

Regeneration of *Adiantum capillus-veneris* L. : an
homosporous fern species

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to God Almighty and to my family.

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ABSTRACT

Spore and tissue culture studies of *Adiantum capillus-veneris*, an ornamental and medicinal fern species were carried out to raise new fernlets, study the growth rate and develop a protocol for rapid vegetative propagation of the fern *in-vitro*.

Sterilized spores were sown on semi-solid agar containing Parker's and Thompson's nutrient. Agar and soil cultures were established to study spore germination and gametophyte development. The germination rate was scored at an interval of four days for thirty days. Sporophyte and gametophyte explants were sterilized and cultured on Murashige and Skoog (MS) medium supplemented with different auxins (IBA and NAA) and cytokinins (BA and Kinetin).

Callus regeneration was obtained from leaflet explants cultured on MS medium supplemented with 0.50 mg/L NAA. Root regeneration was obtained from rhizome explants cultured on MS medium supplemented with 0.5 mg/L IBA and MS medium supplemented with 0.5 mg/L NAA. Shoot regeneration was obtained from stipe explant cultured on MS medium supplemented with 0.15 mg/L KIN. Crozier explants gave rise to shoots in MS medium supplemented with 0.5 mg/L BA. Direct regeneration of plantlets was obtained from rhizome explants supplemented with 0.25 mg/L BA and crozier explant cultured on MS medium supplemented with 0.5 mg/L IBA. Neither callogenesis nor organogenesis was observed from gametophyte explants.

This study showed that *A. capillus-veneris* can be raised from spores. A protocol for efficient regeneration of *A. capillus-veneris* has been developed using rhizome explant in BA and crozier segment in IBA.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Pteridophytes

Pteridophytes are flowerless, seedless, spore-producing vascular plants. They are characterized by their sporangia being either homosporous or heterosporous. Homosporous ferns produce the same kind of spore and are characterized by exosporic gametophytes in which the germination of spores and development of gametophytes are under the control of external conditions. Also, they give rise to monoecious prothallus (Sporne, 1975).

The heterosporous plants produce different kinds of spores and produce endosporic gametophyte which is independent of external conditions. They give rise to dioecious prothallus. The mode of sporangia development and the level in which they are borne on the sporophyte constitute important taxonomic characters. If the sporogenous tissue is derived from the inner daughter cell, the sporangium is described as eusporangiate and if it is from outside, it is referred to as leptosporangiate. The eusporangiate is more primitive while the leptosporangiate is more advanced (Sporne, 1975).

Pteridophytes are dependent upon the microclimatic conditions of the region for their successful survival in that region. Any kind of disturbance can hinder the evolutionary process leading to their population decline. Factors such as climatic change and anthropogenic activities such as industrialization, encroachment of forest lands, over-exploitation of natural resources, large scale collection of ferns as ornamentals and for medicinal uses pose a major threat to the survival of these groups of plants (FEPA, 1991; FRN, 2010).

Pteropsida is a class of pteridophytes represented by the order Filicales to which ferns and allied plants belong (Carrington, 2003). They vary in size from few centimeters to tree trunk-like structure called tree fern (*Cyathea dicksonia*). They may be found within a wide climatic range but as in seed plants, are mostly diversified in the tropics where aquatic, epiphytic and terrestrial ferns are common. In terms of habits, pteridophytes are classified as erect, succulent, tall, walking, climbing and herbaceous plants. Ferns are considered as most neglected plants. But in recent years, several ferns and ferns-allies have been identified to be economically important as they can be used as pollution indicator and hyper-accumulator of noxious metals and metalloids (Shastri *et al*, 2005).

Odu and Opapeju (1986) reported that in Nigeria, fern species occur abundantly in the rainfall belt of the South. Oloyede and Odu (2011) reported 39 species of ferns in Southern Nigeria.

1.2 The family *Adiantaceae*

It occurs in almost all habitats but most abundant in moist, montane forests particularly along the streams and rivers. Most species are shade-loving and many are epiphytic (Agnew, 1974). It is represented by 5 genera and 11 species in Southwestern Nigeria. The family is characterized by leathery leaflets, polished brown stipes and spores borne in sporangia. It is homosporous with short rhizome which is short-creeping, sub-erect, dictyostelic or solenostelic, indumentum of scales which are neither peltate nor clathrate (Oloyede and Odu, 2011).

1.3 *Adiantum capillus-veneris*

Adiantum is a genus with a cosmopolitan worldwide distribution usually found growing in moist calcareous cliffs, banks, edges of streams, rivers and walls of lime sinks (Jones, 1998). Alston (1959) reported nine species of *Adiantum* in West Africa, six in Northern and Southern

Nigeria while Oloyede and Odu (2011) reported only *Adiantum capillus-veneris* in southwestern Nigeria.

Adiantum capillus-veneris has water repelling compounds on the foliage with the result that water runs off the leaves and even when the plant is immersed in water the leaves remain dry. This strong water repelling property is the scientific basis for its botanical name – *Adiantum* which is derived from the Greek word ‘Adiantos’ translated as ‘unwetted’ while the specific epithet *capillus-veneris* is taken from the Latin words ‘capillus’ meaning hair and ‘veneris’ the generative form of Venus, the goddess of love, so named for its beautiful fronds and supplying the plant with one of its common names, the Venus hair fern. *Adiantum capillus-veneris* is commonly called Maidenhair fern. It is native to America, Eurasia, Western Asia, Australasia (Cundall, 2004) and Nigeria (Nwosu, 2002). It is a small slow growing fern found throughout the world in moist places (Leslie, 2005).

A. capillus-veneris is an homosporous fern because it is characterized by spores which are morphologically similar unlike Selaginellaceae, Isoetaceae, Azollaceae, Marsiliaceae which produce microspores and megaspores. Spores are borne under false indusia (rolled flaps of tissue) at the edge of the subdivisions of the leaf, a characteristic unique to the genus *Adiantum*.

Spores of *A. capillus-veneris* are circular in shape with regulate-gemmate ornamentation. Its equatorial and polar views are 41.43 and 35.43 μm respectively and has a cell number of annulus which ranges from 19 – 23 (Fahimeh *et al.*, 2011). Spores enhance the continuity and distribution of the species because of the regenerative ability of each spore to form a new (whole) plant. Successful propagation needs proper nutrition, protection and free dispersal (Oloyede *et al.*, 2011).

1.4. Economic Importance

Adiantumcapillus-veneris is cultivated as a popular garden fern and as an outdoor and indoor house plant (Oloyede, 2012). *A. capillus-veneris* has many medicinal purposes ranging from its use as hair tonic and treatment for dandruff (Foster and Duke, 1990).

Leaves and pinnae oils of *A. capillus-veneris* was reported to have antimicrobial activity (Victor *et al.*, 2003). Tonic for the treatment of cough, throat infection, visual tumours and

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