Phytoprotection

Antagonistic potential of native agrocin-producing non-pathogenic *Agrobacterium tumefaciens* strain UHFBA-218 to control crown gall in peach

Potentiel antagoniste de la souche indigène non pathogène UHFBA-218 d'*Agrobacterium tumefaciens* produisant de l'agrocine pour lutter contre la galle du collet chez la pêche

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Article abstract

A non-pathogenic agrocin-producing native isolate of *Agrobacterium tumefaciens* strain UHFBA-218 was tested as a biological control agent against the peach crown gall. This strain was compatible with all the recommended pesticides used in stone fruits in the integrated pest management (IPM) module, except for copper oxychloride, which was detrimental to its growth. Upon artificial co-inoculation of 4-wk-old plants of tomato var. Solan Gola with *A. tumefaciens* strain UHFBA-218 and tumorigenic *A. tumefaciens* strain Peach 2E-10, out of the 27 isolates recovered, six were transconjugants showing selective acquisition of tumorigenic factors as made evident by amplification with *ipt* and *virD2* primers, whereas the rest of the isolates did not acquire any of these tumorigenic factors. A white stone powder-based formulation of this isolate (103.3×10^8 cfu g^{-1}) retained appreciable viability for up to 6 months at room temperature. When peach roots and seeds were soaked in cell suspensions of different doses of a white stone powder-based bioformulation of UHFBA-218 before planting in the field, the number of plants with tumours was reduced, with the lowest incidence of crown gall being observed in the 0.1% UHFBA-218 root dip treatment, i.e. 1.48% and 0.80% during the years 2013 and 2014, respectively. No incidence of crown gall was observed in the three seed dip treatments, i.e. 30-min dip in UHFBA-218 followed by 1 h of shade drying, stratified seeds dipped for 30 min in 0.1% suspensions of strains UHFBA-218 or K84 followed by 1 h of shade drying before sowing, as compared with 14.76% incidence in untreated plants.

Antagonistic potential of native agrocin-producing non-pathogenic *Agrobacterium tumefaciens* strain UHFBA-218 to control crown gall in peach

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Keywords: antagonistic potential, bioformulation, dose, incidence, native agrocin-producing isolate, non-pathogenic *Agrobacterium tumefaciens*, selective acquisition, transconjugants

[Potentiel antagoniste de la souche indigène non pathogène UHFBA-218 d'*Agrobacterium tumefaciens* produisant de l'agrocine pour lutter contre la galle du collet chez la pêche]

Un isolate indigène non pathogène de la souche UHFBA-218 d'*Agrobacterium tumefaciens* produisant de l'agrocine a été testé comme agent de lutte biologique contre la galle du collet de la pêche. Cette souche était compatible avec tous les pesticides recommandés pour les fruits à noyaux dans le module de lutte antiparasitaire intégrée (LAI), à l'exception de l'oxychlorure de cuivre qui était nuisible à sa croissance. Parmi les 27 isolats recouvrés, la co-inoculation artificielle de plants de tomate var. Solan Gola âgés de 4 sem avec la souche UHFBA-218 d'*A. tumefaciens* et la souche Peach 2E-10 d'*A. tumefaciens* tumorigène a résulté en six isolats transconjugants pouvant faire l'acquisition sélective de facteurs tumorigènes tel que démontré par l'amplification des amorces *ipt* et *virD2*, tandis que le reste des isolats ne possédaient pas de facteurs tumorigènes. Un mélange à base de poudre de pierre blanche de cet isolat (103.3×10^8 cfu g^{-1}) a conservé une durabilité appréciable pendant jusqu'à 6 mois à température ambiante. Lorsque les racines ou les graines de pêche était trempées dans une suspension cellulaire comprenant différentes doses du biomélange d'UHFBA-218 à base de poussière de pierre blanche avant d'être plantées en champs, le nombre de plants avec tumeurs était réduit, la plus faible incidence de la galle du collet étant observée avec le traitement de trempage des racines dans 0,1 % de UHFBA-218, soit 1,48 % et 0,80 % en 2013 et 2014, respectivement. Aucune incidence de galle du collet n'a été observée dans les trois traitements de trempage de graines, soit un trempage de 30 min dans UHFBA-218 suivi d'un

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séchage à l'ombre pendant 1 h, un trempage de 30 min des graines stratifiées dans des suspensions de 0,1 % des souches UHFBA-218 ou K84 suivi d'un séchage à l'ombre pendant 1 h avant l'ensemencement, comparativement à une incidence de 14,76 % dans les plants non traités.

Mots-clés : acquisition sélective, *Agrobacterium tumefaciens* non pathogène, agrocine, bioformulation, dose, incidence, isolat indigène, potentiel antagoniste, transconjugants

INTRODUCTION

Bacteria-inducing tumours or hairy roots on the underground part of stone and pome fruit plants have been classified in the genus *Agrobacterium* causing crown gall. The disease is responsible for extensive economic loss in the nursery production of fruit trees, roses and grapevines in many countries (Garrett 1973; Kennedy and Alcorn 1980; Sobiczewski *et al.* 1991). Infection of plants by *Agrobacterium* has a genetic character and, in the case of tumorigenic strains, it consists of the transfer of a fragment of bacterial Ti plasmid (T-DNA) into the plant cell and its incorporation into the plant genome. This transfer is controlled by virulence (*vir*) genes, which are located on the Ti plasmid, but not in the T-DNA region. Expression of T-DNA genes, which code for auxin synthesis [*tms1* (*iaaM*) and *tms2* (*iaaH*)] and cytokinin synthesis [*tmr* (*ipt*)], causes abnormal cell division resulting in tumour formation (Zhu *et al.* 2000). Tumours inhibit plant physiological functions such as transport of water and nutrients. Large tumours partly girdle the bigger roots or crown; plants show reduced growth and become severely stunted. Tumour breakdown creates wounds on the roots and the wounds become entry points for other soil-borne pathogens (e.g. *Pseudomonas syringae* and *Armillarea mellea*) or pests, such as insect borers (Escobar and Dandekar 2003). Infected plants in the nursery, especially those with tumours on the main roots and collar, become unfit for marketing and must be disposed of. The disease seldom kills plants, but it can elicit a lack of vigour and reduced growth.

The non-pathogenic *Agrobacterium rhizogenes* (Riker *et al.* 1930) Conn 1942 (=*Agrobacterium radiobacter* biovar-2) *Rhizobium rhizogenes* (Young *et al.* 2001) strain K84 has been used successfully to control crown gall in many plant species (Moore and Warren 1979; Penyalver *et al.* 2000). Bacteriocin produced by K84 (agrocin 84), which is thought to be the primary control factor (Kerr 1980), targets RNA synthetase in tumorigenic *Agrobacterium* strains (Reader *et al.* 2005). Recombinant DNA techniques have been used to construct a new biological control strain, K1026, that is identical to K84 except for the 5.9-kb deletion overlapping the Tra region of pAgK84 (Jones and Kerr 1989). K1026 is unable to transfer its mutant agrocin 84 plasmid, called pAgK1026, to other agrobacteria, but it remains inhibitory to strains sensitive to agrocin 84 (Penyalver and López 1999). The commercial formulation of *A. radiobacter* K1026 is called NOGALL™; it is marketed by Bio-Care Technology Pty Ltd (Davis, CA, USA) and is recommended as dip treatment (as inoculant) for germinating seeds, roots, stems and cuttings in nurseries and greenhouses. The NOGALL™ culture of strain K1026 contains no less than 1 billion colony-forming units (cfu) per gram of moist peat medium. NOGALL™ is shipped as packs of 100 g or 500 g of concentrated moist peat bacterial cultures. Webster *et al.* (1986) reported that *A. tumefaciens* J73, a biotype 2 strain harbouring a nopaline Ti plasmid, was found to produce an agrocin active against a broad range of *A. tumefaciens* strains, including grapevine isolates. Sensitivity to J73 is not encoded by a Ti plasmid. As strain J73 belongs to biotype 2, it does not form galls on grapevines. Another non-pathogenic *A. vitis* strain, VAR03-1, was also found to be effective against crown gall in grapevine, rose and tomato (Kawaguchi *et al.* 2008). In the present study, we are reporting a non-pathogenic agrocin-producing *A. tumefaciens* strain effective against crown gall in peach. SI-PFGE analysis of strain UHFBA-218 indicated a possible tripartite genomic makeup, with a primary circular chromosome, a secondary linear chromosome, and the presence of a megaplasmid. Based on BLASTn analysis, a 149-kb cluster of contigs (n = 12) represented a megaplasmid that showed 99% homology (51% query coverage) to the tumour-inducing plasmid pTiC58. However, no virulence genes have been found on the putative megaplasmid, which makes it an interesting prospect for future research (Dua *et al.* 2013). The UHFBA-218 strain (MCC 2101, National Centre for Cell Science, Pune, India; NCBI: KC488176) retained maximum viable counts (103.3 $\times$ 10^8 cfu g^{-1}) in a white stone powder (WSP)-based formulation stored at room temperature for up to 6 months. Thus, this formulation of native isolate was applied at different doses in the field as seed and root dip treatment in peach (*Prunus persica* L.) seedlings in a randomised block design to evaluate the efficiency of the isolate and to find out the optimum dose that should be applied under field conditions to control crown gall in peach. Higher doses of bacterial antagonist often lead to negative results owing to the phenomenon of quorum sensing that is most prevalent in Gram-negative bacteria.

MATERIALS AND METHODS

The bacterial culture and its maintenance

The bacterial culture of the non-pathogenic agrocin-producing *A. tumefaciens* UHFBA-218 was originally isolated from galls collected in October 2008 in the crown portion of infected cherry (*Prunus avium* L.) Colt rootstock plants raised in nursery beds having a history of high incidence of crown gall, ranging from 40% to 65% in the past 5 yrs. Strain K-84 has been maintained in the culture collection (BCC-68) of the Department of Plant Pathology of our university since 1989. The isolates are being maintained in slant on yeast extract mannitol agar (YEMA) medium periodically subcultured every third month and kept at 4°C in the refrigerator for further use, in addition to being preserved as glycerol stocks at -80°C.

In vitro evaluation for antagonistic activity and agrocin production

The native *A. tumefaciens* strain UHFBA-218 and strain K84 (used as reference strain for comparison purposes) were evaluated for their antagonism against *A. tumefaciens* strains C58 (belonging to biovar-2 and used as reference strain) and Peach 2E-10 (the native tumorigenic *A. tumefaciens* strain belonging to biovar-1) following the method described by New and Kerr (1972). Strains UHFBA-218 and K84 (test antagonist) were spot-inoculated on mannitol glutamate agar medium supplemented with biotin (2 μL^{-1}) and the plates were incubated for 3 d at 27°C in a BOD incubator (Patel Scientific Instruments PVT LTD, Ahmedabad, Gujarat, India). In one set, the test antagonist was killed by chloroform and the plates were then misted with tumorigenic *A. tumefaciens* (grown in mannitol agar broth medium supplemented with biotin 2 μL^{-1} with 10^8 cfu per mL after incubation at 27°C for 3 d). In the other set, plates were misted with *A. tumefaciens* without killing the test antagonist. The plates were further incubated for 3 d at 27°C in a BOD incubator. The presence of a zone of inhibition (diameter measured in cm) in plates that were not exposed to chloroform indicated that the isolate had an antagonistic activity against *A. tumefaciens*, and the zone of inhibition in plates exposed to chloroform suggested the production of bacteriocin.

Bacteriocin activity assays

The bacteriocinogenic activity of strains UHFBA-218 and K84 was monitored against *A. tumefaciens* C58 to check the inhibitory spectrum by agar-well diffusion assay (Geis *et al.* 1983).

Preparation of crude bacteriocins

The strains that were selected as potential bacteriocin producers were grown in nutrient broth at 25°C for 96 h. Cells were separated by centrifugation (6000 g, 30 min and 4°C). The cell-free supernatant was maintained at pH 7.0. Bacteriocin activity in the supernatant was then tested by agar-well diffusion assay.

Compatibility of *Agrobacterium* strains with recommended pesticides

The effect on the growth of *A. radiobacter* of the recommended pesticides for stone fruits was studied at three different concentrations (i.e. oxyfluorfen 0.025%, 0.05% and 0.1%, glyphosate 0.05%, 0.1% and 0.15%, atrazine 0.25%, 0.5% and 0.1%, chlorpyriphos 0.1%, 0.15% and 0.2%, phorate 0.025%, 0.05% and 0.1%, carbofuran 0.025 %, 0.05% and 0.1%, mancozeb 0.2%, 0.25% and 0.30%, carbendazim 0.025%, 0.05% and 0.1%, and copper oxychloride 0.2%, 0.25% and 0.3%) using a food poisoning technique along with a control.

The desired concentration of each recommended pesticide was achieved by mixing a double concentration of each pesticide and thoroughly mixing it with an equal volume of double strength YEMA. In each Petri plate, 25 mL of the medium containing the desired concentration of pesticide was poured under aseptic conditions. A 5-mm disc (taken from the periphery of 3-d-old bacterial lawn grown on YEMA) was kept in the centre of Petri plates that contained YEMA and the desired concentration of pesticide. Petri plates were kept at 25 ± 1°C in a BOD incubator and bacteria growth (UHFBA-218) was measured after 5 d. Petri plates on which bacteria (UHFBA-218) had grown on the medium containing no pesticide served as controls. Similar experiments were also performed with strain K84. The percentage of inhibition was calculated using the following formula:

$$\text{Percentage of growth inhibition} = \frac{\text{Growth of bacterium (cm diam) in control - Growth of bacterium (cm diam) in treatment}}{\text{Growth of bacterium (cm diam) in control}} \times 100$$

Co-inoculation of *Agrobacterium* strains for the production of transconjugants

Strain UHFBA-218 was inoculated by inserting a toothpick into the stem of 4-wk-old tomato (*Solanum lycopersicum* L.) var. Solan Gola plants. The following day, the *A. tumefaciens* tumorigenic isolate, i.e. Peach 2E-10, a native *A. tumefaciens* strain belonging to biovar-1 (since we did not encounter any biovar-2 strains of *A. tumefaciens* in studies conducted over the past 15 yrs; Kamal and Gupta 2003) was inoculated at the same place in the wound. Tumour formation was visually assessed 4 wk after inoculation. Inoculation was replicated five times on each plant and 150 plants were inoculated to retrieve 27 isolates from developed galls. The 27 isolates were presumed to be transconjugants; they were screened for agrocin production, for pathogenicity on tomato plants, and for the occurrence of virulence genes.

Analysis of virD2 *and* ipt *regions of transconjugant strains*

Among the 27 isolates, six produced agrocin, but none of the 27 isolates were pathogenic on tomato plants. Total DNA was isolated from six bacterial isolates by growing them at 28°C in yeast extract mannitol (YEM) broth at 200 rpm. The cells were harvested and processed for DNA isolation. Genomic DNA was isolated using a total DNA isolation kit (real genomic isolation kit as per the manufacturer's instructions). The isolated DNA was finally suspended in 100 µL of elution buffer and quantified on 1% agarose gel.

PCR reaction mixtures (50 µL) contained primer oligonucleotides at 0.4 µM each, deoxynucleotide triphosphates at 200 µM each, 1 U of thermostable DNA polymerase (PerkinElmer; Waltham, MA, USA [Taq] or Epicenter Technologies; Thane, Maharashtra, India [Tft]) reaction cocktail supplied by the manufacturer (PerkinElmer: 10 mM Tris [pH 8.3 at 25°C], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin [sigma G2500]; Epicenter Technologies: 50 mM Tris [pH 9.0 at 25°C], 20 mM ammonium sulphate, 1.5 mM $MgCl_2$), and 50 to 250 ng of purified template DNA. Amplification was initiated by incubation at 94°C for 1 min, followed by 40 cycles at 94, 50 and 72°C for 1 min at each temperature.

A PCR-based virulence assay was carried out based on *virD2* and *ipt* genes as previously described by Haas *et al.* (1995). Amplification of *virD2* gene was carried out using one sense-strand oligonucleotide, primer A, and two antisense-strand oligonucleotides, primer C' and primer E' (Table 1). These primers were used in two different pairs to produce PCR products of 338 bp (A-E') and 224 bp (A-C'). The *ipt* gene of 427 bp was amplified using a sense-strand primer, CYT, and an antisense-strand primer, CYT' (Table 1).

Table 1. Oligonucleotide primers and PCR cycling conditions used in this study.

Gene name	Primer	Sequence 5′–3′	Anneal. temp. (°C)	Reference
virD2	A	ATGCCCGATCGA GCT CAA GT	52	Haas *et al.*1995
	E′	CCTGACCCAAACATCTCGGCTGCCCA		
	C′	TCGTCTGGCTGACTTTCGTCATAA		
lpt	CYT	GATCG(G/C)GTCCAATG(C/T)TGT	55	
	CYT′	GATATCCATCGATC(T/C)CTT		

Mass multiplication of strains UHFBA-218 and K84.

The WSP-based formulation of strains UHFBA-218 and K84 was prepared in a 14 L capacity fermenter (LFS-14, Murhopye Scientific Company, Mysore, India). Optimum growth conditions, i.e. YEM broth at pH 7.0, temperature at 25°C, an incubation period of 96 h and an air supply of 20 L m^{-1}, were used to obtain maximum viable counts of *A. radiobacter* strains. The fermenter vessel was filled with 5 L of YEM broth, sterilized *in situ* and inoculated aseptically with 50 mL of seed culture of the individual strain, with 36×10^{12} cfu mL^{-1} viable counts in strain K-84 and 42×10^{12} cfu mL^{-1} in the native strain UHFBA-218. Agitation at 150 rpm was maintained for 4 d and the product was taken off the fermenter vessel for a final estimation of cfu mL^{-1} by serial dilution in three replications. For each replication, cfu mL^{-1} was counted using a digital colony counter meter (Labsol Enterprises, New Delhi, India).

Preparation of the formulation

The fermented cultures of the native *A. tumefaciens* UHFBA-218 and K84 having initial inoculums of 10^{12} cfu mL^{-1} were mixed at the ratio of 1:2 in UV sterilized carrier medium, i.e. WSP (an initial 254 nm wavelength of 30 min before turning it, followed by another exposure of 30 min to UV rays of 8000 µW.s/cm^2). This was done for two consecutive days before mixing the bacterial inoculums with WSP, the carrier medium. The pH of WSP was measured as per the method of Okereke and Okeh (2007) by mixing it at the ratio of 1:2 (WSP:water). The mixture was first shaken on an orbital shaker at 100 rpm for 30 min before it was allowed to stand for 30 min prior to pH reading using a LI 127 ELICO pH meter (Saintifik Jaya, Shah Alam, Selangior, Malaysia). The moisture content of the formulation at the time of mixing it with WSP was estimated using a standard procedure (weighing a fixed quantity before and after keeping it at 60°C for 12 h in a hot air oven) and the formulation was stored in milky polypropylene bags (50 µm thickness; 30 × 20 cm^2) for further use.

Biological control in field experiment

Experiments on the biological control of the peach crown gall were carried out in the field at the experimental farm of the Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, India, in 2013 and 2014. There had been a high incidence of crown gall over the past 5 yrs; therefore, the experiments were conducted with the native *A. tumefaciens* strain UHFBA-218 to minimize this incidence, using strain K84 as a positive control. The field trial was conducted using concrete-frame plots (1.5 m × 1.6 m $plot^{-1}$, 1.0 m tall) filled with soil (pH = 6.8, NO_3–N = 6.8 mg 100 g^{-1} of soil, P_2O_5 = 120 mg 100 g^{-1} of soil, K_2O = 67 mg 100 g^{-1} of soil, CaO = 250 mg 100 g^{-1} of soil, cation exchange capacity = 13.5 meq 100 g^{-1} of soil, organic matter content = 1.8%). Farm yard manure at 60 MT ha^{-1}, N at 90 kg ha^{-1}, in two equally split doses (first dose applied at the time of transplanting and second dose applied during the month of August), P at 30 kg ha^{-1} and K at 50 kg ha^{-1} applied a month before transplanting were applied to the nursery soil.

Root dip treatment

Cell suspensions of strains UHFBA-218 and K84 were each adjusted to about 10^8 cells mL^{-1}. One third of the roots of peach seedlings (1-yr-old) were pruned and soaked for 30 min in a cell suspension of antagonistic isolates with different doses of WSP-based formulation. The different concentrations/doses of WSP-based formulation for UHFBA-218 were 0.01%, 0.05%, 0.1%, 0.25%, 0.5%, 1% and 5%, with K84 serving as positive control. Treated plants were shade dried for 1 h, kept in the wet sawdust heap overnight and planted the next day (Johnson and Dileone 1999). In each plot (8.0 m × 3.0 m $plot^{-1}$), 50 seedlings per treatment were planted in the centre with a 15-cm space between each plant in February 2013 and 2014. Each treatment was replicated three times in a randomized block design. In each plot, the topsoil was covered with a sheet of non-woven fabric used as weed covering material after planting. The plants were subsequently irrigated at 3-d intervals in the first month of the research trial and, later, at weekly intervals. Tumour formation was investigated in the month of December in 2013 and 2014. The temperature ranged from 10 to 33°C, and no severe damage by weather or insects was observed during cultivation.

Field evaluation of different modes of seed treatment with strains UHFBA-218 and K84

The field trial was laid out in 2014. Seeds were stratified in the pits for 2 mo and the treatment details were as follows:

T1. Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, stratified for 60 d, then sown in the field.
T2. Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, shade dried for 1 h, stratified for 60 d, then sown in the field.
T3. Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain K84, stratified for 60 d, then sown in the field.
T4. Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain K84, shade dried for 1 h, stratified for 60 d, then sown in the field.

T5. Stratified seed dipped for 30 min in a 0.1% suspension of strain UHFBA-218, shade dried for 1 h, then sown in the field.
T6. Stratified seed dipped for 30 min in a 0.1% suspension of strain K84, shade dried for 1 h, then sown in the field.
T7. Seed soaked for 3 consecutive d in plain water (water changed every day), dipped in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, shade dried for 1 h, then sown in the field.
T8. Seed soaked for 3 consecutive d in plain water (water changed every day), dipped in a 0.1% suspension of WSP-based formulation of strain K84, shade dried for 1 h, then sown in the field.
T9. Control – stratified untreated seeds.

Data analysis

In this study, statistical analyses were performed using the software SPSS (IBM Analytics, Armonk, NY, USA). In the field experiments, data were analysed by randomized block design. The values of disease incidence were applied to square root transformation. A correlation analysis was carried out to determine the degree of association between two variables. The degree of relationship between disease incidence and each of the other parameter characteristics was determined using Pearson's correlation coefficient (Snedecor and Cochran 1972). The significance of the correlation coefficient was tested using the *t*-statistic. A stepwise regression was used to select a small subset from the larger set so that the resulting regression model had good predictive ability. The variables were added to the regression equation one at a time, using the statistical criterion of maximizing the R^2 of the variables included.

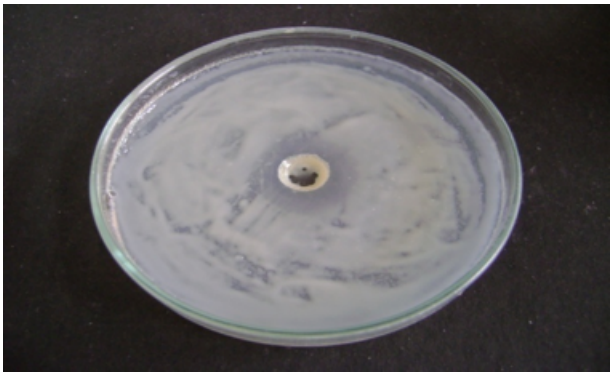


Fig. 1. Activity of crude agrocin K84.

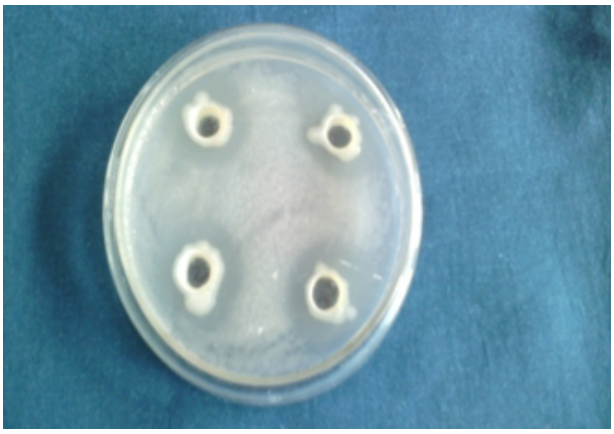


Fig. 2. Agrocin UHFBA-218 against *A. tumefaciens* C58.

RESULTS

In vitro evaluation for antagonistic activity and agrocin production

Agrobacterium strains K84 and UHFBA-218 were able to inhibit the growth of pathogenic *A. tumefaciens* strain C58 with and without exposure to chloroform vapours. In the native strain UHFBA-218, a maximum zone of inhibition (4.16 cm) was observed without exposure to chloroform. The native strain was able to inhibit the growth of pathogenic *A. tumefaciens* even after exposure to chloroform vapours and a maximum zone of inhibition was observed, i.e. 3.57 cm. The *A. radiobacter* strain K84 used the world over had a 1.21 cm and 2.92 cm zone of inhibition with and without exposure to chloroform, respectively.

Bacteriocin activity assay

The crude bacteriocin of K84 and UHFBA-218 was tested for inhibitory spectrum against *A. tumefaciens* C58 and Peach 2E-10. It was observed that the crude extract was able to inhibit the growth of *A. tumefaciens* C58 with an inhibition zone of 2.0 cm and 2.1 cm in K84 and UHFBA-218, respectively (Figs. 1 and 2). Similar results were also obtained for Peach 2E-10.

Compatibility of *A. tumefaciens* strain UHFBA-218 and *A. radiobacter* strain K84 with the recommended pesticides

The data presented in Table 2 reveal that minimum inhibition, i.e. 3.44%, was observed in glyphosate (0.05%), followed by 13.79% in oxyfluorfen (0.025%). Copper oxychloride at all three tested concentration (0.20, 0.25 and 0.30%) completely inhibited the growth of UHFBA-218. Except for copper oxychloride, all the recommended pesticides inhibited the growth of UHFBA-218, ranging from 3.44% to 46.20%. Similarly, copper oxychloride at all three concentrations was also inhibitory to strain K-84 (Table 3).

Molecular analysis of transconjugants *A. tumefaciens* UHFBA-218 × Peach 2E-10

In total, 27 transconjugants were recovered after 4 wk of inoculation on tomato plants with non-pathogenic agrocin-producing *A. tumefaciens* strain UHFBA-218 and from the same wounds inoculated the following night with tumorigenic *A. tumefaciens* Peach 2E-10. Among these, six produced agrocin. The possibility of transfer of virulence genes in these six isolates/transconjugants was confirmed by conducting a pathogenicity test on the same host plants; none were pathogenic. The transfer of virulence genes was again confirmed using specific primers of the *ipt* (CYT-CYT′) region and *virD2* (A-C′ and A-E′) specific

Table 2. Effect of different recommended pesticides for stone fruits on the growth of *Agrobacterium tumefaciens* strain UHFBA-218.

Pesticide	Concentration (%)	Diametric growth (cm)	Inhibition (%)
Glyphosate	0.05	2.86	3.44
	0.10	2.43	16.20
	0.15	1.86	35.86
Chlorpyriphos	0.10	2.36	18.62
	0.15	2.00	31.03
	0.20	1.93	35.92
Carbendazim	0.025	2.30	20.68
	0.05	2.16	25.51
	0.10	1.73	30.00
Phorate	0.025	2.03	19.65
	0.05	2.13	30.00
	0.10	2.00	31.03
Copper oxychloride	0.20	0.00	100.00
	0.25	0.00	100.00
	0.30	0.00	100.00
Carbofuran	0.025	2.00	31.03
	0.05	1.76	39.31
	0.10	1.56	46.20
Atrazine	0.25	2.06	28.96
	0.50	2.03	30.00
	0.10	1.63	44.82
Oxflurofen	0.025	2.50	13.79
	0.05	1.86	35.86
	0.10	1.83	36.89
Mancozeb	0.20	2.20	24.13
	0.25	2.00	31.03
	0.30	1.93	33.34
$CD_{0.05}$		12.53	
S.E. ±		6.25	

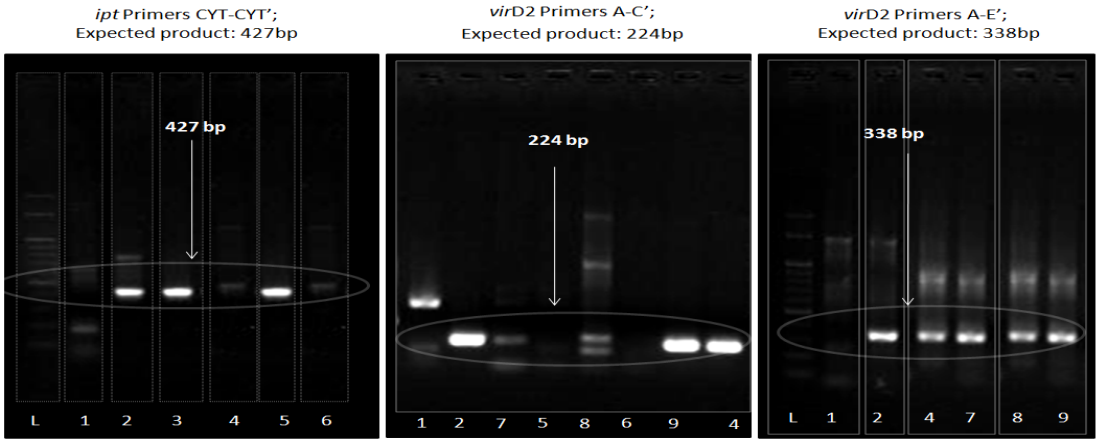
Table 3. Compatibility of *Agrobacterium radiobacter* strain K84 with recommended pesticides for stone fruits.

Pesticide	Concentration (%)	Diametric growth (cm)	Inhibition (%)
Glyphosate	0.05	2.43	10.00
	0.10	2.36	12.59
	0.15	2.13	21.11
Chlorpyriphos	0.10	2.56	5.18
	0.15	2.06	23.70
	0.20	1.8	33.33
Carbendazim	0.025	2.26	16.29
	0.05	1.93	28.51
	0.10	1.76	34.81
Phorate	0.025	1.96	27.40
	0.05	1.96	27.40
	0.10	1.56	42.22
Copper oxychloride	0.20	0.00	100.00
	0.25	0.00	100.00
	0.30	0.00	100.00
Carbofuran	0.025	1.86	31.11
	0.05	1.63	39.62
	0.10	1.56	42.22
Atrazine	0.25	1.90	29.62
	0.50	1.83	32.22
	0.10	1.66	38.51
Oxflurofen	0.025	1.93	28.51
	0.05	1.53	43.33
	0.10	1.50	44.44
Mancozeb	0.20	1.46	45.92
	0.25	1.43	47.03
	0.30	1.43	47.03
$CD_{0.05}$		13.79	
S.E. ±		7.28	

primers yielding a 427 bp amplification product with *ipt* primers and 224 and 338 bp amplification product with *virD2* A-C′ and A-E′ primers, respectively. None of these transconjugants yielded a detectable amplified product with any of the primers. However, transconjugant O-12 showed the desired amplification with both *virD2* primers, and partial amplification with *ipt*. Transconjugant O-18 resulted in amplification with *ipt*, while transconjugants O-6 and O-21 showed amplification of the desired intensity with *virD2* A-E′ primers and partial amplification with *virD2* A-C′ primers. Transconjugant O-16 showed partial amplification with *ipt* only, whereas O-19 resulted in detectable amplification with both *virD2* primer pairs (Fig. 3).

Biological control in field experiments

Biological control assays under field conditions using the method of soaking the roots in a cell suspension of the antagonist were conducted in 2013 and 2014, before planting peach seedlings in soil naturally infested with tumorigenic strains of *A. tumefaciens*. Healthy plant's height, root length, number of leaves per plant, leaf area, stem girth, disease incidence, number of galls per plant, gall size, gall weight, diseased plant height and diseased plant root length were observed at the time of uprooting of plants in the last wk of December of each yr.



O: Overnight L: Ladder (100 bp)

1. *A. radiobacter* (K84)	5. *A.tumefaciens* UHFBA-218 (0-18)
2. *A. tumefaciens* (C58)	6. *A. tumefaciens* UHFBA-218 (0-16)
3. *A. tumefaciens* (Peach 2E-10)	7. *A. tumefaciens* UHFBA-218 (0-21)
4. *A. tumefaciens* UHFBA-218 (0-12)	8. *A. tumefaciens* UHFBA-218 (0-6)
	9. *A. tumefaciens* UHFBA-218 (0-19)

Fig. 3. PCR analysis of transconjugants *A. tumefaciens* UHFBA-218 × Peach 2E-10, *A. tumefaciens* C58 and *A. radiobacter* K84.

Fig. 4. Effect of non-pathogenic agrocin-producing *Agrobacterium tumefaciens* strain UHFBA-218 (0.1%) on crown gall incidence.

Fig. 5. Incidence of crown gall in untreated peach seedlings.

Growth parameters and disease incidence in root dip-treated peach seedlings in 2013 and 2014

Pooled data for 2013 and 2014 indicated a maximum plant height of 116.23 cm for healthy plants, root length of 30.43 cm, 121.32 leaves per plant, leaf area of 13.41 cm^2 and stem girth of 1.13 cm in T3 (Table 4). In the control, plant height, root length, number of leaves per plant, leaf area and stem girth were 101.93 cm, 24.48 cm, 11.74, 11.98 cm^2 and 0.72 cm, respectively.

Crown gall disease incidence was evaluated during the last wk of December in 2013 and 2014. T3 significantly reduced the incidence of crown gall in 2013, i.e. 1.48 %, and it was statistically on par with T4, T2, T8, T1, T5 and T6 with 2.67%, 3.00%, 3.05%, 3.25%, 3.33% and 5.54%, respectively. There was minimum incidence in 2014 in T3, i.e. 0.80%, followed by 2.00% in T8 and 2.17% in T4. Pooled data for both years indicate that T3 resulted in minimum (1.14%) incidence of crown gall in peach plants (Fig. 4) for both yrs compared with high incidence (16.34%) in control plants (Fig. 5). Pooled data also indicate that the minimum number of galls per plant, gall size and gall weight observed in T3 were 0.83, 0.51 cm and 0.65 g, respectively (Table 5).

Table 4. Effect of different doses of WSP-based formulation of *Agrobacterium tumefaciens* strain UHFBA-218 applied as root dip on the growth of peach seedlings.

Treatment	Healthy plant height (cm)		Pooled	Healthy plant root lenght (cm)		Pooled	Number of leaves per plant		Pooled	Leaf area (cm^2)		Pooled	Stem girth (cm)		Pooled
	2013	2014		2013	2014		2013	2014		2013	2014		2013	2014	
T1	104.40	121.40	112.90	20.46	33.06	26.76	39.40	182.66	111.03	12.03	12.43	12.23	0.68	1.22	0.95
T2	84.60	125.33	104.97	18.13	26.73	22.43	33.84	191.28	112.56	12.60	13.46	13.03	0.63	1.47	1.05
T3	95.40	137.06	116.23	28.66	32.20	30.43	44.74	197.91	121.32	12.76	14.06	13.41	0.83	1.44	1.13
T4	88.86	125.33	107.10	23.53	29.73	26.63	38.22	193.33	115.77	12.63	12.76	12.70	0.78	1.25	1.01
T5	81.00	124.33	102.66	21.60	26.86	24.23	37.64	191.24	114.44	12.66	13.20	12.93	0.55	1.25	0.90
T6	77.33	120.40	98.86	24.13	28.46	26.30	38.16	190.57	114.37	12.56	12.43	12.50	0.46	1.04	0.75
T7	74.00	122.13	98.06	21.60	31.16	26.38	36.50	187.35	111.93	12.80	13.00	12.90	0.39	0.88	0.63
T8	92.46	120.16	106.31	18.53	22.33	20.43	35.24	189.22	112.23	12.83	13.70	13.26	0.40	0.95	0.67
Control	84.53	119.33	101.93	20.73	28.23	24.48	39.53	183.96	111.74	11.93	12.03	11.98	0.49	0.95	0.72
SE ±	8.05	4.74	4.95	2.28	2.75	2.24	2.45	3.85	2.27	0.27	0.56	0.36	0.10	0.09	0.06
$CD_{0.05}$	17.07	10.05	10.51	4.83	5.83	4.76	5.21	8.17	4.82	0.57	1.18	0.77	0.23	0.19	0.15

Note: T1 = UHFBA-218 at 0.01%; T2 = UHFBA-218 at 0.05%; T3 = UHFBA-218 at 0.1%; T4 = UHFBA-218 at 0.25%; T5 = UHFBA-218 at 0.5%; T6 = UHFBA-218 at 1%; T7 = UHFBA-218 at 5%; T8 = K84 at 0.1% serving as positive control.

Table 5. Effect of different doses of WSP-based formulation o*f Agrobacterium tumefaciens* strain UHFBA-218 applied as root dip on crown gall of peach seedlings.

Treatment	Disease incidence (%)		Pooled	No. of galls/ plant		Pooled	Gall size (g)		Pooled	Gall weight (cm)		Pooled	Diseased plant height (cm)		Pooled	Diseased plant root lenght (cm)		Pooled
	2013	2014		2013	2014		2013	2014		2013	2014		2013	2014		2013	2014	
T1	3.25	2.77	3.01	1.33	2.22	1.78	1.09	0.75	0.92	1.07	0.83	0.95	75.00	111.71	93.35	19.57	25.47	22.52
T2	3.00	3.27	3.13	1.00	2.50	1.75	1.29	1.10	1.19	1.33	1.23	1.28	75.00	104.20	89.60	20.33	26.85	23.59
T3	1.48	0.80	1.14	0.67	1.00	0.83	0.57	0.46	0.51	0.74	0.56	0.65	73.67	111.67	92.67	21.33	29.67	25.50
T4	2.67	2.17	2.42	1.00	1.67	1.33	0.95	1.10	1.03	1.00	1.23	1.12	74.00	115.33	94.67	21.00	26.67	23.83
T5	3.33	2.33	2.83	1.67	3.00	2.33	1.70	1.06	1.38	1.83	1.20	1.52	73.67	104.33	89.00	20.44	26.47	23.46
T6	5.54	3.70	4.62	2.00	2.87	2.43	1.78	1.67	1.72	1.87	1.73	1.80	74.33	112.53	93.43	20.00	26.73	23.37
T7	6.23	4.00	5.12	3.00	3.33	3.17	2.00	1.49	1.75	2.33	1.60	1.97	75.00	112.00	93.50	22.00	24.86	23.43
T8	3.05	2.00	2.52	1.00	1.33	1.17	0.77	0.78	0.77	0.80	0.87	0.83	73.00	117.33	95.17	21.16	24.60	22.88
Control	16.85	15.83	16.34	5.66	8.67	7.17	4.00	5.00	4.50	4.83	5.33	5.08	70.00	99.00	84.50	16.58	18.67	17.63
SE	2.85	1.50	1.67	0.51	0.04	0.44	0.65	0.39	0.36	0.86	0.53	0.41	1.08	5.06	2.57	1.12	2.45	1.29
$CD_{0.05}$	6.04	3.18	3.55	1.07	0.09	0.94	1.39	0.83	0.76	1.82	1.13	0.87	2.29	10.73	5.45	2.38	5.20	2.74

Note: T1 = UHFBA-218 at 0.01%; T2 = UHFBA-218 at 0.05%; T3 = UHFBA-218 at 0.1%; T4 = UHFBA-218 at 0.25%; T5 = UHFBA-218 at 0.5%; T6 = UHFBA-218 at 1%; T7 = UHFBA-218 at 5%; T8= K84 at 0.1% serving as positive control.

Correlation studies of disease incidence with different parameters for 2013 and 2014

The different parameters were correlated with disease incidence in 2013 and 2014. The correlation data indicated that disease incidence was positively correlated with the number of galls per plant, gall size and gall weight, with values of 0.89, 0.97 and 0.98, respectively. The healthy plant parameters analyzed did not show any relationship with disease incidence. Diseased plant height, diseased plant root length, healthy plant height, healthy plant root length, stem girth, number of leaves per plant and average leaf area were all negatively correlated with disease incidence, with values of -0.24, -0.62, -0.23, -0.16, -0.32, -0.12 and -0.59, respectively (Table 6).

Table 6. Correlation of different parameters with disease incidence.

Parameters	Correlation with disease incidence
Number of galls per plant (X_1)	0.89*
Gall size (X_2)	0.97*
Gall weight (X_3)	0.98*
Diseased plant height (X_4)	-0.24
Diseased plant root length (X_5)	-0.62
Healthy plant height (X_6)	-0.23
Healthy plant root length (X_7)	-0.16
Stem girth (X_8)	-0.32
Number of leaves per plant (X_9)	-0.12
Leaf area (X_{10})	-0.59

* Significantly correlated.

Multiple regressions

A stepwise regression analysis was applied and the proposed model was:

$$Y = -0.915 - 3.344x_2 - 6.278x_3 \quad R^2 = 0.967$$

(0.342) (1.792) (1.594) Adj $R^2 = 0.963$

The results indicate that predicting disease incidence using gall size and gall weight is possible, while other morphological characteristics, i.e. diseased plant height, diseased plant root length, healthy plant height, root length, stem girth, number of leaves per plant and leaf area, do not contribute significantly towards multiple regression.

Effect of the different modes of seed treatment with strains UHFBA-218 and K84 on the growth of peach plants and incidence of crown gall

Healthy plant maximum height (171.46 cm) was observed in T5, and it was statistically on par (169.40 cm) with T3. T5 showed maximum mean leaf area, i.e. 11.9 cm^2, and it was statistically on par (11.76 cm^2) with T4. There was no incidence of disease in T2, T5 and T6. Disease incidence was found to be highest (14.76%) in control plants in 2014, at the time of uprooting. The number of galls per plant was found to be equal in four treatments, i.e. 0.66 in T1, T3, T4 and T7, and was highest (1.27) in the control. Gall size and gall weight were found to be highest (1.50 g and 1.66 g, respectively) in T1 (Table 7), while it was not recorded for treatments where no incidence of crown gall was observed. Diseased plant height was highest (92.16 cm) in T8.

DISCUSSION

Major emphasis has been put on the biological control of crown gall using strain K84 and its genetically-engineered derivative K1026 around the world for over a decade as up until now, no other management strategy had proven to be effective. However, these strains are ineffective in many instances owing to the transfers of pAgK84 plasmid to *A. tumefaciens* and Ti plasmid from *A. tumefaciens* to strain K84 and even to strain K-1026. Raio *et al.* (2009) gave evidence of pAgK84 transfer from *Agrobacterium rhizogenes* K84 to natural pathogenic *Agrobacterium* species. They

Table 7. Effect of the different modes of seed treatment with strains UHFBA-218 and K84 on the growth of peach plants and incidence of crown gall in 2014.

Treatment	Healthy plant height (cm)	Healty plant root length (cm)	Stem girth (cm)	Number of leaves/ plant	Leaf area (cm^2)	Disease incidence (%)	Number of galls/ plant	Gall size (cm)	Gall weight (g)	Diseased plant height (cm)	Diseased plant root length (cm)
T1	164.26	32.20	0.60	265.86	11.23	1.56	0.66	1.50	1.66	79.66	13.66
T2	157.20	33.53	0.59	227.10	10.46	0	0	0	0	0	0
T3	169.40	33.26	0.61	205.99	10.36	2.02	0.66	0.93	0.96	79.66	22.33
T4	144.33	29.86	0.55	182.53	11.76	4.22	0.66	0.83	0.86	85.16	18.33
T5	171.46	31.80	0.54	222.57	11.90	0	0	0	0	0	0
T6	151.33	33.73	0.63	229.97	10.80	0	0	0	0	0	0
T7	139.73	28.93	0.78	165.06	9.43	1.33	0.66	0.65	0.66	43.33	10.66
T8	133.40	27.13	0.86	161.72	9.66	4.89	1.00	1.15	1.19	92.16	14.16
Control	141.60	28.20	0.74	155.66	8.43	14.76	1.27	1.14	1.17	90.81	19.37
SE	12.58	3.06	0.05	23.24	0.89	4.72	0.64	0.81	0.87	52.87	11.56
$CD_{0.05}$	26.68	6.48	0.11	49.27	1.89	10.02	1.37	1.72	1.85	112.07	24.50

Note: T1 = Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, stratified for 60 d, then sown in field; T2 = Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, shade dried for 1 h, stratified for 60 d, then sown in field; T3 = Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain K84, stratified for 60 d, then sown in field; T4 = Seed dipped for 30 min in a 0.1% suspension of WSP-based formulation of strain K84, shade dried for 1h, stratified for 60 d, then sown in field; T5 = Stratified seed dipped for 30 min in a 0.1% suspension of strain UHFBA-218, shade dried for 1 h, then sown in the field; T6 = Stratified seed dipped for 30 min in a 0.1% suspension of strain K84, shade dried for 1 h, then sown in the field; T7 = Seed soaked for 3 consecutive d in plain water (water changed every day), dipped in a 0.1% suspension of WSP-based formulation of strain UHFBA-218, shade dried for 1 h, then sown in the field; T8 = Seed soaked for 3 consecutive d in plain water (water changed every day), dipped in a 0.1% suspension of WSP-based formulation of strain K84, shade dried for 1 h, then sown in the field; Control (T9) = Stratified untreated seeds.

reported both virulent and avirulent transconjugants that represent a threat for biological control by K84, and their survival in the soil could also make future applications of K84 ineffective. Under natural conditions, transconjugants of *Agrobacterium* may occur as either of the two abovementioned factors or even after mutations of Ti-plasmid resulting in alteration and loss of virulence factors. Should these mutants acquire agrocin-producing capability they would be inhibitory to strains carrying nopaline-, octopine-, and agropine-Ti-plasmids carrying *A. tumefaciens*. Non-pathogenic agrocin-producing *A. tumefaciens* belonging to biovar-2 have been found to be effective against tumorigenic *A. tumefaciens* belonging to biovar-1, -2 and -3. Webster *et al.* (1986) also reported a South African non-pathogenic isolate of *A. tumefaciens* strain J73 isolated from infected *Prunus salicinia* Lindl. tree. Agrocin of this nopaline utilizing *Agrobacterium* belonging to biovar-2 was inhibitory to a biovar-3 grapevine isolate. Thus, it was further confirmed that bacteriocins of non-pathogenic *A. tumefaciens* are effective against pathogenic strains under laboratory conditions. We also encountered an agrocin-producing nopaline utilizing the non-pathogenic *A. tumefaciens* strain UHFBA-218 belonging to biovar-1 and carrying a putative megaplasmid that showed 99% homology (51% query coverage) to the tumour-inducing plasmid pTiC58. However, no virulence genes were found on the putative megaplasmid (Dua *et al.* 2013). The crude bacteriocin of this strain showed inhibitory effect against *A. tumefaciens* C58 and other native tumorigenic isolates belonging to both biovar-1 and biovar-2.

The compatibility of strain UHFBA-218 and of the reference strain K84 with most commonly used pesticides in stone fruits showed minimum growth inhibition (3.44%) in glyphosate (0.05%), followed by 13.79, 16.20, 18.62, 19.65, 20.68 and 24.13% in mancozeb (0.20%). Copper oxychloride at all three tested concentrations completely inhibited the growth of the native *A. tumefaciens* strain UHFBA-218 and of strain K84. These results indicate that in stone fruit nurseries, chlorpyriphos, carbendazim, glyphosate, atrazine, oxyflurofen, phorate and carbofuran can be applied at the recommended doses even after the application of *Agrobacterium* strains; they are not detrimental to these biocontrol agents and thus have no adverse effect on disease control. Therefore, these chemicals are safe for use with antagonistic strains of *Agrobacterium*.

None of the agrocin-producing transconjugants of *A. tumefaciens* strain UHFBA-218 × Peach 2E-10 were tumorigenic, suggesting the absence of virulence gene acquisition. The phenomenon of selective acquisition was made evident by the fact that the O-12 isolate both showed amplification with *virD2* and partial amplification with *ipt* primers. As an *ipt* region corresponding to a length of 17 bp leading to the formation of cytokinin is needed for gall formation, partial amplification with *ipt* suggests that transconjugants acquired only some of the cytokinin-producing genes. In transconjugants O-6, O-19 and O-21, the presence of *virD2* and the absence of *ipt* suggest that these isolates arose from *A. tumefaciens* through a deletion in the T-DNA. The presence of detectable *ipt* genes in O-16 and O-18 and the absence of *virD2* genes suggest that these non-pathogenic transconjugants arose from the alteration of putative non-virulent megaplasmid of UHFBA-218 rather than the loss of Ti-plasmid in tumorigenic *A. tumefaciens* at a significant frequency (Hass *et al.* 1995).

Under field conditions, the inhibitory spectrum of these strains was checked for antagonistic action against *A. tumefaciens*. Field trials were conducted in 2013 and 2014 to assess the potential effect of native *A. tumefaciens* strain UHFBA-218 in reducing the incidence of crown gall on peach. This non-pathogenic strain has been studied as a biological control agent of peach crown gall and is effective against tumorigenic strains of *A. tumefaciens*. In a 2-yr research trial of biological control, soaking the roots of peach in a cell suspension of UHFBA-218 and K84 before planting in soil infested with tumorigenic *A. tumefaciens* significantly reduced the percentage of plants with tumours. Root dip-treated peach seedlings with UHFBA-218 at 0.1% have an astonishingly high specificity in controlling crown gall incidence in nursery plants. More field experiments are being performed under different environmental conditions to confirm the stability of the inhibitory effect. A minimum incidence of crown gall was observed in 2014 when UHFBA-218 was applied at 0.1% as root dip treatment to peach seedlings. K84 applied at 0.1% as positive control reduced disease incidence by up to 3.05 and 2.00% in 2013 and 2014, respectively. The number of galls per plant, gall size and gall weight were positively correlated with disease incidence and were reduced most effectively when UHFBA-218 at 0.1% was applied as root dip as well as seed treatment. This particular dose (0.1%) of native isolate was significantly on par with a 0.25% dose of UHFBA-218. There was a very high incidence of crown gall (16.85%) in untreated peach seedlings in 2013, and strain UHFBA-218 at a dose of 0.1% was effective in controlling the incidence of crown gall.

Kawaguchi *et al.* (2008) also reported that treatment with VAR03-1 bacteriocin-producing strains of *A. tumefaciens* significantly reduced the number of plants with tumours and disease severity. The inhibitory effects of treatment with VAR03-1 and the non-pathogenic *A. rhizogenes* strain K84 on crown gall of rose and tomato were almost identical, and the inhibitory effect of VAR03-1 on grapevine was superior to that of K84. The isolate was bacteriocinogenic and inhibited *A. tumefaciens* isolates. Kawaguchi (2013) also reported a non-pathogenic strain of *Rhizobium vitis* ARK-1 as biological control agent for grapevine crown gall. Grapevine roots were soaked in a cell suspension of strain ARK-1 before planting in the field, and the number of plants with tumours decreased.

Seed dip treatment with strains UHFBA-218 and K84 was more effective in controlling disease incidence as compared with root dip treatment. There was no incidence of crown gall in the three seed dip treatments, i.e. T2, T5 and T6, as compared with 14.76% incidence in untreated plants.

Multiple regression and correlation studies showed that disease incidence of the crown gall can be predicted by gall size and gall weight, while other variables related to the normal growth of plants do not contribute much to the disease.

This is the first study to report that the non-pathogenic agrocin-producing native *A. tumefaciens* strain UHFBA-218 effectively controlled peach crown gall in the field as compared with strain K84 used as positive control. The results of field trials comparing the effectiveness of strains UHFBA-218 and K84 indicated that UHFBA-218 was superior to K84. Furthermore, this study showed that the agrocin produced by UHFBA-218 not only inhibited the growth of the pathogenic strain under laboratory conditions, it also efficiently controlled losses caused by the crown gall under field conditions. The applicability of UHFBA-218 to other plants in the field will also be investigated further.

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