
UNIVERSITI PUTRA MALAYSIA

# IMPROVEMENT IN ORGANOGENESIS AND THE DEVELOPMENT OF A TRANSFORMATION PROCEDURE FOR CUCUMBER AND MUSKMELON

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# IMPROVEMENT IN ORGANOGENESIS AND THE DEVELOPMENT OF A TRANSFORMATION PROCEDURE FOR CUCUMBER AND MUSKMELON

By

A. K. M. MOHI UDDIN

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*Specially Dedicated*
*To My*

*Father (Alhajj Md. Lutful Hoque)*
*Mother (Tahera Hoque)*
*Father-in-law (Md. Salimullah)*
*Mother-in-law ( Masuma Salim)*
*and*
*Wife (Nazneen Salim)*

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## LIST OF ABBREVIATION

| | |
|---|---|
| ANOVA | analysis of variance |
| BAP | 6-benzylaminopurine |
| Bp | base pair |
| CaMV | cauliflower mosaic virus |
| CAT | chloramphenicol acetyl transferase |
| Cf | Cefotaxime |
| CPA | p-chlorophenoxyacetic acid |
| cv | cultivar |
| cvs | cultivars |
| d | day |
| DMRT | duncan multiple range test |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| g | gram |
| GUS | β-glucuronidase |
| h | hour |
| IAA | indole acetic acid |
| IBA | indole-3-butyric acid |
| 2iP | 6-(γ-γ-dimethylallylamino) purine |
| Kg | Kilogram |
| Km | kanamycin |
| L | liter |
| LB | left border |
| LB medium | Luria-Bertani medium |
| M | molar |
| Min | minute |
| ml | millilitre |

| | |
|---|---|
| MS | Murashige and Skoog |
| MUG | 4-methyl umbelliferyl glucuronide |
| NAA | naphthalene acetic acid |
| NOS | nopaline syntheses |
| *nptII* | neomycin phosphotransferase II |
| OD | optimal density |
| PCR | polymerase chain reaction |
| $P^H$ | hydrogen ion concentration |
| RB | right border |
| Rf | rifampicin |
| Ri | root-inducing |
| SAS | statistical analysis system |
| s | second |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid |
| TDZ | thidiazuron |
| Ti | tumour-inducing |
| v/v | volume/volume |
| w/v | weight/volume |
| *X-gluc* | 5-bromo-4-chloro-3-indolyglucuronide |
| µ | micro |

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

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**Chairman: Dr. Suhaimi Napis**

**Faculty: Food Science and Biotechnology**

A comprehensive study was carried out to optimise and improve a direct shoot organogenesis system to facilitate the transfer of reporter genes (*GUSINT* and *nptII*) into cucumber (*Cucumis sativus* L.) and muskmelon (*C. melo* L.) plants. The studies carried out were: (i) a comparative study on direct and indirect shoot regeneration (ii) the improvement of a direct shoot regeneration system by using an ethylene action inhibitor, silver nitrate and an ethylene biosynthesis inhibitor, cobalt chloride (iii) identification of suitable strains of *Agrobacterium tumefaciens* and *A. rhizogenes* that are able to infect cucumber and muskmelon plants (iv) development of an *intron* containing gene transformation system for both cucurbit species through *A. tumefaciens* and (v) analysis of transformants.

For the comparative study of direct and indirect shoot regeneration, four different types of explants from five elite commercial cucumber cultivars namely Spring Swallow, Suyo Cross, Suyo Long, Tasty Glory, Tasty Green and one muskmelon cultivar called Birdie were used. Explants from both species were cultured onto Murashige and Skoog medium containing different concentrations of *6*-benzylaminopurine alone and in combination with either 2,4-dichlorophenoxyacetic acid or naphthalene acetic acid. *6*-benzylaminopurine alone at 1.0 or 2.0 mg/l significantly ($p<0.05$) induced shoot primordia

from the largest number of proximal cotyledon and hypocotyl explants of all cucumber cultivars and muskmelon explants tested. However, the addition of either 2,4-dichlorophenoxyacetic acid or naphthalene acetic acid to *6*-benzylaminopurine, resulted in a reduction of the shoot primordia induction rate.

Higher number of shoots induced from explants as well as high numbers of morphological normal shoots were obtained when explants were cultured on medium containing *6*-benzylaminopurine alone. Specific concentrations of indole-butyric acid and naphthalene acetic acid significantly ($p<0.05$) contributed to root initiation from the largest number of cucumber and muskmelon shoots, respectively. Dark treatment was sufficient to significantly induce root formation from the non-rooting cucumber and muskmelon shoots.

The addition of either silver nitrate or cobalt chloride to the seed germination medium or shoot primordia induction medium caused a significant ($p<0.05$) enhancement of shoot regeneration rate from cucumber cv. SS and TG explants compared to the control. The regeneration rate was further enhanced when these two ethylene inhibitors were added to both SGM and SPI media. Furthermore, the number of shoots induced from explants of both Spring Swallow and Tasty Green cultivars was also enhanced upon the same treatment. However, muskmelon shoot induction and regeneration were reduced when the same treatment was employed.

In the *Agrobacterium*-mediated transformation experiments inoculation of cucumber cultivar Spring Swallow and muskmelon cultivar Birdie explants with *A. tumefaciens* and *A. rhizogenes* wild type strains revealed the different degrees of virulence of both bacteria. It was found that the virulence of both *Agrobacterium* species was enhanced when acetosyringone was added to the culture of inoculum