

# The Establishment of Two Efficient Transformation Systems in Quinoa

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## Research Article

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# Abstract

Quinoa (*Chenopodium quinoa* Willd.) provides a gluten-free food with abundant nutrition and is potential to become a major crop in future. Quinoa has a lot of unique features including the high tolerance to multiple stresses, of which the underlying mechanisms may help improve other crops. Genetic manipulation will provide powerful tools to investigate the function of key genes in regulation of quinoa development and stress responses, which will further improve quinoa planting in the fields. However, the efficient transformation system for quinoa has not been well developed yet. Here, we established two rapid and efficient transformation systems for quinoa by using hairy root and leaf agroinfiltration, which provide useful tools for quick analyses of genes function in quinoa. Hairy roots were obtained from three types of explants, including cotyledon-nod with hypocotyl, cotyledon itself, and hypocotyl pieces. Interestingly, explants of cotyledon-nod with hypocotyl showed the highest transformation efficiency at 67.9%, and cotyledon displayed medium efficiency at 42.2%, while hypocotyl with the lowest at 31.6%. We also obtained transgenic quinoa roots successfully *in-vivo*, which showed low efficiency, but this provides a potential method to test gene function in live plants. By using young leaves for agroinfiltration, direct injection showed a better transgenic effect compared with vacuum penetration. Overall, the transformation systems using both hairy root and leaf agroinfiltration provide efficient and convenient ways to manipulate and analyse gene functions in quinoa.

## Key Message

The cotyledon-nod with hypocotyl was the most suitable explant for quinoa hairy roots induction. Direct injection displayed a much better result than vacuum penetration in leaf agroinfiltration.

## Introduction

Quinoa, which belongs to the *Chenopodiaceae* family, contains abundant nutrition and is used as the staple food for the indigenous inhabitants of South America, including the Andean regions for more than 7000 years (Wilson 1990; Jacobsen et al. 2003). Quinoa, considered as a potential future major crop, has attracted intense public attention since the Food and Agriculture Organization of the United Nations announced “International Year of Quinoa” in 2013 (Bazile et al. 2016). Previous analyses showed that quinoa constitutes all of the essential amino acids required for human health, and contains more protein content than rice, barley, corn, rye and sorghum (R. et al. 2003; Bodner-Montville et al. 2006; Abugoch et al. 2010; Gonzalez et al. 2012; Escuredo et al. 2014; Aloisi et al. 2016; Filho et al. 2015). Moreover, quinoa can sustainably endure well in the extreme growth conditions, including drought, salinity and frost (Ruiz et al. 2014; Ruiz et al. 2016). For instance, some varieties of quinoa can tolerate the salinity around 700 mM NaCl, which is higher than the salinity of seawater (Hariadi et al. 2011; Orsini et al. 2011; Adolf et al. 2012). These unique properties of quinoa, including stress tolerance and high nutrition, result in its considerable roles in global food security due to climate change paradigm shifts. Recently, there is a surge in the functional analysis of quinoa genes own to the availability of the genome draft and sequence ((Zou et al. 2017; Jarvis et al. 2017). However, all the functional verifications were only through

the comparison of gene expression profiles based on the relative phenotypes. Moreover, it is crucial to develop a method to study the gene function through genetic manipulation.

At present, *Agrobacterium tumefaciens*-mediated stable transformation is a prominent method that is used to analyze gene function regulating plant development or environmental adaptation. Although the stable transformation in quinoa has been studied since last century, it is still unsuccessful (Komari 1990; López-Marqués et al. 2020). The function analyses are alternatively done in model plant systems such as *Arabidopsis thaliana* or *Nicotiana benthamiana* (Imamura et al. 2019). However, there is still a significant barrier referring to unique genes and pathways of quinoa. Thus, both transient and stable transformation systems are critically needed to be established to investigate the function of valuable genes in quinoa.

Agroinfiltration is *A. tumefaciens*-mediated instant transformation method for gene function related studies, which is fast, real-time and economical. Agroinfiltration is a convenient tool in plant gene function research without resorting to stable transformation (Del Toro et al. 2014). Through the injection of *A. tumefaciens*, extraneous genes are transiently expressed in leaves, fruits, flowers, petals, roots, needles and other tissues (Ma et al. 2020). Compared with stable transformation that produces minimal transgenic lines and needed to consider the effect of T-DNA insert position, agroinfiltration infects a large number of cells with high efficiency in a short time and does not require T-DNA integration (Yang et al. 2000; Gelvin and Stanton 2010).

*Agrobacterium rhizogenes*-mediated transformation system has many advantages compared with *A. tumefaciens*-mediated transformation which is time-consuming, genotype dependent and laborious. *A. rhizogenes* can potentially infect numerous varieties of dicotyledonous plants and subsequently induce transgenic hairy root lines (Gomes et al. 2019; Lam et al. 1984). The hairy root system can excellently characterize root development and related gene functions, which has been extensively applied in metabolic engineering, rhizosphere physiology, environmental stress response, etc. However, when the target genes or biological pathways don't work in the plant roots only, the hairy root system will not be applicable. The composite plant consisting of the wild-type shoots and transgenic hairy roots can be used to study root-shoot interactions and resistance against the various environmental and abiotic stresses, especially when roots are in stress conditions, such as salinity, drought or heavy metal (Mellor et al. 2012; Xue et al. 2017; Kajikawa et al. 2010; Cho et al. 2000; Cai and D. 1997; Savka 1990). Besides, the composite plants were employed in previous studies of hormone transport, symbiosis, secondary metabolites and root nutrient uptake. Up to date, various plant species have been established ex-vitro composite plant transformation system, such as pea (Clemow et al. 2011), soybean (An et al. 2018), tea (Alagarsamy et al. 2018) and peanut (Guimaraes et al. 2017). Both hairy roots in-vitro and composite plants could be derived in a cost-effective, fast and efficient way, which were considered as a practical protocol, especially for gene function study in roots. In this study, we developed the hairy root system and leaf agroinfiltration system, which were used to analyse gene function rapidly in quinoa.

## Materials And Methods

## Plant materials preparation

Four quinoa cultivars: MS509/N, QN108B, 141R and 20ALC were used. All the seeds were stored at 4-6 °C with 15% humidity.

Healthy seeds were carefully selected and then surface sterilized with chlorine gas (produced by mixing sodium hypochlorite and HCl) in a desiccator for 4-6 h. Sterilized seeds were then moved to a clean bench for air blowing for one day and germinated in half strength Murashige and Skoog medium (MS) at 50  $\mu\text{mol/s/m}^2$  light exposure 16 h light/8 h dark at  $25\pm 1$  °C for 5-6 days to obtain suitable explants from seedlings for hairy roots induction.

For *in-vivo* seedlings, healthy seeds were carefully selected and sowed in a mixture of soil: vermiculite: perlite (5:3:2) in the greenhouse at 540  $\mu\text{mol/s/m}^2$  light exposure 16 h light/8 h dark, two-week-old plants with only primary leaves spread out were used for agroinfiltration, two-month-old plants were used for *in-vivo* hairy roots induction.

## Preparation of the Agrobacterium strains for infection

*A. rhizogenes* K599 carrying the binary vector PTF102 harbouring the *GUS* gene was used to induce positive hairy roots. The hairy roots of negative control were induced using the same vector but without the *GUS* gene. The K599 was streaked on solidified YEP medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 15 g/L agar, pH 6.8) with a disposable plastic inoculation loop. After being incubated at 28 °C for two days, a half loop of the cultures were collected and restreaked on a new YEP medium then incubated for another one day. The *A. rhizogenes* cultures were collected and suspended to  $\text{OD}_{660}$  of 0.8 with infection medium (MS medium, Acetosyringone (AS 19.6 mg/L), 2-(4-Morpholino) ethanesulfonic acid (MES 500 mg/L), 3% Sucrose, pH 5.4). *A. tumefaciens* EHA105 carrying the binary vector pBI121 harbouring the *GUS* gene was used for agroinfiltration of leaves. EHA105 was prepared in the same way as K599. The cultures were collected and suspended to  $\text{OD}_{660}$  of 1.0 with infection medium (1/10 MS medium, 19.6 mg/L AS, 100 mg/L MES, 2% Sucrose, pH 5.6).

The vector pX11 harbouring *CYP76AD6*, *CYP76AD1*, *BvDODA1* and *cDOPA5GT* (kindly provided by Dr. Asaph Aharoni, Weizmann Institute of Science, Israel) was also used to test and verify the hairy root system. The transgenic hairy roots could produce red pigment betacyanins, which showed red-violet colour and could be visually observed (Polturak et al. 2016).

## Hairy roots induction from three types of explants

Three types of explants were obtained from five-day-old aseptic seedlings (Fig. 1-A) (MS509/N, QN108B, 141R, 20ALC), including the cotyledons with hypocotyl explant (CHE), were excised approximately 1 cm below the node of the cotyledons; the cotyledon explants (CE) excised from the seedlings; the hypocotyl explants (HE) were cut into a length of 1-1.5 cm.

CHEs were gently pierced near the cotyledonary node with a syringe tip for two to three times, CEes were pierced in the middle of the abaxial side (Fig. 1-B), while the HEs were obtained by just cutting off the hypocotyls into pieces. The explants were immediately incubated in the infection medium for 30 min at 25 °C (Fig. 1-C), then transferred to the co-culture medium at 22 °C in the dark (Fig. 1-D). Two days later, the explants were transferred to hairy roots induction medium (Gamborg B-5 basal medium, 500 mg/L MES, 3% Sucrose, pH 5.6) at 25 °C in the dark for two weeks.

### **Hairy roots induction *in-vivo***

*In-vivo* hairy roots were induced by injecting the Agrobacterium culture into the taproots of two-month-old seedlings (MS509/N) (Fig. 3-A). Part of the soil was moved away to expose the taproot. The *A. rhizogenes* K599 infection culture of OD<sub>660</sub> of 1.0 was slowly injected into the taproots with a 2.5 mL syringe (Fig. 3-B). After injection, the taproot was covered with wet vermiculite and a plastic film to maintain humidity (Fig. 3-C). The seedlings were grown at 540 μmol/s/m<sup>2</sup> light exposure 16 h light/8 h dark at 25°C until the hairy roots emerged.

### **Agroinfiltration of quinoa leaves**

Two-week-old seedlings (MS509/N) with only primary leaves spread out were chosen for agroinfiltration (Fig. 4-A). Two methods were developed for agroinfiltration of quinoa leaves, one was syringe injection, and the other was vacuum penetration.

The cotyledons and one of the primary leaves were removed. The *A. tumefaciens* EHA105 infection culture was injected into the other primary leaf remained on the seedlings with a 2.5 mL needle-free syringe (Fig. 4-B). The leaf infiltrated with EHA105 carrying pBI121 without *GUS* gene was used as the negative control. After injection, the treated seedlings were covered with a plastic jar and grew at 100 μmol/s/m<sup>2</sup> light intensity with the photoperiod of 16 h light/8 h dark at 22 °C for 4 days (Fig. 4-C).

In vacuum penetration, the remaining primary leaf was gently damaged by pressing a wire brush on the abaxial surface while placing a rubber cork wood on the adaxial surface (Fig. 4-D). The damaged leaf was immediately incubated in Agrobacterium culture and vacuumed at 0.07 MPa for 1 min in a tightly sealed desiccator (Fig. 4-E). After infiltration, the seedlings were treated for recovery in the same way as injection method.

### **Histochemical staining assay of GUS activity**

The hairy roots produced from three types of explants and *in-vivo* injection were collected for GUS staining assay. The leaves after agroinfiltration were subjected to GUS assay then soaked in 100% ethanol to remove and destain the chlorophyll.

### **Polymerase chain reaction (PCR) verification of *GUS* gene**

When hairy roots grew out from the explants, they were collected for PCR analysis of *GUS* gene. The plasmid of PTF102 was used as a positive control. To confirm the insertion of *GUS* gene, PCR was performed with a pair of primers specific to *GUS* gene (5'-GCAGGTCCTGGATTTTGGT-3'; 5'-ACGGCAGAGAAGGTAAGTGGGA-3'). The amplification conditions were: a 3-min melting step at 95 °C, followed by 30 cycles of a 30-s melting step at 95 °C, a 30-s annealing step at 55 °C, and a 25-s elongation step at 72 °C and a 5-min elongation step at 72 °C after cycles. PCR products were analyzed by electrophoretic separation on 1% agarose gels.

## Results

### Hairy roots induction *in-vitro*

We tested hairy roots induction in four quinoa cultivars, and found all four cultivars were able to produce hairy roots. We mainly used MS509/N for hairy roots induction in this study. All three types of explants, including CHE (Fig. 1-E), CE (Fig. 1-F) and HE (Fig. 1-G), produced hairy roots after two-week induction. GUS staining assay showed that CHE got the highest transformation efficiency at 67.9% (Fig. 2-A), followed by CH at 42.2% (Fig. 2-B) and the lowest was HE at 31.6% (Fig. 2-C). Hairy roots from the negative control didn't show GUS expression (Fig. 2-D). PCR analysis also confirmed the *GUS* gene expressed successfully in hairy roots (Fig. 2-I). The transformation efficiency was calculated by the ratio of the number of explants produced positive hairy roots to the total explants number (Table 1). The transformation efficiency differed among these explants, CHE and CE showed relatively higher transformation efficiency, which suggested they may be suitable explant types for transformation.

In order to test whether this method can be used for gene function analysis in quinoa, we transformed pX11 containing betacyanins biosynthesis genes to hairy roots, and found the accumulation of red colour in the hairy roots (Fig. 2-E, F, G, H). This suggested hairy root might be a good system to test gene function in quinoa.

### Hairy roots induction *in-vivo*

Many hairy roots initiated from the injection sites grew out from soil two weeks after the injection (Fig. 3-D). By using GUS reporter assay, although most of the roots showed negative results in GUS staining, we still could get positive ones (Fig. 3-E). Taproot injection was a tedious, time-consuming method with lower transformation efficiency compared with *in-vitro* system, however, this provided potential to study some genes expressed in root and regulated the shoot development.

### Leaf Agroinfiltration

For leaf agroinfiltration, we injected agrobacterium carrying pBI121 plasmids with *GUS* gene, the whole leaf showed blue colour in GUS staining assay (Fig. 4-G), while no blue colour appeared in the negative control (Fig. 4-F). We also tested the vacuum penetration system, and found only partial wounded spots appeared (Fig. 4-H). Considering the homogeneity of leaf age and Agrobacterium concentration (OD<sub>660</sub> of

1.0), the result indicated that vacuum penetration had much less throughput compared with syringe injection. For the cultivar MS509/N in this study, the primary spread leaves from two-week-old seedlings were the most suitable for agroinfiltration. Leaves from younger seedlings were too small and delicate to carry out the syringe technique and thus resulted in severe damages from the injection leading to necrosis, while older leaves had lower transformation efficiency.

## Discussion

### The hairy roots induction *in-vitro* and *in-vivo*

Hairy roots could be induced from different organs of quinoa, such as cotyledonary node, cotyledon, hypocotyl and *in-vivo* taproot in soil. Both CHE and CE had cotyledons and higher transformation efficiency. It was inferred that cotyledons provided more nutrition for hairy roots induction and subsequent elongation. CHE was considered to be the optimal explant type for its highest transformation efficiency according to our experiments.

For the *in-vivo* system, the composite plant with transgenic roots is an excellent research material to study genes expressed in roots and regulated the upper part of the plant. In this study, it was quite difficult to get positive hairy roots by injecting the taproot. It is necessary to optimize this protocol to obtain more composite plants. Many factors should be further explored such as the seedling age, the temperature and the soil humidity after injection (Alagarsamy et al. 2018; Guimaraes et al. 2017; Cao et al. 2009).

Hairy roots grow fast even without hormonal supplements, which can be used as bioreactors to produce bioactive substances more efficiently and economically. Quinoa contains abundant bioactive substances including saponins, polyphenols and flavonoids (Alvarez-Jubete et al. 2010). These secondary metabolites accumulate richly in roots (Lim et al. 2020). In our research, transgenic hairy roots produced betacyanins due to the expression of the genes from pX11, which will be ideal approach for commercially production of nutritious betacyanins from quinoa hairy roots.

For quinoa, *A. tumefaciens*-mediated stable transformation has not been developed yet. Hairy root is originated from a single cell and usually non-chimeric, which can be chosen as explant to regenerate stable transgenic plantlets (Chen et al. 2018). Up to date, some species have regenerated successfully from hairy root cultures, such as tobacco (Gurusamy et al. 2017), alfalfa (Jin et al. 2003), maize (Xu et al. 2006), potato (Butler et al. 2020). Especially for spinach, which is closely related to quinoa, the transgenic shoots have been regenerated from the hairy roots (Ishizaki et al. 2002). Therefore, the hairy root system here provides a new way to explore quinoa genetic transformation.

### Agroinfiltration of quinoa leaves

Agroinfiltration is an efficient system during the research of gene expression, promoter activities, metabolism, and protein-protein interaction (Levy et al. 2005; Mooney and Graciet 2020). To our

knowledge, this is the first report of agroinfiltration application in quinoa. The whole agroinfiltration process could be done within one month and independent of regeneration, which was efficient compared with tedious and laborious stable transformation.

Based on our research, seedling stage, humidity and recovery time were crucial to the system. In our protocol, the first pair of spread leaves from two-week-old seedlings had higher agroinfiltration efficiency, the explant older than one-month could hardly be injected. It was similar to *Nicotiana benthamiana*, *Arabidopsis thaliana*, *Brassica oleracea* and *Thellungiella salsuginea*, in which the younger leaves proved to be better for agroinfiltration (Zhang et al. 2020). Keeping high humidity was vital for quinoa seedlings to survive from agroinfiltration damage, the treated leaves were easy to wither or even to die if plants were not covered with jars to keep humidity.

The duration of recovery after agroinfiltration also influenced the assay result. It was inferred that genes started to express a few hours after transformation (Jones et al. 1989). Many reports showed that in plant tissues, gene expression level usually reached a peak 2-4 days after agroinfiltration (Lacroix and Citovsky 2013; Krenek et al. 2015; Marion et al. 2008). In GUS assay, light blue colour started to appear on the 2nd day and the dark blue appeared on the 4th day, so four-day recovery was considered suitable for quinoa seedlings.

## Conclusions

Two rapid and efficient systems for gene function analysis were established in this report: hairy roots system and leaf agroinfiltration system. Hairy roots could be obtained from three types of explants in two weeks with different efficiencies. The CHE showed the highest efficiency at 67.9%, thus was the recommended explant type for hairy roots induction in quinoa. The first pair of spread leaves of two-week-old seedlings were the most suitable material for agroinfiltration. The leaf injection displayed a much better result than vacuum penetration in transient expression assay.

## Abbreviations

MS: Murashige and Skoog; AS: Acetosyringone; MES: 2-(4-Morpholino) ethanesulfonic acid; CHE: Cotyledons with hypocotyl explant; CE: Cotyledon explant; HE: Hypocotyl explant; PCR: Polymerase chain reaction

## Declarations

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### Conflicts of Interest

All authors read and approved the final manuscript. The authors have no conflict of interest.

### **Availability of data and material**

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

### **Code availability**

Not applicable.

### **Authors' Contributions**

Wang YF and Zhang YZ performed most of experiments and wrote the manuscript; Ma J helped with experimental design and data analysis; Dai CJ, S. Jaikishun, Zhou YW and Yan JH helped with transformation procedure and plasmid preparation. Yang ZB provided valuable suggestions for transformation strategy. Song SK and Xu TD initiated and designed this project, and wrote the manuscript.

### **Ethics approval**

Not applicable

### **Consent to participate**

Not applicable

### **Consent for publication**

Not applicable

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## **References**

Abugoch L, Castro E, Tapia C et al (2010) Stability of quinoa flour proteins (*Chenopodium quinoa* Willd.) during storage. *Int J Food Sci Technol* 44:2013–2020

Adolf VI, Shabala S, Andersen MN et al (2012) Varietal differences of quinoa's tolerance to saline conditions. *Plant Soil* 357:117–129. <https://doi.org/10.1007/s11104-012-1133-7>

Alagarsamy K, Shamala LF, Wei S (2018) Protocol: High-efficiency in-planta *Agrobacterium*-mediated transgenic hairy root induction of *Camellia sinensis* var. *sinensis*. *Plant Methods* 14:1–8. <https://doi.org/10.1186/s13007-018-0285-8>

- Aloisi I, Parrotta L, Ruiz KB et al (2016) New insight into quinoa seed quality under salinity: Changes in proteomic and amino acid profiles, phenolic content, and antioxidant activity of protein extracts. *Front Plant Sci* 7:1–21. <https://doi.org/10.3389/fpls.2016.00656>
- Alvarez-Jubete L, Wijngaard H, Arendt EK, Gallagher E (2010) Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem* 119:770–778
- An J, Cheng C, Hu Z et al (2018) The *Panax ginseng* PgTIP1 gene confers enhanced salt and drought tolerance to transgenic soybean plants by maintaining homeostasis of water, salt ions and ROS. *Environ Exp Bot* 155:45–55. <https://doi.org/10.1016/j.envexpbot.2018.06.025>
- Bazile D, Jacobsen S, Verniau A (2016) The Global Expansion of Quinoa: Trends and Limits. *Front Plant Sci* 7:1–6. <https://doi.org/10.3389/fpls.2016.00622>
- Bodner-Montville J, Ahuja JKC, Ingwersen LA et al (2006) USDA Food and Nutrient Database for Dietary Studies: Released on the web. *J Food Compos Anal* 19:100–107. <https://doi.org/10.1016/j.jfca.2006.02.002>
- Butler NM, Jansky SH, Jiang J (2020) First-generation genome editing in potato using hairy root transformation. *Plant Biotechnol J* 18:2201–2209. <https://doi.org/10.1111/pbi.13376>
- Cai D (1997) Positional Cloning of a Gene for Nematode Resistance in Sugar Beet. *ence* 275:832–834
- Cao D, Hou W, Song S et al (2009) Assessment of conditions affecting *Agrobacterium rhizogenes*-mediated transformation of soybean. *Plant Cell Tissue Organ Cult* 96:45–52. <https://doi.org/10.1007/s11240-008-9458-x>
- Chen L, Cai Y, Liu X et al (2018) Soybean hairy roots produced in vitro by *Agrobacterium rhizogenes*-mediated transformation. *Crop J* 6:162–171. <https://doi.org/10.1016/j.cj.2017.08.006>
- Cho HJ, Farrand SK, Noel GR, Widholm JM (2000) High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. *Planta* 210:195–204. <https://doi.org/10.1007/PL00008126>
- Clemow SR, Clairmont L, Madsen LH, Guinel FC (2011) Reproducible hairy root transformation and spot-inoculation methods to study root symbioses of pea. *Plant Methods* 7:1–15. <https://doi.org/10.1186/1746-4811-7-46>
- Del Toro F, Tenllado F, Chung BN, Canto T (2014) A procedure for the transient expression of genes by agroinfiltration above the permissive threshold to study temperature-sensitive processes in plant-pathogen interactions. *Mol Plant Pathol* 15:848–857. <https://doi.org/10.1111/mpp.12136>
- Escuredo O, González Martín MI, Wells Moncada G et al (2014) Amino acid profile of the quinoa (*Chenopodium quinoa* Willd.) using near infrared spectroscopy and chemometric techniques. *J Cereal Sci*

60:67–74. <https://doi.org/10.1016/j.jcs.2014.01.016>

Filho AMM, Pirozi MR, Borges JTDS et al (2015) Quinoa: Nutritional, functional, and antinutritional aspects. *Crit Rev food ence Nutr* 57:1618–1630

Gelvin, Stanton B (2010) Plant proteins involved in *Agrobacterium*-mediated genetic transformation. *Annu Rev Phytopathol* 48:45–68

Gomes C, Dupas A, Pagano A et al (2019) Hairy Root Transformation: A Useful Tool to Explore Gene Function and Expression in *Salix* spp. Recalcitrant to Transformation. *Front Plant Sci* 10:1–6. <https://doi.org/10.3389/fpls.2019.01427>

Gonzalez JA, Konishi Y, Bruno M et al (2012) Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions. *J Sci Food Agric* 92:1222–1229

Guimaraes LA, Pereira BM, Araujo ACG et al (2017) Ex vitro hairy root induction in detached peanut leaves for plant-nematode interaction studies. *Plant Methods* 13:1–10. <https://doi.org/10.1186/s13007-017-0176-4>

Gurusamy PD, Schäfer H, Ramamoorthy S, Wink M (2017) Biologically active recombinant human erythropoietin expressed in hairy root cultures and regenerated plantlets of *Nicotiana tabacum* L. *PLoS One* 12:1–23. <https://doi.org/10.1371/journal.pone.0182367>

Hariadi Y, Marandon K, Tian Y et al (2011) Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. *J Exp Bot* 62:185–193. <https://doi.org/10.1093/jxb/erq257>

Imamura T, Isozumi N, Higashimura Y et al (2019) Isolation of amaranthin synthetase from *Chenopodium quinoa* and construction of an amaranthin production system using suspension-cultured tobacco BY-2 cells. *Plant Biotechnol J* 17:969–981. <https://doi.org/10.1111/pbi.13032>

Ishizaki T, Hoshino Y, Masuda K, Oosawa K (2002) Explants of Ri-transformed hairy roots of spinach can develop embryogenic calli in the absence of gibberellic acid, an essential growth regulator for induction of embryogenesis from non-transformed roots. *Plant Sci* 163:223–231. [https://doi.org/10.1016/S0168-9452\(02\)00097-3](https://doi.org/10.1016/S0168-9452(02)00097-3)

Jacobsen SE, Mujica A, Jensen CR (2003) The Resistance of Quinoa (*Chenopodium quinoa* Willd.) to Adverse Abiotic Factors. *Food Rev Int* 19:99–109

Jarvis DE, Ho YS, Lightfoot DJ et al (2017) Corrigendum: The genome of *Chenopodium quinoa*. *Nature* 545:510

Jin H, Jia JF, Hao JG (2003) Protoplasts from *Agrobacterium rhizogenes*-transformed cell line of *Medicago sativa* L. regenerated to hairy roots. *Vitr Cell Dev Biol - Plant* 39:208–211.

<https://doi.org/10.1079/IVP2002367>

Jones H, Ooms G, Jones MGK (1989) Transient gene expression in electroporated *Solanum* protoplasts. *Plant Mol Biol* 13:503–511. <https://doi.org/10.1007/BF00027310>

Kajikawa M, Morikawa K, Abe Y et al (2010) Establishment of a transgenic hairy root system in wild and domesticated watermelon (*Citrullus lanatus*) for studying root vigor under drought. *Plant Cell Rep* 29:771–778. <https://doi.org/10.1007/s00299-010-0863-3>

Komari T (1990) Transformation of cultured cells of *Chenopodium quinoa* by binary vectors that carry a fragment of DNA from the virulence region of pTiBo542. *Plant Cell Rep* 9:303–306

Krenek P, Samajova O, Luptovciak I et al (2015) Transient plant transformation mediated by *Agrobacterium tumefaciens*: Principles, methods and applications. *Biotechnol Adv* 33:1024–1042. <https://doi.org/10.1016/j.biotechadv.2015.03.012>

Lacroix B, Citovsky V (2013) The roles of bacterial and host plant factors in *Agrobacterium*-mediated genetic transformation. *Int J Dev Biol* 57:467–481

Lam S, Lam B, Harrison L, Strobel G (1984) Genetic information on the Ri plasmid of *Agrobacterium rhizogenes* determines host specificity. *Plant ence Lett* 34:345–352

Levy M, Rachmilevitch S, Abel S (2005) Transient *Agrobacterium*-mediated gene expression in the *Arabidopsis* hydroponics root system for subcellular localization studies. *Plant Mol Biol Report* 23:179–184

Lim JG, Park HM, Yoon KS (2020) Analysis of saponin composition and comparison of the antioxidant activity of various parts of the quinoa plant (*Chenopodium quinoa* Willd.). *Food Sci Nutr* 8:694–702. <https://doi.org/10.1002/fsn3.1358>

López-Marqués RL, Nørrevang AF, Ache P et al (2020) Prospects for the accelerated improvement of the resilient crop quinoa. *J Exp Bot* 71:5333–5347. <https://doi.org/10.1093/jxb/eraa285>

Ma Z, Liu JJ, Zamany A, Williams H (2020) Transient gene expression in western white pine using agroinfiltration. *J For Res* 31:1823–1832. <https://doi.org/10.1007/s11676-019-00938-5>

Marion J, Bach L, Bellec Y et al (2008) Systematic analysis of protein subcellular localization and interaction using high-throughput transient transformation of *Arabidopsis* seedlings. *Plant J* 56:169–179. <https://doi.org/10.1111/j.1365-313X.2008.03596.x>

Mellor KE, Hoffman AM, Timko MP (2012) Use of ex vitro composite plants to study the interaction of cowpea (*Vigna unguiculata* L.) with the root parasitic angiosperm *Striga gesnerioides*. *Plant Methods* 8:1–12. <https://doi.org/10.1186/1746-4811-8-22>

- Mooney BC, Graciet E (2020) A simple and efficient *Agrobacterium* -mediated transient expression system to dissect molecular processes in *Brassica rapa* and *Brassica napus*. *Plant Direct* 4
- Orsini F, Accorsi M, Gianquinto G et al (2011) Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: functional elements of successful halophytism. *Funct Plant Biol* 38:818–831
- Polturak G, Breitel D, Grossman N et al (2016) Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants. *New Phytol* 210:269–283. <https://doi.org/10.1111/nph.13796>
- Repo-Carrasco R, C., et al (2003) Nutritional Value and Use of the Andean Crops Quinoa (*Chenopodium quinoa*) and Kaiwa (*Chenopodium pallidicaule*). *Food Rev Int* 19
- Ruiz KB, Biondi S, Martínez EA et al (2016) Quinoa – a Model Crop for Understanding Salt-tolerance Mechanisms in Halophytes. *G Bot Ital* 150:357–371
- Ruiz KB, Biondi S, Oses R et al (2014) Quinoa biodiversity and sustainability for food security under climate change. A review. *Agron Sustain Dev* 34:349–359. <https://doi.org/10.1007/s13593-013-0195-0>
- Savka MA (1990) Induction of hairy roots on cultivated soybean genotypes and their use to propagate the soybean cyst nematode. *Phytopathology* 80:503–508
- Wilson HD (1990) Quinoa and Relatives (*Chenopodium* sect. *Chenopodium* subsect. *Celluloid*). *Econ Bot* 44:92–110. <https://doi.org/10.1007/BF02860478>
- Xu H, Zhou X, Lu J et al (2006) Hairy roots induced by *Agrobacterium rhizogenes* and production of regenerative plants in hairy root cultures in maize. *Sci China Ser C Life Sci* 49:305–310. <https://doi.org/10.1007/s11427-006-0305-1>
- Xue R, Wu X, Wang Y et al (2017) Hairy root transgene expression analysis of a secretory peroxidase (PvPOX1) from common bean infected by *Fusarium* wilt. *Plant Sci* 260:1–7. <https://doi.org/10.1016/j.plantsci.2017.03.011>
- Yang Y, Li R, Qi M (2000) In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. *Plant J* 22:543–551. <https://doi.org/10.1046/j.1365-313X.2000.00760.x>
- Zhang Y, Chen M, Siemiatkowska B et al (2020) A Highly Efficient *Agrobacterium*-Mediated Method for Transient Gene Expression and Functional Studies in Multiple Plant Species. *Plant Commun* 1:100028. <https://doi.org/10.1016/j.xplc.2020.100028>
- Zou C, Chen A, Xiao L et al (2017) A high-quality genome assembly of quinoa provides insights into the molecular basis of salt bladder-based salinity tolerance and the exceptional nutritional value. *Cell Res* 27:1327–1340. <https://doi.org/10.1038/cr.2017.124>

# Tables

Table 1  
The efficiency of three types of explants

Explant type	Total number	Number of explant with positive hairy roots	Infection efficiency
Cotyledon node	56	38	67.9%
Cotyledon	64	27	42.2%
Hypocotyl	57	18	31.6%

# Figures

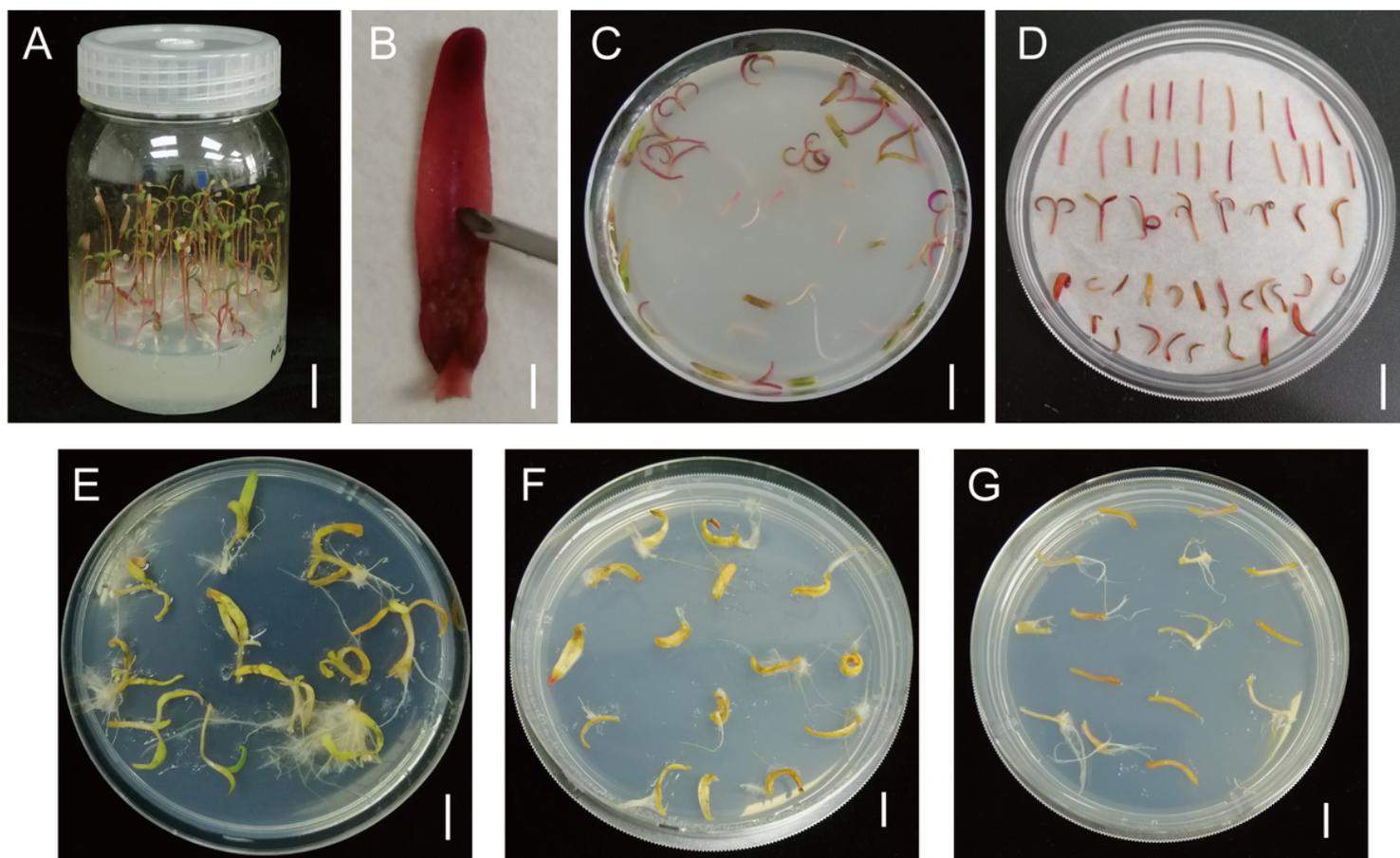
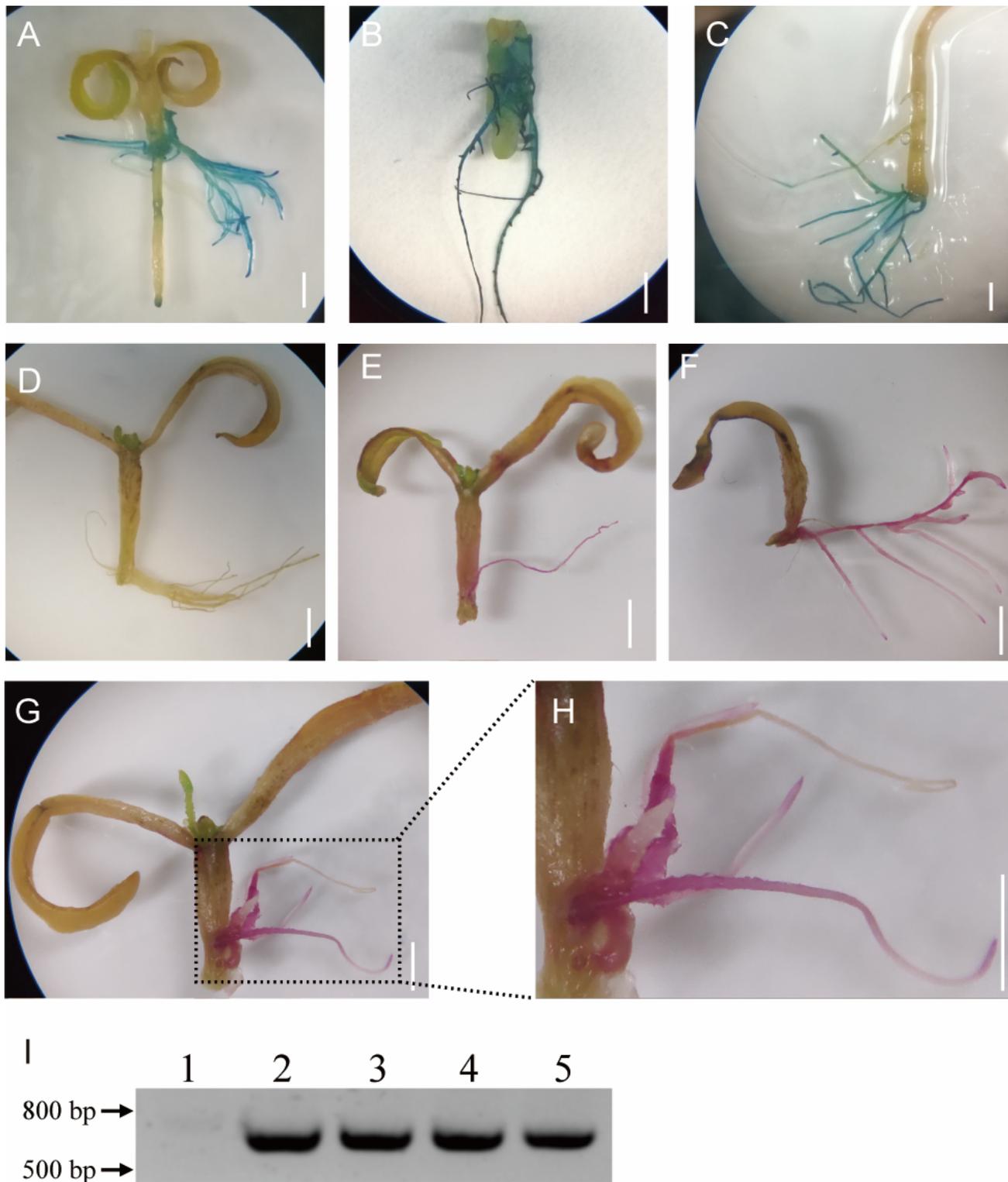


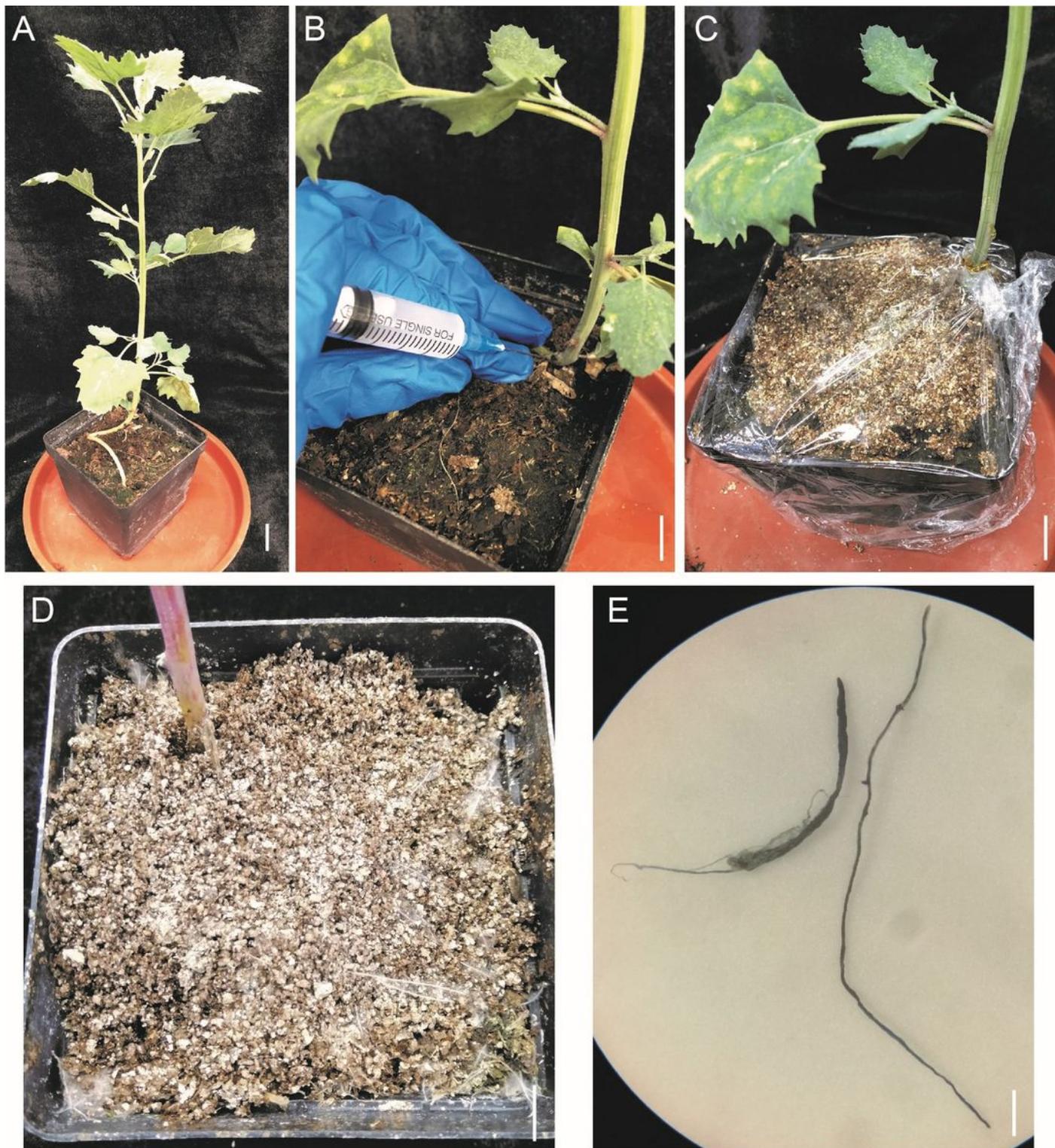
Figure 1

Hairy roots induction in-vitro. 5-day-old seedlings were obtained from half-strength MS medium(a), cotyledon was pierced with a syringe tip at the abaxial side (b), three types of explants were immersed into K599 culture (c), explants were transferred to co-culture medium covered with sterile filter paper (d), hairy roots from CHE in hairy root induction medium after two weeks (e), hairy roots from CE (f), hairy roots from HE (g). Scale bar a, c-g: 1 cm; b: 0.15 cm



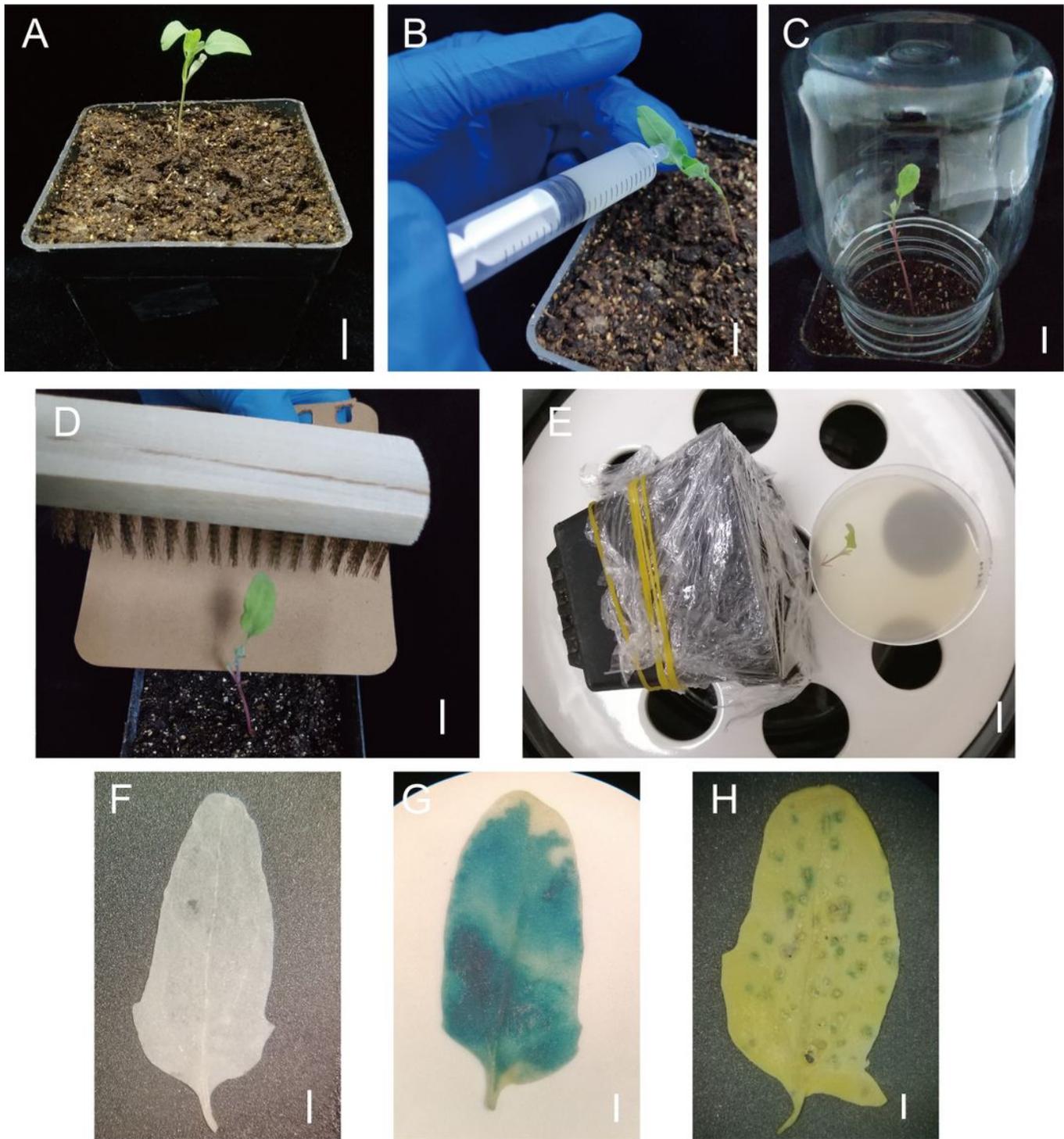
**Figure 2**

GUS assay with hairy roots from three types of explants: CHE (a), CE (b), HE (c), GUS assay with hairy roots from the negative control (d); red pigment in hairy roots—CHE (e), CE (f), CHE (g) and magnified picture of hairy roots (h); PCR analysis of GUS gene: 1 DNA from hairy roots of negative control, 2 plasmid of PTF102 as a positive control, 3-5 DNA from hairy roots of CHE, CE, HE (i). Scale bar a-c: 0.2 cm, d-h: 0.25 cm



**Figure 3**

Hairy roots induction in-vivo. 2-month-old seedling was used for injection (a), the taproot was injected with a syringe needle (b), covered with wet vermiculite and plastic film to keep humid (c), hairy roots of quinoa injection grew out from quinoa's tap root after two weeks (d), hairy roots cut from the tap root and assay for  $\beta$ -glucuronidase activity (e). Scale bar a: 0.5 cm; b-c: 1 cm; d-e: 0.25 cm



**Figure 4**

Agroinfiltration of quinoa leaves. Leaf was used for agroinfiltration from two-week-old seedling(a), leaf was injected at the abaxial side with a needleless syringe (b), the treated seedlings were covered with a plastic jar for recovery for 4 days (c), leaf was pressed at the abaxial side by the brush (d), leaf was incubated in *Agrobacterium* culture and vacuumed at 0.07 MPa for 1 min (e), GUS staining of leaf injected with 1/10 MS culture (negative control) (f), GUS assay of leaf injection (g), GUS assay of leaf vacuum penetration (h). Scale bar a-e: 1cm; f-h: 0.2 cm