Regeneration and Genetic Transformation of *Arabidopsis Pumila*: An Ephemeral Plant Suitable for Investigating the Mechanisms for Adaptation to Desert Environments

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Abstract

Arabidopsis pumila is a type of cruciferous ephemeral plant, which in China mainly grows in the desert environments of northern Xinjiang. *A. pumila* not only has a short growth duration, but also has high photosynthetic efficiency, seed yield, salt tolerance, and drought resistance. It is an ideal species for the study of environmental adaptations in ephemeral plants. We induced callus tissue formation on the roots and hypocotyls of 8-day-old seedlings, and on the leaves and petioles of 4-week-old seedlings, and obtained multiple adventitious shoots on these tissues grown on Murashige and Skoog induction medium supplemented with 0.5 mg/L 6-Benzylaminopurine and 0.1 mg/L α-Naphthalene acetic acid. Young roots, hypocotyls, leaves, and petioles could all induce calluses, but the induction rate was highest on young roots. In addition, the leaves and petioles of 4-week-old seedlings were used as explants, the Δ1-pyrroline-5-carboxylic acid synthase gene 1 of *A. pumila* controlled by 35S promoter of cauliflower mosaic virus was used as target gene, and hygromycin B was used as screening antibiotic to explore *Agrobacterium tumefaciens* GV3101 mediated transformation. The results showed that the callus induction rate of petiole explants was the highest when they were treated with *Agrobacterium* suspension (OD_{600} = 0.6) for 10 min and thenco-cultured in dark for 2 d. The qRT-PCR results showed that the *ApP5CS1.1* gene was overexpressed in the transgenic plants. These protocols provide working research methods for exploring the cellular level adaptative mechanisms of this species to desert environments.

Key Message

Establishment of tissue culture in ephemeral plant *Arabidopsis pumila* and efficient *Agrobacterium*-mediated genetic transformation for investigating the mechanisms for adaptation to desert environments.

Introduction

Efficient *in vitro* plant propagation and genetic transformation techniques are the key to effective fundamental and applied plant research. However, establishing stable genetic transformation techniques in plants remains challenging. At present, *Agrobacterium* transformation is still the leading transformation technique, and is common in many research areas such as investigations into plant gene function (Bertier et al. 2018; Lee et al. 2019), crop breeding and improvement (Gerszberg et al. 2015; Gambhir et al. 2017), rapid propagation and cultivation of commercial plants (Weckx et al. 2019), germplasm preservation of certain vegetatively propagated plants (Bömer et al. 2019), and rescue of rare/endangered plant species (Sadeq et al. 2016). In addition, certain plant cell and organ cultures can produce secondary metabolites for use in industry and medicine (Lu et al. 2016; Tatsis and O'Connor 2016; Loyola-Vargas and Ochoa-Alejo 2018). Omics research (i.e., genome, proteome, transcriptome, and metabolome) combined with tissue culture technologies provides a powerful means of understanding the complex developmental processes of plants (Imin et al. 2005; Wickramasuriya et al. 2015; Loyola-Vargas and Ochoa-Alejo 2018).

In recent years, there has been an increasing number of studies on regeneration and cultivation techniques for different plants, especially crops such as soybean (*Glycine max*) (Raza et al. 2017, 2019), corn (*Zea mays*) (Garrocho-Villegas et al. 2012), cabbage (*Brassica oleracea*) (Kumar et al. 2015), sugar beet (*Beta vulgaris*) (Kagami et al. 2016), coriander (*Coriandrum sativum*) (Ali et al. 2017), and potato (*Solanum tuberosum*) (Fossi et al. 2019). *Agrobacterium*-mediated genetic transformation techniques allow the production of transgenic plants and require genetic engineering methods using plant regeneration systems (Cao et al. 2018; Li et al. 2019; Wang et al. 2019). *Agrobacterium*-mediated gene transformation has obvious advantages compared with other gene transformation methods. It is relatively cheap and fast to perform and allows: the transfer of large fragments of DNA; the integration of low copy number transgenes; the ability to efficiently and stably integrate genes into the nuclear genome; and the preferential insertion of transcriptional activity zones (Cha et al. 2012; Gasparis 2017; Bruce et al. 2019). *Agrobacterium*-mediated genetic transformation techniques based on tissue culture have been developed for many plants, including strawberry (*Fragaria vesca*) (Oosumi et al. 2006), rice (*Oryza sativa*) (Hiei et al. 2008; Saika et al. 2010), wheat (*Triticum aestivum*) (Hu et al. 2003), corn (Zhong et al. 2018), sorghum (*Sorghum bicolor*) (Kuriyama et al. 2019), hybridized poplar (*Populus Alba* × *Populus glandulosa* Uyeki) (Song et al. 2019), and Prymnesiophycae such as *Isochrysis galbana* (Prasad et al. 2014).

The current most widely used gene editing technologies also rely to a great extent on tissue culture and *Agrobacterium*-mediated transformation technologies, which can directly edit the plant genome at a specific site without introducing foreign genes. This technology has produced good mutants of tomato (*Solanum lycopersicum*) (Soyk et al. 2017), wheat (Wang et al. 2014), rice (Li et al. 2016; Srivastava et al. 2017), corn (Feng et al. 2016), and other crops.

Ephemeral plants have only one or several generations each year, and only sprout and grow during favorable periods (i.e., when sufficient water is available) and can survive unfavorable periods as seeds (Shi et al. 2006). In China, ephemeral plants are mainly distributed in the deserts of the Junggar Basin and the Ili River Valley in northern Xinjiang, bordering the eastern edge of the Junggar Basin (Shi et al. 2006; Huang et al. 2017). They germinate in mid-March or early April using rainwater and snowmelt from the Tianshan Mountains and complete their life cycle about two months later in mid-June. This period includes the season with the strongest winds in the northern Xinjiang deserts. Therefore, ephemeral plants play an important role as windbreaks stabilizing sandy soils, conserving soil and water, and improving microhabitat quality (Shi et al. 2006; Jin et al. 2019). They are subject to a variety of harsh environments during their life cycle, including extreme temperature, drought, and high soil salinity. Soil salinity is one of the main abiotic factors restricting plant growth and crop production. Therefore, understanding the genetic basis of plant salt tolerance in plants, especially in crops, is an extremely important area of research. The extremely harsh ecological environment in Xinjiang means that many salt-tolerant and drought-resistant organisms inhabit its saline and semi-saline soils. Among them, *Arabidopsis pumila* is a short-lived desert plant which grows quickly in the brief favorable season when the rain falls and snows melt in early spring, and benefits from its fast growth, high yield, high photosynthetic efficiency, and stress tolerance (Tu et al. 2016; Jin et al. 2019). Understanding the

molecular mechanism behind *A. pumila's* rapid growth and adaptations to harsh environments is therefore of great significance in protecting and using its stress tolerant genes to improve crop resistance.

We constructed a cDNA library of *A. pumila* seedlings, carried out sequencing analysis of expressed sequence tags, and cloned a number of salt-tolerant-related genes such as the H^+ inorganic pyrophosphatase gene, the delta-1-pyrroline-5-carboxylic acid synthase 1 (*P5CS1*) gene, and the Na^+/H^+ antiporter (Zhao et al. 2013; Huang et al. 2017). Transcriptome sequencing analysis of *A. pumila* seedlings stressed with 250 mM NaCl revealed that many salt stress-responsive genes showed dynamic changes. These results will enable a better understanding of the salt adaptation mechanisms of short-lived plants, such as proline metabolism which enables plants to respond to certain abiotic stresses (Yang et al. 2018).

A large amount of proline accumulates in plants under abiotic stress conditions, such as drought, high salt levels, strong light, and oxidative stress (Szabados et al. 2010). *P5CS* participates in catalyzing the first step of proline synthesis in which it acts as the rate-limiting enzyme (Anton et al. 2020). We found that the *ApP5CS1* homologous gene actively responds to high salt stress (Huang et al. 2017) (Supplementary Fig. 1), suggesting that *P5CS1* also plays an important role in *A. pumila's* salt tolerance response. In-depth study of gene function can better explain the adaptive mechanisms enabling *A. pumila* to grow in desert environments. Although many important salt-tolerant genes are known, detailed studies of gene function in *A. pumila* have been limited due to the lack of suitable tissue culture and transformation techniques. The establishment of a tissue culture regeneration system for *A. pumila* is therefore important for exploring the cellular level adaptative mechanisms and evolution of this species in desert environments. Understanding these issues will bring significant improvements to modern biotechnology, the development of genetic resources, and the preservation of germplasm.

Materials And Methods

Plant materials

Arabidopsis pumila seeds were preserved in our laboratory. The seeds were sterilized by rinsing with absolute ethanol for 1 min, and then with 20% sodium hypochlorite for 10 min, and finally with sterile water six times. Sterilized seeds were sown on Murashige and Skoog (MS) agar medium with 3.0% (w/v) sucrose and 0.8% (w/v) plant agar added. Seedlings were grown in climate-controlled conditions at 22 ± 1 °C, 300 μmol m^{-2} S^{-1} of light intensity, 16 hours light/8 hours dark, and 70% relative humidity. Pieces of young root (1 cm long, Fig.1a, c), hypocotyl (1 cm long, Fig.1a, b), leaf (1 cm^2, Fig.1d, e) and petiole (1 cm long, Fig.1d, e) tissue were collected from 8-day-old and 30-day-old seedlings to serve as explants (Fig. 1).

Adventitious shoot induction

Adventitious shoots were induced under conditions of 22 ± 1 °C, 300 μmol m^{-2} S^{-1} light intensity and 70% relative humidity. Explants of young root, hypocotyl, leaf, and petiole were cultivated on induction medium containing 0.5 mg/L 6-Benzylaminopurine (6-BA) and 0.1 mg/L α-Naphthalene acetic acid (NAA).

The induction medium was supplemented with 3.0% (w/v) sucrose and 0.8% (w/v) agar powder, and the pH was adjusted to 5.8. The medium was autoclaved at 121 °C for 20 min and then cooled to 55 °C before adding the plant hormones, and was finally placed into 150 mL sterilized flasks (about 25 mL per flask). The basic MS medium and hormones (6-BA and NAA) used in this study were purchased from Beijing Boao Tuoda Biotechnology Co., Ltd. (Boao Tuoda, Beijing, China).

Root induction and plant domestication

The elongated adventitious shoots (1–2 cm) were transferred for cultivation to MS medium (pH 5.8) containing 0.1 mg/L NAA and 500 mg/L carbenicillin (Carbenicillin, Carb) at 22 ± 1 °C with a 16/8 h (light/dark) photoperiod with 300 μmol m^{-2} S^{-1} light intensity until roots were induced. In order to adapt the cuttings to the growth environment, the flasks containing the test tube plantlets were moved from the culture room to a greenhouse for 4–5 d. The seedlings were then carefully removed from the flasks, rinsed with tap water, and transferred to a culture pot containing 1:1 (*w/w*) nutrient soil to vermiculite.

Agrobacterium culture and transformation process

Based on previous studies of the full-length transcriptome of *A. pumila* under salt stress, it was found that two *P5CS* homologous genes exhibited elevated expression characteristics while under salt stress (Yang et al. 2018) (Supplementary Fig. 1). We cloned the *P5CS1.1* gene, and after sequencing (the length of ORF is 2154 bp), and then connected it to the hygromycin-resistant *pCAMBIA1301* vector. After double digestion with *Nco* I and *Bst* EII to verify the plasmid, the correctly constructed *35S:ApP5CS1.1* vector plasmid (Supplementary Fig. 2) was transferred into *Agrobacterium tumefaciens* GV3101. A single colony of *Agrobacterium tumefaciens* was cultured at 28 °C in Luria-Bertani liquid medium containing 50 mg/L kanamycin, 25 mg/L rifampicin, and 50 mg/L gentamicin until the culture density reached an OD_{600} of 0.8. *Agrobacterium tumefaciens* cells were obtained by centrifugation at 5000 rpm for 10 min, and the cells were suspended in a 1/2 MS solution (pH 5.8) containing 3.0% (w/v) sucrose and 20 mg/L acetosyringone.

The petiole and leaf explants were cut on an ultra-clean workbench and infected in infection solution of OD_{600} = 0.6 for 5 min and 10 min, and an infection solution with OD_{600} = 1.0 for 5 min and 10 min, respectively. The experimental design comprised a total of four treatments, each with three replicates. Sterile filter paper was used to absorb excess bacteria, and the samples were incubated on symbiotic medium (MS basal medium containing 0.5 mg/L 6-BA, 0.1 mg/L NAA, and 20 mg/L AS; pH 5.8) for 2 d in the dark. The petiole and leaf explants were then transferred to selection medium (MS basal medium

containing 0.5 mg/L 6-BA, 0.1 mg/L NAA, 10 mg/L Hygromycin B, and 500 mg/L Carb; pH 5.8) for shoot induction. The samples were transferred to fresh selection medium once a week. The induction status of the calluses and adventitious shoots were recorded every 7 d during the culture period. The shoot samples (1–2 cm long) were then cut and transferred to MS agar medium (pH 5.8) and supplemented with 0.1 mg/L NAA and 500 mg/L Carb for root induction.

Genomic DNA extraction and PCR identification

The genomic DNA was isolated from leaves of transgenic and wild-type *A. pumila* plants via the Fastpure plant DNA isolation Mini Kit (Vazyme, Nanjing, China). For PCR analyses, the forward primer 5´-ATGGAGGAGCTAGATCGTTCA-3´, reverse primer 5´-TTAGGCTTGGATGGGAATGTC-3´ were designed according to the CDS of *ApP5CS1.1* (GenBank accession number: MW227238). The final reaction mixture (20 μL) contained 2 μL 10 × Taq buffer, 1.5 μL 2.5 mmol/L dNTP, 0.5 μL forward and reverse primers respectively, 0.5 μL Taq DNA polymerase (Takara, Dalian, China), and ddH_2O supplement. The reaction conditions were 94 °C 2 min, followed by 35 cycles of 30s at 94 °C, 30s at 56 °C, 2 min 20s at 72 °C, and 72 °C 10 min for extension.

Quantitative real-time PCR (qRT-PCR) assays

The seedlings of wild type and transgenic *A. pumila* plants were collected and immediately stored in liquid nitrogen. Total RNA was extracted using RNAprep Pure Plant Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. After purification using DNase without RNase (Tiangen, Beijing, China), the cDNA synthesis reactions were performed using the PrimeScript II first Strand cDNA Synthesis Kit MIX (Bioteke, Beijing, China) following the manufacturer's instructions with 300 ng of total RNA per reaction. qRT-PCR was carried out on an Applied Biosystems 7500/7500-Fast Real-Time PCR system (ABI, Foster City, CA, United States) with the 2 × ChamQ Universal SYBR Green qPCR Master Mix (Vazyme, Nanjing, China). The conditions of PCR were performed according the methods by Huang et al. (2017). The gene-specific forward primer of *ApP5CS1.1* was 5´-GGAAGAATCGTTGGTGGCTC-3´, and the reverse primer was 5´-ACAAGTGCATCAGGTCGAGA-3´. Glyceraldehyde-3-phosphate dehydrogenase gene (MW227237) was used as an internal reference control (Jin et al. 2019). Three biological replicates were performed with RNA isolated independently and each RT reaction had three replicates. The initial denaturation time was 94 °C 30s, followed by 40 cycles at 95 °C 15s, 60 °C 15s, 72 °C 15s, and finally extended at 72 °C for 10 min. The relative gene expression levels were calculated by $2^{-\Delta\Delta Ct}$ method (livak and schmittgen 2001).

Results

Regeneration induction of four different explants of A. pumila

Young root, hypocotyl, leaf, and petiole explants were cultured in MS induction medium containing 0.5 mg/L 6-BA and 0.1 mg/L NAA. Of the four explant tissues, roots showed the highest callus induction rate (99.42%), followed by petioles (97.04%) and leaves (96.58%), while hypocotyls had the lowest callus induction rate (93.53%) (Table 1).

Table 1
Callus formation and adventitious bud induction rates of four different explants from *A. pumila*

Explants	Total number	Callus number	Induction rate (%)	Shoot formation number	Shoot formation (%)	Callus formation (d)	Shoot Formation (d)
Youg root	343	341	99.42	335	97.7	7.33 ± 0.48^{d}	32 ± 2.15^{d}
hypocotyl	232	217	93.53	208	89.7	10.05 ± 0.74^{c}	59.05 ± 2.16^{a}
petiole	405	393	97.04	356	87.9	13.24 ± 0.77^{a}	44.25 ± 4.07^{b}
leaf	322	311	96.58	298	92.5	12.76 ± 0.77^{b}	41.95 ± 2.37^{c}

Different lowercase letters in the same column represent significant differences among the average values (mean ± SD, $P < 0.05$, Duncan's multiple range test).

Young Roots

The young white root explants began to form calluses after one week on the induction medium (Table 1); somatic embryos formed at the cuts at both ends of the explants, and the embryogenic callus appeared green, spherical, and clustered (Fig. 2a). Embryogenesis of somatic cells is asynchronous and can occur anywhere on the explant after 7–20 d of induced culture. After 20 d of callus induction, 341 of 343 explants were successfully induced, and the total callus induction rate was 99.42%. At 30 d after induction, embryogenic calluses began to form adventitious shoots (Fig. 2b). The number of explants inducing adventitious shoots was 335, accounting for 97.7% of all explants (Table 1). The adventitious shoots (1–2 cm long) were cut off and transferred to a flask containing rooting medium to induce rooting. Rooting occurred after about 20 d (Fig. 2c, d). Regenerated seedlings with strong roots eventually grew into complete plants (Fig. 2e, f).

Hypocotyls

Arabidiopsis pumila hypocotyl explants were incubated on induction medium for about 10 d to form embryogenic calluses. Embryogenic calluses generally appeared on the cut surface of the explant, or

along the sides in contact with the culture medium (Fig. 3a). Some somatic embryos formed shoots on the upper surface of the explants, and clustered shoots produced roots (Fig. 3b). Of the 232 hypocotyl explants, a total of 217 were induced to form calluses, and the callus induction rate was 93.53% (Table 1). Adventitious shoots on hypocotyls began to form after explants had been cultured for about two months, and the adventitious shoot induction rate was 89.7% (Table 1). The adventitious shoots (1–2 cm long) were cut off and transferred to a rooting induction medium to induce the rooting process (Fig. 3c). Regenerated shoots with many strong roots were able to grow into complete plants (Fig. 3d).

Petioles

Embryogenic calluses were observed on petiole explants after 13 d of culture on induction medium. Green embryogenic callus cell clusters formed at the point of incision of the explants, and these embryos developed well. The petiole tissues of *A. pumila* can form a large number of healthy green embryogenic calluses after about 20 d (Fig. 3e). Of the 405 petiole explants, 393 were successfully induced to produce adventitious shoots, and the callus induction rate was 97.04% (Table 1). The calluses began to form adventitious shoots after about 44 d, and the adventitious shoot induction rate was 87.9% (Fig. 3f, Table 1). Adventitious shoots were transferred to rooting medium to induce rooting (Fig. 3g) until strong root systems and complete plants developed (Fig. 3h, 3k, and 3l).

Leaves

The leaf explants swelled and bent after 12 d of induction culture, and somatic embryos were formed directly at the cut edges of the explants (Fig. 3i). Embryonic cells appeared in green globular clusters. However, somatic embryogenesis was asynchronous, varying over two to six weeks. Some embryos germinated on the starting medium, or when subcultured on the same fresh medium, and proliferated into larger embryos by producing secondary embryos (Fig. 3i). Of 322 leaf explants, 311 successfully induced calluses, and the callus induction rate was 96.58% (Table 1). After forming sufficient calluses, callus was further induced to produce adventitious shoots after about 42 d (Fig. 3j), with an induction rate of 92.5%. The adventitious shoots appeared earlier, and at a higher induction rate, than on the petioles (Table 1). Adventitious shoots (1–2 cm long) were cut and transferred to a rooting medium to induce rooting and sufficient strong roots were produced to enable growth into complete plants (Fig. 3k, l).

Establishment of an A. pumila genetic transformation system

We optimized the concentration and duration of *Agrobacterium* infection during the transformation process. At an OD_{600} of 0.6, infection for 10 min produced a higher callus induction rate than infection for 5 min, for both petiole and leaf explants. On the contrary, when the OD_{600} was 1.0, the callus induction rate was lower (Table 2). Of the four treatments, OD_{600} at 0.6 with an infection time of 10 min produced a petiole explant callus percentage of 34.23%, significantly higher than that of the other three treatments.

The percentage of callus formation on leaf explants was highest (31.23%) at OD_{600} at 1.0 with an infection time for 5 min.

Table 2
Leaf and petiole explants were treated with different concentrations of infection solution

Concentration/Time	Petioles			Leaves		
	Number	Callus	Callus rate (%)	Number	Callus	Callus rate (%)
OD_{600} = 0.6/5 min	35.33 ± 0.58	6.33 ± 0.58	17.91 ± 1.33	40 ± 1	4.33 ± 0.58	10.84 ± 1.46
OD_{600} = 0.6/10 min	39 ± 1	13.33 ± 0.58	34.23 ± 2.3	39 ± 2	7 ± 1	17.89 ± 1.6
OD_{600} = 1.0/5 min	43.33 ± 1.5	10 ± 1	23.04 ± 1.5	33 ± 2.65	10.33 ± 1.5	31.23 ± 2.6
OD_{600} = 1.0/10 min	37 ± 1	8 ± 1	21.61 ± 2.44	34.67 ± 52	7 ± 2	19.98 ± 4.35

A simple, rapid *Agrobacterium*-mediated genetic transformation system was established for *A. pumila*. The genetic transformation for petiole and leaf explants was carried out according to the following protocol: the petiole explants were first infected in an *Agrobacterium* suspension with OD_{600} at 0.6 for 10 min (Fig. 4a) and then co-cultured for 2 d in the dark (Fig. 4b). Adventitious shoot induction was performed for three months (Fig. 4c–f) and rooting induction for two months (Fig. 4g–i), to allow for further seedling development (Fig. 4h, i), and transgenic *A. pumila* plants were eventually obtained (Fig. 4j, k).

The leaf explants were infected in the *Agrobacterium* suspension with OD_{600} at 1.0 for 5 min (Fig. 5a) and co-cultured in the dark for 2 d (Fig. 5b). Adventitious shoots were induced for three months (Fig. 5c, d) and rooting induction for two months (Fig. 5e). After seedling development (Fig. 5f), transgenic *A. pumila* plants were eventually obtained.

Identification of positive plants and analysis of ApP5CS1.1 gene expression

Using transgenic plant leaf genomic DNA as a template for PCR amplification, bands of the expected size could be amplified in the transgenic plants, suggesting that the *ApP5CS1.1* gene was successfully transferred into *A. pumila* (Fig. 6a). Gene expression analysis by qRT-PCR showed that the *ApP5CS1.1* gene was up-regulated to varying degrees in the transgenic plants tested. In the three different transgenic lines, the expression level of *ApP5CS1.1* was 7.68, 3.27, and 1.27 times that of the wild-type plant, respectively (Fig. 6b). This result confirmed that the expression of *ApP5CS1.1* was differentially overexpressed in the different transgenic lines.

Discussion

The selection of plant explants is the key to adventitious shoot regeneration. To date, there have been reports of regeneration of different explants using plant apical meristems (Agrawal et al. 1997), stem tips (Sidky 2017), cotyledons (Wright et al. 2006), roots (Valvekens et al. 1988), leaf segments (Meijer et al. 1985), petioles (Kumar et al. 2015; Gambhir et al. 2017), and hypocotyls (Roussy et al. 1996). In this study, we used leaf and petiole explants of flask cultivated seedlings, and the hypocotyls and roots of 8-day-old seedlings for efficient adventitious shoot regeneration. It was found that calluses were generally formed either directly at the point of incision of the root and hypocotyl explants, on the cut surface of the cut end of the leaf or petiole, or along the side of the petiole and leaf explants. Previous studies have shown that different explants and explants of different ages will affect the speed and efficiency of callus formation. For example, the hypocotyls of cabbage are more suitable for regeneration than the cotyledons (Gerszberg et al. 2015; Gambhir et al. 2017). We compared the regeneration ability of young roots and hypocotyls over the same time period. The callus induction rate (99.42%) and adventitious shoot induction rate (97.7%) of *A. pumila* young roots were significantly higher than the rate for hypocotyls, which were 93.53% and 89.7%, respectively. In addition, the time taken to form calluses (7.33 ± 0.48 d) and adventitious shoots (32 ± 2.15 d) of young roots were also significantly shorter than for hypocotyls (10.05 ± 0.74 d and 59.05 ± 2.16 d, respectively) (Table 1).

Therefore, the 8-day-old seedling root explants of *A. pumila* were more suitable for inducing calluses and adventitious shoots than hypocotyl explants. The growth times of *A. pumila* leaf and petiole explants were longer than young roots and hypocotyls, with longer callus formation times. However, petiole and leaf adventitious shoot formation times (44.25 ± 4.07 d and 41.95 ± 2.37 d, respectively) were shorter than for young roots and hypocotyls. The percentage of leaf explants forming calluses (92.5%) was slightly lower than that of young roots (97.7%). Also, the adventitious shoots appeared sooner on leaf explants than on petioles (Table 1). Adventitious shoots were produced on explants grown on MS medium containing growth regulators, and the shoots elongated and developed roots to produce plants which grew well in a mixture of nutrient soil and vermiculite (*w/w*, 1:1).

This study showed that the root, hypocotyl, leaf and petiole explants of *A. pumila* can be efficiently induced to form calluses on MS medium supplemented with 6-BA and NAA. These four types of explant can quickly induce callus within 1–2 weeks and can induce adventitious shoots within one to two months (Table 1). *Arabidopsis pumila* plants have a large quantity of leaf and petiole tissues, thus providing excellent explants for *Agrobacterium*-mediated genetic transformation experiments. The concentration of *Agrobacterium* and the infection time usually have significant effects on the ease of infection of explants (Han et al. 2013; Movahedi et al. 2014). The most suitable *Agrobacterium* concentration and infection time required for the infection of petiole and leaf explants are different. The callus rate of petiole explants and leaf explants reached 34.23% and 31.23% with an *Agrobacterium* infection solution of OD_{600} at 0.6 and 1.0, and an infection time of 10 min and 5 min, respectively. In this study we performed *35S:ApP5CS1.1* genetic transformation on petiole and leaf explants of *A. pumila*, and successfully obtained transgenic generation plants. A qRT-PCR analysis showed that the *ApP5CS1.1* gene

were significantly differentially expressed in the two lines (Fig. 6), which provides a foundation for subsequent in-depth studies of the function of the *ApP5CS1.1* gene.

We compared the regeneration capacity of *A. pumila* roots, hypocotyls, leaves, and petioles. The induction time of root adventitious shoots was the shortest and the induction rate was the highest when grown on MS medium with 0.5 mg/L 6-BA and 0.1 mg/L NAA added. We did not use different hormone concentrations and parameter combinations in this study, and this will be the subject of future investigations. There are problems with the process of *Agrobacterium* transformation of petioles and leaf explants, such as the ease of contamination and slow callus formation. Future work will further improve and optimize the *A. pumila* regeneration cultivation and genetic transformation systems. Our findings suggested that our regeneration system is feasible. Using the four different explants of *A. pumila* (roots, hypocotyls, leaves, and petioles), the number of regenerated seedlings can be easily counted. Transgenic plants can be obtained by genetic transformation of leaves and petioles. These results further indicated that *A. pumila* is a suitable model material for studying the adaptations of ephemeral plants to the environment, and provides a basis for experiments to identify the stress tolerance genes of ephemeral plants and explore the biological mechanisms behind rapid growth and development as adaptions to the unique desert climate in Xinjiang.

Abbreviations

MS	Murashige and Skoog
6-BA	6-Benzylaminopurine
NAA	α-Naphthalene acetic acid
Carb	Carbenicillin
P5CS1	delta-1-pyrroline-5-carboxylic acid synthase 1
qRT-PCR	Quantitative real-time PCR

Declarations

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Authors' Contributions

XH and YJ conceived and designed research. YJ and LG conducted the experiments. DL, YL, and HA analyzed the data and revise the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Competing interests The authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

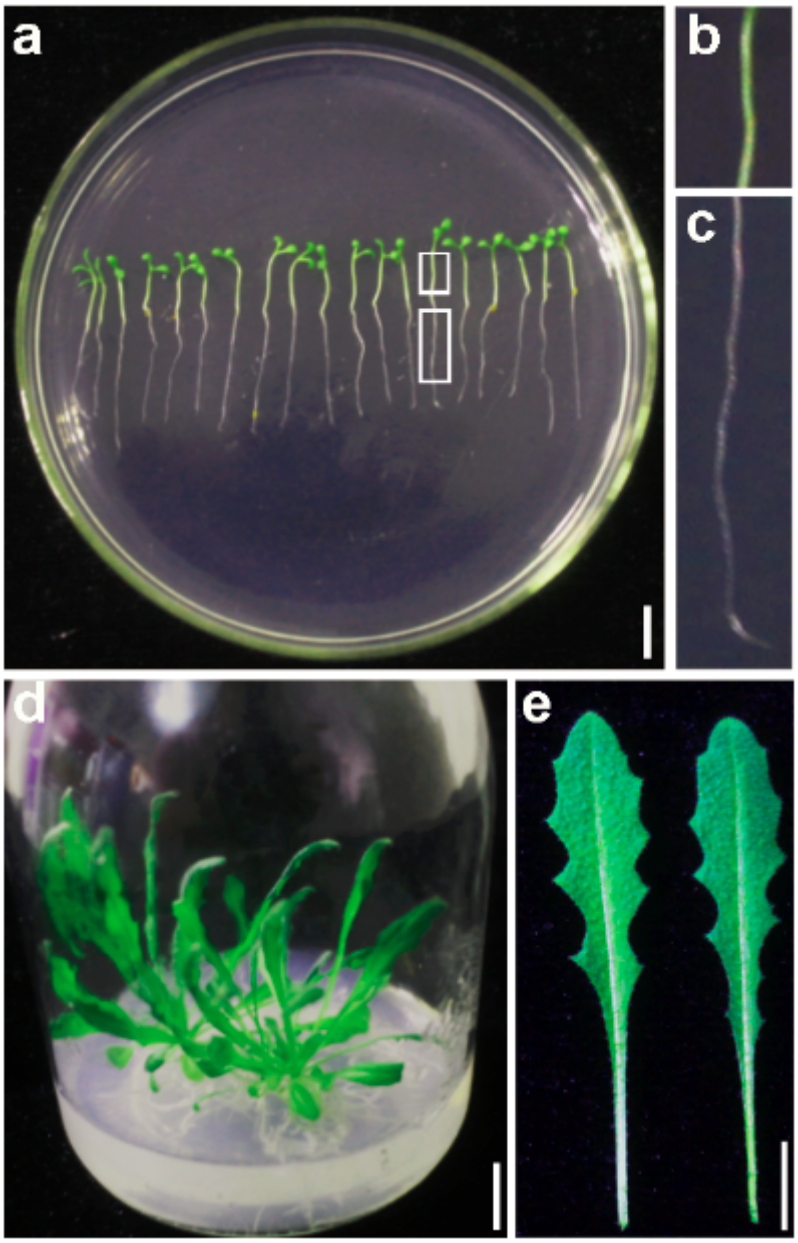

Figure 1

Observation on different explants of Arabidopsis pumila during greenhouse cultivation. a The seedlings were grown on MS medium for 8 d. b and c are enlarged images of hypocotyls and young roots of seedlings in a plot, respectively. d A. pumila seedlings were cultured in greenhouse for 30 d. e Leaves and petioles of A. pumila. Scale bar, 1 cm.

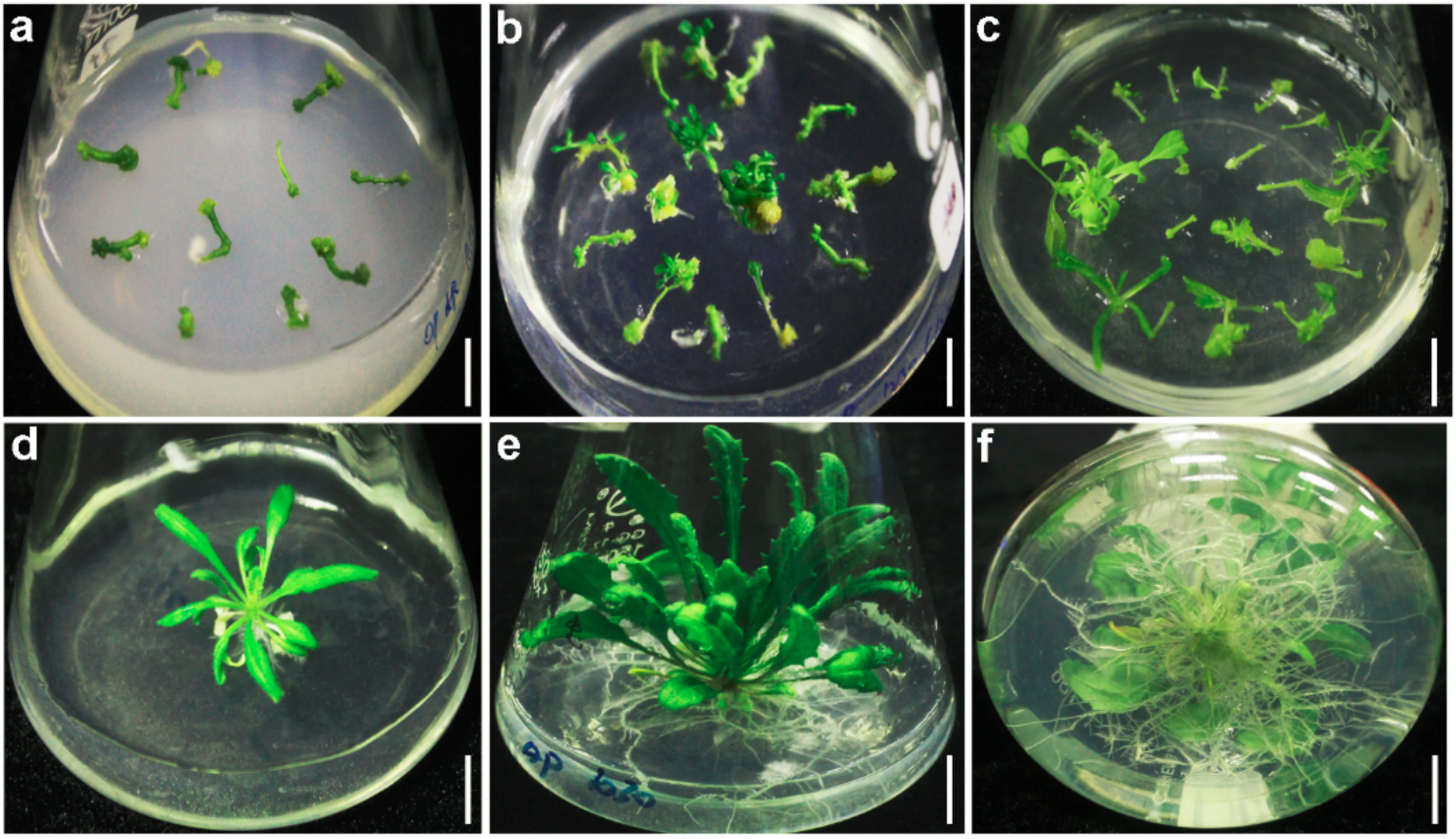

Figure 2

In vitro regeneration system of young root explants from Arabidopsis pumila. a Young root formed embryogenic callus. b Embryogenic callus induced adventitious shoots. c embryogenic callus formed a large number of adventitious shoots. d Rooting medium to induce rooting. e and f Tissue culture seedlings grew a large number of roots. Scale bar, 1 cm.

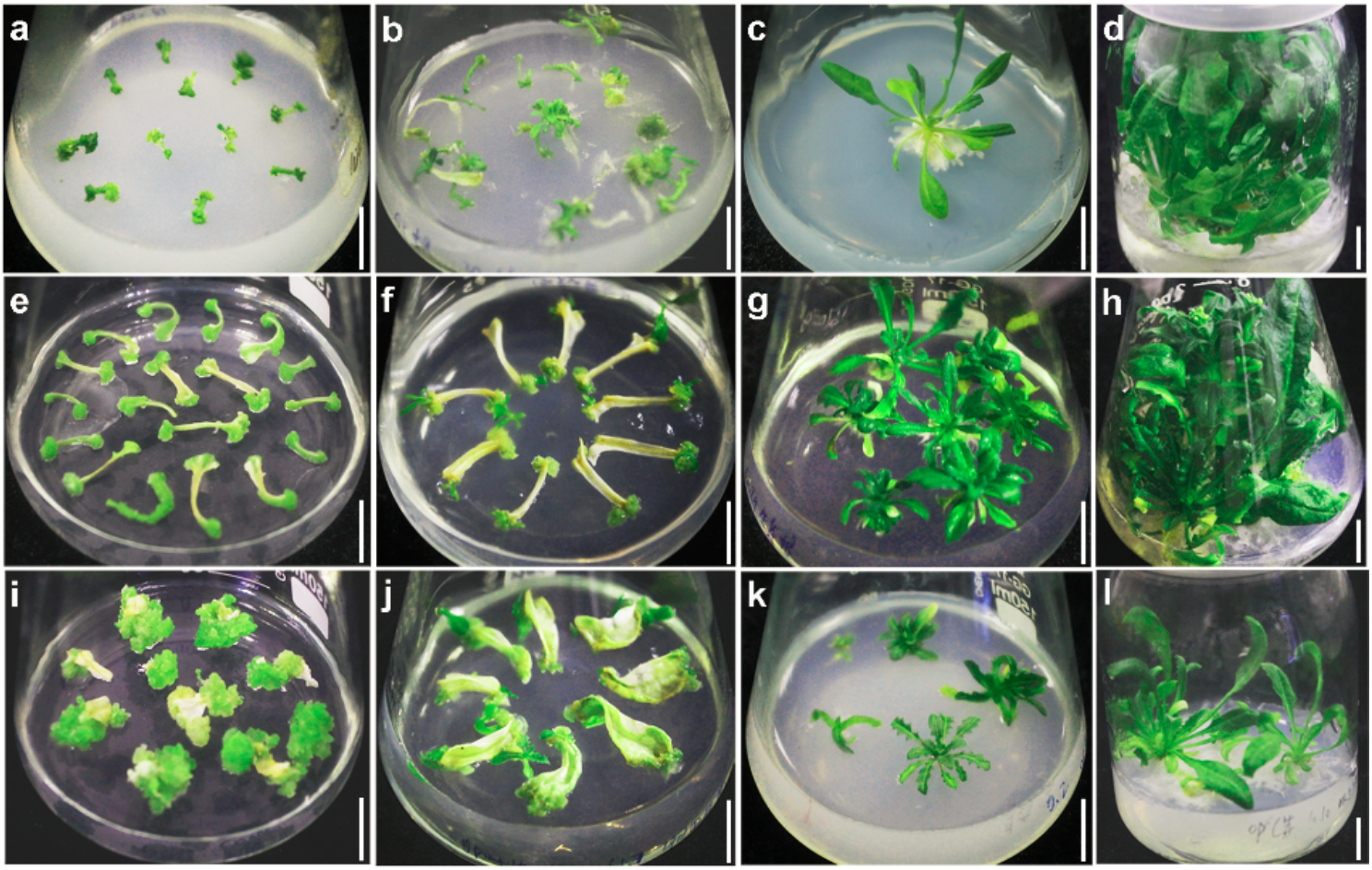

Figure 3

In vitro regeneration system of hypocotyl, petiole, and leaf explants from Arabidopsis pumila. a, e, i Calluses induced from hypocotyl, petiole, and leaf explants; b, f, j Calluses formed adventitious shoots. c, g, k Rooting medium to induce adventitious shoots rooting. d, h, l Regenerated seedlings induce a large number of roots. Scale bars, 1 cm.

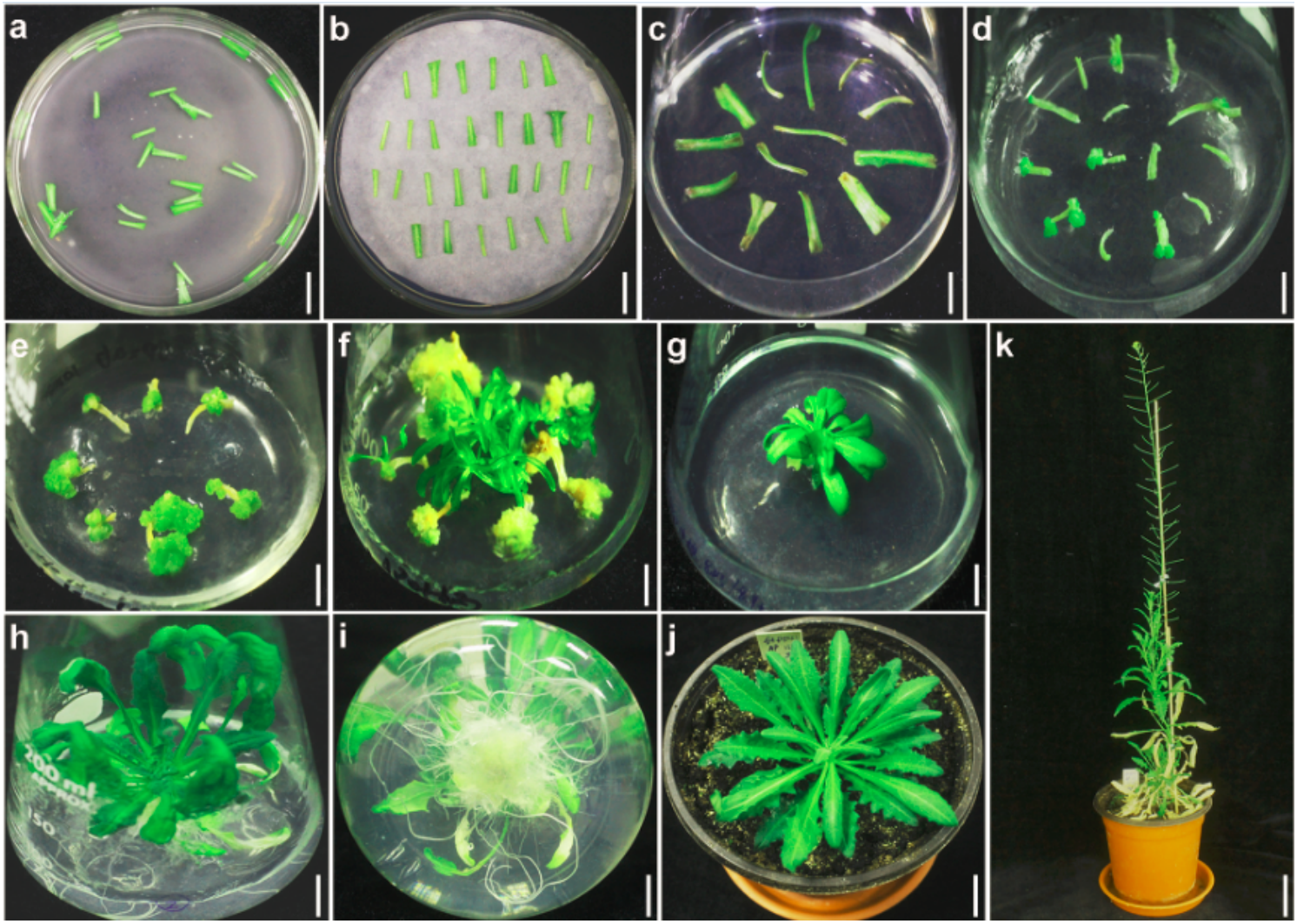

Figure 4

Agrobacterium-mediated transformation of 35S:ApP5CS1.1 into petiole explants of A. pumila. a Petioles were infected with A. tumefacienis with an OD600 value of 0.6 for 10 min. b The petiole explants were co-cultured for 2 d. c Petiole explants were cultured for 7 d after infection. d The petiole explants formed calluses at 35 d. e The calluses of petiole explants for 53 d. f Petiole explants formed adventitious shoots at about 3 months. g Rooting medium induced adventitious shoots rooting. h Adventitious shoots induced to form a large number of roots in 2 months and 15 d. i Observation on the roots of transgenic seedlings. j Transplanting to soil for growth. k Adventitious shoots produced a large number of siliques. Scale bars in Fig. a–i represent 1 cm, and scale bar represents 5 cm in the Fig. k.

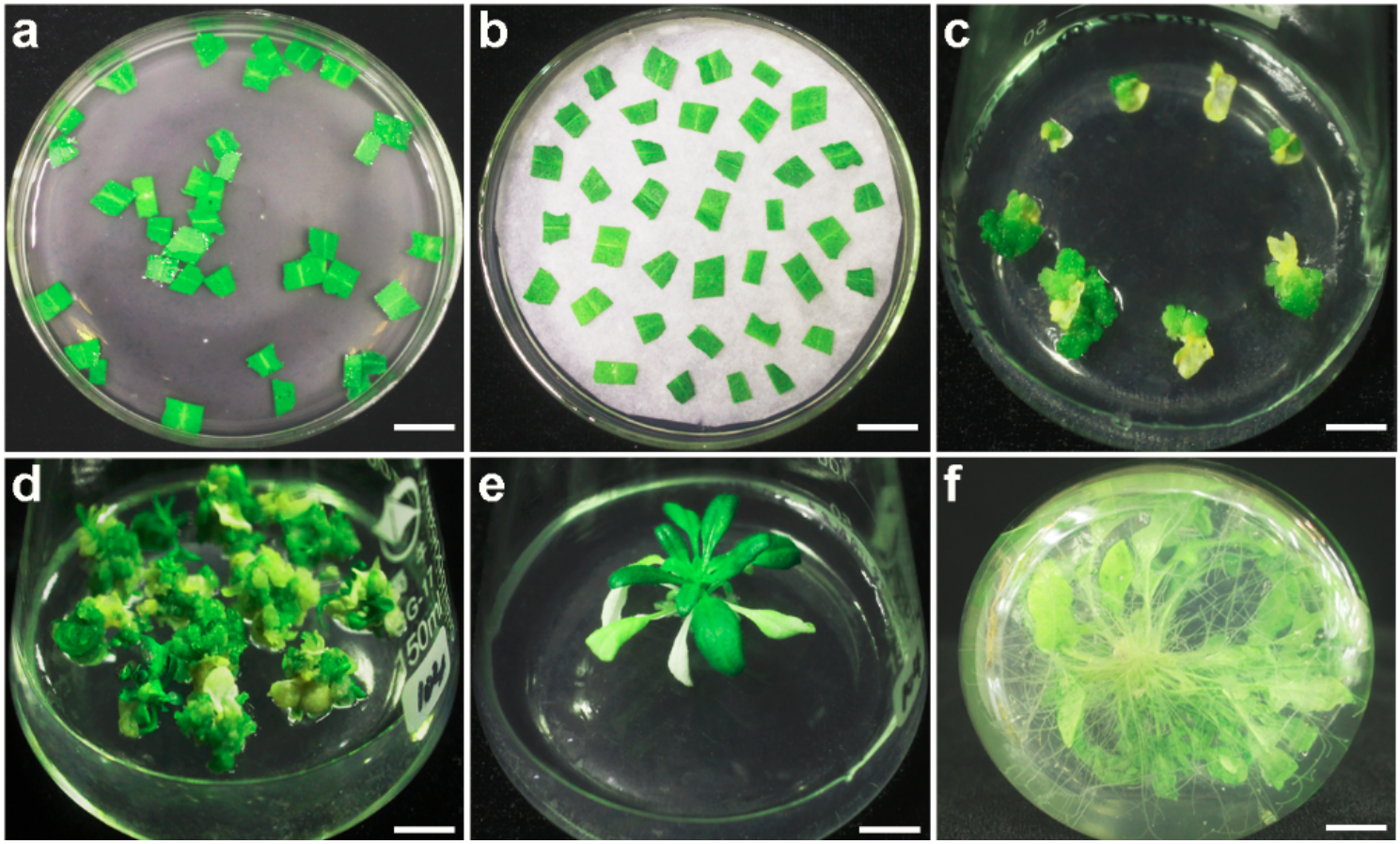

Figure 5

The flowchart of the Agrobacterium-mediated transformation system of 35S:ApP5CS1.1 into leaf explants of for A. pumila. a Leave explants were infected with A. tumefacien strain GV3101 harboring 35S:ApP5CS1.1 plasmids with an OD600 value of 1.0 for 5 min. b The leaf explants were co-culture for 2 d. c Callus status of leaf explants for 53 d. d Leaf explants formed adventitious shoots at about three months and 20 d. e Rooting medium induced adventitious shoots rooting. f Adventitious shoots were induced to form a large number of roots in two months and 15 d. Scale bars indicate 1 cm.

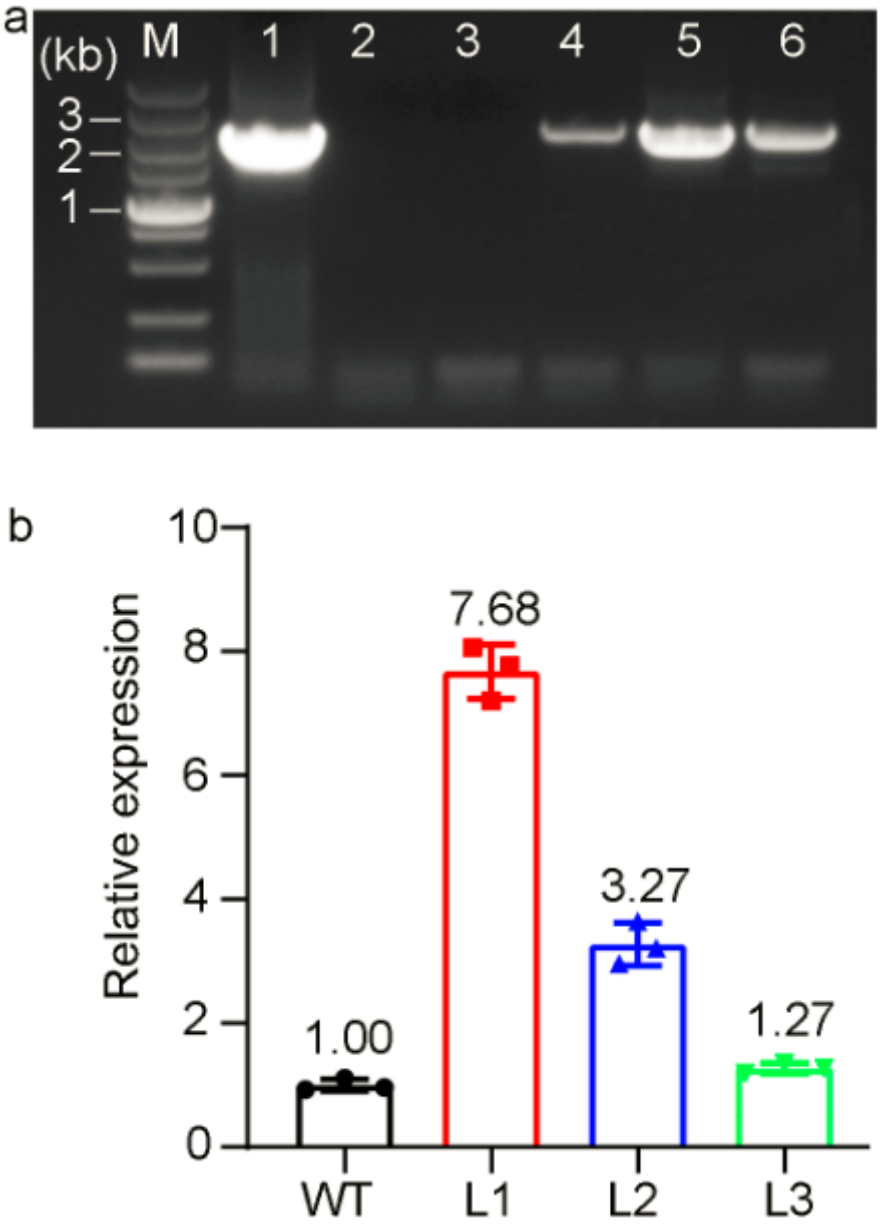

Figure 6

Gene expression analysis by qRT-PCR showed that the ApP5CS1.1 gene was up-regulated to varying degrees in the transgenic plants tested. In the three different transgenic lines, the expression level of ApP5CS1.1 was 7.68, 3.27, and 1.27 times that of the wild-type plant, respectively (Fig. 6b)

Supplementary Files

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