MICROPROPAGATION OF *Holmskioldia sanguinea* Retz.Plant

By

AHMED FARGHALY HASSANEAN

B.Sc. Agric. Sci. (Horticulture) , Fac. Agric., Alazhar Univ., 2001

THESIS

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APPROVAL SHEET

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Title of Thesis: Micropropagation of *Holmskioldia sanguinea* Retz.Plant

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ABSTRACT

This study was carried out in the Plant Tissue Culture Laboratory, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, during the period from 2011 to 2014. An efficient direct shoot regeneration micropropagation protocol has been established successfully for commercial *in vitro* propagation of *Holmskioldia sanguinea*, a rare plant with ornamental and medicinal values. The optimized strilization condition for the single node stem cutting explants was exposure to 30% Clorox for 20 min (70.83 survival %, 4.17 mortality % and 25.00 contamination %). In establishment stage full strength of Murashige and Skoog medium (MS) supplemented with NAA at 0.5 mg/l was superior in *in vitro* growth of explant in respect of shoot length (2.13 cm) and number of leaves / explant (11.33 leaves). The multiplication stage should be carried out, with cutting the shoot apex, on MS medium supplemented with 1.0 mg/l BA which recorded 5.33 shoots at the end of the third subculture. For rooting the regenerated shoots, 1/8 strength MS medium supplemented with 2.0 mg/l IBA was effective and economically treatment, which resulted in 100% rooting percentage, 9.67 roots/plantlet, 8.00 cm root length, 14.67 leaves/plantlet and 8.00 cm plantlet length. Growing medium consised of peatmoss and vermiculite at the ratio of 2:1 (v/v) was the best for acclimatization of regenerated plantlets giving 100% survival percentage which grew into mature plants , recording highest values (after 6 and 12 weeks) for plantlet length (10.83 and 15.67 cm, respectively), number of leaves/plantlet (14.67 and 22.33 leaves, respectively), stem diameter (1.80 and 1.97 cm, respectively), and number of shoots/plantlet (2.33 shoot for each). Inter simple sequence repeats (ISSR) analysis detected similarity (13.7%) to the mother plant in the in *vitro* regenerated plants from the nodal explants, that the direct regeneration protocol will be useful for *Holmskioldia sanguinea* production. This *in vitro* plant developmental protocol could be used for large scale regeneration of *Holmskioldia sanguinea.*

Key words: *Holmskioldia sanguinea*, tissue culture, IBA, NAA, BA, ISSR analysis .

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LIST OF ABBREVIATIONS

AC: Activated charcoal.

BA: 6- Benzyl adenine.

BAP: Benzyl amino purine.

Clorox: 5.25 % soudium hypochlorite (commercial bleach).

2,4-D: (2,4-Dichlorophenoxy acetic acid).

GA_3: Gibberellic acid, (Gibberellin A_3).

IAA: Indole-3- acetic acid.

IBA: Indole -3- butyric acid.

Kin: 6-Furfuryl- amino purine (Kinetin).

MS: Murashige& Skoog medium.

NAA: Naphthalene acetic acid.

PVP: Polyvinyl pyrrolidone.

TDZ: Thidiazuron.

Tween 20: Polyoxyethlene sorbitan monolaurate.

V/V: Volume by volume.

WPM: Woody plants medium.

Gl: Glutamine

AdS: Adenine sulphate.

PGR: Plant growth regulators

PPFD: Photosynthetic photon flux density

PG: Phloroglucinol

MSBs: Multiple shoot buds

TIS: Temporary immersion system

DNA**:** Deoxyribonucleic acid

ISSR: Inter simple sequence repeats

ISSR-PCR: Inter simple sequence repeat **-** Polymerase chain reaction

SSRs**:** Simple sequence repeats

PCR: Polymerase chain reaction

McCown's: McCown’s woody plant medium.

CK: Cytokinin

RAPD: random amplified polymorphic DNA

UPGMA: unweighted pair group method with arithmetic means

(DAMD): directed amplification of minisatellite DNA

CONTENTS