Callus induction of tartary buckwheat and enhancement of its flavonoids *via FtCHS1* overexpression

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Tartary buckwheat (*Fagopyrum tataricum*), a popular and traditional health care-related cereal, has recently been the focus of research because of its metabolic regulation of flavonoids. Elicitingtissues in *vitro*culture is an effective way to explore flavonoid biosynthesis mechanisms in tartary buckwheat. In the present study, we developed an *in vitro* genetic transformation system using the tartary buckwheat variety 'Xiqiao No. 2'. The results showed thattherate of callus induced from hypocotylexplants on Murashige and Skoog (MS) medium containing 0.8 mg/L 6-BA and 3.5 mg/L 2,4-D was 100%. Much greater amounts of calli could then be obtained by repeated subculture on MS medium supplemented with 3.0 mg/L 6-BA and 1.0 mg/L KT. Furthermore, transgenic calli expressing the *FtCHS1* gene were obtained *via*Agrobacterium-mediatedtransformation. Overexpressing *FtCHS1* in tartary buckwheat callus led tothe marked promotion of flavonol ($P<0.01$) and anthocyanin accumulation ($P<0.05$) due to the dramatic upregulation of the transcription of *FtCHI*, *FtCHS2*, *FtFLS1*, *FtFLS2*, *FtFLS3* and *FtDFR1*, the genes of key enzymes involved in the flavonol and anthocyaninbiosynthesispathways ($P < 0.01$). This study provides solidsupportfor further transgenic manipulation of calli as part of a system for regenerating tartary buckwheat.

Callus Induction of Tartary Buckwheat and Enhancement of its Flavonoids *via FtCHS1* Overexpression

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Abstract

Tartary buckwheat (*Fagopyrum tataricum*), a popular and traditional health care-related cereal, has recently been the focus of research because of its metabolic regulation of flavonoids. Eliciting tissues in *vitro* culture is an effective way to explore flavonoid biosynthesis mechanisms in tartary buckwheat. In the present study, we developed an *in vitro* genetic transformation system using the tartary buckwheat variety 'Xiqiao No. 2'. The results showed that the rate of callus induced from hypocotyl explants on Murashige and Skoog (MS) medium containing 0.8 mg/L 6-BA and 3.5 mg/L 2,4-D was 100%. Much greater amounts of calli could then be obtained by repeated subculture on MS medium supplemented with 3.0 mg/L 6-BA and 1.0 mg/L KT. Furthermore, transgenic calli expressing the *FtCHS1* gene were obtained *via* Agrobacterium-mediated transformation. Overexpressing *FtCHS1* in tartary buckwheat callus led to the marked promotion of flavonol ($P<0.01$) and anthocyanin accumulation ($P<0.05$) due to the dramatic upregulation of the transcription of *FtCHI*, *FtCHS2*, *FtFLS1*, *FtFLS2*, *FtFLS3* and *FtDFR1*, the genes of key enzymes involved in the flavonol and anthocyanin biosynthesis pathways ($P < 0.01$). This study provides solid support for further transgenic manipulation of calli as part of a system for regenerating tartary buckwheat.

Keywords: Tartary buckwheat; Callus; Chalcone synthase; Flavonoids

Introduction

Fagopyrum tataricum, also known as tartary buckwheat, is a traditional healthy cereal with abundant flavonoids. As an ancient producer of coarse grains, tartary buckwheat is cultivated and consumed in Asia and Europe(Fabjan et al. 2003). In China, its main production area is in the southwestern region, including Sichuan, Guizhou, Yunnan and Tibet provinces. In particular, the Liangshan area, which is located in western Sichuan, produces among the most excellent-quality tartary buckwheat worldwide (SabineScheucher 2004; Zhao & Al 1998). Amazingly, investigations have revealed that the local Yi people, who compose a major ethnic minority in Liangshan, have much lower hypertension and cardiovascular patients than do people in other areas (Liu et al. 2012). Additional studies indicated that the Yi people consume tartary buckwheat as their staple food (Wang et al. 2010; Xing 2002). Rutin, a flavonoid compound that is abundant in tartary buckwheat, has been widely used as a health product and medicine because of its many pharmacological effects, such as anti-ageing, hypoglycaemic, antibacterial and anti-inflammatory effects (Yang et al. 2012). At present, tartary buckwheat, a kind of functional health food rich in flavonoids, has attracted widespread attention worldwide, especially among vegetarians. Therefore, revealing the regulation of flavonoid metabolism has become a hot topic in the study of tartary buckwheat.

In recent years, Logacheva *et al*. (Logacheva et al. 2011) completed the transcriptome sequencing of tartary buckwheat and common buckwheat (*Fagopyrum esculentum* Moench.) at the flowering stage. Gupta *et al*. (Nidhi et al. 2011)subsequently compared the flavonoid biosynthesis genes and rutin content variation between tartary buckwheat and common buckwheat at different growth stages. Suzuki *et al*. (suzuki et al. 2005) profiled the rutin concentration and rutin glucosidase activity in tartary buckwheat under stress conditions. Moreover, Li *et al.* and Park *et al.* (Li et al. 2012c; Nam Il et al. 2011) compared the expression profiles of flavonoid biosynthetic genes and the accumulation of rutin and anthocyanin in different varieties and mutants of tartary buckwheat. More importantly, many R2R3-MYB transcription factors from tartary buckwheat have been suggested to be involved in flavonoid biosynthesis regulation, such as FtMYB1 and FtMYB2 to enhance proanthocyanidin biosynthesis, *FtMYB15* to improve anthocyanin and proanthocyanidin accumulation, and four

jasmonate-responsive MYB factors to repress rutin biosynthesis (Li et al. 2012a; Li et al. 2012b; Yao et al. 2016). However, the abovementioned functional studies were mainly performed in heterologous hosts such as Arabidopsis(Gao et al. 2016) and tobacco(Zhou et al. 2019), which may not effectively and accurately reflect the actual biological effects of the transcription factors in tartary buckwheat. Although a hairy root system is a better choice for flavonoid metabolism research in tartary buckwheat (Zhang et al. 2018), its application is also limited due to the inability to differentiate into different cell types and develop into a complete plant(Guillon et al. 2006; Kim et al. 2009). Therefore, a genetic transformation system based on calli is the best technique for gene functional studies in tartary buckwheat so far. In actuality, there are some reports about the successful induction of calli and even the subsequent development of plantlet regeneration of tartary buckwheat *in vitro*(Soojeong et al. 2013; Wang et al. 2016; Zhang et al. 2019). Regretfully, no functional genes from tartary buckwheat have been successfully transformed into its callus so far(Betekhtin et al. 2017; Huang et al. 2016; Kumar 2018).

In this study, stable and effective calli were induced successfully from the hypocotyl of tartary buckwheat. The *FtCHS1* gene, which encodes chalcone synthase, the enzyme that catalyses the first committed step in the flavonoid biosynthetic pathway in tartary buckwheat, was transformed by *Agrobacterium tumefaciens* and overexpressed in calli. The results strongly indicated that *FtCHS1* plays a significant positive role in flavonol biosynthesis in tartary buckwheat. Additionally, some possible factors affecting callus formation and transformation are also further discussed.

Materials & Methods

Plant Materials and Growth Conditions

With respect to tartary buckwheat, ‘Xi Qiao No. 2’ was donated by Prof. Anhu Wang of Xichang College and planted at Sichuan Agricultural University. The tartary buckwheat seeds were disinfected with 75% alcohol and 0.1% $HgCl_2$ and then sown on 1/2-strength MS media (0.7-0.75% agar, 2.37 g/L MS, 3% sucrose, pH 5.8-6.2.). The seeds were incubated in a light incubator for 10-12 days (12 h of light culture, 12 h of dark culture, light intensity of 2.0 klux, temperature of 23 ± 2 °C). After 12 days, tartary buckwheat seedlings were obtained at the cotyledon stage. The hypocotyl of the tartary buckwheat seedlings was cut into 0.5 cm × 0.5 cm

pieces and inserted into MS media (0.7-0.75% agar, 4.74 g/L MS, 3% sucrose, pH 5.8-6.2.). The explants were then placed in a dark incubator 1-3 days before infection.

Tartary buckwheat callus culture

Tartary buckwheat explants were induced in solidified MS media (MS + 0.8 mg/L 6-BA +3.5 mg/L 2,4-D) supplemented with 300 mg/L cefotaxime sodium. Calli were obtained in a 16 h/8 h light/dark incubator after 10 days, and then the large number of calli were obtained on subculture media (MS+3.0 mg/L 6-BA+1.0 mg/L KT+50 mg/L kanamycin).

Preparation of transfection solution

Agrobacterium tumefaciens (GV3101) containing the pCHF3-YFP-FtCHS1 plasmid was grown at 28 °C on 1.5% (w/v) agar-solidified YEB medium with 50 mg/L rifampicin and 35 mg/L spectinomycin for two days. Positive clones were identified by colony PCR using the primer pair MYFP-F/MYFP-R. A single positive strain of *A. tumefaciens* was inoculated in 20 ml of YEB liquid medium with 50 mg/L rifampicin and 35 mg/L spectinomycin and then cultured at 28 °C under oscillation (180 rpm/min). When the Agrobacterium liquid reached OD_{600} = 1.5, 1 ml was absorbed and put into 50 ml of YEB medium. The liquid was centrifuged (4000 rpm, 5 min), after which the bacteria were collected under aseptic conditions. Afterward, 1/2-strength MS liquid medium supplemented with 15 g/L sucrose and *acetyleugenol* 200 mmol/L was used to flush the bacteria, which were then re-suspended; the infection system density OD_{600} = 0.6. The re-suspended bacterial solution was used to infect the hypocotyls of tartary buckwheat as mentioned above, and positive identification was carried out after the calli grew to a diameter of 3-5 cm after bactericidal culture. The tartary buckwheat hypocotyls were dipped into transfection solution for 20 min. The treated explants were then placed on filter paper for 30 and grown on agar-solidified MS media in the dark in a 25°C incubator. The control group calli were infected by *Agrobacterium tumefaciens* containing an empty vector (pCHF3-YFP).

Positive callus identification and phenotypic observations

Genomic DNA of tartary buckwheat calli was extracted by the CTAB procedure (Sun et al. 2003). Positive calli were identified by PCR using the specific primers FtCHS1-F-KpnI/FtCHS1-R-SalI. Furthermore, the FtCHS1-YFP fusion protein expressed in the calli was observed by fluorescence microscopy under blue excitation light. The localization of the *FtCHS1* gene in onion epidermal cells was observed under the same conditions by means of instantaneous infection.

Determination of the flavonol content in calli by HPLC analysis

Tartary buckwheat calli (1 g) were frozen in liquid nitrogen for 10 min, ground carefully into a fine powder, extracted twice with 50 ml of methanol, and then transferred to 4 °C refrigerators for 24 h. The extracts were dried at 60 °C, and 10 ml of methanol was then added. The solution was filtered through a polyfilter (pore size 0.45 um) and diluted by adding double-volume methanol. The contents of flavonols including quercetin, kaempferol, myricetin and rutin were analysed by high-performance liquid chromatography with a C_{18} column 250 mm x 4.6 mm 5 μm; Agilent 1260（Changsha Kemei Analytical instrument Co., Ltd.） at 30 °C. The mobile p hase consisted of acetonitrile (solvent C) and water (solvent B), and the flow rate of the machine was 1 ml/min. The solvent gradient was 40% solvent A and 60% solvent B, which were converted to 65% solvent A and 35% solvent B. The injection volume was 20 μL. Quercetin, kaempferol, myricetin and rutin were detected and quantified with standard products from Nanjing Yuan Zhi Biotechnology Co., Ltd. Three biological repeat samples were used in the present study.

Determination of anthocyanins in calli

Two hundred milligrams of fresh callus material was dissolved in 1 ml of acid-methanol (1% HCl, v/v). The samples were shaken at 30 r/min at 25°C for 18 hours. Two millilitres of water dilution extract was then added, after which 2 ml of chloroform was added. The sample was rotated gently for a few seconds and then centrifuged for 5 min with a centrifuge at 12 000 rpm/min. The water phase was extracted and used for spectrophotometric determination at 530 and 657 nm. The following equation was used: $Q_{Anthocyanins}=(A_{530}-0.25\times A_{657})\times M^{-1}$, where A_{530} and A_{657} represent the absorption at the indicated wavelength and M is the weight of the plant material used for extraction. Three independent biological replicates were measured for each sample.

qRT-PCR of flavonoid synthesis-related genes in calli

An RNeasy Plant Mini Kit (Aidlab Biotech, Beijing, China) was used to extract the total RNA from tartary buckwheat calli, after which the RNA was enzymatically convert to first-strand cDNA using ReverTra Ace (Toyobo, Osaka, Japan). Next, semi-quantitative RT-PCR was performed to measure the expression levels of flavonoid-related genes in the flavonoid synthesis pathway, including *FtCHS1* (GenBank: GU172165), *FtCHS2* (GenBank: KT284884), *FtCHS3* (GenBank: KT284885), *FtCHI* (GenBank: KF831243), *FtFLS1* (GenBank: JF274262), *FtFLS2*

(GenBank: JX401285), *FtFLS3* (GenBank: KJ094503), *FtDFR1* (GenBank: GU169468) and *FtDFR2* (GenBank: LC216399). The amplification primers for the abovementioned genes were designed using Primer Premier 5 software (Table S1). Moreover, the *FtH3* gene (GenBank ID: HM628903), a housekeeping gene, was used as a reference gene in this study[47]. qRT-PCR was performed with a CFX Connect system using a SYBR Premix EX Taq Kit (TaKaRa, Japan). The reaction procedure was as follows: 40 cycles of 95°C for 3 min, 95°C for 15 s, 60°C for 15 s, and 72°C for 20 s. The data were ultimately evaluated using the $2^{-\Delta\Delta CT}$ method. Three independent biological replicates were measured for each sample.

Results

Callus induction of tartary buckwheat

To establish a stable genetic transformation system of tartary buckwheat, we explored the conditions for inducing callus formation from the hypocotyl of tartary buckwheat without *A. tumefaciens* infection. After many different hormone concentrations and combinations were tested, tartary buckwheat hypocotyls could be effectively induced to form transparent white calli after 7 d only on MS plates with 3.5 mg/L 2,4-D and 0.8 mg/L 6-BA (Fig. 1 B-E). Furthermore, a relatively large biomass of callus was harvested approximately 30 d later (Fig. 1 F-O); a relatively shorter time-consuming method was carried out for callus subculture in MS medium mixed with 3.0 mg/L 6-BA and 1.0 mg/L KT. The whole process of callus formation is shown from induction to subculture in Fig. 1. At 4-5 d after the explant culture induction was started, yellow and white calli began to appear approximately one week later (Fig. 1 B-E). During the development from 12 d to 25 d, the calli began to expand at both ends of the stem first and then promoted the formation of callus in the middle of the stem; as the cells expanded, the callus colour gradually turned yellow and brown (Fig. 1, F-G). More than a month later, a large number of calli with loose granules were obtained and identified as non-embryogenic calli that tended not to differentiate (Fig. 1a K-O).

Positive identification of FtCHS1 transgenic calli

After the *FtCHS1* gene was introduced into the tartary buckwheat calli, three positive calli, #1, #3, and #6, were screened from six candidates *via* specific PCR primers for identification of the exogenous *FtCHS1* gene (Fig. 2). Two different PCR products represented the exogenous

FtCHS1 cDNA sequence (1.2 kb) and the endogenous *FtCHS1* DNA sequence, including an intron that was 1.6 kb in length. On the basis of visual observations, the transgenic calli appeared transparent with a slight red colour on a light yellowish-brown background compared with the grey control group (Fig. 3). As far as the size of the tissue block was concerned, the experimental group (Fig. 3 A-C) showed a visibly lower proliferation rate of cells than did the control group (Fig. 3 E-F) at the same culture time (25 days after the end of co-culture). Moreover, the transgenic calli appeared to be loose and fluffy.

To confirm the expression of the exogenous *FtCHS1* gene, the transgenic calli were observed by fluorescence microscopy. The results indicated that the callus mass with the *FtCHS1* gene showed an obvious bright yellow fluorescence under both the dark field and merged field (Fig. 4 (a) D-I). In contrast, there was no visible fluorescence observed in the wild type (Fig. 4 (a) A-C). Because of the stacking of callus cells, the FtCHS1-YFP fusion protein was not clearly localized in the cells. Another more accurate experiment was performed to verify the FtCHS1-YFP distribution in onion epidermal cells (Fig. 4 (b) A-I). Fluorescence microscopy showed that the FtCHS1-YFP fusion proteins were transiently expressed and distributed throughout the whole cytoplasm, whereas no fluorescence was observed in the control cells.

Effects of FtCHS1 overexpression on flavonoid accumulation in tartary buckwheat

To describe the effects of the *FtCHS1* gene on flavonol accumulation in tartary buckwheat, high-performance liquid chromatography (HPLC) was first performed to measure the components in the flavonol branch pathway. The results showed that flavonols including rutin, quercetin, kaempferol and myricetin accumulated in the transgenic calli extremely significantly more than they did in the wild type ($P < 0.01$) (Fig. 5A-D). Specifically, four flavonols constituted more than 2.5 mg/g dry weight in the experimental samples, but less than 0.5 mg/g of those same flavonols was present in the controls. Interestingly, quercetin was the predominant element in transgenic calli, although rutin was the main component in non-transgenic calli as well as in tartary buckwheat seeds and other issues. Additionally, anthocyanin contents in all positive lines were approximately 40% greater than those in the control group ($P < 0.05$) (Fig. 5 E), which was consistent with phenotypic observations.

Expression of Flavonoid Biosynthesis-Related Genes in Tartary Buckwheat

To elucidate the relationship between gene expression and the increase in flavonoids in the calli, we evaluated the expression levels of flavonoid-related genes by real-time quantitative

PCR (Fig. 6). We found that the exogenous *FtCHS1* gene had almost no effect on endogenous *FtCHS1* gene expression ($P<0.05$). However, the overexpression of *FtCHS1* altered the expression of many other flavonoid biosynthesis-related genes at the transcriptional level. Concretely, the expression of *FtFLS1*, *FtFLS2*, *FtFLS3* and *FtDFR1*, which are genes of the key enzyme involved in the flavonol and anthocyanin biosynthesis pathway, was dramatically higher in the transgenic calli than in the control group ($P < 0.01$), as was that of *FtCHI* and *FtCHS2*, which are early flavonoid biosynthesis genes ($P < 0.05$). In contrast, the homologous genes *FtCHS3* and *FtDFR2* were significantly downregulated ($P < 0.01$). In general, the expression of the major homologous genes of these key enzymes in the flavonoid pathway was upregulated to promote the increased metabolic flux from the phenylpropanoid pathway to both the flavonol and anthocyanin branches.

Discussion

Tartary buckwheat is not only the main food crop in the Liangshan Yi nationality area in Southwest China but also one of the traditional staple foods in Asian and Europe countries, such as Nepal, Japan, Slovenia and Russia. Currently, tartary buckwheat is a popular functional food for a much broader group of people because consumers can benefit greatly from its abundance of flavonoids. Hence, the industrial driving force has promoted the research focus on the molecular mechanisms of flavonoid metabolism in tartary buckwheat. To date, the main research on tartary buckwheat has been conducted at the molecular level in several different ways. *Arabidopsis thaliana* is undoubtedly the most common model plant used for gene functional studies because of the relatively easy transformation protocol and the relatively large amount of genetically modified seeds (Gao et al. 2016; Luo et al. 2017). Tobacco represents another general genetic transformation system because of its abundance of secondary metabolites and observable changes in flower colour (Yao et al. 2017). Furthermore, to avoid functional differences caused by heterologous hosts, the hairy root system of tartary buckwheat is an effective and reliable tool for studies (Aye Aye et al. 2014; Huang et al. 2016). In addition, tartary buckwheat callus, an embryogenic tissue, has been prepared successfully in many previous reports because it has the potential to differentiate and regenerate an entire plant. Regretfully, there are still no studies that have achieved success with transgenic calli or plant lines of tartary buckwheat. In this study, we

not only effectively produced growth-induced tartary buckwheat callus tissue but also successfully obtained positive transgenic lines overexpressing *FtCHS1*.

Fortunately, tartary buckwheat calli could be successfully inducted from hypocotyls with a 100% rate on an optimized MS medium with 3.5 mg/L 2,4-D and 0.8 mg/L 6-BA in this study; this rate was at least 24% higher compared with that in previous reports (Wang et al. 2000; Zhan-Qi & Zi-Qin 2006). Furthermore, the MS media with 3.0 mg/L 6-BA and 1.0 mg/L KT were replaced every 15 d for subculture, which effectively prevented callus browning. In addition, *germetin*, an antibiotic, inhibited bacterial contamination well, and the calli were washed in aseptic water 8-10 times to avoid fungal contamination(Akulov et al. 2010).

As the first key enzyme in the pathway from phenylpropanoid metabolism to flavonoid metabolism, chalcone synthase plays an important role in flavonoid synthesis and accumulation and even colour modification (Feinbaum & Ausubel 1988; Krol et al. 1988). In *Silybum marianum*, *SmCHS* gene overexpression could increase the total flavonoid contents in hairy roots (Rahnama et al. 2013). Similarly, ectopic expression of *FhCHS1* from *Freesia hybrid* could not only fully restore the pigmentation phenotype of the seed coats in Arabidopsis *tt4* mutants but also clearly altered the flower colour from white to pink in transgenic petunia plants(Wei et al. 2015). In actuality, a previous study confirmed that, among its three homologous genes in tartary buckwheat, *FtCHS1* was the major gene in stems and flowers (Yao et al. 2016). In the current study, *FtCHS1* was mainly located in the cell cytoplasm, and both flavonols and anthocyanins were significantly increased in transgenic calli overexpressing *FtCHS1*. According to the expression of key enzyme genes, the major homologous genes of *FtCHI*, *FtFLS* and *FtDFR* were significantly enhanced at the transcriptional level (Li et al. 2012a; Zhang et al. 2017). DFR and FLS generally compete for three dihydroflavonols as their common enzymatic substrates (Davies et al. 2003). However, *FtCHS1* overexpression would cause more upstream metabolic flow than downstream flow to increase the flavonol and anthocyanin biosynthesis simultaneously.

Overall, a stable and effective callus system was established for transgenic tartary buckwheat. Overexpression of *FtCHS1* in calli could markedly increase flavonol and anthocyanin accumulation by the increased expression of major genes in the flavonoid metabolic pathway. It is difficult to obtain transgenic plants from tartary buckwheat, so using the callus platform represents a simple method to verify the function of tartary buckwheat genes and to

perform follow-up experiments, and this experiment provides a research method for the study of the metabolism of tartary buckwheat.

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Author Contributions:

Qixin Dong and Haixia zhao conceived the original screening and research plans; Qixin Dong., Qi Li., Bingbing Li., Chenglei Li, Xiaoli Wang, and Hui Chen performed most of the experiments; Qixin Dong. and Qi Wu analyzed the data and wrote the article; Xuerong Zhao provided assistance to this research. All authors read and approved the final manuscript.

Abbreviations: 2,4-D: 2,4-Dichlorophenoxyacetic acid; 6-BA: 6-Benzylaminopurine; KT: Kinetin *CHS*: Chalcone synthase; *CHI*: Chalcone isomerise; *FLS*: Flavonol synthase; *DFR*: Dihydroflavonol reductase; YFP: yellow fluorescent protein.

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Competing interests

The authors declare that they have no competing interests.

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Figure

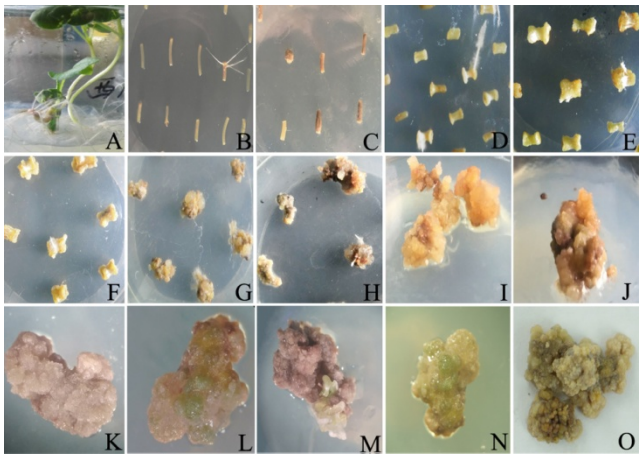

Figure 1 tartary buckwheat callus induction process. A shows the growth status of tartary buckwheat aseptic seedlings at 15 d. B-E show the tartary buckwheat callus induction at 7 d; F-J show tartary buckwheat calli at 25 d after induction; K-O show tartary buckwheat calli at 40 d after induction.

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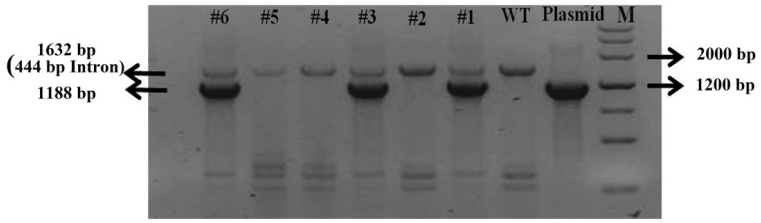

426

427 **Figure 2** Positive identification pf tartary buckwheat calli *via* electrophoresis. The positive samples
428 #1, #3 and #6 have two strips of 1.2 kb and 1.6 kb. The negative samples #2, #4, #5 and #7 have
429 only one 1.2 kb strip. M represents marker III.

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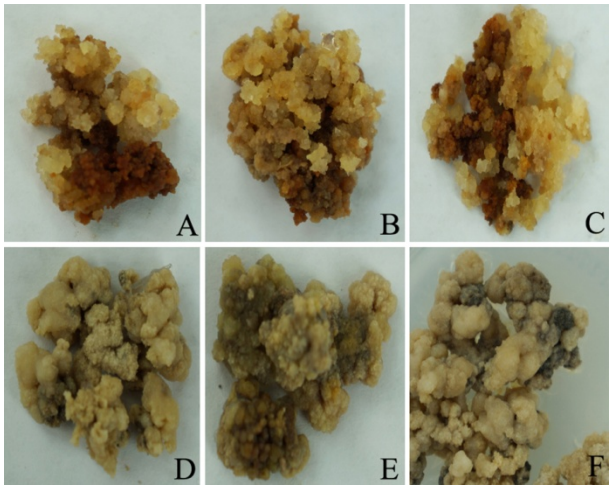
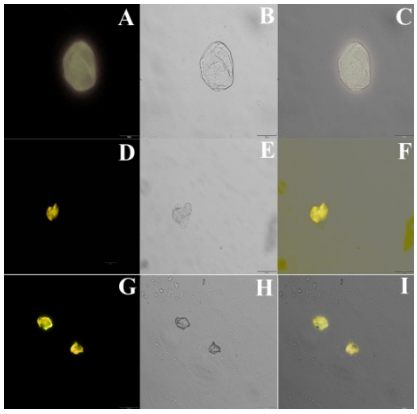


Figure 3 Transgenic calli. A-C show *FtCHS1* overexpression calli at 25 d after induction; D-F show the wild-type tartary buckwheat induced calli at 25 d after induction.

(a)



(b)

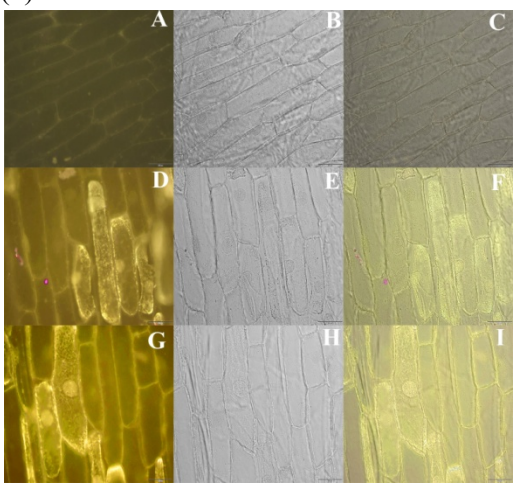


Figure 4 Localization of the *FtCHS1* protein. (a) The individual panels show YFP alone (A) and *FtCHS1* (D, G) in tartary buckwheat callus cells, corresponding to bright-field (B, E and H) and merged images (C, F and I). (b) The individual panels show YFP alone (A) and *FtCHS1* (D, G) in onion epidermal cells, corresponding to bright-field (B, E and H) and merged images (C, F and I) (200 μm).

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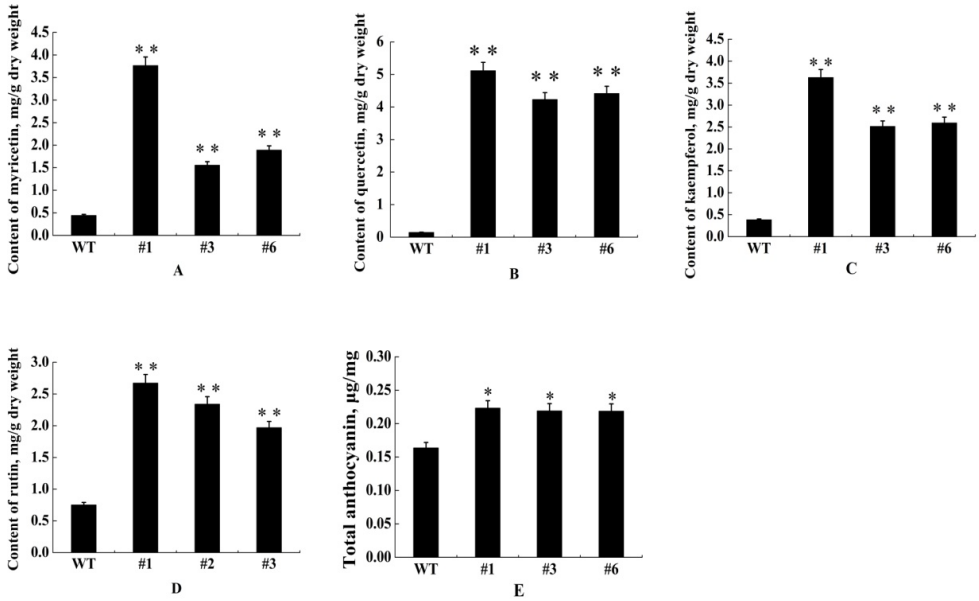

449

450 **Figure 5** Flavonol and anthocyanin accumulation quantities in calli of tartary buckwheat. Each
451 experimental group was repeated three times, and the standard deviations are expressed as error bars. WT
452 represents wild-type tartary buckwheat induced callus; #1, #3 and #6 are transgenic tartary callus lines. (*
453 $P<0.05$, ** $P<0.01$)

454

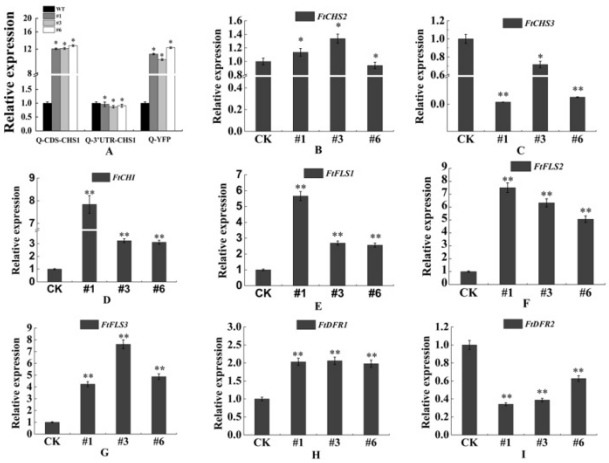

Figure 6 Gene analyses of FtCHS1-overexpressing tartary buckwheat calli. A is the relative expression level of *FtCHS1*. The Q-CDS-CHS1 group represents the total expression level of *FtCHS1*; the Q-3'UTR-CHS1 group represents the individual original expression level of *FtCHS1*; the Q-YFP group represents the expression level of the pCHF3-EYFP vector in the calli; and B-I represent the relative expression levels of *FtCHS2*, *FtCHS3*, *FtCHI*, *FtFLS1*, *FtFLS2*, *FtFLS3*, *FtDFR1* and *FtDFR2*. CK represents wild-type tartary buckwheat induced calli; #1, #3 and #6 are transgenic tartary callus lines (* $P<0.05$, ** $P<0.01$).

Figure 1

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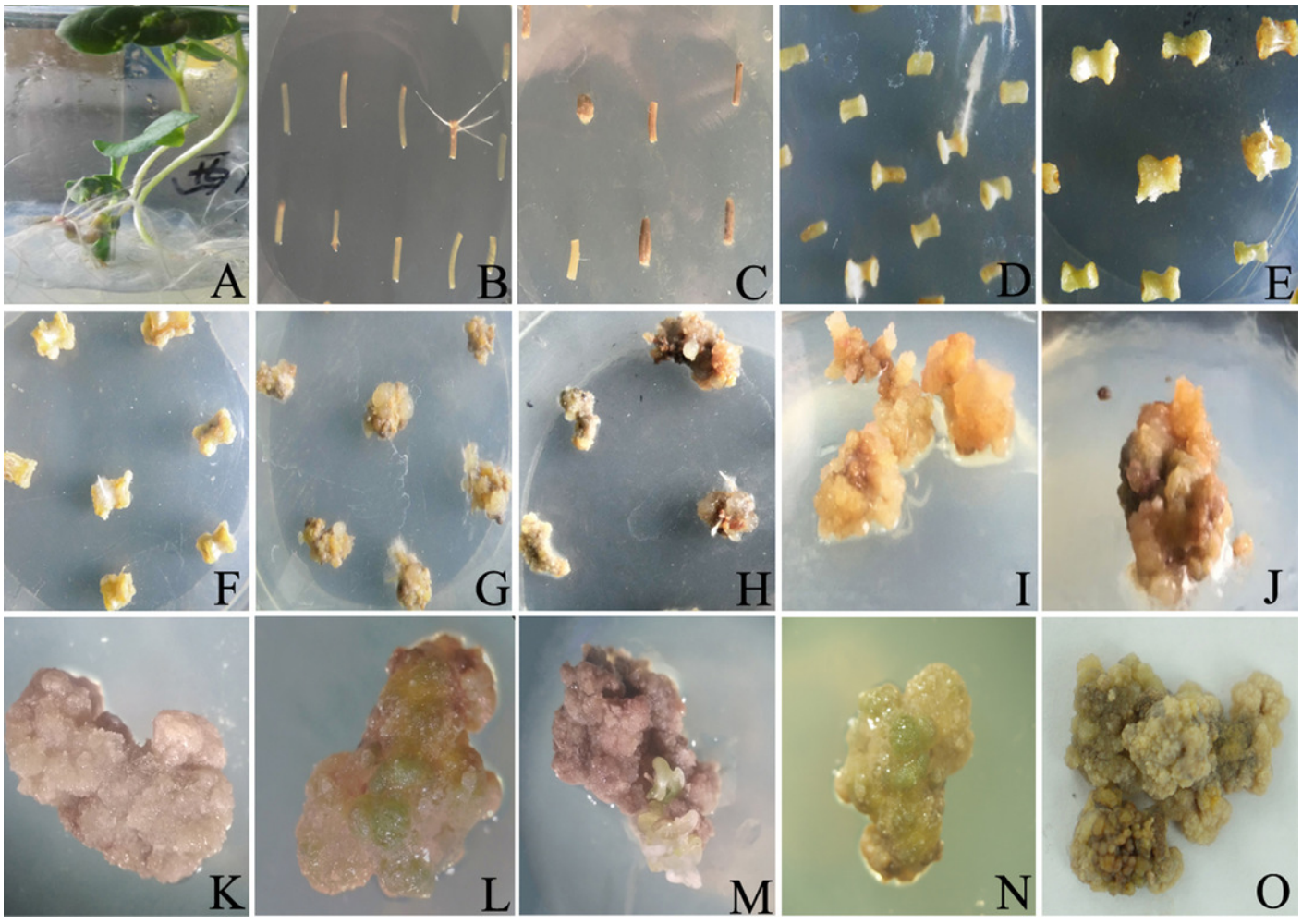

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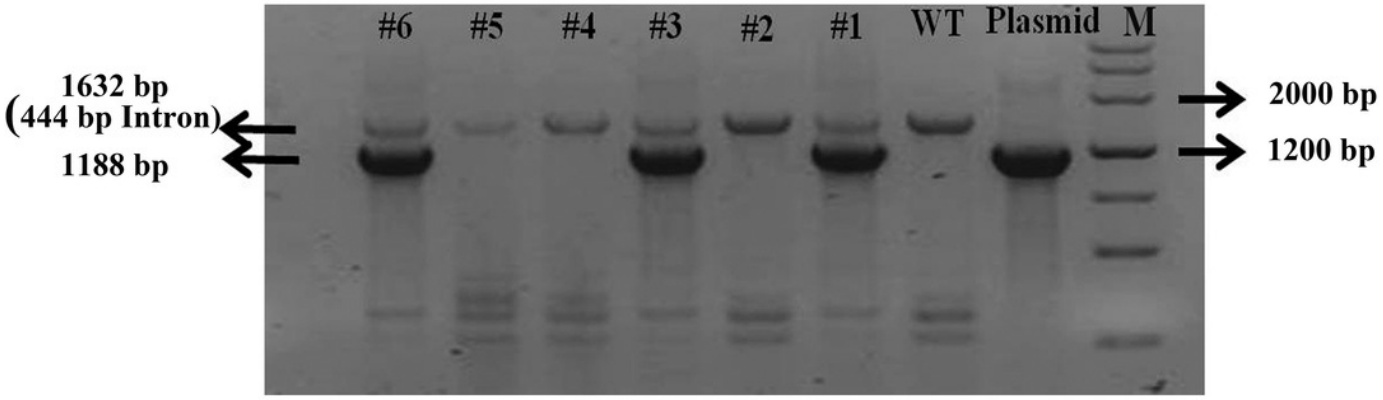

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A-C show *FtCHS1* overexpression calli at 25 d after induction; D-F show the wild-type tartary buckwheat induced calli at 25 d after induction.

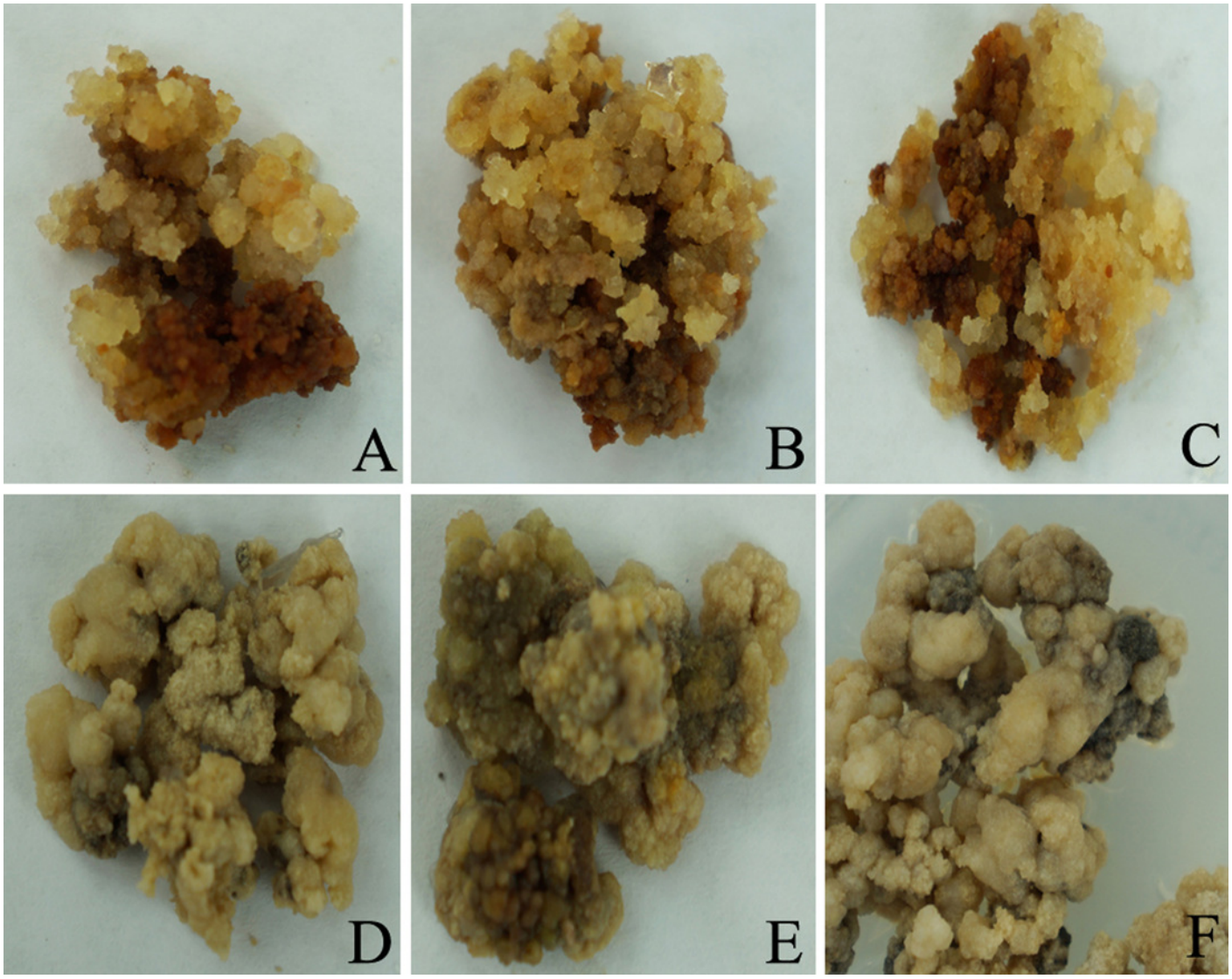

Figure 4

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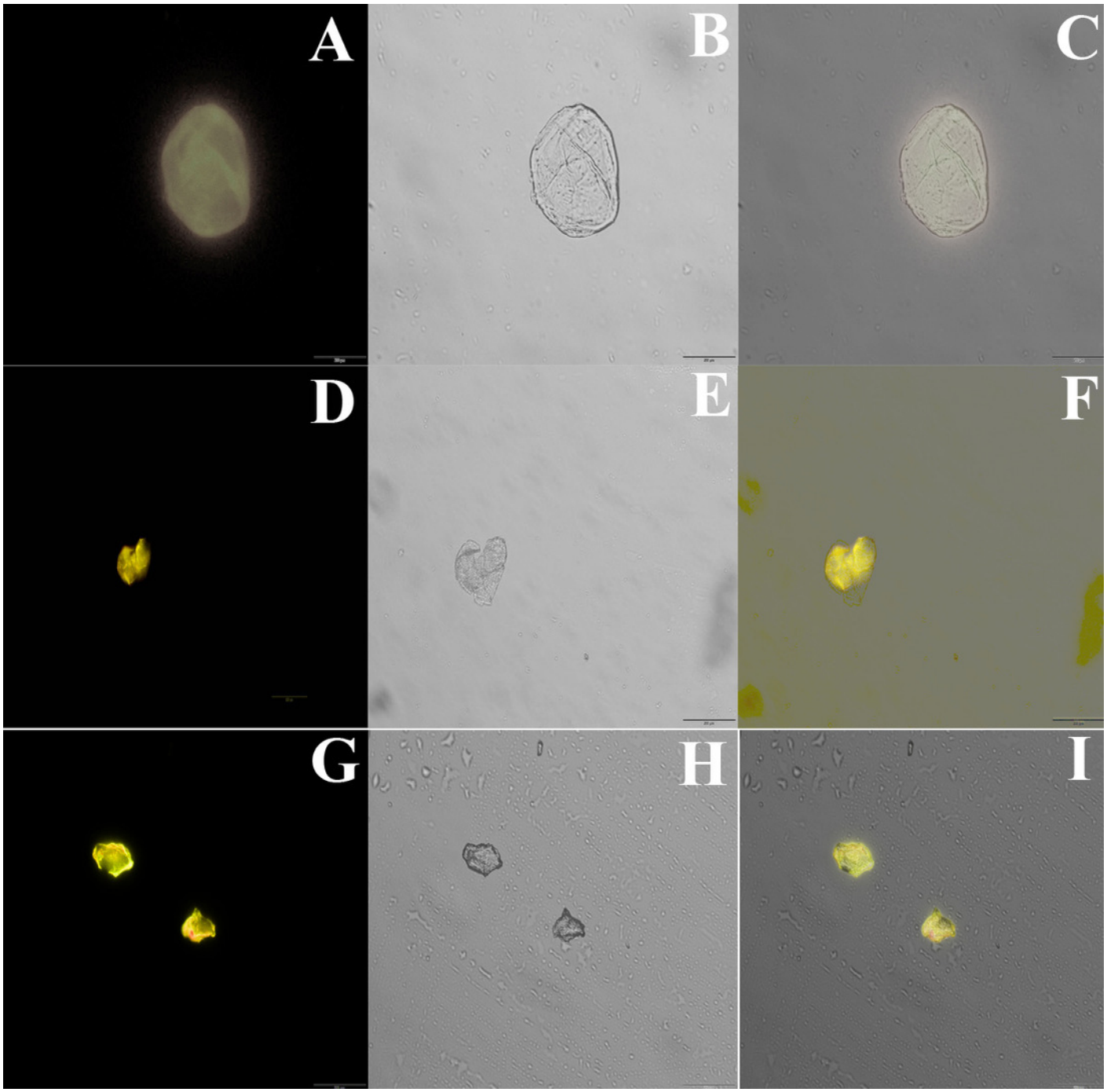

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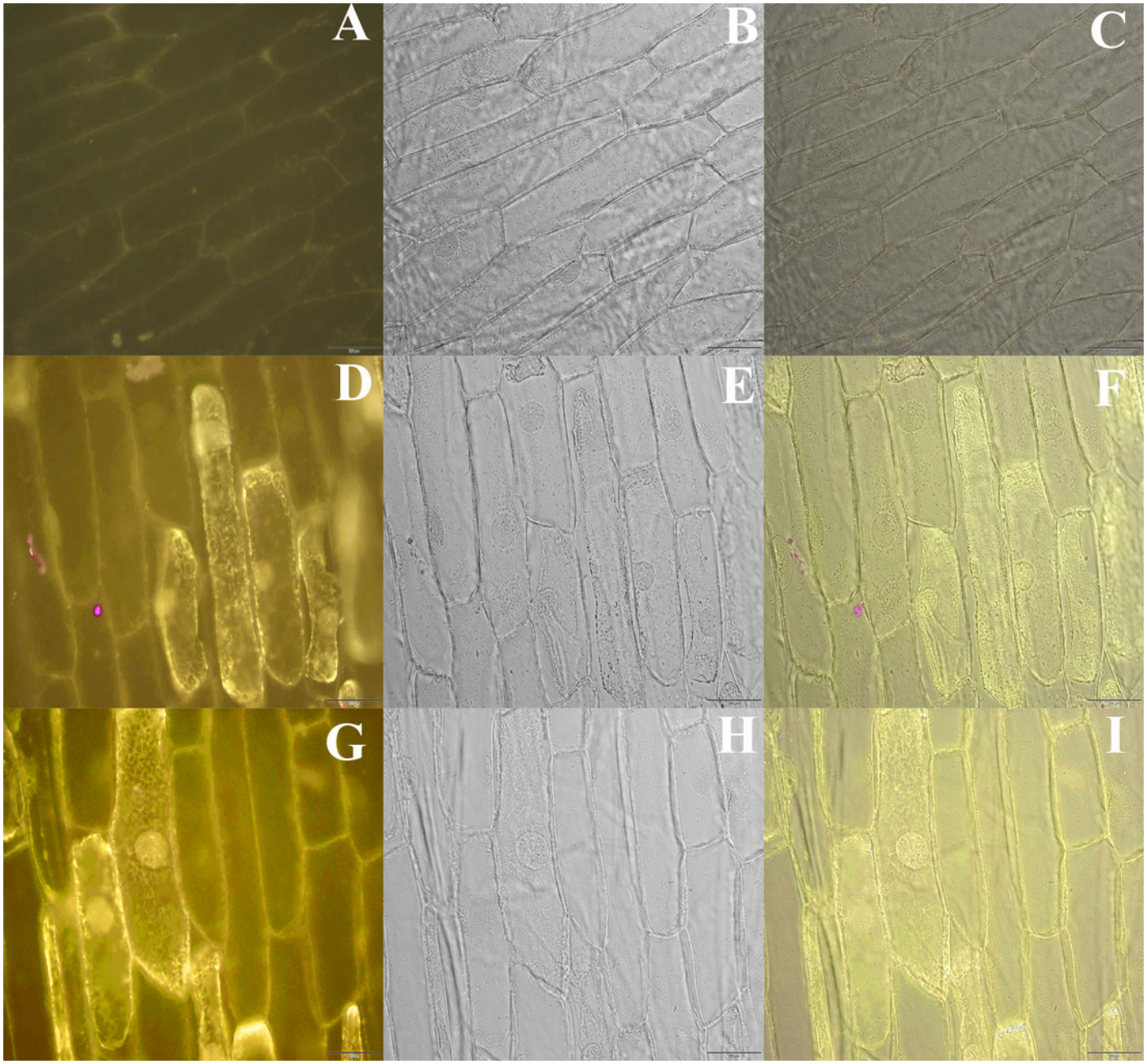

Figure 6

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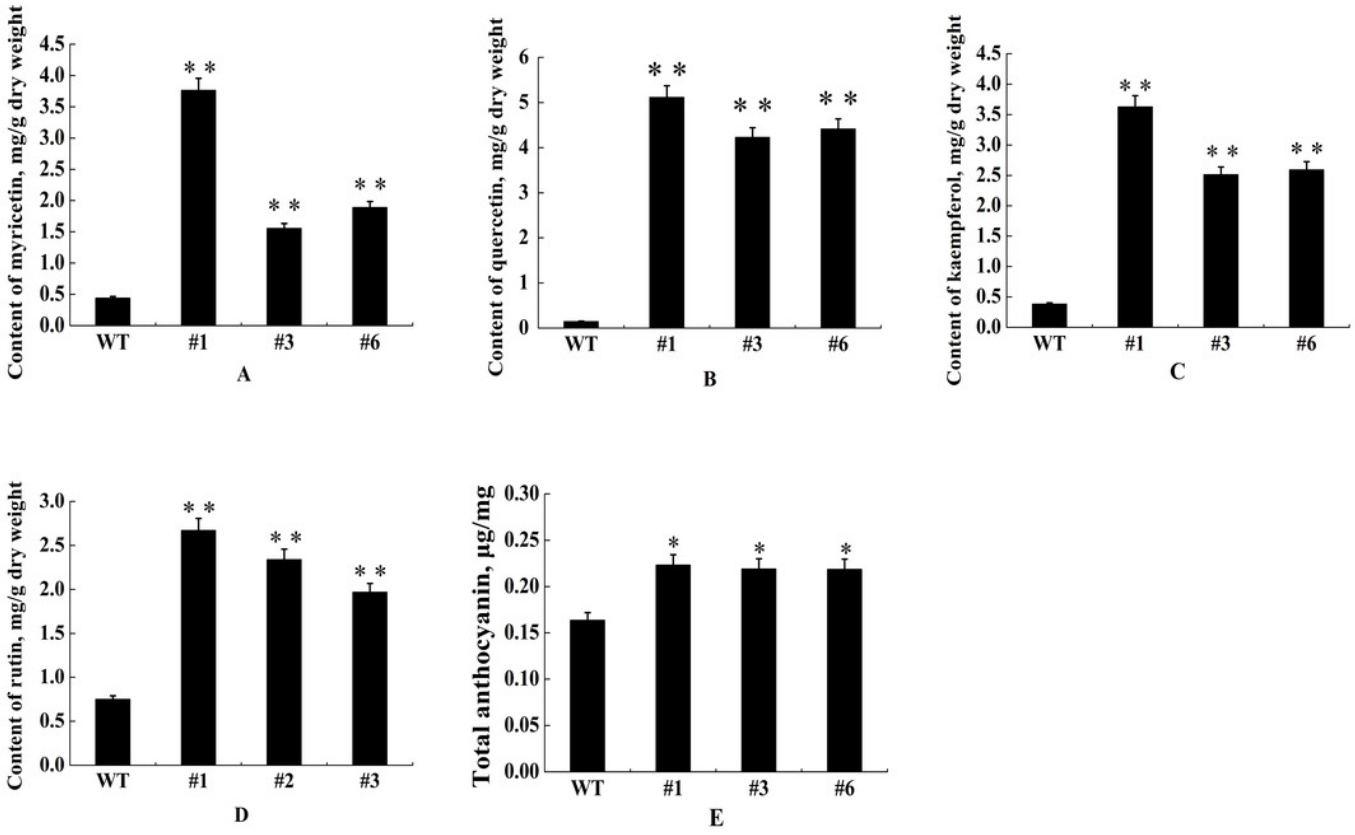
Each experimental group was repeated three times, and the standard deviations are expressed as error bars. WT represents wild-type tartary buckwheat induced callus; #1, #3 and #6 are transgenic tartary callus lines. (* $P<0.05$, ** $P<0.01$)



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