Improved Agrobacterium Tumefaciens-Mediated Transformation of Cucumber via Modified use of Antibiotics and Acetosyringone

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Research

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Improved *Agrobacterium tumefaciens*-mediated transformation of cucumber via modified use of antibiotics and acetosyringone

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Abstract

Background: Cucumber (*Cucumis sativus*) is one of the most important vegetable crops in the world. As conventional breeding of cucumber is very challenging, genetic engineering is an alternative option to introduce important traits such as enhanced stress resistance and nutritional value. However, the efficiency of the transformation system depends on genotypes, transformation conditions, selection agents, etc. This study aims to speed up the process of *Agrobacterium*-mediated transformation of cucumber. '*Xintaimici*', a very popular and typical north China-type cucumber variety, was transformed with *Agrobacterium* GV3101. The strain carried the pCAMBIA2300s plasmid, a double vector with the marker gene neomycin phosphotransferase II (*npt* II).

Results: The research results indicated that cefotaxime sodium was suitable for inhibiting

Agrobacterium in the screening and bud elongation stages. Timentin was best used during the rooting stage. Furthermore, 25 mg/L kanamycin was used in the early stage of screening and increased to 50 mg/L for further screening. At the bud elongation and rooting stages, 75 and 100 mg/L kanamycin was used, respectively, to improve the screening efficiency. To obtain the highest regeneration frequency of resistant buds, 50, 150, and 100 μM acetosyringone was added in the pre-culture medium, infection solution, and co-culture medium, respectively. To confirm the presence of the transgenes, DNA from *npt* II transgenic cucumber plants was analysed by polymerase chain reaction after transplanting resistant regenerated plants.

Conclusions: We finally achieved an 8.1% conversion, which is among the highest values reported to date using the cucumber '*Xintaimici*'. Thus, an effective protocol for *Agrobacterium tumefaciens*-mediated genetic transformation of cucumber was optimized.

Keywords: Cucumber, *Agrobacterium*, Kanamycin, Bacteriostatic antibiotics, Acetosyringone, Genetic transformation

Background

Cucumber (*Cucumis sativus*) is one of the most important vegetable crops widely grown in the world. Studies on the gene function and genetic breeding of cucumber have been conducted worldwide[1, 2]. Abnormal expression of genes in transgenic cucumber plants can help us to engineer and select more robust crop species, such as resistance to biotic and abiotic stress, improved fruit

quality[3], growth and development[4, 5]. Since the advent of cucumber tissue culture and genetic transformation technology[6, 7], researchers have made great achievements in the transformation of genes through the improvement and optimization of transformation methods. At present, the *Agrobacterium tumefaciens*-mediated transformation system of cucumber is still one of the most mature and popular genetic transformation methods[8]. However, the genetic transformation efficiency of cucumber remains low. The key factors affecting the infection efficiency of *Agrobacterium* include the type of explant[9], *Agrobacterium* species[10], exogenous hormone[11, 12], selection agent[13], *Agrobacterium* inhibitors[14] and phenols[15]. In addition, the cutting method for explants[16] and the mode of infection, such as vacuum infiltration treatment[13, 15], also have an impact on the transformation of cucumber to varying degrees.

In the selective culture stage of cucumber genetic transformation, selection antibiotics and antimicrobial antibiotics are often used together[17]. Kanamycin, hygromycin and glyphosate commonly are used for selection markers[13]. It is generally believed that the resistance of cucumber explants to selection antibiotics is closely related to their genotype and culture stages. Instead, antibiotics that have little effect on explants and a certain inhibition effect on *Agrobacterium* are often selected and used[17, 18], such as cefotaxime sodium, carbenicillin, and timentin[15]. However, they are often used at a high concentration, which also has a certain impact on the regeneration and growth of explants[19]. Many studies found that phenolics had a significant effect on the regeneration frequency of resistant buds when added to the pre-culture, infection and co-culture stages of genetic transformation[15, 20]. It is widely recognized that phenolics, such as

65 acetosyringone, can activate the *vir* gene of *Agrobacterium* and promote the introduction of foreign
66 genes into the plant genome, and then the efficiency of genetic transformation is improved[21].
67 However, acetosyringone is often dissolved in toxic organic solvents or has toxic effects at high
68 concentrations and interacts with infection modes and other transformation conditions[22].
69 Therefore, the specific addition stages and concentrations of acetosyringone were different in
70 existing reports[1].

71 Nonconformity between the infection site of *Agrobacterium* and the regeneration site of
72 explants is the most important reason for the low efficiency of the genetic transformation of
73 cucumber. Additionally, the inconsistent sites are affected by various conditions and factors[1, 23].
74 In addition, the selection and the concentration of antibiotics are the main factors affecting the
75 regeneration of positive buds[14]. However, these parameters were different in various reports in
76 the literature and lack sufficient detail in existing studies. All of these reasons lead to browning,
77 *Agrobacterium* pollution, vitrification shoots, low efficiency of genetic transformation[24], gene
78 expression and genetic instability, which still perplex researchers. To improve the efficiency of
79 genetic transformation, the effects of different concentrations of kanamycin on the regeneration of
80 cotyledonary nodes applied in different transformation periods were analysed in the present study.
81 Meanwhile, the inhibitory effects of three different antibiotics on *Agrobacterium* and the effects on
82 regenerated buds were evaluated, and the effects of acetosyringone on the regeneration frequency
83 of resistant buds in different stages of cucumber genetic transformation were investigated. This
84 study hopes to provide a reference for future research on transgenic cucumber.

Results

Effects of kanamycin on the regeneration frequency and browning of explants

The untransformed explants were placed in media with different kanamycin concentrations for 28 d (Fig. 1-a). The regeneration frequency of buds and the browning level showed opposite trends with the increase in the kanamycin concentration. The regeneration frequency of 0 mg/L treatment was 90.0% and was significantly higher than that of any other treatments. Prominent differences were observed between 25, 75, and 100 mg/L treatments. However, the regeneration frequencies of 50, 75, and 100 mg/L treatments were not notably different. Browning began to appear with 50 mg/L treatment and reached the peak with 100 mg/L treatment (90.0%). Except for 100 mg/L treatment, there was no obvious difference among the other treatments (Fig. 1-b).

Effects of bacteriostatic antibiotics on *Agrobacterium* and explants

The explants were cultured in selective media containing 300 mg/L cefotaxime sodium, carbenicillin, or timentin for 28 d (Fig. 2-a). The experiment identified that the three antibiotics at 300 mg/L could inhibit the growth of *Agrobacterium* on the explants. Cefotaxime sodium treatment had the highest resistant bud frequency (76.7%) and shared the same level of difference with timentin treatment. The lowest resistant bud frequency (43.3%) came from carbenicillin treatment and showed no significant difference with timentin treatment (Fig. 2-b). Part c of Fig. 2 shows the growth of *Agrobacterium* on different types of antibiotics. When the culture time reached 96 h, the highest diameter of the inhibition zone was observed with timentin treatment (6.4 cm). In contrast, carbenicillin treatment was the lowest (5.6 cm). Significant differences were seen in all treatments.

Another interesting finding was that at the culture time of 30 d, the maximum diameter of the inhibition zone (5.8 cm) in response to timentin treatment was observed. Furthermore, the result was similar to the timentin and cefotaxime sodium treatments. However, the diameter of the inhibition zone with carbenicillin treatment decreased significantly and reached the lowest level (4.4 cm) over time (Fig. 2-d). In another experiment, uninfected explants were cultured with different concentrations of cefotaxime for 28 d (Fig. 2-e). With the increase in cefotaxime sodium concentration, the number of regenerated buds in each explant decreased from 7.1 to 2.7, which was a reduction of almost 4 times. Compared with 0 mg/L treatment, the 100 mg/L treatment did not significantly alter the number of regeneration buds. The number of regeneration buds with the 0 and 100 mg/L treatments was significantly higher than that with any other treatments. There was no significant difference among the 200, 300 and 400 mg/L treatments (Fig. 2-f).

Effects of acetosyringone on the regeneration of resistant buds

Different concentrations of acetosyringone were added to four important stages of genetic transformation: the pre-cultivation (Fig. 3-a), infection (Fig. 3-b), co-cultivation (Fig. 3-c), and selective culture stages (Fig. 3-d). The regeneration frequencies of resistant buds were counted after 28 d and are shown as follows.

In the test with added acetosyringone in the pre-cultivation stage, the frequency of resistant buds increased initially and then decreased with an increase in the acetosyringone concentration. Treatment at 50 μM had the highest frequency of resistant buds (53.3%), which was significantly higher than that observed with the 100, 200, and 400 μM treatments. However, acetosyringone

greater than or equal to 100 μM showed severe inhibition of the frequency of resistant buds. There was no notable difference between the 0 and 50 μM treatments (Fig. 3-e). As shown in Fig. 3-f, the final frequency of resistant buds increased first and then decreased with the increase in acetosyringone concentration in the *Agrobacterium* inoculation process. The regeneration frequency of resistant buds reached the peak (53.3%) under 150 μM treatment, which was significantly lower than that under 200 μM treatment (20.0%). Other than 150 μM treatment, no significant difference was found between any other treatments. From Fig. 3-g, we know that the frequency of resistant buds increased first and then decreased with the increase in acetosyringone concentration in the co-cultivation stage. Treatment at 100 μM had the highest regeneration frequency of resistant buds (80.0%), which differed from the 50 and 200 μM treatments to the same degree and was significantly higher than that with the 0 and 400 μM treatments. The lowest regeneration frequency of resistant buds was with the 0 and 400 μM treatments (33.3%), which showed no obvious difference compared with the 50 and 200 μM treatments. The explants grew in a screening medium with additional acetosyringone, and the results are shown in Fig. 3-h. By increasing the concentration of acetosyringone from 0 to 200 μM, the frequency of resistant buds decreased from 53.3% to 23.3%. The frequencies of resistant buds under the 0, 50, and 100 μM treatments showed the same degree of difference, and the 50, 100, 150, and 200 μM treatments shared another difference level. Taken together, these results suggested that extra acetosyringone could increase the regeneration frequency of resistant buds while in the early stages of transformation but not after screening (Fig. 3).

Polymerase chain reaction (PCR) analysis

The regenerated plants of cucumber were cultivated, and the total DNA of the ninth tender leaf was extracted. The primers for the reporter gene *npt* II were used to identify the transformed plants (Fig. 4-a), and a 480 bp product was amplified, which was the same as the positive control. The primers of the *Agrobacterium* genome were used to eliminate the contamination of plants from *Agrobacterium*, and the total DNA of regenerated plants was not amplified except for lanes 12, 17, and 18 (Fig. 4-b). DNA of wild-type plants and negative controls was not amplified (Fig. 4).

Discussion

This study was conducted to establish an *Agrobacterium tumefaciens*-mediated transformation system for cucumber. We evaluated the optimal dosage of kanamycin and various antimicrobial antibiotics by observing the growth of explants and *Agrobacterium* in different concentrations. The amount of added acetosyringone was changed when applied to different culture stages. Then, the optimized regeneration protocol was adapted for transformation of cucumber.

Effects of kanamycin on explants

Kanamycin has a great inhibitory effect on untransformed explants, especially the growth of roots[25]. Therefore, kanamycin was widely used as a selection marker with successful results[26, 27]. The screening concentrations of different cucumber varieties needed to be explored owing to their differential sensitivity to kanamycin. As shown in Fig. 1, the regeneration of the buds was inhibited completely at 75 mg/L kanamycin, but the explants began to brown at the same time. In order not to affect the regeneration of the delicate explants, 25 mg/L kanamycin was used after co-culture and then raised to 50 mg/L for further screening without browning. In addition, the

concentration of kanamycin could be increased to 75 and 100 mg/L to prevent the emergence of false-positive plants[28] at the bud elongation and rooting culture stages. The dynamic concentration of kanamycin (50–100 mg/L) agreed with relevant reports[29, 30].

Effects of bacteriostatic antibiotics on *Agrobacterium* and explants

Different strains of *Agrobacterium* had different sensitivities to antibiotics[31]. The specific effect of antimicrobial antibiotics was not reported in cucumber transformation. Therefore, it is very important to select antibiotics that can effectively inhibit the pollution of *Agrobacterium* GV3101 and have little effect on cucumber regeneration buds simultaneously. There were significant differences in the effects of three commonly used antimicrobial antibiotics (cefotaxime sodium, carbenicillin, and timentin) on the explants and *Agrobacterium*. These data demonstrated that the highest regeneration frequency of resistant buds could be obtained by using cefotaxime sodium (Fig. 2-a and b). This result might be due to its chemical structure, which is related to auxin and could interact with other substrates in the culture medium[32]. Timentin had the best antibacterial effect and the longest duration relative to the others (Fig. 2-c and d) due to its high resistance to β-lactamases produced by bacteria[33], but it was not as cheap and common as cefotaxime sodium for practical use. Additionally, we found that the medium containing cefotaxime sodium (Fig. 5-II) would turn yellow gradually within 2 weeks in the bud elongation culture stage, while this was not observed in the medium with timentin (Fig. 5-I). It was reported that the yellowed medium, which contained harmful substances, was caused by the accumulation of o-quinones through enzymatic browning[34]. On the other hand, the toxic effect of cefotaxime sodium on shoot rooting had been

reported[17]. In this sense, cefotaxime sodium was more suitable for cucumber genetic transformation and could be added to the medium in the selective and bud elongation culture stages, while timentin could be added to the rooting culture stage because of its long lasting effect[35]. Additionally, carbenicillin was unsuitable for the genetic transformation of cucumber '*Xintaimici*'. The effect of cefotaxime sodium on the explants further indicated that the 100 mg/L concentration of cefotaxime sodium had little effect on explants, while 500 mg/L showed significant inhibition (Fig. 2-e and f). The suitable concentration of cefotaxime sodium was 200–400 mg/L, which could be adjusted according to the extent of pollution from *Agrobacterium*.

Effects of acetosyringone on the regeneration of resistant buds

Yadav et al. indicated that twelve low-molecular-weight phenolic compounds and salicylic acid were the main substances secreted after explants of chickpea (*Cicer arietinum* L.) were injured, and polyphenol oxidase was activated to oxidize phenols[34]. These processes led to the decrease in phenol and the increase in o-quinones gradually with time, which was one of the factors that resulted in the difficulty of T-DNA transport. Additionally, secretions such as salicylic acid and gallic acid inhibited the growth and transformation of *Agrobacterium*. In many studies, the transformation efficiency of cucumber was improved by adding an additional phenol, such as acetosyringone[20, 27]. Improved efficiency could also be achieved by inhibiting the oxidation of phenols, such as via the addition of antioxidants including α-caprylic acid, L-cystine, dithiothreitol, and $Na_2S_2O_3$[34]. In this study, adding different concentrations of acetosyringone in four key steps of genetic transformation showed that the concentration of acetosyringone had a significant effect on the

regeneration of resistant buds. The analysed results showed that the addition of acetosyringone had a significant effect on the regeneration of resistant buds in different stages (Fig. 3). In the pre-cultivation stage, only 50 μM acetosyringone was needed to improve the regeneration frequency of resistant buds. The regeneration frequency of resistant buds was lower than the control level (0 μM treatment) with an acetosyringone concentration that was too high. The best concentration of acetosyringone in the infection liquid of *Agrobacterium* was 150 mg/L. Furthermore, the high concentration made the regeneration frequency of resistant buds drop sharply. Similarly, the optimum concentration of acetosyringone in the co-culture medium was 100 mg/L, and the highest regeneration frequency of resistant buds was 80.0%. In the stage of selective culture, the extra acetosyringone did not help increase the regeneration frequency of resistant buds. In contrast, the presence of acetosyringone decreased the differentiation resistance of explants. Through these experiments, we successfully determined the content of acetosyringone in the process of cucumber genetic transformation as a basis for improving the transgenic efficiency of the cucumber '*Xintaimici*'.

Conclusions

In recent years, new genetic transformation methods have been reported, such as nanoparticle-mediated genetic transformation[36]. New technologies such as CRISPR/Cas9[1, 37] and selection markers with no antibiotic[38] were also applied to the genetic transformation of cucumber. The *Agrobacterium tumefaciens*-mediated transformation system of cucumber is still one of the most relevant transformation methods, and improvement is still the focus. However, cucumber is one of

the most difficult species to transform, although it has been carried out for 39 years[7]. The highest transformation efficiency of cucumber was 26%[2], while the lowest was only 0.1%, and most reports are between 1% and 10%[2]. Moreover, there have been few reports about the transformation of cucumber '*Xintaimici*'[39].

The transformation method reported here is a modification and improvement scheme building on previous reports in cucumber. The complete transformation method was used in the study, and the main steps are shown in Fig. 5. We increased the infection depth through the vacuum system, and we set a gradient concentration of kanamycin to prevent damage to tender plants and the emergence of false-positive and chimeric plants. We used three antimicrobial antibiotics in different stages and compared their effects on the growth of *Agrobacterium* GV3101 and explants. By changing the usage of acetosyringone in the important genetic transformation stages, we improved the regeneration frequency of resistant buds by increasing the content of phenolics. We successfully obtained transgenic plants from the cotyledonary nodes of cucumber '*Xintaimici*' after 3 months of application of the improved genetic transformation system (Fig. 5). From 223 explants, 134 resistant buds were regenerated, and 42 rooting regenerated plants were obtained. At last, 18 plants were identified as positive. The positive rate was 42.8%, and the transgenic efficiency was 8.1%, which was improved compared with other reports[39]. This project was undertaken to improve the *Agrobacterium*-mediated transformation of cucumber with a similar genetic background as '*Xintaimici*' and laid a foundation for other gene transformation work.

Materials and Methods

Plant materials and media

The seeds of cucumber '*Xintaimici*'(a north China-type cucumber variety) were soaked for 2–3 h in water, and the seed coats were peeled. The unclad seeds were dipped in 75% ethanol for 1 min and 15% sodium hypochlorite for 15 min and then were rinsed 4 times in sterile distilled water. The sterilized seeds were germinated in the dark at 28℃ for 48 h and light for 24 h with medium-I (2.21 g/L M519 + 15 g/L sucrose + 2.5 g/L phytagel, pH=6.8). Cotyledonary nodes were first cut in half, 2 mm hypocotyls were retained, and the distal 2/3 parts and growth point were removed. Cotyledonary nodes were cultured in medium-II (4.43 g/L M519 + 30 g/L sucrose + 2.5 g/L phytagel + 0.5 mg/L 6-benzylaminopurine + 1.0 mg/L abscisic acid + 1.0 mg/L $AgNO_3$, pH=6.8) with varying concentrations (0, 50, 100, 150, and 200 μM) of acetosyringone in the dark at 28℃ for 24 h.

Agrobacterium strain and vector

The *Agrobacterium* strain GV3101 was used for transformation. The binary vector was pCAMBIA2300s, including the neomycin phosphotransferase II (*npt* II) selection marker, driven by the CaMV-35S promoter. The *Agrobacterium* was resuscitated in Luria-Bertani (LB)-I medium (5 g/L yeast extract + 10 g/L tryptone + 10 g/L NaCl + 15 g/L agar, pH=5.8) with 50 mg/L kanamycin and 25 mg/L rifampicin at 28℃ until single colonies appeared. The *Agrobacterium* single colonies were added to 1 mL of LB II (5 g/L yeast extract + 10 g/L tryptone + 10 g/L NaCl, pH=5.8) with 50 mg/L kanamycin and 25 mg/L rifampicin at 28℃ until turbid. Then, the *Agrobacterium* was cultured with 100 mL of LB II containing 50 mg/L kanamycin and 25 mg/L rifampicin at 28℃ until an

optical density at 600 nm (OD_{600}) of 0.6–0.8 was achieved. The *Agrobacterium* culture was centrifuged and resuspended in medium-III (2.21 g/L M519 + 15 g/L sucrose, pH=6.8), and the final concentration of the *Agrobacterium* (Measured by OD_{600}) was adjusted to 0.2–0.3. Varying concentrations (0, 50, 100, 150, and 200 μM) of acetosyringone were added to medium-III. Before inoculation, the resuspended *Agrobacterium* inoculum was shaken for the induction of *vir* genes at 28℃ for 1 h.

Effects of kanamycin and bacteriostatic antibiotics on *Agrobacterium* and explants

Explants that were not being exposed to *Agrobacterium* were placed on medium-II. Varying concentrations of kanamycin (0, 25, 50, 75, and 100 mg/L) and cefotaxime sodium (0, 100, 200, 300, 400, and 500 mg/L) were added in medium-II. Petri dishes were placed in the tissue culture room at 28℃, 4000 Lx, 16 h/d. The medium was changed every 2 weeks for 28 d. A total of 100 μL of the *Agrobacterium* (OD_{600}=0.7) was added to LB-I medium. A piece of sterile filter paper 6 mm in diameter with 0.5 mg of bacteriostatic antibiotics (cefotaxime sodium, carbenicillin, and timentin) was placed in the centre. Petri dishes were placed in 28℃ bacterial incubators for 96 h and 30 d.

Inoculation, co-cultivation, screening, and regeneration

Explants were immersed in *Agrobacterium* inoculum in sterile Erlenmeyer flasks with breathable filter membranes. Erlenmeyer flasks were placed in a vacuum system at 0.094 MPa for 5 min. The vacuum was relieved slowly to prevent damage to explants caused by stress transients. The infected explants were cultured in medium-II with a sterile filter paper in the dark at 28℃ for 48 h[40, 41]. Varying concentrations (0, 50, 100, 200, and 400 μM) of acetosyringone were added

284 in medium-II. After co-cultivation, explants were washed 5 times with sterilized distilled water.
285 Explants were blotted dry on sterile filter paper. Then, explants were transferred to medium-II.
286 Kanamycin at 50 mg/L, varying concentrations of acetosyringone (0, 50, 100, 200, and 400 μM)
287 and bacteriostatic antibiotics (cefotaxime sodium, carbenicillin, and timentin, 300 mg/L) were
288 added in medium-II. Petri dishes were placed in the tissue culture room at 28℃, 4000 Lx, 16 h/d.
289 The medium was changed every 2 weeks until 2-cm-high regenerating buds were grown.

290 Resistant regenerating buds were cut off and transferred to medium-IV (4.43 g/L M519 + 30
291 g/L sucrose + 2.5 g/L phytagel + 0.2 mg/L 6-benzylaminopurine + 1.0 mg/L $AgNO_3$, pH=6.8)
292 containing 75 mg/L kanamycin and 300 mg/L cefotaxime sodium or timentin to grow for 2 weeks.
293 Then, the resistant regenerating buds were transferred to medium-V (2.21 g/L M519 + 15 g/L
294 sucrose + 2.5 g/L phytagel + 1.0 mg/L $AgNO_3$, pH=6.8) containing 100 mg/L kanamycin and 300
295 mg/L timentin to induce rooting for 2 weeks. The regeneration plants with flourishing roots were
296 transferred into the matrix (V_{Peat}: $V_{Perlite}$=1: 1) and cultivated in an artificial climate chamber (Day:
297 28℃, 6000 Lx, 16 h; Night: 18℃, 8 h; Relative humidity: 65%)[42]. Each regeneration cucumber
298 was covered with cling film to maintain humidity for 1 week. Then, the cucumber plants were
299 managed by normal water and fertilizer[43].

300 **DNA isolation and PCR analysis**

301 Cucumber '*Xintaimici*' was genetically modified with the improved genetic transformation
302 system. After obtaining regenerated plants, the ninth new leaf of the cucumber regeneration plants
303 was removed, rapidly frozen with liquid nitrogen, and their total DNA was extracted by the CTAB

304 method[44]. PCR was used to confirm the presence of the transgene in primary transformants. PCRs
305 were carried out in a 20 μL volume containing 2 μL of 10 ×PCR Buffer, 200 μM of each dNTP,
306 0.4 U *Taq* DNA polymerase, 100 ng template DNA, and 1 μM of each primer. The primer sequences
307 were *npt* II Forward 5'-TCGGCTATGACTGGGCACAACAGA-3' and *npt* II Reverse 5'-
308 AAGAAGGCGATAGAAGGCGATGCCT-3', yielding an amplification product of 480 bp.
309 *Agrobacterium* genome exclusion primers were *HrcA* Forward 5'-
310 CATCGTCGAAGGTTATCTCGATACG-3' and *HrcA* Reverse 5'-
311 TATAATCGACCATCGGTACGATACG-3'[15], yielding an amplification product of 800 bp. PCR
312 amplification was performed as follows: 94℃ for 3 min; 35 cycles of 94℃ for 30 s, 58℃ for 30 s,
313 and 72℃ for 1 min; followed by a final extension of 72℃ for 10 min. PCR products were separated
314 on a 1% agarose gel and visualized by ethidium bromide staining.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed to publish this manuscript.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

C. D. and H. F. conceived and designed the study. L. C., C. L., and Y. Y. S. performed the experiments. L. C. wrote the paper with input from all authors.

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Figures

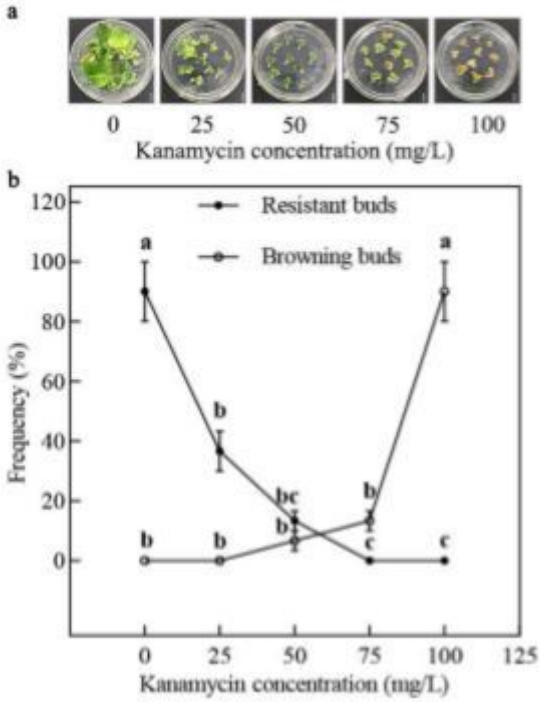

Figure 1

Browning and regeneration frequency of explants at different kanamycin concentrations. a, Growth and browning of explants at different kanamycin concentrations. Bar: 1 cm. b, Analysis of variance of regeneration frequency and browning frequency. Values are the means of 3 replicates ± SE, and 10 explants were repeated each time. Different lowercase letters show significant differences at $p< 0.05$ by Tukey's test after analysis of variance.

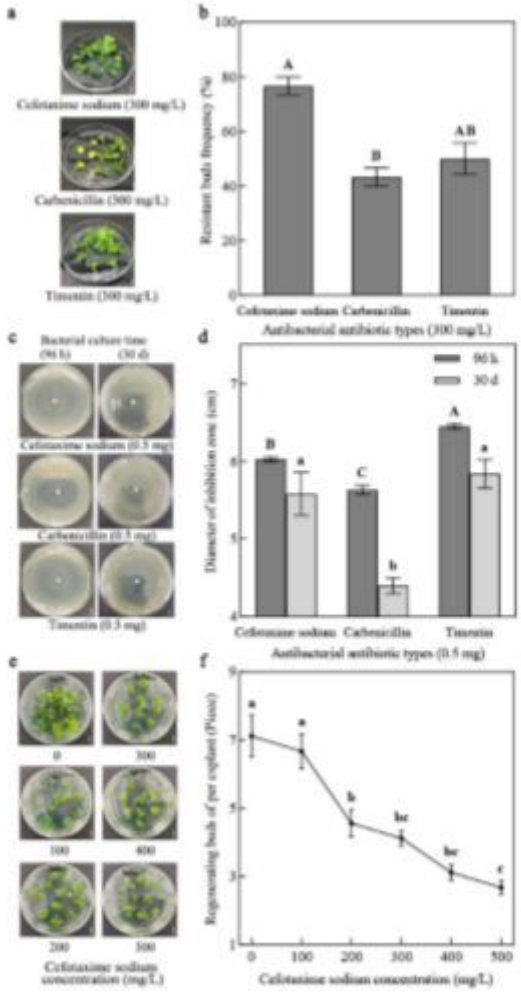

Figure 2

Effects of bacteriostatic antibiotics on Agrobacterium and explants. a, Growth of explants with different bacteriostatic antibiotics at 300 mg/L. b, Analysis of variance of regeneration frequency with different bacteriostatic antibiotics at 300 mg/L. c, Inhibitory effect of 0.5 mg of different bacteriostatic antibiotics on Agrobacterium in 96 h and 30 d. d, Analysis of variance of diameter of inhibition zone in 96 h and 30 d. e, Regeneration of explants at different concentrations of cefotaxime sodium. Bar: 1 cm. f, Analysis of variance of regeneration buds per explant at different concentrations of cefotaxime sodium. Values are the means of 3 replicates ± SE, and 10 explants were repeated each time. Different lowercase letters show significant differences at $p< 0.05$, and different capital letters show high significance at $p< 0.01$ by Tukey's test after analysis of variance.

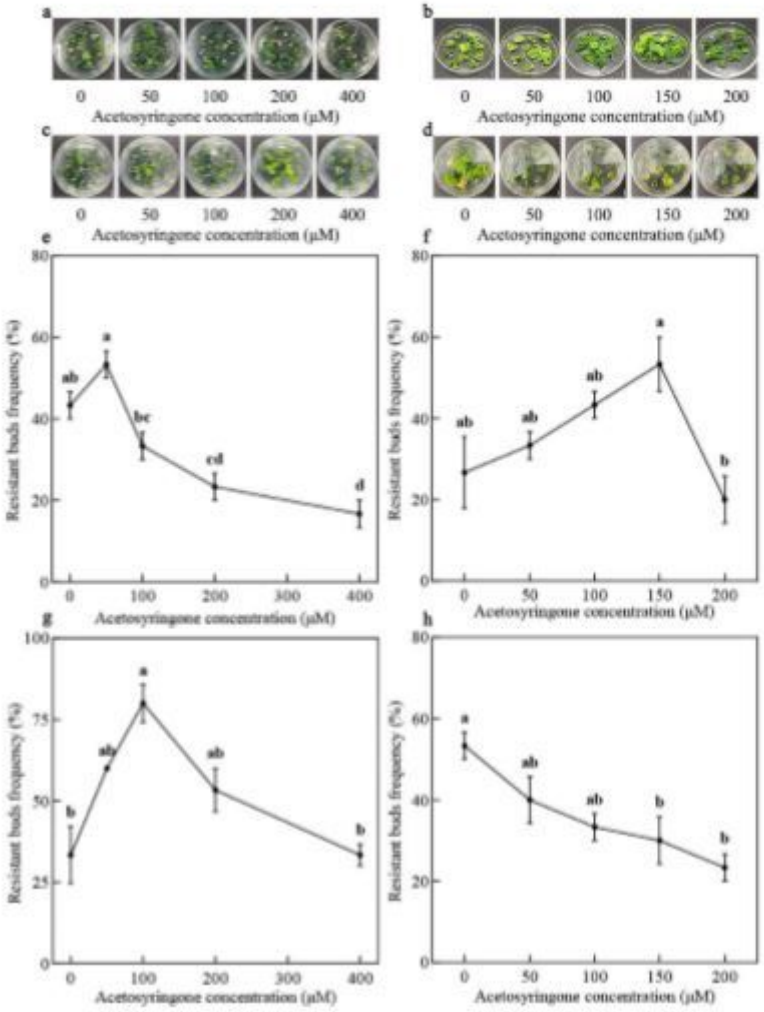

Figure 3

Effect of acetosyringone on the regeneration of resistant buds. a, Growth of explants in the pre-cultivation stage with different concentrations of acetosyringone. b, Growth of explants in the infection stage with different concentrations of acetosyringone. c, Growth of explants in the co-cultivation stage with different concentrations of acetosyringone. d, Growth of explants in the selective culture stage with different concentrations of acetosyringone. Bar: 1 cm. e, Analysis of variance of resistant bud frequency in the pre-cultivation stage with different concentrations of acetosyringone. f, Analysis of variance of resistant bud frequency in the infection stage with different concentrations of acetosyringone. g, Analysis of variance of resistant bud frequency in the co-cultivation stage with different concentrations of acetosyringone. h, Analysis of variance of resistant bud frequency in the selective culture stage with different concentrations of acetosyringone. Values are the means of 3 replicates ± SE, and 10 explants were repeated each time. Different lowercase letters show a significant difference at $p < 0.05$ by Tukey's test after analysis of variance.

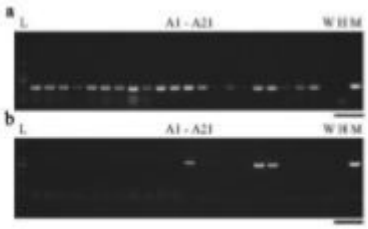

Figure 4

Gel electrophoresis of PCR products. a, Gel electrophoresis results for PCR products from npt II primers. b, Gel electrophoresis results for PCR products from HrcA primers. Lane L is a line DNA marker of 2000 bp. Lanes A1–A21 are different independently regenerated plants. Lane W is a wild-type plant. Lane H is without template DNA (negative control). Lane M is genomic DNA of positive Agrobacterium (positive control). Bar: 1 cm.

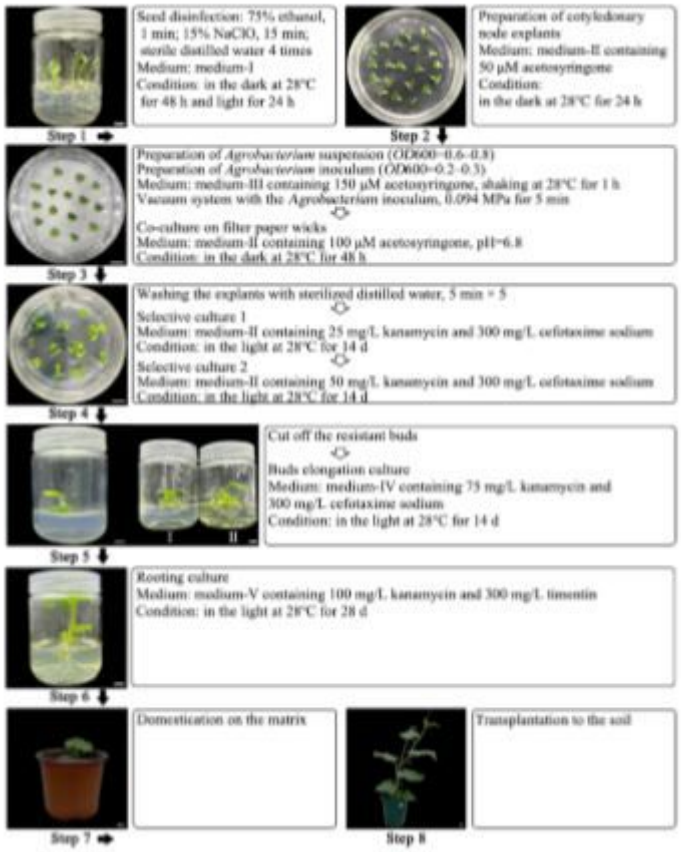

Figure 5

Steps in the transformation of cucumber ‘Xintaimici’ shoot organogenesis from cotyledonary node explants. Step 5-I, 300 mg/L timentin in culture medium as a bacteriostatic antibiotic. Step 5-II, 300 mg/L cefotaxime sodium in culture medium as a bacteriostatic antibiotic. Bar: 1 cm.