAAPs*

Eight *StAAP* proteins are searching for their collinearity in hole potato protein sequences by MCSanX. Simultaneously, 27 AAPs, from *S. lycopersicum*, *N. tabacum*, *Z. may*, *S. tuberosum* and *A.thaliana*, are used to construct the phylogenetic tree by MEGA 7 software and these protein sequences are downloaded from Sol Genomics Network⁸ and Phytozome⁹. Finally, these results are combined and showed through circos-0.69¹⁰.

The expression analysis of *StAAPs* based on RNA-seq data

We select 12 tissues, 4 phytohormone treatments, 4 biotic treatments and 3 abiotic treatments from transcriptome sequencing results. And reference the data treatment from Zhang to visualize the expression [34].

Plant material growth conditions

The potato material (*Desiree*, which is self-preserved by the laboratory's resource library) was grown with 20 g/L sugar on solid MS medium with vitamins (MS, USA) as culture medium for four weeks in a plant incubator at 25±1°C under 10 000 Lx in light for 16 h and 20±1°C under 0 Lx for 8h.

The seeds of *N. tabacum*, which is self-preserved by the laboratory's resource library, was planted in the greenhouse for 4-6 weeks at 23°C under 10 000 Lx in light for 16 h and 20°C under 0 Lx for 8h.

Subcellular location of *StAAP1* and *StAAP8*

Designing the primer consisted of some vector sequences which were next to the restriction enzyme cutting site, *SpeI*, and some purpose gene sequences which include initiation codon but no termination codon (*StAAP1*-GFP-F: ACCATGGTAGATCTGACTAGTATGGCACCTGAATTTCAAAA; *StAAP1*-GFP-R: GCTCACCATCCTAGGACTAGTTTGATGAGTTGAGAAAGGCT; *StAAP8*-GFP-F:

ACCATGGTAGATCTGACTAGTATGGCATCAGAATTTGAGAA; StAAP8-GFP-R
GCTCACCATCCTAGGACTAGTATCATGATCTTGAGGTT). The cDNA of *Desiree* was used as the substrate for PCR reaction with KOD- Plus-Neo. Using the one step cloning kit to recombine the pCEGFP to acquire the new fusion expression vector. And called *pCEGFP-StAAP1* and *pCEGFP-StAAP8*. Selected 3 to 4 tobacco plants injected Agrobacterium to infect tobacco leaves and observed the green fluorescence excitation position by confocal laser scanning microscope (CLSM) in 2 days later to ensure the protein expression site.

Overexpression of StAAP1 and StAAP8

Using same method to construct the recombine vectors and the primers were (*StAAP1*-OE-F:GAGAACACGGGGGACTCTAGAATGGCACCTGAATTTCAAAA; *StAAP1*-OE-R:CGATCGGGGAAATTCGAGCTCTTATTGATGAGTTGAGAAAG; *StAAP8*-OE-F:GAGAACACGGGGGACTCTAGAATGGCATCAGAATTTGAGAA; *StAAP8*-OE-R:CGATCGGGGAAATTCGAGCTCTCAATCATGATCTTGAGGTT) based on pCambia1300 which was cut by *XbaI* and *SacI*. The new vectors named *OX-StAAP1* and *OX-StAAP8*, and used method for transforming potato leaves by Agrobacterium to induce callus to acquire the transgenic plants.

The transgenic plants were identified by PCR through the vector-specific primers and the sequence was hygromycin coding region (*HYG*-F: GAAGAAGATGTTGGCGACCTC; *HYG*-R: ACATTGGGGAGTTTAGCGAGA) belonged to pCambia1300. The real-time PCR was used to confirm the expression changes by CFX96 Touch. (*StAAP1*-F: ATCGCTGTCCACCTTGTCGG; *StAAP1*-R: GAACTCGCATCGCGTATTCTG; *StAAP8*-F: CATTGCTCGCCGATTGTTATC; *StAAP8*-R: ATTATTTGTATTATTGCAAAAATAA; *EF1a*-F: ATTGGAAACGGATATGCTCCA; *EF1a*-R: TCCTTACCTGAACGCCTGTCA)

Analysis of the phenotypic changes in transgenic plants

We selected some highly expression transgenic tube plantlet planted into feeding block which contained half vermiculite and half nutrient soil in the greenhouse at 25±1°C under 10 000 Lx in light for 16 h and 20±1°C under 0 Lx for 8h. Each 7 days added the Hoagland's medium keeping the soil wet. Observing the growth phenotype and the tuber form about 3 months later. Compared the stem height, leaves and tubers of transgenic and wild potatoes to analyze phenotypic changes.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Conflict of Interests

None of the authors have any actual or potential conflicts of interest.

Authors' contributions

C.Z., Q.C. and N.K. designed experiments; C.Z., N.K. and J.S. carried out experiments; C.Z., N.K. and D.W. analyzed experimental results. C.Z. and N.K. wrote the manuscript. Q.C. provided financial support. All authors have read and approved the manuscript.

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Table

Table 1 Physicochemical properties of the corresponding amino acid sequence of *StAAPs*

Gene name	CDS sequence length	Number of amino acids	Theoretical pI	Aliphatic index	Instability index	GRAVY
<i>StAAP1</i>	1446bp	481	8.81	98.11	32.99	0.440
<i>StAAP2</i>	1395bp	464	8.34	106.31	35.58	0.476
<i>StAAP3</i>	1407bp	468	8.54	106.0	35.76	0.544
<i>StAAP4</i>	1422bp	473	8.41	101.23	33.15	0.520
<i>StAAP5</i>	1371bp	456	8.55	104.17	32.02	0.478
<i>StAAP6</i>	1419bp	472	8.95	97.92	34.72	0.489
<i>StAAP7</i>	1440bp	479	8.81	102.38	32.64	0.471
<i>StAAP8</i>	1467bp	488	8.92	100.92	39.86	0.389

Figures

Figure 1

Prediction of transmembrane domain of potato AAP gene family members. The gray rectangle indicated cytomembrane, upside was extracellular and downside was cytoplasm. The yellow rectangles were transmembrane regions and the lines means pore-lining regions. StAAP3, StAAP5 and StAAP6 had 10 transmembranes and others had 9 transmembranes.

Figure 2

A: Protein secondary structure analysis of potato StAAP gene family. The purple, green, red and blue bar respectively denoted Random coil, beta turn (β -turn), Extended strand and Alpha helix (α -helix). Each protein had same structure and α -helix had the largest proportion. B: Prediction of protein tertiary structure corresponding to 8 StAAP genes. Amino (N) termini of proteins and 5' termini of nucleic acids are blue. Carboxy (C) termini of proteins and 3' termini of nucleic acids are red. Between termini, colors follow a spectral rainbow sequence.

Figure 3

Colinearity analysis of StAAP gene and construction of phylogenetic tree. We selected *S. tuberosum*, *N. tabacum*, *S. lycopersicum*, *Z. mays* and *A. thaliana* to build the phylogenetic tree and it clearly divided into 2 subgroups, yellow and blue respectively indicated subgroup I and II. The figure inside the phylogenetic tree was collinearity of the StAAPs. The cycle with different colors was the 12 chromosomes and the lines connecting different genes meant they had a collinear relationship. The gene names of blue

were potato transcript that was not predicted to be AAP, and the red names were StAAPs which distributed in different chromosomes.

Figure 4

Analysis of the expression pattern of potato StAAP genes. the expression pattern was constituted 4 parts, including tissues (leaves, tubers, sepals, stamens, stolons, flowers, petioles, petals, roots, shoots, callus and carpels), biotic (*P. infestans*, BABA and BTH), abiotic (salt, mannitol and heat) and hormone

treatment (BAP, IAA, ABA and GA3). Eight StAAP genes divided into two categories based on expression patterns. StAAP2 and StAAP3 had lower expression in all treatment and tissues and the others were slightly improved.

Figure 5

Subcellular localization of StAAP1 and StAAP8 in tobacco leave cells. The first column was display under green fluorescence, the second column was bright fired and the third column was merged. The first line showed the localization of free GFP, the second line and the third line respectively showed the localization of StAAP1 and StAAP8 in tobacco leave cells.

Figure 6

A: Genetic transformation of potato. a: Induction of leaf resistance callus. b: Adventitious bud differentiation of leaf callus. c: Rooting screening of resistant seedlings with hygromycin. B: PCR detection of StAAP1 and StAAP8 transgenic potato. M: DL10000 DNA Maker. 1: Negative control (wild-type). 2: Positive control (the plasmid of pCambia1300). 3-6: OXStAAP1 transgenic plants. 7-10: OXStAAP8 transgenic plants. The Target electrophoretic band size was 467 bp. All bands have target bands except for the negative control and it could be proved the regenerated plants were transgenic, preliminarily.

Figure 7

Expression of transgenic plants. A: Expression analysis of StAAP1 gene in transgenic plants. B: Expression analysis of StAAP8 gene in transgenic plants. Wt meant the wild-type and set the wild-type as standard.

Figure 8

A: Phenotypes of the transgenic potato plants. WT meant wild-type and OXStAAP1 and OXStAAP8 were indicated the overexpression of StAAP1 and StAAP8. We selected equivalent growth potential plants were

transplanted from culture plantlet which cultivated for about 4 weeks. One month cultivated from greenhouse to observe the growth situation. B: Phenotypes of the transgenic potato. Collected potato tubers after planting in the greenhouse for three months to observe their potato type, size and numbers.

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