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M. Phil

Screening existing black pepper (*Piper nigrum* L. Family Piperaceae) cultivars for superior spice value (high essential oil, oleoresin and piperine content) and their *in vitro* propagation

by

R. M. Dharmadasa

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The work described in this thesis was carried out by me under the supervision of Dr. Y.M.H.B. Yapabandara (Industrial Technology Institute) and Dr. (Mrs). P.L Hettiarachchi (University of Sri Jayawardanapura) and a report on this has not been submitted in whole or in part to any University for another degree.

02.12.2002 .....

Date Signature

(R.M. Dharmadasa)

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation

02-12-2002

Date

Dr. Y.M.H.B. Yapabandara

02/12/2002

Date

Dr. (Mrs) P.L. Hettiarachchi

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Rathnayake Mudiyanselage Dharmadasa

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KEY TO ABBREVIATIONS

AC	: Activated charcoal.
ANOVA	: Analysis of Variance
BA	: 6-Benzylaminopurine (N^6 Benzyladenine).
°C	: Degree of Centigrade
CRD	: Completely Randomized Design
DEA	: Department of Export Agriculture
GLC	: Gas Liquid Chromatography
GLM	: Generalized Linear Model
Ha	: Hectare
HPLC	: High Performance Liquid Chromatography
IAA	: 3-Indoleacetic acid.
IBA	: 3-Indolebutyric acid
Kn.	: Kinetin
MS	: Murasige and Skoog's (1962) medium
NAA	: 1-Naphtalene Acetic acid
PVP	: Polyvinylpyrelidone
SAS	: Statistical Analysis System
SDC	: Sodium diethyldithiocarbonate
2,4-D	: 2,4-Diclorophenoxy Acetic Acid.
2iP	: 2-Isopentenyl adenine

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ABSTRACT

Some selected local and introduced (Panniyur and Kuchin) black pepper, *Piper nigrum* L. cultivars from different pepper growing areas of the country were screened for physical (number of seeds per spike, length of spike, number of pinheads per spike and thousand seed weight) and chemical (oil content and composition, oleoresin content and piperine content) quality parameters in order to determine the cultivars with the best spice value. Subsequently the possibility of mass propagation of these desired cultivars using an *in vitro* techniques and their ability to get acclimatized under normal field conditions were tested with the view of commercializing the technique to supply pepper growers with economic cultivars.

The oil, oleoresin and piperine contents varied in both local and introduced cultivars. Among local cultivars the highest oil content (6.3%) from Handessa-1 (Kandy district), oleoresin (21.78%) from SMD NK (Monaragala district) and piperine (14.51%) from Naranwela (Kandy district) were obtained. Comparatively low content of oil, oleoresin and piperine were observed in introduced cultivars. Pepper oil was analyzed using GLC (Gas Liquid

Chromatography) and found that it is a mixture of about 100 different compounds. The most significant compounds identified were ∝-pinene, β-pinene, Limonene, Sabinene, ∝-phelendrine and β-Caryophyllene. The piperine content of both local and introduced cultivars seems to be similar. However, piperine contents determined by HPLC (High Performance Liquid Chromatography) method were low when compared to the direct UV (Ultra Violet) method. Results of the chemical analysis revealed that spice value was high in local cultivars when compared to the introduced cultivars.

In vitro propagation of black pepper was severely hampered by contamination and browning. However, maintenance of mother plants in the greenhouse, by avoiding overhead watering and spraying systematic fungicide significantly reduced the contamination and browning. Browning of explants caused by a viscous jelly like exudation from the cut surface of the explants was considerably overcome by incorporation of PVP (Polyvinyl Pyrolidone) at the rate of 200 mg/l, initial culture incubation in low light and frequent transferring of explants to the fresh medium.

In vitro propagation protocol for black pepper was developed using nodal and shoot tip explants taken from greenhouse maintained mother plants. Nodal and shoot tip explants collected from actively growing pepper plants were successfully established in MS (Murasige and Skoog's 1962) medium supplemented with 2.5 mg/l BA (6-Benzylaminopurine) after sterilization with 0.1% $HgCl_2$ for 10 minutes in a low speed shaker.

Shoot proliferation was compared using MS medium supplemented with different growth regulators such as BA, Kinetin and 2ip (2-Isopentenyl adenine). BA was found superior over other two cytokinins tested. The suitable BA concentration was 3.5 mg/l. A higher shoot multiplication (6.57) was observed in liquid medium than in solid medium (3.99). About 6-7 fold shoot multiplication was achieved in liquid medium within six weeks. Higher shoot multiplication was observed in some local pepper cultivars i.e. GK 49 and M4 than in introduced cultivars.

Rooting of *in vitro* propagated shoots from nodal and shoot explants in MS medium supplemented with 0.2% activated charcoal (AC) both with and without IBA (3-Indolebutyric acid) were tested separately. The highest number of roots and early rooting were observed in medium with 1 mg/l IBA and charcoal. The rooted plantlets were transferred to pots filled with 1:1:1 coir: compost: sand and the level of successful acclimatization obtained was more than 80%. The acclimatized pepper plants were transferred to normal soil and grown in the garden successfully.

Callus induction from leaf and stem explants was studied in medium containing auxin 2,4-D (2,4-Diclorophenoxy Acetic Acid) or IAA (3-Indoleacetic acid) supplemented with or without BA. Root formation was observed in some of the calli after long period of incubation but shoot regeneration from these calli was unsuccessful.