THE DEVELOPMENT OF *WASABIA JAPONICA* (MIQ.) MATSUMURA *IN VITRO*

Cao Dinh Hung

Thesis submitted for degree of Master of Science

Department of Environmental Sciences,

Faculty of Science,

University of Technology, Sydney

MSc

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student

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ABBREVIATIONS

µl	:	Microlitre
µM	:	Micro molar
µmol $m^{-2}s^{-1}$	:	Micromoles per square meter per second
¼MS	:	Quarter-strength MS (Murashige and Skoog, 1962)
½MS	:	Half-strength MS
2,4-D	:	2,4-dichlorophenoxyacetic acid
AITC	:	Allyl isothiocyanate
ANOVA	:	Analysis of variance
B_5	:	Gamborg's medium (Gamborg *et al*., 1968)
BA	:	N^6-benzyladenine
DMSO	:	Dimethyl sulphoxide
DNA	:	Deoxyribonucleic acid
DW	:	Dry weight
FID	:	Flame ionisation detector
FW	:	Fresh weight
GA_3	:	Gibberellic acid
GC	:	Gas chromatography
GC-MS	:	Gas chromatography–Mass spectrometry
GSLs	:	Glucosinolates
Gy	:	Gray
He	:	Helium
HEPA	:	High-Efficiency Particulate Air
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3- butyric acid
ITCs	:	Isothiocyanates
KC	:	Knudson (1925)
Mf	:	Molecular formula
mM	:	Mili molar per litre
MS	:	Full-strength MS
MU	:	Monitor unit
Mw	:	Molecular weight
N&N	:	Nitsch & Nitsch
N_6	:	Chu (1978) medium

NAA	:	α-naphthalene acetic acid
NaOCl	:	Sodium hypochlorite
Oryzalin	:	3,5-dinitro-N^4,N^4-dipropylsulphanilamide
pA	:	Peak area
PGRs	:	Plant growth regulators
ppm	:	Part per million
rpm	:	Round per minute
rt	:	Room temperature
TDZ	:	Thidiazuron
UTS	:	University of Technology, Sydney
v/v	:	Volume per volume
w/v	:	Weight per volume
WPM	:	Woody plant medium (Lloyd and McCown, 1980)
Zeatin	:	6-(4-hydroxy-3-methyl-2-butenylamino)purine

ABSTRACT

The aim of this project was to investigate the feasibility of the development of *in vitro* methods for assisting in the plant breeding of the important culinary and medicinal plant, *Wasabia japonica* Matsumura, through rapid plantlet production and improved plant quality, by the application of micro-propagation and induced mutation techniques. The synthesis and accumulation of a key valuable chemical component was also examined, through callus culture, tissue culture, and induced mutation.

In vitro propagation systems by means of gelled and liquid culture were developed for *W. japonica*. *In vivo* dormant buds were used as a primary source of explants, following disinfection in alcohol, NaOCl and detergent. Explants were successfully initiated in culture followed by a rapid shoot multiplication on gelled basal media containing nutrient salts of Murashige and Skoog (MS), vitamins, sucrose and growth regulators. Amongst various cytokinins used (BA, TDZ, kinetin and zeatin) BA proved to be the most effective, with the best concentration at 5 µM. Combinations among growth regulators enhanced shoot proliferation. In order to establish a simple and efficient *in vitro* propagation protocol, ½MS liquid culture medium with 5 µM BA was used for effective shoot multiplication of *W. japonica* with reduced labour and cost. The best rooting occurred in shoots cultured on gelled ½MS basal medium supplemented with 10 µM IBA. Over ninety per cent of plantlets were successfully acclimatised to *in vivo* conditions, exhibiting normal development.

The effects of *in vitro* mutation induction by physical and chemical mutagens on wasabi shoot tips were also investigated. Gamma rays and X-rays as physical mutagens in the range of 10 to 40 Gy, reduced the allyl isothiocyanate content of *in vitro* explants, to a greater extent as doses increased. X-ionising irradiation had a more detrimental influence on survival and growth of wasabi tissues than gamma-ionising irradiation. Colchicine and oryzalin as chemical mutagens, however, did not reduce the content of this compound. Oryzalin appeared to be more phytotoxic than colchicine, with a concentration of 15 µM affecting explant growth. Conversely, a low concentration of colchicine at 25 µM appeared to be favourable for the growth and development *in vitro*. Leaf morphology appeared to be altered in several greenhouse-grown plants treated with chemical mutagens, exhibiting typical characteristics of polyploids. Further studies

of morphological effects and assessments of the alteration of allyl isothiocyanate need to be undertaken on *in vivo*-grown plants.

The yield of allyl isothiocyanate was investigated in calluses, *in vitro* mutated- and non-mutated explants, and *in vivo* plants. Light conditions and leaf tissues proved to be more effective than dark conditions and petiole tissues, respectively, for the accumulation of this component in callus cultures. Shoot bases accumulated higher yield than leaves and petioles in the *in vitro* explants over three months in culture. Similarly, roots and rhizomes produced higher yields than leaves and petioles of *in vivo* plants over a period of 12 months. Leaves accumulated greater amounts of allyl isothiocyanate than petioles *in vitro*, but lower amounts than petioles *in vivo*. Thus it appears that he harvest of allyl isothiocyanate in plant leaves and petioles *in vivo* could be most effectively commenced after growth for nine months.

These results lay a scientific foundation on which a more elaborated and effective system of *in vitro* propagation and improvement for *W. japonica* can be achieved. Further studies on the analysis of chemical components need to be conducted for the pharmacautical uses of this plant.

PUBLICATIONS FROM THIS PROJECT

Hung C D, Johnson K, Torpy F. (2006). Liquid culture for efficient micropropagation of *Wasabia japonica* (Miq.) Matsumura. *In Vitro Cellular & Developmental Biology – Plant*; **42**(6): In press.

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