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Effects of squirting cucumber (*Ecballium elaterium*) fruit juice on *Agrobacterium tumefaciens*-mediated transformation of plants

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Abstract: Different concentrations of squirting cucumber (*Ecballium elaterium* (L.) A.Rich.) fruit juice were added to *Agrobacterium tumefaciens* growth, leaf disc inoculation, and cocultivation media, to investigate its effect on the transformation frequency of tobacco and potato. *A. tumefaciens* strain GV2260 harboring p35S GUS-INT and pAoPR1-GUS-INT plasmids were used separately in the transformation experiments. Neomycin phosphotransferase (NPT-II) gene was used as a plant selectable marker at a concentration of 100 mg L^{-1}. The addition of 5–10 mg L^{-1} squirting cucumber fruit juice to bacterial nutrient medium increased *A. tumefaciens* growth significantly by 6 h. Moreover, the use of high concentrations (2.5–20 mL L^{-1}) of fruit juice resulted in excessive bacterial growth on cocultivation and selection media around the explants, which was difficult to eliminate by subculture or higher levels of antibiotics. On the other hand, lower concentrations (0.2–1.6 mL L^{-1}) of squirting cucumber fruit juice significantly increased the transformation frequency in both tobacco and potato. Kanamycin-resistant tobacco shoots, rooted in a medium containing 100 mg L^{-1} kanamycin, were transferred to pots containing organic soil and perlite in growth cabinets for acclimatization. Transgenic plants grew normally and set seeds. The presence of T-DNA in these transformants was confirmed by PCR and GUS analysis.

Key words: Genetic transformation, squirting cucumber, phenolics, potato, tobacco

1. Introduction

Agrobacterium tumefaciens is the most efficient way of introducing foreign genes into plants. Plants such as tobacco, potato, eggplant and other species of the family Solanaceae can easily be transformed by *A. tumefaciens.* However, cereals, legumes, and trees are recalcitrant to *A. tumefaciens*-mediated genetic transformation. Therefore, several strategies have been developed to increase genetic transformation frequency in recalcitrant plant species. These include the use of different bacterial density, inoculation and cocultivation methods, and explants (Kumar et al., 2002; Park, 2006; Sharma et al., 2009). In addition, the application of phenolic compounds to inoculation and cocultivation media also enhances transformation, even in recalcitrant plant species (Dutt and Grosser, 2009; Rashid et al., 2010).

Squirting cucumber (*Ecballium elaterium* (L.) A.Rich.) is a poisonous plant that is used for medicinal purposes (Sezik, 1997). It contains different compounds, such as α-elaterin (cucurbitacin E), β-elaterin (cucurbitacin B), elatericine A (cucurbitacin D), and elatericine B (cucurbitacin I) in different plant organs. It also contains sterols, phenolic compounds, vitamins, flavonoids, alkaloids, resin, starch, amino acids, and fatty acids (Koç, 2002; Memişoğlu and Toker, 2002). Compounds found in leaves, stems, roots, and testa are poisonous and show antibacterial activities (Oskay and Sarı, 2007; Koca et al., 2010; Adwan et al., 2011). Similarly, Adwan et al. (2011) also reported antibacterial activity of ethanol extracts obtained from mature dried fruit of squirting cucumber, which exerted negative effects on the growth of *Staphylococcus aureus* and *Candida albicans*. However, in our unpublished preliminary studies, we found that the juice of mature fruits induced growth of *E. coli* and *Staphylococcus aureus.* Therefore, the present study was designed, for the first time, to check the effects of fresh juice of mature fruits of squirting cucumber on growth of *A. tumefaciens* and its gene transfer efficiency to tobacco and potato plants.

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2. Materials and methods

2.1. Squirting cucumber material

Mature fruits of squirting cucumber were collected from the natural habitat of the Faculty of Agriculture, Ankara University (Turkey), in September 2011. Fruits were squeezed manually, and their juice was collected in glass jars and was filter-sterilized with 0.45-μm filters. It was then stored at –20 °C. Squirting cucumber fruit juice was added to bacterial growth, leaf disc inoculation, and cocultivation media at different concentrations.

2.2. *Agrobacterium tumefaciens* material and growth conditions

This study used *A. tumefaciens* strain GV2260 harboring p35S GUS-INT or pAoPR1 GUS-INT plasmids containing neomycin phosphotransferase II (NPT-II) gene, driven by NOS promoter and β-glucuronidase (GUS) gene under the control of 35S or AoPR1 promoter (Özcan et al., 1993), respectively. Both plasmids contained GUS gene interrupted by plant intron region in order to prevent expression of GUS gene in *A. tumefaciens*. A single colony of *Agrobacterium* was inoculated in nutrient broth (NB) containing rifampicin and kanamycin at a concentration of 50 mg L^{-1}, and incubated overnight at 28 °C. Furthermore, 100 μL of this culture was added to 10 mL of NB broth, supplemented with appropriate antibiotics and squirting cucumber fruit juice at different concentrations of 0, 1.25, 2.5, 5, 10, and 20 mL L^{-1}, and incubated in a shaker at 28 °C. OD_{600} of bacterial growth was measured by Eppendorf Biophotometer after 6, 12, and 24 h.

2.3. Plant culture conditions

MSD4X2 medium consisted of Murashige and Skoog (MS) minerals and vitamins (Murashige and Skoog, 1962), 1.0 mg L^{-1} 6-benzylaminopurine (BAP), and 0.1 mg L^{-1} α-naphthalene acetic acid (NAA), and 30 g L^{-1} sucrose was used in tobacco transformation, while 5 mg L^{-1} gibberellic acid (GA_3) was added to this medium for potato transformation experiments. Agar (0.7%) was added to MSD4X2 medium after adjusting the pH of the media to 5.6–5.8 with 0.1 N KOH or 0.1 N HCl, before autoclaving at 121 °C under a pressure of 15 psi (103.42 kPa) for 20 min. All cultures were maintained under a light intensity of 42 μmol m^{-2} s^{-1} photosynthetic active radiation at 24 ± 1 °C with a 16-h light photoperiod (Aasim et al., 2013).

2.4. Plant transformation

A. tumefaciens GV2260 carrying p35S GUS-INT and pAoPR1 GUS-INT plasmids was grown overnight and diluted with a liquid MSD4X2 medium to 1×10^8 cell mL^{-1}. Tobacco cv. Samsun and potato cv. Innovator leaf disc explants of approximately 0.5 cm, isolated from in vitro-grown plantlets, were inoculated for 30 min in this bacterial inoculation medium. Then explants were transferred to solid MSD4X2 medium for 2 days in a growth chamber at 24 ± 1 °C for cocultivation. In the first experiment, high concentrations (1.25–20 mL L^{-1}) of squirting cucumber fruit juice were added to all bacterial growth, inoculation, and cocultivation media. However, due to excessive bacterial growth in cocultivation and selection media, squirting cucumber fruit juice concentrations were reduced to 0.2–1.6 mL L^{-1} in all media in the second experiment. After 2 days of cocultivation, explants were transferred to solid MSD4X2 selection medium supplemented with 100 mg L^{-1} kanamycin and 500 mg L^{-1} amoklovin (a board spectrum antibiotic to suppress *Agrobacterium* growth). After 4 weeks of culture, kanamycin-resistant shoots were cut and transferred to MS0 (MS medium and 3% sucrose) medium with 100 mg L^{-1} kanamycin and 500 mg L^{-1} amoklovin for rooting. Thereafter, rooted putative transgenic plantlets were transferred to pots containing perlite and organic soil for acclimatization.

2.5. Histochemical GUS assay

Potato callus was developed on selection medium, and after successful acclimatization the newly developed leaves on the top of the plants were cut off and subjected to GUS analysis, as described by Jefferson et al. (1987) and Özcan et al. (1993). All samples were submerged in X-GLUC solution that comprised 100 mM sodium phosphate (pH 7.0), 10 mM EDTA, 0.1% Triton X-100, and 1 mM 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc), and were incubated at 38 °C for 1–2 h. Thereafter, the solution was replaced with concentrated ethanol and GUS positive. Callus and plants were identified with blue staining.

2.6. Confirmation of putative transgenic plants by PCR

Seeds from each individual putative transgenic tobacco plant were ground with liquid nitrogen, and 600 μL of sterile ultrapure distilled water was added before vortexing. The amplification reaction proceeded with NPT-II gene primers F: 5'-TTGCTCCTGCCGAGAAAG-3' and R: 5'-GAAGGCGATAGAAGGCGA-3', following the protocol described in the Phine Plant Direct PCR Kit (Thermo Scientific). The PCR was carried out in 20 μL of reaction mixture containing 1 μL of seed sample, 25 pmol of each primer (2 μL), 10 μL of Phire Plant PCR kit, 0.4 μL of Phire Hot Start II DNA polymerase, and 6.6 μL of water. PCR amplification was performed on a Biometra T-personal thermocycler, and the PCR parameters were 40 cycles of 98 °C for 5 s, 58 °C for 10 s, 72 °C for 20 s, preceded by an initial denaturation at 98 °C for 5 min and extended for a final time at 72 °C for 5 min. Amplified DNA was finally observed on agarose gel.

2.7. Observations and statistical analysis

The number of tobacco leaf disc explants producing kanamycin-resistant callus and shoots was scored after 2 and 4 weeks of culture, respectively. On the other hand,

kanamycin-resistant callus formation was recorded in potato leaf disc transformation experiments after 4 weeks. Each treatment had three replicates containing 10 explants. All data regarding callus and shoot formation were analyzed with SPSS 16.00. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test using an MSTAT-C. All data were converted to percentages in arcsine transformation before statistical analysis (Snedecor and Cochran, 1967).

3. Results

3.1. Effect of squirting cucumber fruit juice on *A. tumefaciens* growth

Results on growth of *A. tumefaciens* GV2260 p35S GUS-INT revealed clear bearings of squirting cucumber fruit juice concentration and time of culture. Results revealed that bacterial growth was significantly different after 6 and 24 h and increased with time irrespective of squirting cucumber fruit juice concentrations. *A. tumefacines* showed an increasing growth pattern with increased fruit juice concentrations after 6 h, and the highest results were obtained in media containing 5–20 mL L^{-1} fruit juice (Figure 1a). After 12 h, bacterial growth was 2.10–2.55 and 2.12–2.60 for the OD_{600} with no significant differences between control and concentrations of fruit juice (Figure 1b). The highest bacterial growth was achieved using 1.25 mL L^{-1} fruit juice after 24 h, although the growth was reduced by higher fruit juice concentrations (Figure 1c).

3.2. Effect of squirting cucumber fruit juice on *A. tumefaciens*-mediated transformation of tobacco

In the first experiment, high concentrations (1.25–20 mL L^{-1}) of squirting cucumber fruit juice were used in bacterial growth, inoculation, and cocultivation media for transformation of tobacco by *A. tumefaciens* GV2260 p35S GUS-INT strain. Bacterial growth could be seen on a cocultivation medium after inoculation (Figures 2a and 2b). However, higher concentrations of squirting cucumber fruit juice induced excessive bacterial growth on a cocultivation medium (Figures 2c and 2d). Although high frequency of kanamycin-resistant callus induction with shoot regeneration was achieved on all culture media, excessive bacterial growth was also observed in cocultivation and selection media, especially at higher concentrations (10 and 20 mL L^{-1}) of fruit juice, which hindered callus formation and shoot regeneration (Figures 2e and 2f). Therefore, explants were subcultured to fresh selection medium containing antibiotics at weekly intervals.

Table 1 shows the effects of high concentrations of squirting cucumber fruit juice added to bacterial growth, inoculation, and cocultivation media on kanamycin-resistant callus and shoot development. All explants produced kanamycin-resistant callus. The highest number of

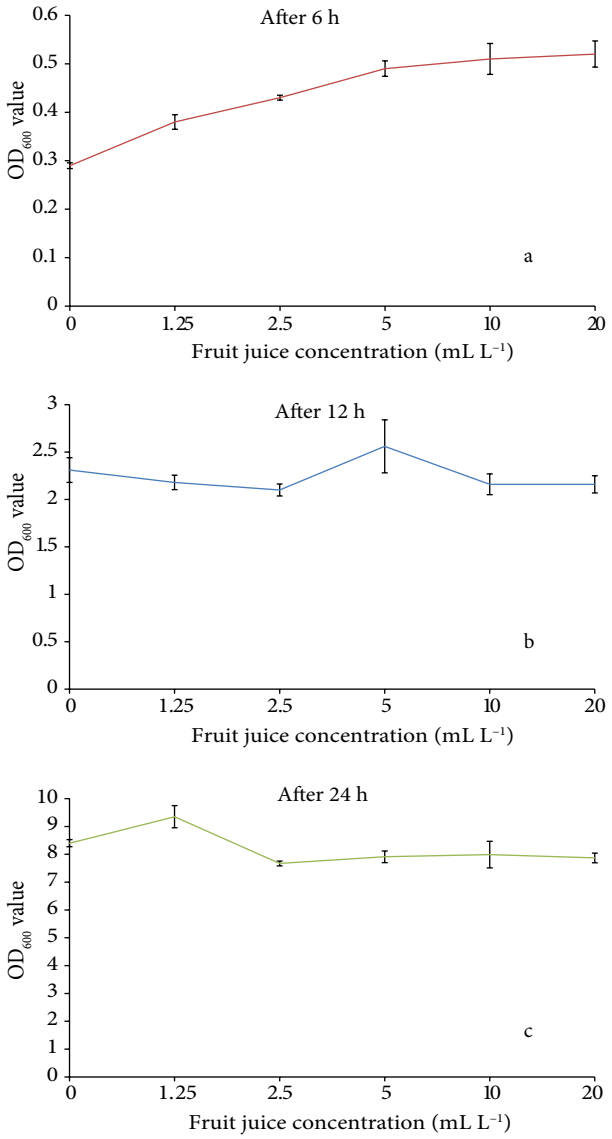

Figure 1. Effect of different concentrations of squirting cucumber fruit juice on the growth of *A. tumefaciens* GV2260 p35S GUS-INT for the OD_{600}.

kanamycin-resistant calli per leaf disc explant was obtained on media containing 1.25 mL L^{-1} fruit juice, whereas the percentage of explants producing shoots was highest with 20 mL L^{-1} fruit juice. On the other hand, the highest number of kanamycin-resistant shoots per explant and petri dish compared to the control was achieved on media containing 2.5 mL L^{-1} fruit juice. Higher concentrations of fruit juice (10 and 20 mL L^{-1}) in media reduced the kanamycin-resistant shoot regeneration frequency significantly due to bacterial overgrowth (Table 1).

In order to prevent excessive *Agrobacterium* growth in culture, the concentration of squirting cucumber fruit juice was lowered to 0.2–0.8 mL L^{-1} in bacterial

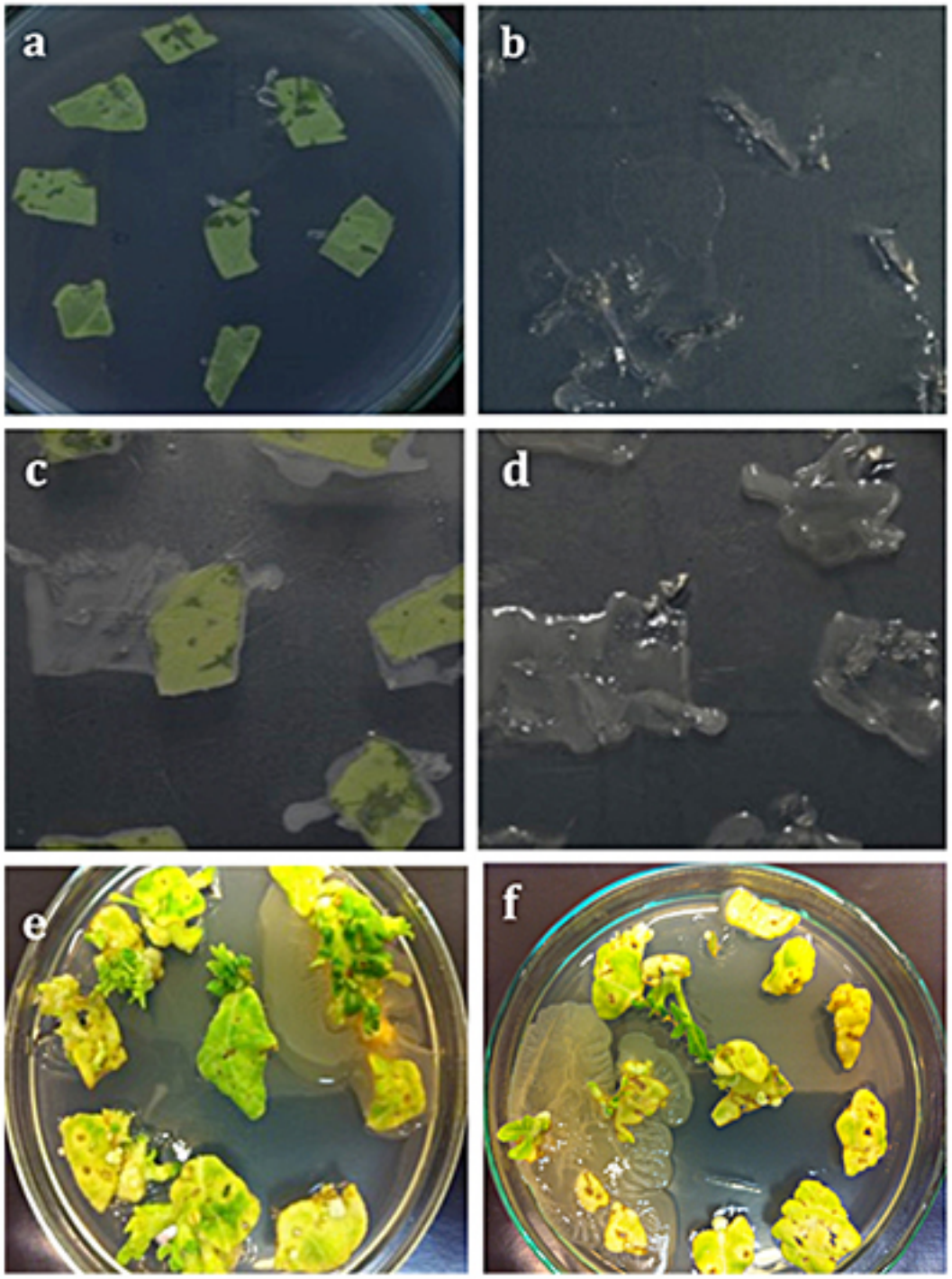

Figure 2. Excessive *A. tumefaciens* GV2260 p35S GUS-INT growth at high concentrations of squirting cucumber fruit juice added to bacterial growth, inoculation, and cocultivation media of tobacco cv. Samsun leaf discs. (a, b) On a cocultivation medium without fruit juice after 2 days of inoculation, (c, d) on a cocultivation medium with 20 mL L^{-1} fruit juice after 2 days of inoculation, (e, f) on a selection medium with the use of 10 and 20 mL L^{-1} fruit juice after 3 weeks of inoculation.

Table 1. Effect of high concentrations of squirting cucumber fruit juice added to bacterial growth, inoculation, and cocultivation media on kanamycin-resistant callus and shoot development from tobacco cv. Samsun leaf discs explants inoculated with *A. tumefaciens* GV2260 p35S GUS-INT strain.

Fruit juice concentration (mL L^{-1})	Number of individual calli/explants[B]	Explants producing shoots (%)	Number of shoots/ explant[B]	Number of shoots/ petri dish
0.00	14.7 ± 3.28 a[A]	66.7 ± 3.33 b	6.4 ± 0.42 bc	42.3 ± 1.20 b
1.25	18.3 ± 1.67 a	73.3 ± 8.82 b	7.7 ± 0.42 c	57.3 ± 10.04 ab
2.50	15.3 ± 1.45 a	66.7 ± 3.33 b	11.7 ± 1.28 a	78.7 ± 11.41 a
5.00	11.4 ± 1.76 ab	66.7 ± 6.67 b	10.8 ± 0.60 a	72.3 ± 11.02 a
10.0	6.6 ± 1.76 bc	73.3 ± 3.33 b	5.2 ± 0.16 c	38.0 ± 2.65 b
20.0	4.2 ± 0.88 c	93.3 ± 3.33 a	4.5 ± 0.069 c	42.3 ± 1.45 b

[A]Values in a column followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.
[B]From leaf disc explants that produced callus or shoots.
All explants produced kanamycin-resistant callus.

growth, inoculation, and cocultivation media. Excessive bacterial growth was not observed on cocultivation and selection media in any lower fruit juice concentrations, and weekly subculture of explants on selection medium was unnecessary. In general, kanamycin-resistant callus and shoot development were lower in this experiment than in the experiment with high concentrations of squirting cucumber fruit juice. However, in all fruit juice concentrations, kanamycin-resistant callus and shoot formation frequencies were still much higher than the control without fruit juice (Table 2; Figures 3a and 3b). Kanamycin-resistant shoots were removed from leaf disc explants after 4 weeks of culture initiation and were cultured in MS0 medium supplemented with 100 mg L^{-1} kanamycin and 500 mg L^{-1} amoklovin for rooting. Approximately 45% of shoots rooted and developed a good rooting system within 2 weeks (Figure 3c). Over 50 rooted plantlets were subsequently transferred to compost (Figure 3d) and set seeds in growth cabinets.

For histochemical GUS analysis, newly developed leaves were collected randomly from 10 putative transgenic plants in growth cabinets and subjected to GUS assay. The results of the GUS analysis confirmed the presence of the GUS gene in all plant genomes (Figure 3e). PCR amplification was carried out using NPT-II marker gene primers and PCR reaction was loaded on agarose gel. PCR results showed the presence of the NPT-II gene amplified at an internal fragment of 459 bp in all 5 transformants, whereas there was no amplification in the negative control (Figure 3f). Both GUS and PCR results confirmed the presence of intact T-DNA insertion in the plant genome.

Table 2. Effect of lower concentrations of squirting cucumber fruit juice added to the bacterial growth, inoculation, and cocultivation media on kanamycin-resistant callus and shoot development from tobacco cv. Samsun leaf discs explants inoculated with *A. tumefaciens* GV2260 p35S GUS-INT strain.

Fruit juice concentration (ml L^{-1})	Explants producing calli (%)	Number of individual calli/ explants[B]	Number of calli/petri dish	Explants producing shoots (%)	Number of shoots/explant[B]	Number of shoots/ petri dish
0.0	20.0 ± 5.77 b[A]	1.5[ns]	3.0 ± 1.15 b	23.3[ns]	1.6 ± 0.87 b	5.7 ± 3.48 b
0.2	83.3 ± 3.33 a	3.7	30.7 ± 3.53 a	56.7	4.7 ± 0.07 a	27.0 ± 7.23 a
0.4	66.7 ± 8.82 a	2.9	19.8 ± 4.33 a	50.0	5.2 ± 0.14 a	36.3 ± 5.53 a
0.8	73.3 ± 8.82 a	4.0	29.7 ± 5.61 a	63.3	4.8 ± 0.24 a	29.7 ± 5.81 a

[A]Values in a column followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.
[B]From leaf disc explants that produced callus or shoots.
ns: not significant.

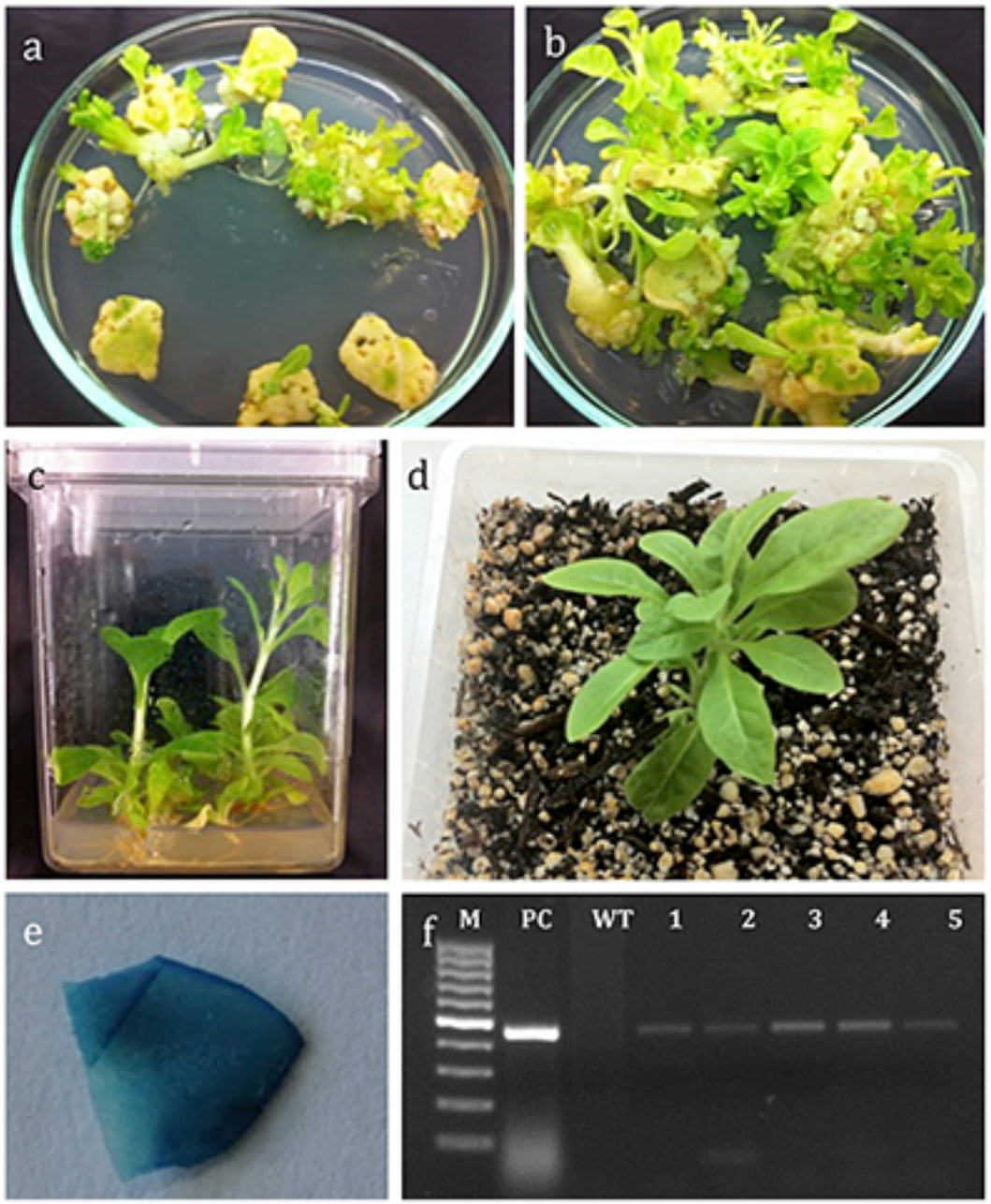

Figure 3. Effect of squirting cucumber fruit juice added to the bacterial growth, inoculation, and cocultivation media on transformation of tobacco cv. Samsun inoculated by GV2260 p35S GUS-INT strain and confirmation of transformants by GUS and PCR analysis. (a) Kanamycin-resistant shoot development on selection medium without using fruit juice after 4 weeks of inoculation, (b) kanamycin-resistant shoot development with no excessive bacterial growth on selection medium with the use of 0.4 mL L^{-1} fruit juice after 4 weeks of inoculation, (c) rooting of kanamycin-resistant shoots on rooting medium with 100 mg L^{-1} kanamycin, (d) acclimatization of rooted plantlets, (e) histochemical GUS activity in leaves of individual transgenic plants, (f) PCR analysis to detect the NPT-II gene in independent tobacco transformants (M: 100 bp DNA ladder, PC: positive control p35S GUS-INT plasmid, WT: nontransgenic wild type, lanes 1–5: independent transgenic plants).

3.3. Effect of squirting cucumber fruit juice on *A. tumefaciens*-mediated transformation of potato

A. tumefaciens strains GV2260 harboring plasmid p35S GUS-INT and pAoPR1 GUS-INT were used in potato transformation. In preliminary experiments, as in the case of tobacco, the addition of 2.50–10.0 mL L^{-1} squirting cucumber fruit juice in bacterial growth, inoculation, and cocultivation media resulted in excessive bacterial growth in cocultivation and selection media (Figures 4a and 4b). This bacterial overgrowth could not be controlled by subculture and higher concentrations of antibiotics. Therefore, lower concentrations (0.2–1.6 mL L^{-1}) of cucumber fruit juice were used in the second experiment. Results revealed statistically significant bearings of low squirting cucumber fruit juice concentration on callus induction frequency and calli per explant without any contamination in the medium (Figure 4c). Use of 0.8 and 1.6 mg L^{-1} fruit juice significantly improved transformation efficiency compared to the control, and the highest frequency of kanamycin-resistant callus formation was achieved when 1.6 mg L^{-1} fruit juice was added to bacterial growth, inoculation, and cocultivation media in both plasmids (Table 3). After 4 weeks of inoculation, histochemical GUS analysis was carried out on randomly selected kanamycin-resistant calli, which were mostly GUS positive (Figure 4d). This result confirmed the T-DNA transfer from *Agrobacterium* to potato leaf disc explants. However, kanamycin-resistant shoot regeneration was not obtained from leaf explants of potato, irrespective of fruit juice concentration.

4. Discussion

A. tumefaciens-mediated genetic transformation is the most popular and efficient technique for introducing foreign genes into plants (Zupan et al., 2000; Joubert et al., 2002). For efficient genetic transformation, activation of *chv* (chromosomal) and *vir* (virulence) genes are of immense importance for the recognition and

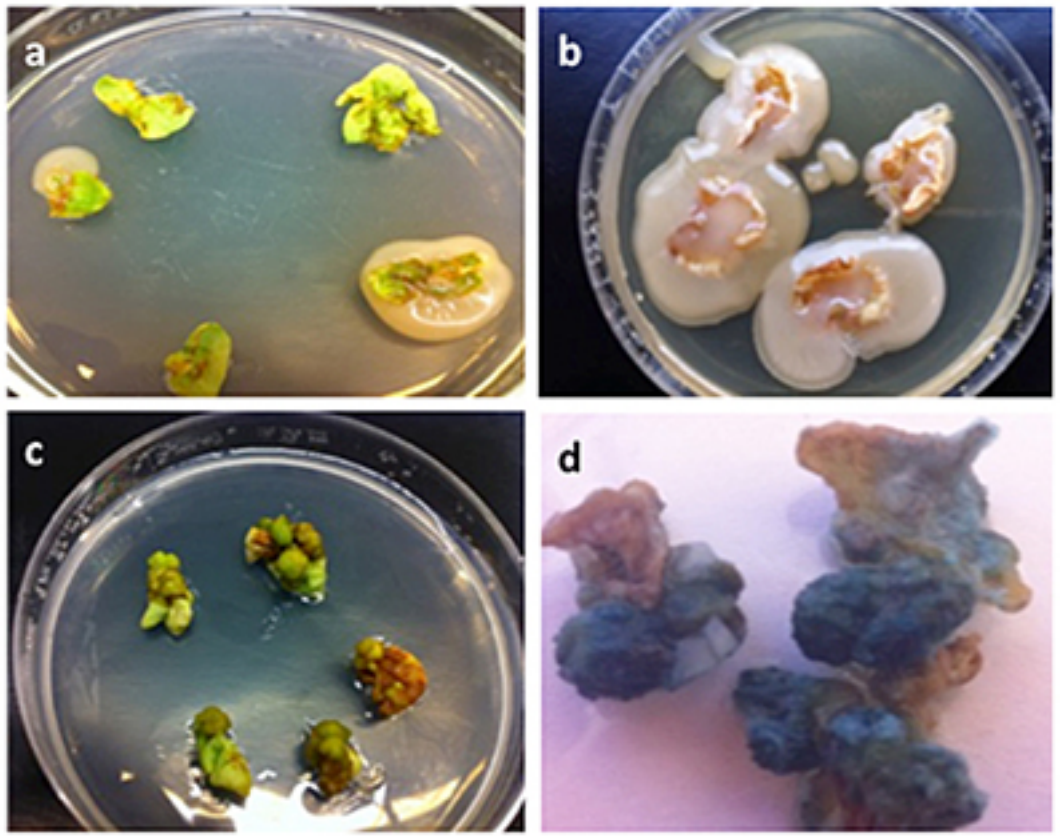

Figure 4. Effect of squirting cucumber fruit juice on transformation of potato cv. Innovator leaf discs inoculated GV2260 p35S GUS-INT strain and confirmation of kanamycin-resistant calli by GUS analysis. (a, b) Excessive *A. tumefaciens* growth on selection medium with the use of 20 mg L^{-1} concentration of squirting cucumber fruit juice in the bacterial growth, inoculation, and cocultivation media, (c) kanamycin-resistant callus development on a selection medium with no excessive growth after 4 weeks of inoculation, with the use of 1.6 mL L^{-1} fruit juice in the bacterial growth, inoculation, and cocultivation media, (d) histochemical GUS activity in kanamycin-resistant calli developed from leaf discs.

Table 3. Effect of lower concentrations of squirting cucumber's fruit juice added to the bacterial growth, inoculation and cocultivation media on kanamycin-resistant callus development from potato cv. Innovator leaf discs explants inoculated with *A. tumefaciens* GV2260 p35S GUS-INT and GV2260 AoPR1 GUS-INT strains.

Fruit juice concent. (mL L^{-1})	GV2260 p35S GUS-INT			GV2260 AoPR1 GUS-INT		
	Explants producing calli (%)	Number of individual calli/ explants[B]	Number of calli/petri dish	Explants producing calli (%)	Number of individual calli/ explant[B]	Number of calli/petri dish
0.0	46.6 ± 6.67 c[A]	4.3 ± 0.33 a	10.3 ± 2.33 b	26.7[ns]	0.9 ± 0.49 b	2.0 ± 1.53 b c
0.2	33.3 ± 13.33 c	3.0 ± 1.15 ab	5.0 ± 2.31 b c	33.3	1.5 ± 0.29 b	2.3 ± 0.33 b
0.4	26.7 ± 6.67 c	1.3 ± 0.33 b	2.0 ± 1.00 c	26.7	2.0 ± 0.00 ab	2.7 ± 0.67 b
0.8	80.0 ± 11.55 b	5.1 ± 0.58 a	20.3 ± 3.28 a	53.3	3.6 ± 0.65 a	10.3 ± 4.37 b
1.6	100.0 ± 0.00 a	5.2 ± 0.41 a	25.7 ± 2.03 a	80.0	6.7 ± 0.82 a	26.7 ± 7.51 a

[A]Values in a column followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.
[B]From leaf disc explants that produced callus.
ns: not significant

immobilization of bacteria on the epidermal plant surface (Douglas et al., 1982). Phenolic compounds are especially responsible for *vir* gene induction. Phenolic compounds, such as acetosyringone I (AS) and hydroxy-AS, which result from plant wounds or exogenic application at low concentrations, are chemoattractants or *vir* inducers at high concentrations (Joubert et al., 2002). A number of studies have revealed the increased transformation frequency by providing acetosyringone (Sunilkumar et al., 1999; Yamada et al., 2001; Polowick et al., 2004) or other related molecules (Morris and Morris, 1990; Delay et al., 1992; Dyé et al., 1997; Cha et al., 2011).

Squirting cucumber is an important poisonous medicinal plant with antibacterial activity when fruit or plant extracts are used (Oskay and Sarı, 2007; Koca et al., 2010; Adwan et al., 2011). However, in our unpublished preliminary studies, we found that the juice of mature squirting cucumber fruits induced growth of *E. coli* and *Staphylococcus aureus*. We hypothesized from our preliminary data that plant seed, seed coat, or other plant organs can exhibit antibacterial activity, except for fruit juice, which induced bacterial growth. Furthermore, no report of antibacterial activity of fruit juice has been established before. To support our hypothesis, the present study was designed to check the efficiency of squirting cucumber fruit juice on *A. tumefaciens* growth and the genetic transformation of tobacco and potato. Several studies have reported on genetic transformation in plants by using plant extracts to induce *vir* genes (Gelvin, 2003).

Results showed positive effects of squirting cucumber fruit juice on bacterial growth after 6 h, compared to the control. In addition, increased concentration of squirting cucumber fruit juice in the culture media also exerted positive effects on bacterial growth. However, bacterial growth was found statistically insignificant after 12 h, which might be due to the stationary growth phase of bacteria, followed by death due to loss of basal medium and increased concentrations of toxic substances. After 24 h, bacterial growth was reduced at higher concentrations of fruit juice, which can be attributed to the fact that bacteria had reached the stationary growth phase earlier.

Results on the use of squirting cucumber fruit juice for genetic transformation revealed the clear bearings of fruit juice concentration. Higher concentration of squirting cucumber fruit juice in the culture medium led to excessive bacterial growth, which ultimately hindered shoot regeneration. These results also confirm the clearly induced effect of fruit juice on *A. tumefeciens* growth. On the other hand, provision of low concentrations (0.2–1.6 mL L^{-1}) of squirting cucumber fruit juice was found efficient for kanamycin-resistant callus and shoot regeneration without any bacterial overgrowth in the culture medium. Results emphasized the importance of modification of the regeneration protocol along with transformation experiments in order to achieve desirable characteristics (Siemens and Schieder, 1996; Tang et al., 1999; Jaime and Teixeira, 2003; Bakhsh et al., 2014).

Kanamycin-resistant tobacco shoots rooted in a medium containing a high concentration (100 mg L^{-1}) of kanamycin. High concentrations of kanamycin in the rooting medium efficiently prevented the rooting of escaped shoots and, therefore, of approximately 45% of the shoots rooted, as reported earlier (Teixeira da Silva and Fukai, 2001; Aasim et al., 2014). These rooted plantlets

were successfully acclimatized in pots under greenhouse conditions and set seeds. Both GUS and PCR analysis confirmed that all raised plants were transgenic.

In conclusion, in the present study, squirting cucumber fruit juice was used for the first time, to increase the transformation efficiency of plants by *A. tumefaciens*. The data presented here clearly indicate that the addition of squirting cucumber fruit juice to bacterial growth, inoculation, and cocultivation media significantly improved the bacterial growth and transformation frequency of tobacco and potato. We conclude that squirting cucumber fruit juice induces certain phenolic compounds that can chemotactically attract *Agrobacterium*, hence inducing *vir* genes that ultimately lead to increased transformation efficiency. Thus, the use of squirting cucumber fruit juice can be valuable for the *Agrobacterium*-mediated genetic modification of crops. However, the exact mechanism of increased bacterial growth and transformation efficiency due to the addition of squirting cucumber fruit needs to be fully understood. This study also opens a new window for researchers to check the efficacy of natural plant extracts for improved genetic transformation studies in other commercial crops.

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