UNIVERSITY OF GHANA
COLLEGE OF BASIC AND APPLIED SCIENCES

# PRODUCTION OF POTATO (*Solanum tuberosum* L) PRE-BASIC SEED THROUGH TISSUE CULTURE IN KATIBOUGOU, MALI

By
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WEST AFRICA CENTRE FOR CROP IMPROVEMENT (WACCI)
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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE AWARD OF DEGREE OF MASTER OF PHILOSOPHY IN SEED SCIENCE AND TECHNOLOGY

WEST AFRICA CENTRE FOR CROP IMPROVEMENT (WACCI)
JULY, 2018

# DECLARATION

I, Moussa ABDOULAYE, do hereby declare that this thesis is my original work and that no previous submission for a degree in the university or elsewhere has been made. All sources of information have been duly acknowledged.

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(Supervisor) Date……

# DEDICATION

This work is dedicated to my family for their never-ending support, guidance and blessings.

# ACKNOWLEDGMENTS

My ultimate gratitude goes to the Almighty Allah for his protection and guidance throughout my thesis research period.

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# ABSTRACT

In Mali, the main problems limiting the productivity and production of potatoes is the non-availability of quality seeds in adequate quantities and at affordable prices for the farmers. To meet quantity and quality requirements for potato seeds in Mali, this study proposes to undertake two experiments to improve the techniques adopted in the IPR/IFRA plant biotechnology laboratory in Mali. The evaluation of the effects of three (3) concentrations of coconut water and two (2) concentrations of potassium nitrate on potato plantlets growth *in vitro* and the effects of two physiological ages and three substrates on potato *in vitro* plants' establishment, post *in vitro* growth and mini tubers production *in vivo*.

The first experiment was laid out in a Completely Randomized Design (CRD) with twelve (12) treatments replicated 4 times. The second experiment was a factorial experiment with 2 factors (physiological ages: 2 levels and substrate: 3 levels) laid out in a Randomized Complete Block Design (RCBD) with 6 treatments replicated 4 times.

The results in the first experiment showed that the culture medium M7 (MS+40 ml/l of coconut water +250 mg of potassium nitrate) had promoted all plant growth parameters (shoot emergence, plant height, number of nodes, leaves and roots and plant fresh and dry weight) after 30 days of *in vitro* culture. The lower concentrations of coconut water (40 ml) and potassium nitrate (250 mg) per liter of MS medium had significant and positive effects on all the *in vitro* growth parameters after 30 days of culturing.

The results in the second experiment showed that the plantlet weaning age of 25 days and the post-flask culture substrate S1 (only soil) provided the best plant survival percentage at 20 days after transplanting *in vivo*. The substrate S2 (soil and cow dung 2:1) positively affects plant stem length, stem diameter, plant fresh and dry biomass formation, tuber yield, tuber numbers per plant and tuber grading size B (tubers with a diameter of less than 28 mm). The substrate compositions S1(only soil) has significantly affected the weight loss (12.50%) of

tubers stored within 8 weeks. The weaning age 45 days and the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) conditions significantly reduced the number of sprouts per tuber and sprouts number per eye on tubers.

In addition, the results indicate that for better and more rapid growth of potato plantlets *in vitro* culture, the coconut water concentration used as supplement to MS medium should be 40ml per liter of medium. The proportion of cow dung used in substrate composition should not exceed the soil and cow dung 2:1 mixture for maximum post transplanting plant re-establishment rate *in vivo* and for rapid maturity of mini-tubers.

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| | |
|---|---|
| ACW | Agroscope Changins-Wädenswil |
| AMATEVI | Association Malienne d'Assistance Technique Villageoise |
| BHEARD | Borlaug Higher Education for Agricultural Research and Development |
| BNDA | Banque Nationale de Développement Agricole du Mali |
| CARA | Chambre d'agriculture de région Alsace |
| CEE-ONU | Commission économique des Nations Unies pour l'Europe |
| CFIA | Canadian Food Inspection Agency |
| CIDES | Centre d'Information et de Developpement Experimental en Serriculture |
| CIP | International Potato Center |
| CW | Coconut Water |
| DGESCO-ENS | Direction générale de l'enseignement scolaire / École Normale Supérieure de Lyon |
| FAO | Food and Agriculture Organization of the United Nations |
| IAA | Indole-3 acetic acid |
| IBGE | Institut Bruxellois pour la Gestion de l'Environnement |
| IPR/IFRA | Institut Polytechnique Rural de Formation et de Recherche Appliquée |
| INRA | Institut National de la Recherche Agronomique, France |
| ITCMI | Institut Technique des Cultures Maraichères et Industrielles |
| $KNO_3$ | Potassium Nitrate |
| M | Media |
| MINRESI-IRAD | Ministère de la Recherche Scientifique et de l'Innovation/ Institut de Recherche Agricole pour le Developpement |
| MS | Murashige and Skoog |
| NPK | Nitrogen (N), Phosphorus (P) and Potassium (K). |

| | |
|---|---|
| OECD | Organisation for Economic Co-operation and Development |
| OEPP | Organisation Européenne et Méditerranéenne pour la Protection des Plantes |
| PMTV | Potato mop-top virus |
| PLRV | Potato leafroll virus |
| PVX | Potato virus X |
| PVY | Potato virus Y |
| LSD | Least Significant Difference |
| LSEP-IER | Laboratoire Sol-Eau-Plant/Institut d'Economie Rural, Mali |
| S | Substrate |
| WACCI | West Africa Centre for Crop Improvement |

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1. Background

The potato (*Solanum tuberosum* L) is one of the important crops in the sub-Saharan region of Africa. It is a tuberous herbaceous plant originating in Latin America (MINRESI-IRAD, 2012).

The potato is the main non-cereal food commodity in the world. Its global production was estimated in 2016 at 376.8 million tons out of 19.2 million hectares with an average yield of 20 tons per hectare. Africa produced 24.5 million tons (FAO, 2016).

The potato is grown in all regions of Mali. In 2016 potato production was estimated at 210,209 tons on an area of 10,525 hectares with an average yield of 20 tons per hectare (FAO, 2016).

The national market consumes 80% of the volume and the rest by the sub-region mainly Ivory Coast, Ghana, Burkina Faso, Togo and Benin. The most important foreign market is the Ivory Coast, which absorbs more than 90% of the potato exported (Diakité and Zida, 2003).

The potato is an important commodity and a cash crop because of its high-energy content and ease of production for millions of farmers in Africa, Asia and Latin America. It is also an important element of urban agriculture, which provides employment and food security to some 800 million people (FAO, 2009).

However, it is remarkable to note that in developed countries production has declined in recent years, particularly in Europe, with a decline of nearly 20% but it is Africa that develops the most with an increase of over 55% of production (Vanderhofstadt, 2011).

Two types of seeds are generally used by Malian producers: seeds produced by the non-certified farmers themselves, and certified seed imported from Europe; A farmer uses an average of 20% local seed and 80% of imported seed (Diakité and Zida, 2003). In Mali, the timely supply of quality seed (certified seed) of potato remains dependent on the imports from France and Holland (Coulibaly *et al*., 2002).

The potato crop production in Mali has been increasing since 1973. From 2010 to 2014 the cultivated area increased by 4,843 hectares. It was estimated at 3,700 hectares in 2010 and 8543 hectares in 2014 (FAOSTAT, 2016; Vanderhofstadt, 2011).

The quantity of potato seeds required is 1 to 2.5 tons per hectare in Mali; With a minimum of 1,000 kg of seeds per hectare and an average price of 1,000 CFA francs per kg of seed (BNDA, 2014). The quantity of potato seeds used by Malian farmers is estimated at 8,543 tons in 2014, with a turnover of 8.5 billion CFA francs (around US$14.2 million) for the seed companies in the seed potato sector.

Increased potato production will depend on quality seed, i.e. potato varieties that are more resistant to pests and diseases, and capable of adapting to future climate change (FAO, 2009).

The development of potato cultivation in Mali requires the search for high yielding varieties and the local multiplication of these varieties. To meet the challenge of improving the quality and quantity of potato seeds, this involves the use of plant biotechnology.

Potato is one of the first major food crops where plant biotechnology has been successfully used for virus elimination (Bajaj and Sopory, 1986). Tissue culture technology produced disease-free plants, as well as microtubers, were disseminated from the laboratory to the field and multiplied on a wide scale in many countries (Bajai and Sopory, 1986).

The establishment of a seed supply chain requires the production of potato pre-basic seeds in the laboratory. The IPR/IFRA of Katibougou through its activities at the plant biotechnology laboratory has initiated the micropropagation of potato in Mali since 2000. Some studies carried out in Mali show that it is preferable to produce potato seed through tissue culture in a 4-year scheme of seed production (from the *in vitro* plantlet through generations G0, G1, G2 to G3 seed) to limit pathogens importation (Coulibaly *et al*., 2002).

An *in vitro* study has been conducted to investigate the effects of coconut water on the growth of *in vitro* plantlets of potato variety Desiree in Pakistan (Muhammad *et al*,. 2015). Coconut water has been used by Overbeek, (1941) as a growth regulator in culture media for growth and development of very young *Datura stramonium* embryos. The cytokinin found in coconut water promotes cell division. When it is used as a supplement to the chemical components it promotes plant growth. (Jackson *et al.*, 2004).

A study conducted in Algeria on four physiological ages (20, 30, 40 and 50 days after culturing) of potato *in vitro* plants concluded that the 50 day-old plantlets produced significantly higher number of mini tubers *in vivo* than the other treatments (ITCMI, 2012).

It is known that the nature of soil mixes used for transplantation can influence the re-establishment of *in vitro* plantlets *in vivo* (Anderson, 1978). Good aeration of the substrate used for transplantation of the *in vitro* plantlets is known to be important for the post transplanting survival and growth of plantlets in some species (Gorst *et al*., 1978).

### 1.2. Problem statement

Unfortunately, non-availability of quality seeds in adequate quantities and at affordable prices is one of the main problems limiting the productivity and production of potatoes in Mali.

The seeds imported carry a number of pathogens such as viruses (PVY and PLRV) and bacteria (*Ralstonia solanacearum*) which have affected potato production in Mali (Vanderhofstadt, 2011).

The quantities of quality seed supplied do not meet the country's needs. There is low production of potato pre-basic seed through tissue culture due to a low rate of multiplication of plantlets *in vitro*, a general lack of research addressing problems associated with post-flask re-establishment of plantlets *in vivo* after transplantation and a lack of an identified appropriate physiological age for the production of pre-basic seed under the current climatic conditions in Mali.

### 1.3. Justification

This study was done in Mali because of:

- ✓ imported seeds contain viruses and bacteria;
- ✓ low production of pre-basic seed in quantity needed for production;
- ✓ a method to multiple improved potato pre-basic seeds quickly;
- ✓ and the need to set up reliable protocols in vitro and in vivo establishment.

### 1.4. Objectives of study

#### 1.4.1. General objective

The general objective is to develop reliable protocols for the production of potato pre-basic seeds via tissue culture in Mali.

#### 1.4.2. Specific objectives

The specific objectives of this study are to:

1. identify the culture media with a high plantlets multiplication rate after 30 days of *in vitro* culture;

2. evaluate the effects of coconut water and potassium nitrate supplementation to MS medium on plantlets growth parameters after 30 days of *in vitro* culture;
3. evaluate the effects of physiological age at transplanting and substrate composition on re-establishment, growth and mini tubers production of *in vitro* raised plantlets of potato;
4. assess the impacts of physiological age and substrate composition on mini tubers quality in storage 3 months after harvest.

# CHAPTER TWO

# 2.0. LITERATURE REVIEW

## 2.1. Botany, Ecology and origin

### 2.1.1. Botany

The white potato domestic (*Solanum tuberosum* L) is highly heterozygous autotetraploid (2n = 4x = 48). It belongs to the genus *Solanum* and the Solanaceae family which includes about 2000 species (CFIA, 2015). The potato has eight cultivated species and 228 wild species, divided into 21 taxonomic series, comprising 19 series of tuber bearing species and two series of no tuberous species (Spooner *et al*., 2014).

**Table 2.1: Systematic classification of potato according to Spooner *et al*., 2014.**

| | |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Viridaeplantae |
| Division | Tracheophyta |
| Subdivision | Spermatophytina |
| Class | Magnoliopsida |
| Order | Solanales |
| Family | Solanaceae |
| Genus | *Solanum* |
| Species | *Solanum tuberosum* L. |

The plant is an herbaceous perennial species but in Agriculture, it is grown as an annual crop for its tubers (Rousselle *et al*., 1996; Vreugdenhil *et al*., 2007) It has both aerial stems and underground stems (Darpoux and Debelley, 1967). Fruits are berries that can hold up to 200 seeds. Tubers are both the organ of multiplication and consumption. All its morphological characters are highly variable and constitute varietal characteristics more or less influenced by the environment (Gallais, and Bannerot, 1992).

The stem is erect in the juvenile stage, but spreads in the advanced stage. The leaves are compound and alternate, irregularly odd pinnate (Patil *et al.*, 2016).

The flowers grouped in cyme are rarely fruiting, however the abundance of fruiting depends on the variety. The flowers are usually white, pink, blue or purplish lilac. They are composed of 5 sepals welded at their base, 5 equally welded petals, 5 free stamens, contiguous to each other and a pistil with a single style. Flowering begins on the branches near the base of the plant and produces it upwards. Each flower will generally remain open for 2 to 4 days, with the stigma being receptive and pollen being produced for about 2 days (Plaisted, 1980). Fertilization takes place about 36 hours after pollination (Clarke, 1940). Viable seeds require a minimum of 6 weeks to develop (CFIA, 2015). The flowers give berries-shaped fruits with whitish and flat seeds, each berry may contain dozens of seeds. The seeds of the potato are generally used in breeding of new varieties (Bamouh and Chibane, 1999).

The potato plants produce stolons that have rudimentary leaves and are typically hooked at the tip. Stolons from the nodes of the basal stem, usually underground, have up to three runners per node (Struik, 2007). The tubers are reserves accumulated by the plant which are formed at the extremity of the stolons. They are multiform (spherical, oval etc.), the flesh can be of various colors (yellow, white, red etc.). The skin of the tuber is smooth or rough and its color can be white, yellow, tan or red. The tuber has a number of buds called eyes.When tubers are planted, eyes develop into stems to form the next vegetative generation (Spooner & Salas, 2006).

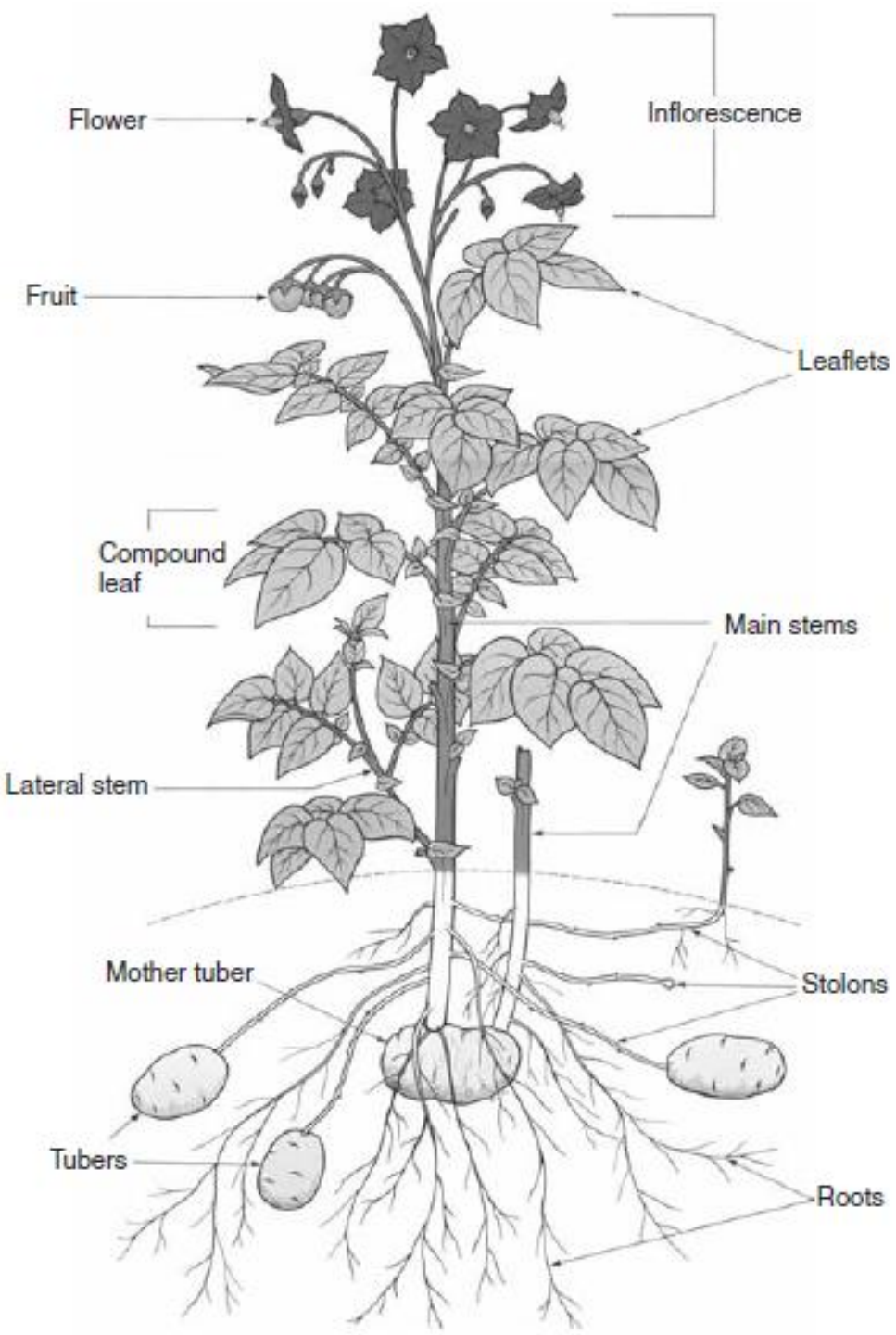


**Figure 2. 1: Schematic representation of potato plant (Spooner and Salas, 2006)**

### 2.1.2. Ecology of potato

The potato is successfully grown at an altitude of 1000 m. Its adaptation area ranges from subtropical to colder regions. it is more resistant to temperate and humid climates (Laumonnier, 1979).

Potato is characterized by a minimum growth temperature between 6 and 8 ° C. Optimum temperatures for growth and tuberization are 18 to 20 ° C during the day and 12 to 15 ° C during the night. A soil temperature above 25 ° C is unfavorable for tuberization. High temperatures stimulate stem growth, whereas low temperatures favor the growth of tubers. The vegetative growth of the potato is favored by day length from 14 to 18 hours. Photoperiod less than 12 hours promotes tuberization (Bamouh and Chibane, 1999).

Potatoes require deep, healthy soils that are always fresh, nutrient-rich and well-drained. It grows well in sandy or humus soils. It prefers soils with low acidity pH: 6 to 6.5 (Rousselle *et al.*, 1996).

### 2.1.3. Origin of potato

The origin of the potato (*Solanum tuberosum*) is South America (Ducreux *et al*., 1986). Its history began around 8000 years ago, near Lake Titicaca, which is at an altitude of 3800 meters between Bolivia and Peru. A wide array of potato varieties with morphological and genetic diversity is present in the Andean cordillera of South America. This distribution extends from western Venezuela to northern Argentina and southern Chile (CFIA, 2015; Patil *et al*., 2016).

The density of wild potatoes is particularly high in the tropical highlands of Central and South America, with clear peaks between regions 88 S and 208 S and around 208 N, in the northern Andes. Argentina, Bolivia, Ecuador and Peru, and central Mexico. Peru is

distinguished by the high number of wild potato species, and for the number of rare wild potato species (Hijmans and Spooner, 2001).

### 2.2. Reproductive biology

#### 2.2.1. Sexual Reproduction

The cultivated form of *Solanum tuberosum* subsp. (tetraploid) is self-compatible (OECD, 1997). The potato can be reproduced by botanical seeds, commonly known as "true potato seeds". True potato seed is used in breeding programs. The degree and duration of flowering are strongly influenced by environmental conditions and the potato cultivar (Burton, 1989).

The germination of the seed is epigeal and the cotyledons are carried above ground by the development of the hypocotyl. The first leaves are simple under favorable conditions, the young plant is only a few centimeters high, the stolons begin to develop, first at the cotyledons and then at the nodes located above, and sink into the ground to give tubers (Rousselle *et al*., 1996).

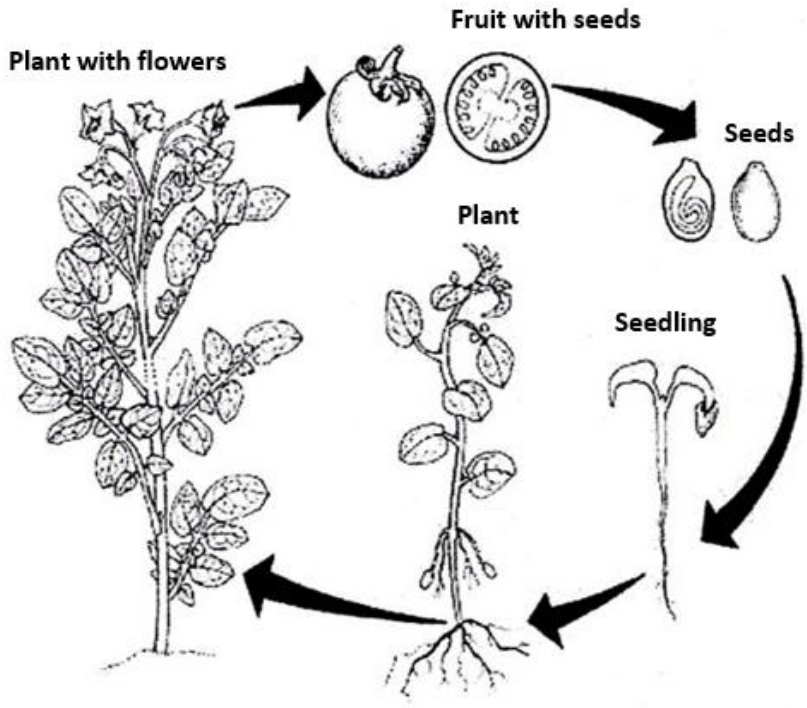


**Figure 2. 2: Sexual method of multiplying the potato (Rousselle *et al*.,1996)**

### 2.2.2. Asexual Reproduction

The potato crop is commonly propagated vegetatively using sections of tubers called seed potatoes (CFIA, 2015). The vegetative development cycle of the potato is three to four months and comprises five main stages of development, from dormancy to destruction of the vegetative organism.

#### 2.2.2.1. Vegetative rest stage

At harvest, the tubers are usually dormant. The dormancy period varies depending on the variety. During cold storage, an internal evolution of the tuber leads to the loss of dormancy, and the production of sprouts from the eyes. The dormancy period of the tubers offers a great advantage in storage. It allows several months of storage with or without application of germination control products (Patil *et al.*, 2016).

Dormancy refers to the vegetative rest of the tubers. Endodormancy, paradormancy and ecodomancy are distinguished (Lang *et al.*, 1987).

Endodormancy: period during which no germination occurs in the tubers, even preserved in ideal germination condition (Reust, 1982). The endodormancy is regulated by internal physiological factors meristem (Lang *et al.*, 1987). It begins at the initiation of the tuber on the mother plant (Delaplace, 2007).

Paradormancy: paradormancy is regulated by physiological factors outside the structure concerned (Lang *et al.*, 1987). The inhibition of germination of the proximal germs by the apical germ is an illustration of this (Delaplace, 2007).

Ecodormancy: Ecodomancy can be maintained by environmental conditions (Lang *et al.*, 1987). Low temperatures (4 ° C) can for example prolong dormancy of tubers (Reust, 1986).

The lot of tubers is sprouted when 80% of tubers component show one or more upper sprouts 1-3 mm (Reust, 1982), 2 mm (Fauconnier *et al*., 2002), 3mm (O'Brien *et al*., 1983) or 5 mm (Reust, 1986 ; Caldiz *et al*., 2001) This can be further refined using the average germination dates of individual tubers (Caldiz *et al*., 2001). The dormancy time of small size (35 mm) tubers is longer than that of larger (50 mm) tubers (Reust, 1982).

Viruses do not seem to affect dormancy of tubers; but they only increase the variability in the measurement of the incubation period for contaminated tubers (Emilsson, 1949).

The dormancy of tubers can be maintained at a temperature below 3 ° C, by use of antigerm substances or by gamma radiation at low doses. When a tuber is placed under favorable environmental conditions with a temperature of 16 to 20 ° C and a relative humidity of 60 to 80 % and by application of chemical substance (rindite), dormancy can be broken immediately and the tubers will start to sprout (Madec and Perennec, 1962).

#### 2.2.2.2. Germination stage

Sprouts develop from the tuber eyes (buds). Sowing of these sprouted tubers promotes root formation in a very short time.

#### 2.2.2.3. Vegetative growth stage

After sprouts emergence, the formation of the plants' vegetative parts such as leaves, stems, roots and stolons begin (Patil *et al*., 2016).

#### 2.2.2.4. Tuberization stage

The process of tuberization in the potato shows a succession of events that are divided into three essential phases:

- induction of stolons;
- initiation of tubers;

- enlargement of the tubers.

Stolons stop their elongations and their extremities bulge to form the tubers. There is continued aerial growth of the plant, followed by tuber bulking and flowering. Tubers increase in volume by accumulation of water and nutrients especially carbohydrates. The tuberization is favored by short days and low temperatures. The physiological age of the tuber depends not only on its date of initiation but also on the storage conditions (Gallais and Bannerot, 1992)

### 2.2.2.5. Maturation stage

The senescence of the plant generally results in the progressive yellowing of the leaves from the base to the top of the plant and leads to drying out (Rousselle *et al*., 1996).

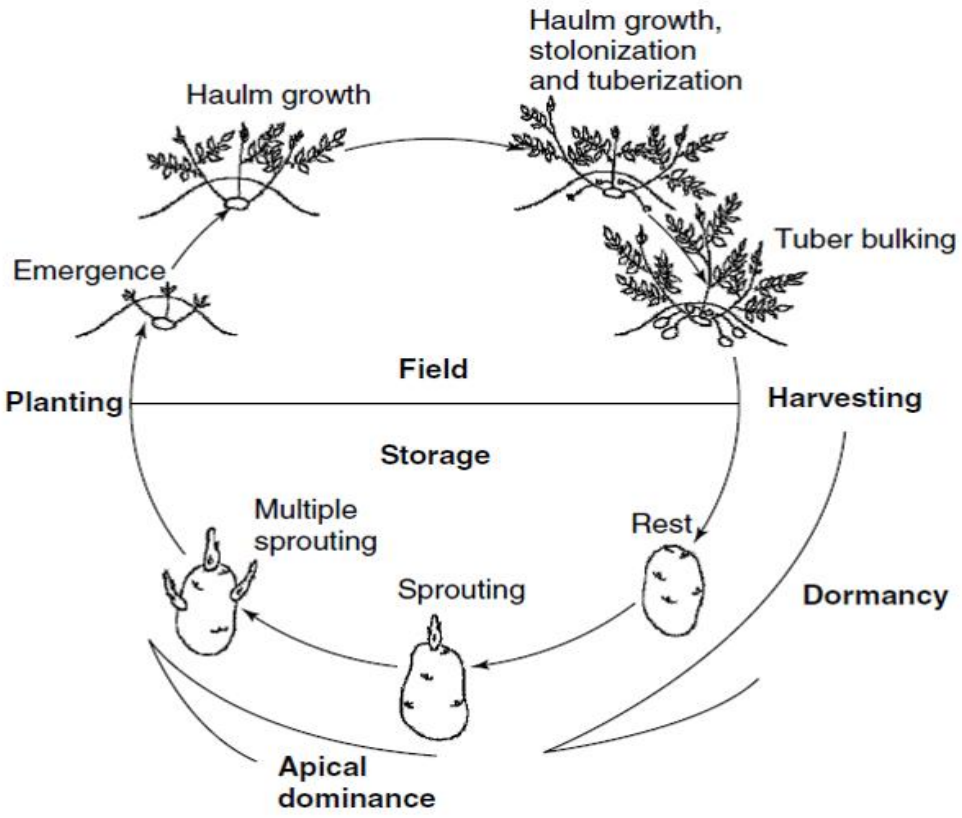


**Figure 2. 3: Asexual method of multiplying the potato (Vreugdenhil *et al*., 2007)**

### 2.3. Major Potato Diseases, Insect and Nematodes

As in all crops, the potato (*Solanum tuberosum*) is attacked by several diseases and pests, sometimes causing serious damage.

#### 2.3.1. Major Diseases of potato

##### 2.3.1.1. Fungal diseases of potato

**a) Late blight of potato:** the sworn enemy of the tuber crops in the world is caused by an aquatic mold (*Phytophthora infestans*) which destroys leaves, stems and tubers. Temperature of 10 to 25 ° C accompanied by heavy dew or rains promote the spread of this disease. Infected tubers are brown on the surface (CIP, 1996).

**b) Early Blight:** This fungal disease is caused by the fungus (*Alternaria solani*). The disease causes damage especially in continental climate, hot and dry, but is accentuated in irrigated culture. the attacked leaves show circular or angular brown spots. Affected tubers show dark brown to black circular to elliptic spots. These spots penetrate the flesh to a depth of 1 to 2 mm (Jean, 2002) .

**c) Black Scurf:** The black scurf disease is caused by a fungus (*Rhizoctonia solani*) which develops from black sclerotia on the mother tuber or in the soil. The most common symptoms are the black encrustations of fungal sclerotia on tubers (Patil *et al*., 2016).

**d) Fusarium Dry Rot:** Fusarium is caused by a fungus of the genus Fusarium (*Fusarium caeruleum*). The leaves of the plant turn yellow and wither and the plant is subsequently completely dried. The disease is also manifested during storage. At the spots where tubers have injuries, pale brown rot spots appear. This disease is most feared in very hot weather, when the temperature is around 30 ° C (IBGE, 2014).

#### 2.3.1.2. Bacterial diseases of potato

**a) Bacterial wilt:** The disease is caused by a bacterium (*Ralstonia solanacearum*) that causes severe losses in subtropical and temperate regions. this disease causes drooping of the leaves due to the loss of turgor followed by an unrecoverable total wilting and causes vascular staining of stems (Patil *et al*., 2016). The eyes of the tubers show grayish white slime. Bacterial wilt disease develops rapidly at high temperatures (CIP, 1996).

**b) Common scab:** Common scab is a bacterial infection caused by (*Streptomyces spp.*). Symptoms of common scab occur only on the surface of tubers as spots and crusts (Lambion, 2006).

**c) Blackleg and Soft rot:** Blackleg disease is a bacterial infection caused by (*Erwnia carotovora*). The name "black leg" is derived from the black color of the stem above the ground. this disease causes soft rot of tubers in the soil (Hodgson *et al*., 1993).

#### 2.3.1.3. Viral diseases of potato

Potato Crop is affected at least by 23 viruses, from an economic point of view, the most important viruses are those that cause mosaic and leaf roll diseases. There are several viruses that are associated with potato mosaic. In a plant infected with this disease, viruses A, S, X and Y may be present alone or in combination (Bouchard, 2017).

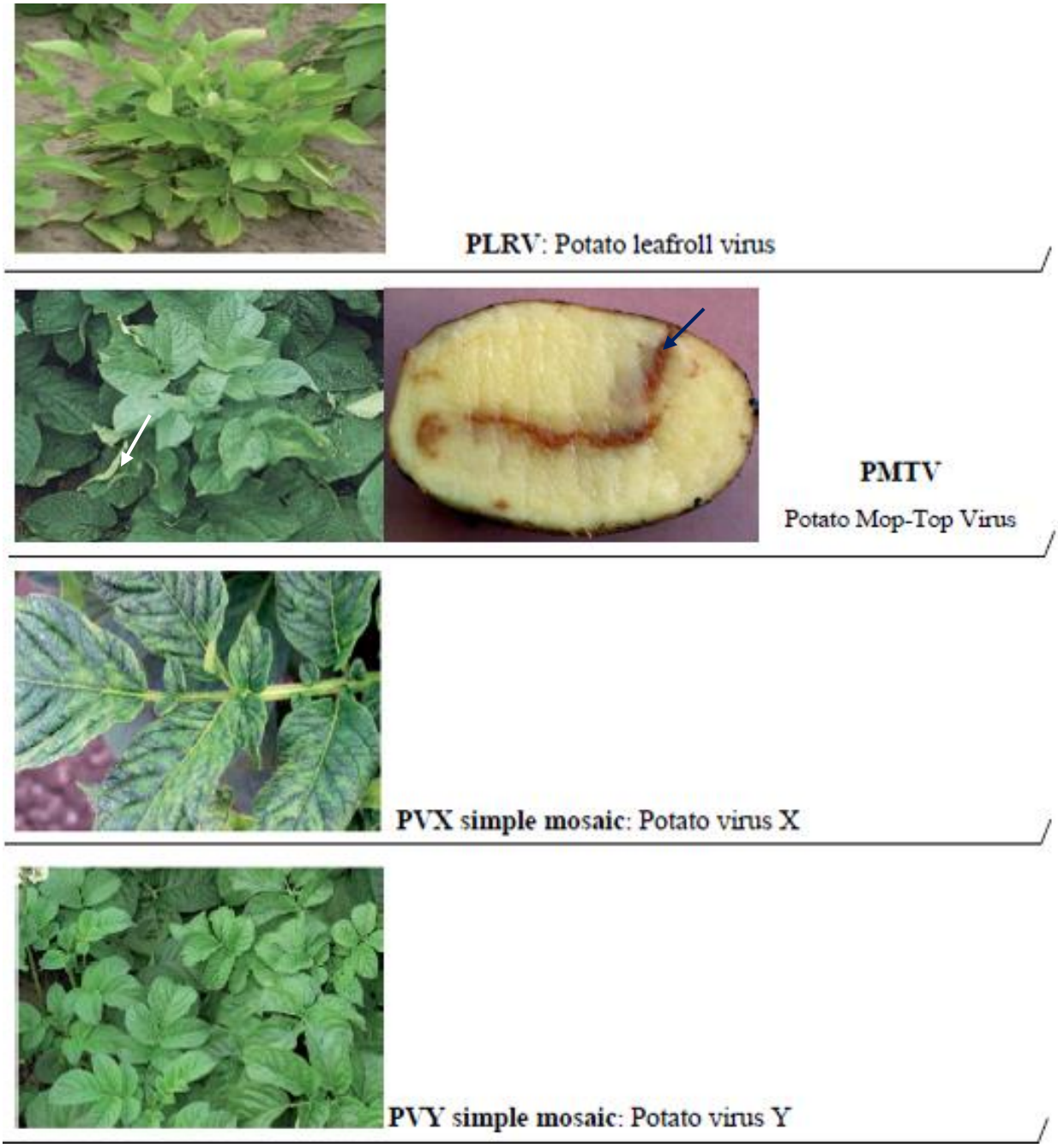


**Figure 2. 4: PLRV, PMTV, PVX and PVY symptoms (CEE-ONU, 2014).**

### 2.3.2. Insect pests of potato

**a) Potato tuber moth** (*Phthorimea operculella* {Zeller}): *Phthorimae operculella* is very prevalent in hot and dry potato growing areas (CIP, 1996). The damage occurs mainly on the tubers in conservation. The larvae dig holes and galleries in the tubers. These attacks depreciate the commercial value and quality of infested tubers. The holes of penetration of the larvae are barely visible and favor tuber rots (OEPP, 2000).

**b) Potato Flea Beetles** (*Epitrix* spp.): Four species of *Epitrix* are associated with potato damage: *E. tuberis, E. cucumeris, E. similaris* and *E. subcrinata* (CEE-ONU, 2014). Adults feed on the foliage of the potato, leaving circular holes less than 3 mm in diameter on the leaflets. The larvae feed on the root system and tubers and burrow to the surface of the tubers. This damage may facilitate the penetration of pathogens present in the soil (CIP, 1996).

**c) Aphids** (*Myzus persicae* and other *Aphididae*): Aphids can be identified in plant terminals and on the underside of leaves in the field (CIP, 1996). The dangerous character of aphids is mainly due to their role as vectors of viral diseases. It is a problematic pest only for seed growers (Duval, 2008).

**d) Wireworms** *Agriotes* spp.: The larvae dig deeper tunnels inside the tuber but they do not live inside the tuber. The damage from wireworms are an entry point for other pathogens, which can cause tuber rot (CEE-ONU, 2014).

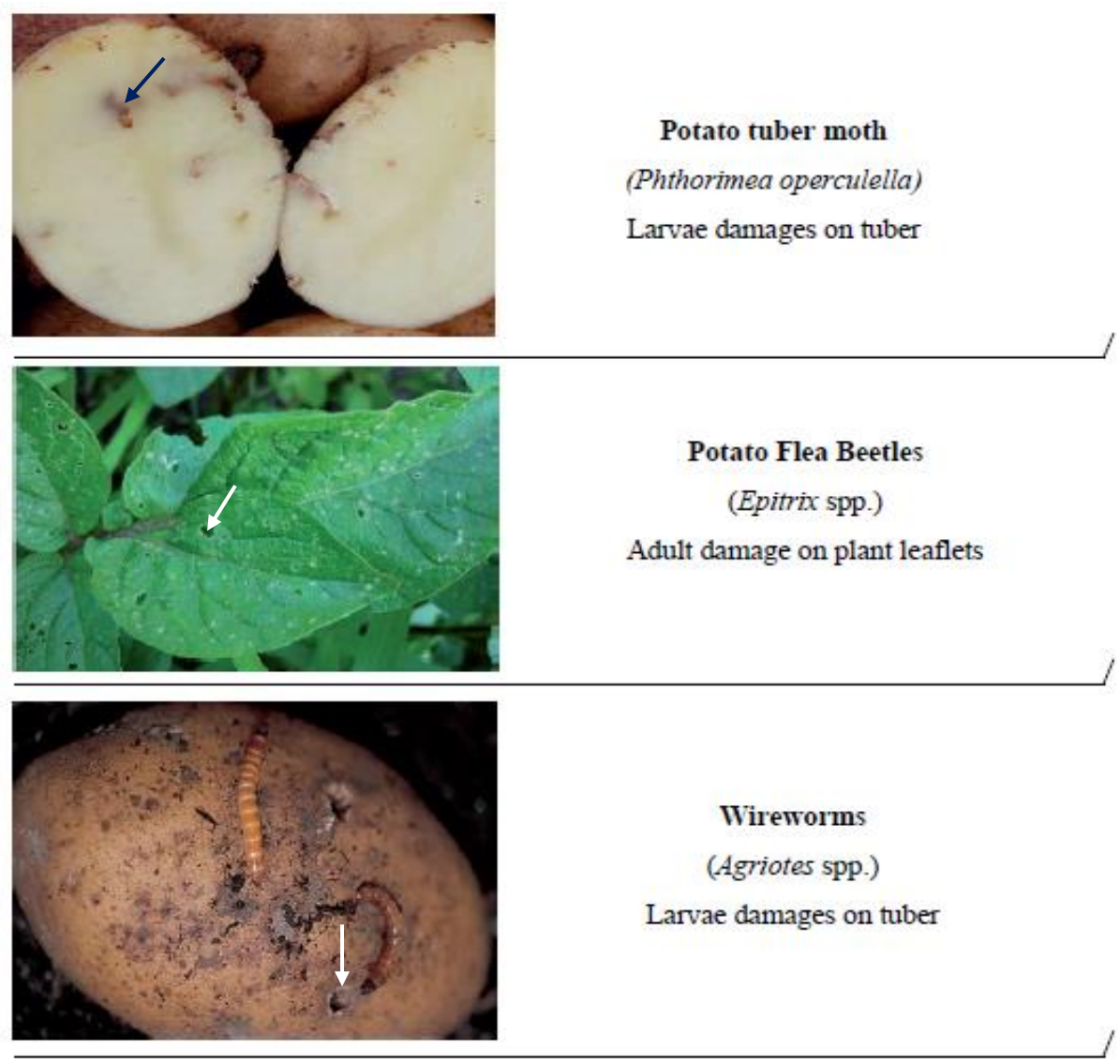


**Figure 2. 5: Potato tuber moth, Flea Beetles and Wireworms symptoms (CEE-ONU, 2014).**

### 2.3.2. Nematodes

**a) Root knot Nematode** (*Meloidogyne* spp.): Several species of *Meloidogyne* cause the appearance of symptoms on the potato. These nematodes develop galls on the tuber surface, depending on the variety. When infested tubers are cut, small brown spots may appear inside the tuber cortex. Each point corresponds to an adult female surrounded by a mass of brown eggs (CEE-ONU, 2014).

**b) Potato cyst nematodes** (*Globodera* spp.): Two species of *Globodera* attack potato *G. rostochiensis* and *G. pallida*. Infestation by potato cyst nematode is characterized by plants

whose foliage is darker in color and sometimes it is possible to see white or golden-yellow cysts developing on the roots (CEE-ONU, 2014).

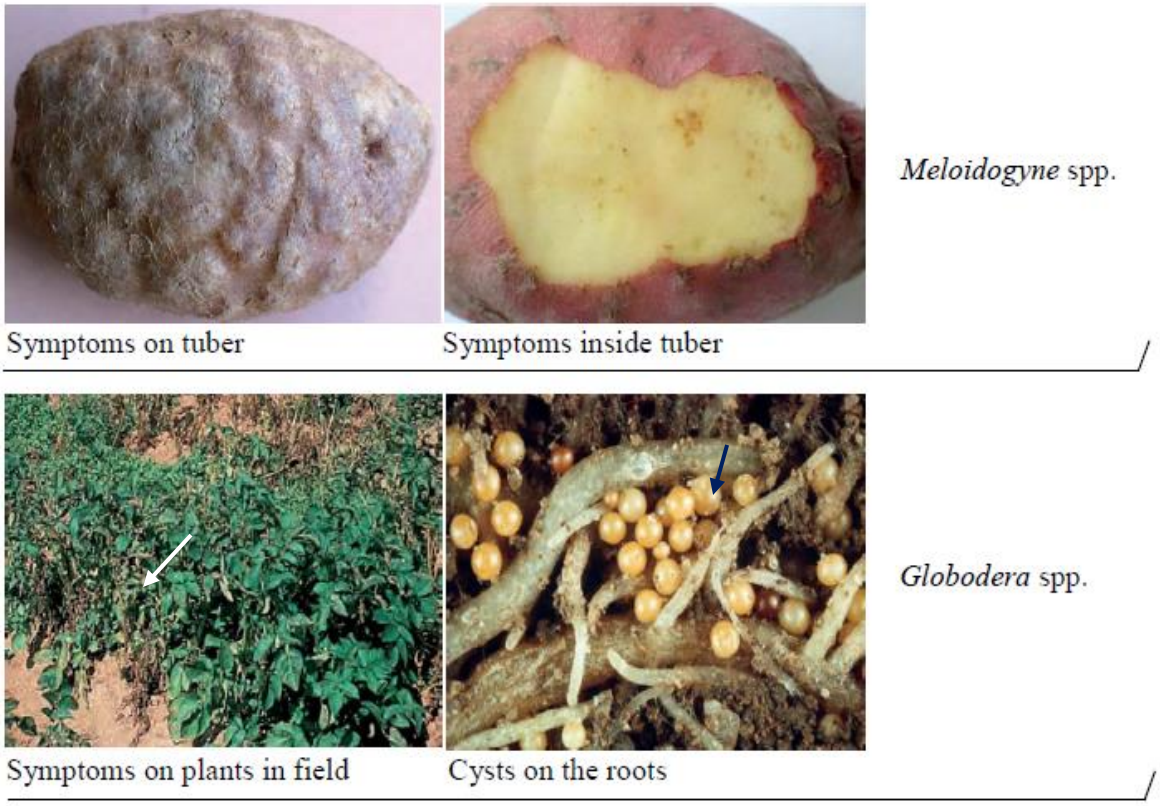


**Figure 2. 6: Potato Root knot and Cyst nematodes symptoms (CEE-ONU, 2014).**

**2.4. Distribution and production**

The potato is cultivated worldwide, it is grown in the mountains of Peru, the northern European plains, Yunnan Plateau in China, the equatorial highlands of Rwanda and subtropical lowlands of India (FOA, 2007).

The spread of the Andean potato to the rest of the world was done by the Spanish conquest. The conquistadors came in search of gold in Peru, but the real treasure they brought to Europe was *Solanum tuberosum*. The first evidence of potato cultivation in Europe dates from 1565, on Spain's Canary Islands. In 1573, the potato was known to be grown on the Spanish mainland and shortly thereafter spread throughout Europe. The potato was grown in London in 1597 and reached France and the Netherlands shortly thereafter. Meanwhile, European co-

lonialism and emigration diffused potato around the globe. They introduced potato cultivation into the flood plains of Bengal and the Egyptian Nile Delta in the Atlas Mountains of Morocco and on the Jos Plateau in Nigeria (FAO, 2009).

In 1971, was founded in Lima in Peru in the historic cradle of the potato, the International Potato Center (CIP) (CIP, 2008). The potato is the world's fourth-largest food crop (after rice, wheat, maize) but the first non-cereal crop, the potato adapts to various environmental conditions, it produces well from the polar circle to the equator by playing on seasons, varieties, altitude, etc. (Boufares, 2012).

World production was estimated in 2016 at over 376.8 million tons. However, it is important to mention that, production decreased in the last 10 years in Europe and Oceania with a decrease of 3.2% and 2% respectively. On the contrary, the Americas increased production by 1.61%, Africa also increased by 32%, but it is Asia that has developed most in production with an increase of 45.88% compared to 2007 production (FAO, 2016).

**Table 2. 2: World potato production from 2007 to 2016 by continents**

| Continent | Years production (Ton) | |
|---|---|---|
| | 2007 | 2016 |
| Africa | 18,558,689 | 24,501,902 |
| Americas | 41,916,749 | 42,592,735 |
| Asia | 130,594,987 | 190,516,292 |
| Europe | 121,443,660 | 117,555,648 |
| Oceania | 1,694,377 | 1,660,390 |

FAO, 2007 and 2016

Table.3 shows that China is the world's leading producer of potatoes in 2016 with 99.07 million tons, followed by India with 43.8 million tons. Also note, the largest yield to United States of America 49.92 t / ha of potato followed by Germany with 44.42 t / ha.

**Table 2. 3: Top 10 potato producing countries in 2016**

| Country | Production (Ton) | Yield (T/ha) |
|---|---|---|
| China, mainland | 99,065,724 | 17.04 |
| India | 43,770,000 | 20.55 |
| Russian Federation | 31,107,797 | 15.32 |
| Ukraine | 21,750,290 | 16.58 |
| United States of America | 19,990,950 | 49.02 |
| Germany | 10,772,100 | 44.42 |
| Bangladesh | 9,474,099 | 19.92 |
| Poland | 8,872,445 | 28.47 |
| France | 6,834,680 | 39.01 |
| Netherlands | 6,534,338 | 42.00 |

FAO, 2016

In Africa, it is Egypt, which was the first producer in 2016 with 5.03 million tons of potato, followed by Algeria with 4.8 million tons. Mali ranks second in West Africa, after Nigeria and 18$^{th}$ in Africa, in potato production in 2016 with 210,209 tons. (FAO, 2016).

### 2.5. Utilization of Potato

#### 2.5.1. Processed products intended for human consumption

Depending on the type of preparation, we can classify the processed products in three main categories:

- Baked goods, sterilized in jars, boxes or plastic bags;
- Dehydrated products (powders and flakes) intended for the preparation of reconstituted purées or used for the manufacture of soups, snacks, etc.;
- Fried products: chips, fresh or frozen French fries, gratins, pancakes, etc (Fauconnier & Delaplace, 2004).

### 2.5.2. Other uses: animal feed and industrial outlets

In addition to its food uses, the potato has multiple non-food destinations, it is widely used in the starch industry. Potato starch has many uses in the non-food industry. The potato starch is used in the composition of certain drugs, in lipstick, diapers, paper, textile and plywood industry manufacture; Biotechnology could find new opportunities for it in the near future (DGESCO-ENS de Lyon, 2009).

## 2.6. Fertilizers requirements of potato

For high yield, the potato requires cool season conditions, reasonable amount of nitrogen (N), phosphorus (P) and potassium (K) in the soil (Suh *et al.*, 2015). Variation in application rate of organic and mineral fertilizers can influence potato yield (Monirul *et al*.,2013).

Nitrogen is a determinant of the yield and quality of potato tubers. the excess of nitrogen promotes an abundant foliage which is favorable for disease development and delays maturity and harvest. its deficiency leads to reduced tuberization (CARA, 2015).

Phosphorus plays a major role in plant growth and development processes (Sultenfuss and Doyle, 1999). Deficiency of phosphorus promotes tuber yield loss in potato (Vhuthu, 2017).

Potassium activates many essential physiological process enzymes and plays a key role in tuber quality. it promotes optimal maturation by causing a decrease in mechanical damage and improved conservation. Potassium deficiency leads to a premature cycle end (foliage wilting), reducing assimilation, quality and yield. The foliage of potatoes dies prematurely which reduces the growing season and yields (KALI, 2015).

Maintaining a good level of organic matter, a well-planned rotation system and an adequate pH are the basis of soil fertility. However, they will not be enough to replace all the nutrients taken by the potato for its growth, then exported by the tubers. The production of 25 tons per

hectare of potatoes will export 92.5 kg of nitrogen, 12.5 kg of phosphorus, 100 kg of potassium, 5 kg of calcium and 5 kg of magnesium per hectare (Fraser, 1998).

The potato does not appreciate fresh manure or young compost, which favors the development of galls. It will apply between 20 and 25 t / ha of well-decomposed compost (Fraser, 1998).

**2.7. Potato cultivation in Mali**

Potato cultivation is an ancient practice in Mali and in Sikasso region. It was be introduced since the 19$^{th}$ century by French colonizers. But the first crops produced by the peasants go back to the aftermath of the First World War in the village of Kafuziela (Sikasso)(Diakité and Zida, 2003).

Potato crop is present in most parts of the country, but the main areas of potato production remain the Sikasso region (80% of total production); Koulikoro and the Segou region in the Office du Niger (Maiga, 2004).

The main period of production is the cold dry season but is also cultivated during the rainy season (Diakité and Zida, 2003).

In the cool season (October to March) most of the tubers produced annually come from the Sikasso region. This period is the most favorable for the production of potato due to the large amplitude of the daily temperatures (Coulibaly *et al.*, 2002).

In rainy season (May to September), the weather conditions are unfavorable for production. Temperatures are high with low amplitude and high intensity of rains or excess water in the soil which cause damage to vegetation. To overcome these problems, the culture is carried out on the hill slopes at high altitude (750 meters) on gravelly soils (Coulibaly *et al.*, 2002).

The most cultivated varieties in Mali are Claustar, Pamina, Spunta, Lola, Aida, Atlas, Sahel, Cosmos, Diamond, Provento, Mondial, Liseta, Iroise, Charlotte, Yasmina and Cynthia (Diakité and Zida, 2003).

Two types of seed are generally used by producers: Seed stored by the producer himself (this seed comes from the previous season's production and is called local seed) and the imported certified seed (Diakité and Zida, 2003).

The potato sector is structured on three levels: seed suppliers, producers and buyers marketers traders. Production techniques remain largely traditional: manual land management, gully watering from small wells. The main problem faced by producers is access to funding for: seed supply, water points, the purchase of improved drainage facilities and the construction of cold storage structures (BNDA, 2014).

Mali potato production was estimated in 2016 at 210,209 tons. However, it is important to mention that, production increased from 2007 to 2016, but the yield is decreased. The data in the table.2.4, show the evolution of Mali's national production in potato from 2007 to 2016 (FAO, 2016).

**Table 2.4: Mali potato production, Area harvested and Yield from 2007 to 2016 by years**

| Year | Production (Ton) | Area harvested (hectare) | Yield (T/ha) |
|---|---|---|---|
| 2007 | 114,478 | 4,759 | 24 |
| 2008 | 167,221 | 6,970 | 24 |
| 2009 | 90,407 | 3,800 | 24 |
| 2010 | 82,470 | 3,741 | 22 |
| 2011 | 124,532 | 6,083 | 20 |
| 2012 | 138,351 | 6,953 | 20 |
| 2013 | 147,401 | 7,615 | 19 |
| 2014 | 157,439 | 8,543 | 18 |
| 2015 | 183,410 | 9,396 | 20 |
| 2016 | 210,209 | 10,525 | 20 |

FAOSTAT, 2007 and 2016

### 2.8. Tissue culture and its application on potato crop

Tissue culture, began after the second world war. In 1952, Morel and Martin from INRA obtained for the first time, the development of a whole plant (*Dalhia pinnata*) from a cultured meristem. The idea was to verify that the meristem, a small mass of cells at the origin of all the plant organs (stems, leaves, roots, etc.), remains generally free from any viral contamination. In fact, when taken from a diseased plant, it gives a perfectly healthy plantlet. In other words, the passage through the meristem culture can rid a diseased plant of the causal organism. Even when a plant totally infested with a virus, the meristems remain unaffected (Ducreux *et al.*, 1986).

Applications of *in vitro* culture are many today both in the field of horticulture and in research (especially in plant breeding), or to maintain varietal diversity (Conservatories) to save menaced species (conservations ex-situ). These techniques require knowledge of the environmental factors (temperature, light, medium composition, etc.) of the plant fragment cultured to direct it to a specific development program (Ghomari, 2015).

*In vitro* culture, can be used to:

- ✓ Reproduce an identical species and multiply it in large quantities, and at a lower cost to bring to market in the shortest time;
- ✓ Preserve ancient and menaced species to conserve biodiversity;
- ✓ Develop new plant varieties in the shortest time;
- ✓ Clean up viral plants and maintain healthy plants (Ghomari, 2015).

This technique is currently used for the production of potato basic seed. From a practical point of view, the seed production system through tissue culture is undoubtedly an effective alternative for rapidly multiplying a large quantity of seeds of irreproachable sanitary quality.

It can be easily integrated as part of a supply of high quality seedlings for the production of certified seed (ACW, 2011).

The tissue culture was successfully used in all potato seed producing countries to accelerate the early stages of seed production.

Usually the process consists of:

- ✓ virus-free potato production plants using meristem;
- ✓ virus-free plants using micropropagation;
- ✓ production of microtubers and minitubers from *in vitro* plants;
- ✓ cultivating healtly seeds using minitubers as planting material (Naik and Karihaloo, 2007).

The *in vitro* micropropagation provides considerable progress over traditional methods with a multiplication rate of 100 to 1000 times higher and in a shorter time. Through *in vitro* multiplication of potato two million plants are easily obtained in less than one year from a single bud. By comparison, it takes 7 to 8 years to achieve the same result with the traditional mode of propagation (Ducreux *et al*., 1986). This provides a perfect guarantee of genetic conformity and stability of characters in successive subcultures (Demol *et al*., 2008).

### 2.9. Potato seed production

Potato crop multiplication can be achieved by botanical seed (true seed) or tuber seed (Cherif, 2011).

#### 2.9.1. Tuber seed production method

##### 2.9.1.1. Conventional techniques of seed potato production

Conventional potato material for planting (potato seed) is the tuber. Conventional method of production of seed potatoes is to multiply several times the tubers free from diseases in a process known as the clonal propagation (Bryan, 1981). The method has a low multiplication

rate, about 6-8 daughter tubers per plant (Otazu, 2010). This means that the tubers must be replanted for many cycles before sufficient seed is available for a cultivar to be formally registered. (Muthoni and Kabira, 2014). Hence, it is expensive and time consuming to produce enough seed tubers (Accatino and Malagamba, 1982). However, the common method for propagation encouraged accumulation of tissue-borne viruses, fungi and bacteria in subsequent seasons (Tsoka *et al*., 2012).

To prevent diseases free plant material, different rapid multiplication techniques such as tissue culture, aeroponics and hydroponics culture were used to bulk mini tubers of potato varieties released and promising clones for distribution to farmers (Abebe *et al*., 2014).

**2.9.1.2. Tissue culture techniques**

Tissue culture technique is one of the important methods of plant propagation available to producers and its use in the production of seeds has allowed the mass production of potato plants in a very short time (Beukema and Van-der-Zaag, 1990).

The culture of the meristem is a procedure in which the tip of the apical growth / axillary (0.1-0.3 mm) is dissected and allowed to develop into plants and a virus-free plant can be produced if a small piece of meristem is propagated (Morel and Martin, 1952; Wang and Hu, 1982). The apical meristem has a number of unique features that have made possible the elimination of the virus and some of the following characteristics: (a) The vascular system through which viruses propagate is not developed in the meristematic region; (b) multiplication of the meristem chromosomes can inhibit viral replication by interfering with the metabolism of viral nucleic acids and (c) the existence of a viral inactivation system with greater activity in the apical region than elsewhere (Naik and Karihaloo, 2007).

Tissue culture techniques used in the micro-propagation of potatoes consists of aseptic cell culture or fragments of plant tissues and organs in an artificial medium under conditions of temperature and light controlled, then transferred to the screen house in pots and aeroponic

conditions used for production of mini tubers. Mini tubers are commonly used in the production of seed potatoes to increase seed tubers (Ozturk and Yildirim, 2010).

One of the advantages of this method is the maintenance of genotype identity since meristemic cells retain their genetic stability more uniformly (Grout, 1990). When the materials were cleaned of pathogens, they can be mass- multiplied for use as planting material (Abebe *et al*., 2014).

### 2.9.2. True potato seed production method

True potato seed or botanical potato seed is produced by crossing two parental potato lines, a substitute for traditional seed tubers in many potatoes producing countries. True potato seed hybrid production requires technical expertise for the selection of male and female parent lines with desired attributes and maintaining their genetic purity/health (Gupta *et al.*, 2004).

True potato seed used to grow commercial potato two methods (transplanting of seedlings and use of seedlings tubers) (Naik and Karihaloo, 2007).

The disadvantages of the technology of true potato seed are that the seeds are not genetically pure and have a high heterogeneity; the harvest is late compared to the crop grown from the seed tubers; and the technology requires a lot of labour (Naik and Karihaloo, 2007).

### 2.9.3. Potato seed production challenges

Potato seed production system via tuber seed is challenging with low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagation. About 30 viruses and virus like agents infect potato (Naik and Karihaloo, 2007).

The main bottleneck of the formal seed supply system is slow multiplication of basic seed into certified seed (Muthoni and Kabira, 2014).

Most of developing countries are failing to maximize their efforts on tissue culture technology to make up the country potato seed system because of the high investment and operating; Cost of a specialized tissue culture laboratory. Moreover, different media components and reagents are expensive (Badoni and Chauhan, 2010).

### 2.9.4. Potato seed production in Mali

The tuber multiplication technique is predominant for crop development, but with the use of micro-propagation of tissue culture plants in the laboratory during the pre-base seed phase (Cherif, 2011).

Considering the increase in potato production in Mali, storage issues, price and quality of imported seed and small quantity of seeds produced locally, a seed production through Tissue culture scheme in Mali was developed from the plant biotechnology Laboratory of IPR / IFRA, Katibougou in 2000. In addition to this production from micropropagation in laboratory, farmers produce potato seeds locally (Coulibaly *et al.*, 2002).

#### 2.9.4.1. Locally produced seed

This is produced from certified seed imported from Europe, usually Class B or A, it is of three types:

***Production of Chikoroni***: From the cold season crops grown in the plains with imported certified seeds, small tuber sizes at harvest in February, are partially shipped to the hills for planting in the rainy season (planting in May-June), these are the "Chikoroni" (old seeds in Bambara language) (Diakité and Zida, 2003).

***Production of Tchikadan***: These are the small tuber sizes from the rainy season crops established on the hills from "Chikoroni". Stored for some time, they are used as seed for the late season crops (December plantings) (Diakité and Zida, 2003).

***Production of "Fassamani":*** Some of the small tubers from dry season crops grown in the plain with certified imported seeds are stored for 7 to 8 months until the next off-season. These are the "Fassamani" (seeds withered or softened in Bambara language). Much of the "Fassamani" is produced from varieties with good storability. The "Fassamani" allow early planting (October) when the imported seeds are not yet available (Diakité and Zida, 2003).

### 2.9.4.2. Certified seeds production through Tissue culture

Three classes of seeds are distinguished in the Malian system:

- ✓ Pre-basic seeds consist of the starting material G0 and G1;
- ✓ Basic seeds (G2) are obtained from the G1 tubers;
- ✓ Certified seeds (G3) are produced from G2 tubers.

The steps of producing potato certified seeds from *in vitro* in Mali are as follows.

#### a) Micropropagation in the laboratory

Tissue culture, *in vitro* culture or micropropagation is the compliant mode of multiplication, which involves starting a tuber culture free from disease.

***Micropropagation of plantlets in vitro:*** Explant derived from healthy material that will first produce plantlets. The developed plantlets will be cut into as many cuttings as there are nodes.

***Microtuberization in vitro:*** Tubers are derived from *in vitro* tuberization of microbuturized plantlets. Such tubers have sizes varying from 3 to 8 mm, which is why they are commonly called "microtubers"(Abdoulaye, 2008).

#### b) First Pre-Basic seed production in tunnel

*In vitro* plants or micro tubers are planted in tunnels where they grow. At the end of 75 to 90 days of cultivation, mini-tubers whose diameters are between 8 and 28 mm, called G0, are obtained (Abdoulaye, 2008).

### c) Three multiplications of seed in field

Tubers G0 from the tunnel are multiplied for three years in the field; In the first year to obtain the Pre-basic second seeds (G1), the Basic seeds (G2) in the second year which in turn is multiplied in the third year to obtain certified seed (G3). The G3 tubers thus obtained is used as seed to produce the potato tubers for consumption (Fig.2.7). The production of the higher quality certified seed requires stricter standards and production guidelines (e.g. protective isolation passages restriction in plot) than the super-elite (Abdoulaye, 2008).

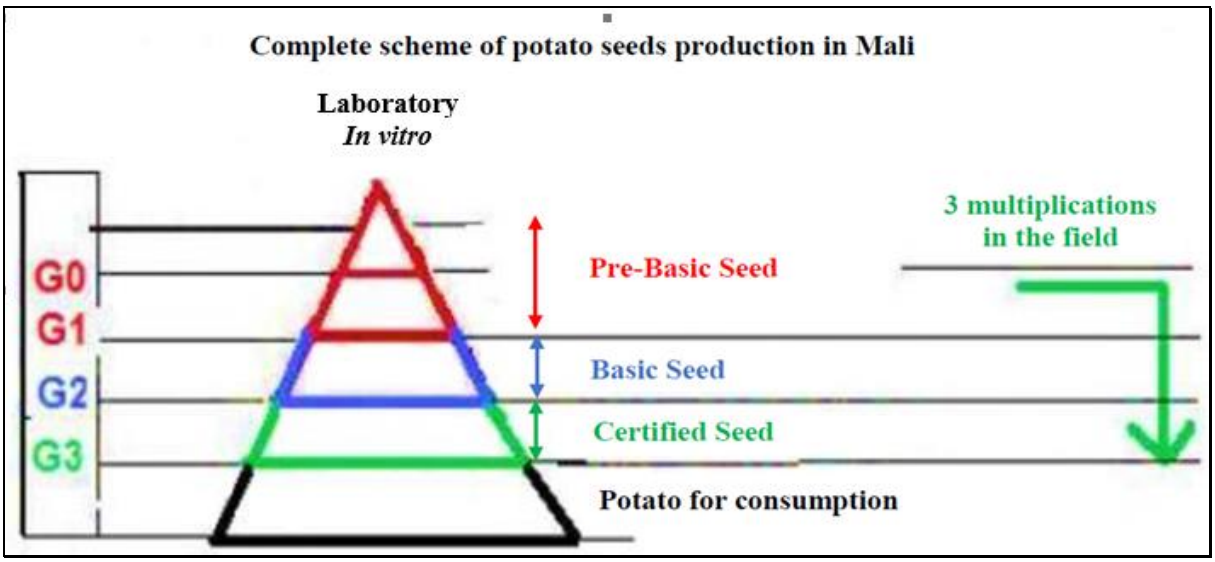


**Figure 2.7: Potato seed production through Tissue culture scheme in Mali (Vanderhof-stadt, 2011).**

# CHAPTER THREE

# 3.0. MATERIALS AND METHODS

## 3.1. Study 1

Evaluation of coconut water and potassium nitrate effects on MS medium as supplement components on potato plantlets growth *in vitro*.

### 3.1.1. Experimental site

The site for the experiment was the IPR/IFRA Plant Biotechnology Laboratory, Mali.

### 3.1.2. Plant material

The plant material was the *in vitro* plantlets one variety of potato (Sahel) already available in the Laboratory of Plant Biotechnology IPR/IFRA of Katibougou. One month old potato *in vitro* plantlets were selected as explants and nodal stem cuttings were used and cultured for 30 days *in vitro*.

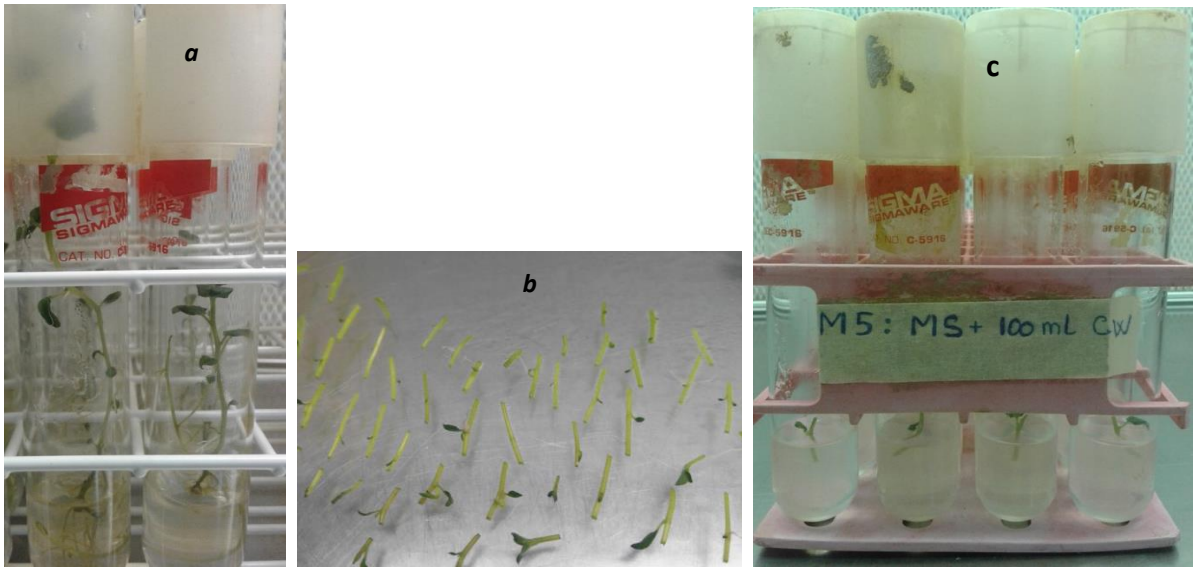


**Figure 2. 8a: Plantlet used as explants; 2.8b: Nodal stem cuttings and 2.8c: Explants on media.**

### 3.1.3. *In vitro* growth conditions

*In vitro* derived plantlets of potato Sahel variety were grown for a period of 30 days on twelve (12) culture media, at the growth temperature of 24 ± 1 °C and at 16 hours photoperiod in a culture chamber.

Nodal explants of potato *in vitro* plants were used for the micro propagation study. They wereobtained by cutting the plants at the internodes. The explant has a stem portion 0.5 to 1 cm long, an axillary bud and an axillary leaf. The explant thus obtained was placed vertically in the culture tube in contact with the solid culture medium. Each culture tube contains 10 ml of solidified culture medium.

### 3.1.4. Culture media

The MS (Murashige and Skoog, 1962) basic medium supplemented with 100 mg per liter of myo-inositol and 30 g per liter of sucrose were prepared. Various combinations of coconut water from the mature dry fruit at three (3) different concentrations (40 ml, 100 ml, and 300 ml per liter) and two (2) different concentrations of potassium nitrate (250 mg and 1000 mg per liter) were used singly or in combinations to generate twelve different treatment media. MS medium with no coconut water and no Potassium Nitrate ($KNO_3$) was used as control.

The pH of each treatment medium was adjusted to 5.7 ± 1 before sterilizing, the different treatment media were solidified with 2 g per liter of Gelrite. The treatment media were sterilized by autoclaved at 115 ± 1 °C for 30 minutes.

The twelve (12) different media for the experiment were:

1. MS (control);
2. MS+250 mg $KNO_3$;
3. MS+1000 mg $KNO_3$;
4. MS +40 ml/l Coconut Water (CW);
5. MS+100 ml/l CW;
6. MS+300 ml/l CW;
7. MS +40 ml/l CW+250 mg $KNO_3$;
8. MS +40 ml/l CW+1000 mg $KNO_3$;
9. MS+100 ml/l CW+25 0mg $KNO_3$;
10. MS+100 ml/l CW+1000 mg $KNO_3$;
11. MS+300 ml/l CW+250 mg $KNO_3$;
12. MS+300 ml/l CW+1000 mg $KNO_3$.

In this study, for the preparations of the different culture media. Commercial bottles of pre-mixed powders, already available in the laboratory were used. The "MS Medium" bottle contained all the mineral salts (macro and micronutrients) and vitamins as prescribed by Murashige and Skoog (1962).

**Table 3. 1: Murashige and Skoog (1962) medium components**

| **Components** | | **mg/l** |
|---|---|---|
| Macronutrients | $CaCl_2 2H_2O$ | 440 |
| | $MgSO_4 2H_2O$ | 370 |
| | $KH_2PO_4$ | 170 |
| | $NH_2NO_3$ | 1650 |
| | $KNO_3$ | 1900 |
| Micronutrients | KI | 0.83 |
| | $H_3BO_3$ | 6.20 |
| | $MnSO_2H_2O$ | 22.30 |
| | $ZnSO_2 7H_2O$ | 8.60 |
| | $Na_2MgO_2 2H_2O$ | 0.25 |
| | $CUSO_4 5H_2O$ | 0.025 |
| | $CoCl_2 6H_2O$ | 0.025 |
| Iron | $Na_2EDTA$ | 37.30 |
| | $FeSO_4 7H_2O$ | 27.80 |
| Vitamins | Myo-inositol | 100 |
| | Nicotinic acid | 0.50 |
| | Pyridoxine Hydrochloride | 0.50 |
| | Thiamine hydrochloride | 0.10 |
| | Glycine | 2 |
| Sugar | Sucrose | 30000 |

### 3.1.5. Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) with twelve (12) treatments replicated 4 times.

### 3.1.6. Treatments

**M1**: MS (control);

**M2**: MS+250 mg $KNO_3$;

**M3**: MS+1000 mg $KNO_3$;

**M4**: MS +40 ml/l Coconut Water (CW);

**M5**: MS+100 ml/l CW;

**M6**: MS+300 ml/l CW;

**M7**: MS +40 ml/l CW+250 mg $KNO_3$;

**M8**: MS +40 ml/l CW+1000 mg $KNO_3$;

**M9**: MS+100 ml/l CW+250 mg $KNO_3$;

**M10**: MS+100 ml/l CW+1000 mg $KNO_3$;

**M11**: MS+300 ml/l CW+250 mg $KNO_3$;

**M12**: MS+300 ml/l CW+1000 mg $KNO_3$.

### 3.1.7. *In vitro* Experiment Layout

A total of 240 culture tubes were used for the trial and each culture tube contained 10 ml of media and one explant with each replication represented by 5 tubes.

### 3.1.8. Data collection

A total of 5 plants were selected per treatment per replication as record plants. Data were collected on the following:

- ✓ Shoot emergence taken 10 days after propagating (D.A.P)
- ✓ Plant height measured 30 D.A.P;
- ✓ Number of leaves per plantlet was taken at 30 D.A.P;
- ✓ Number of roots per plantlet taken 30 D.A.P;
- ✓ Number of nodes per plantlet taken 30 D.A.P
- ✓ Average length of internodes calculated 30 D.A.P;
- ✓ Plantlet fresh and dry weight were taken 30 D.A.P.

### 3.2. Study 2

Evaluation of the effect of age at weaning of plantlets and substrate composition on re-establishment, growth and mini tuber production of potato variety Sahel *in vivo.*

#### 3.2.1. Experimental site

The experiment was conducted at the IPR / IFRA Plant Biotechnology Laboratory, Mali. The work was carried out *in vivo* in a screen house.

#### 3.2.2. Plant material

Plantlets generated *in vitro* from potato variety Sahel were used as the plant material. *In vitro* plantlets characteristics at 25 and 45-days were as follows:

- 5-7 cm height ;
- with 7 leaves ;
- and at least 4 well-developed roots.


**Figure 3.1: *In vitro* plantlets at 25 and 45 days at weaning age.**

#### 3.2.3. *In vivo* culture substrates composition and preparation

The three substrates were composed of a mixture of soil and cow dung (1:0, 2:1 and 1:1) The substrates were steam sterilized for 30 minutes, after cooling they were transferred into the

plots and leveled with a depth of 7cm. The substrate was well watered and allowed to settle for 24 hours before transplanting.


**Figure 3.2: Cow dung used (a), substrate mixture (b), plots ready to receive substrate (c) and substrate transferred into the plots and leveled (d)**

### 3.2.4. Experimental Design

The trial was a factorial experiment with 2 factors (physiological ages 2 levels and substrate 3 levels) laid out in a Randomized Complete Block Design (RCBD) with 6 treatments replicated 4 times. A total of 24 plots with plot size of 0.9 m x 0.8 m (0.72 $m^2$) each were used.

### 3.2.5. Treatments

The treatment factors under consideration included physiological age of the plantlet at weaning and the composition of the substrate on which weaning was carried out.

Two *in vitro* plantlet physiological age at transplanting [25 days (A1) and 45 days (A2)] were evaluated.

The three substrates used were:

1. S1 = Soil and Cow dung, 1:0;
2. S2 = Soil and Cow dung, 2:1;
3. S3 = Soil and Cow dung, 1:1.

**The following were the treatment combinations evaluated in this study:**

1. T1 = Plantlets age 25 days + Soil and Cow dung, 1:0 (A1S1);
2. T2 = Plantlets age 25 days + Soil and Cow dung, 2:1 (A1S2);
3. T3 = Plantlets age 25 days + Soil and Cow dung, 1:1 (A1S3);
4. T4 = Plantlets age 45 days + Soil and Cow dung, 1:0 (A2S1);
5. T5 = Plantlets age 45 days + Soil and Cow dung, 2:2 (A2S2);
6. T6 = Plantlets age 45 days + Soil and Cow dung, 1:1 (A2S3).

### 3.2.6. Layout of *in vivo* experiments

One screen house was prepared for the trial. Plot sizes of 0.9 m x 0.8 m were demarcated to accommodate 56 plants per plot. The spacing adopted was 10 cm x 10 cm.

One thousand three hundred and forty-four *in vitro* plantlets were used for the trial.

### 3.2.7. Agronomic practices *in vivo*

The *in vitro* plantlets were transferred from the culture chamber to the screen house the same day (from 4 to 6 am) because at this period the outside temperature is lower than that of the culture chamber. The *in vitro* plantlets were directly transferred from the *in vitro* culture tubes to growing substrates already disinfected by steam sterilization.

The plants were transplanted at a spacing of 10 cm × 10 cm. Immediately after transplanting the plants were watered.

**Figure 3.3a: *In vitro* plantlets ready to be transplanted and 3.3b: Arrows showing plantlets transplanted at 0.1m x 0.1m spacing.**

Agronomic practices such as water management, fertilizer application and pest and disease control were performed as follows:

**Table 3.2: Cropping operations *in vivo***

| Cultivation Operations | Period | Dose / Quantity |
|---|---|---|
| Plots preparation | before planting | 24 plots of 0.72 $m^2$ |
| First mineral fertilizer application | before planting | 300 kg/ha (NPK 17:17:17) |
| Furadan treatment | before planting | 20 kg/ha |
| Transplantion | 20 December 2017 | 10 cm x 10 cm |
| Watering | every day | - |
| Second mineral fertilizer application | 15 DAT* | 200 Kg/ha (NPK 17:17:17) |
| Third mineral fertilizer application | 25 DAT | 200 Kg/ha (NPK 17:17:17) |
| Fourth mineral fertilizer application | 40 DAT | 300 Kg/ha (NPK 17:17:17) |
| 1st Earthing up | 20 DAT | - |
| 2nd Earthing up | 35 DAT | - |
| 3th Earthing up | 50 DAT | - |
| Disease control | 26 DAT | Ridomil (fungicide) 5.5 g/plot |
| Harvest | 75 DAT | 24 plots |
| Sorting, sizing and counting of tubers | At harvest day | - |

*DAT: days after transplanting

### 3.2.8. Storage conditions

Tubers from the 10 record plants were stored under natural room conditions. The storage room temperature was between 24-34°C.

### 3.2.9. Data collection of *in vivo* experiments

A total of 10 plants were selected per treatment as record plants, boarder plants were excluded.

Data were collected on the following:

**a. Soil and Cow dung analysis**

Soil and Cow dung samples were taken before mixture and sent to the Laboratory of SEP-IER, Mali for analysis to determine chemical properties (pH and nutrient status) for the cow dung and the physical and chemical properties status for the soil.

**b. Substrate samples analysis**

Samples were taken from the three substrates used for the *in vivo* experiments after harvest and sent to the Laboratory of SEP-IER, Mali for analysis to determine the chemical status of the different spent substrates.

**c. Plant re-establishment rate**

The number of surving plant were used to calculate re-establishment rate by counting number of plants that survived at 20 days after transplanting.

**d. Plant height measurements**

Plant height was measured at 15, 40 and 60 days after transplanting. Plant height was measured from the soil level to the top of the plant by using a tape measure.

**e. Stem diameter measuring**

Stem diameter of plants were measured at 15, 40 and 60 days after transplanting; It was taken on the plant stem at the colar by using a pair of calipers.

**f. Number of leaves**

Number of leaves per plant was taken at 40 days after transplanting by counting the leaves on each of the record plants (10 plants per treatment) per replication.

**g. Plant fresh weight at harvest**

Plant fresh weight was recorded at harvest (75 days after transplanting), by weighing each of the ten (10) record plants per treatment per replication.

**h. Plant dry weight at harvest**

The dry weight of the ten (10) record plants for each treatment were recorded after oven drying the plants at $70^{o}$ C for 48 hours. The plants were placed in an envelope before putting in the oven.

**i. Number of tubers per plant**

Number of tubers produced per plant was recorded at harvest by calculating the average number of tubers per plant from the ten (10) record plants per treatment per replication.

**j. Tuber weight per plant at harvest**

Tuber weight per plant was recorded at harvest, by weighing tubers for each of the 10 record plants per treatment per replication.

**k. Mean tuber weight**

The mean tuber weight was taken at harvest, by dividing the total tuber weight per plant by the number of tubers per plant for each treatment.

**l. Tuber grading size**

The tubers from the 10 record plants per treatment were grouped into three (3) grading size groups:

- Grade A: tubers whose diameter were between 28 and 45 mm;
- Grade B: tubers with a diameter less than 28 mm;
- Grade C: tubers with a diameter greater than 45 mm.

After calculation, the percentage of each grade of tubers per treatment was recorded.

**m. Tuber weight loss in storage**

Tubers were stored for 8 weeks after harvest, every 2 weeks the weight of tubers was taken in storage by weighing tubers from the 10 record plants for each treatment.

**n. Number of sprouts per tuber in storage**

Number of sprouts per tuber was taken after three (3) months of storage by counting the sprout on the ten (10) stored tubers per treatment.

**o. Sprout number per eye in storage**

Number of sprouts per eye was taken at three (3) months of storage by dividing the number of sprouts per tuber by number of eye sprouted per tuber for each treatment.

### 3.3. Statistical Analysis

Data were analyzed with GenStat (12$^{th}$ Edition) using ANOVA. Significant treatment means were separated using Fisher's LSD test at 5% significance.

# CHAPTER FOUR

# 4.0. RESULTS

## 4.1. Effects of culture media on plantlets growth *in vitro*

### 4.1.1. Plantlets shoot emergence

The data collected at 10 days after culturing *in vitro* on shoot emergence (Table 4.1), showed significant differences ($p<0.01$) in culture media means. After 10 days of *in vitro* culturing, the maximum shoot emergence (100%) was observed in culture media M7 (MS+40 ml/l CW +250 mg $KNO_3$), M8 (MS+40 ml/l CW+1000 mg KNO3), M10 (MS+100 ml/l CW+1000 mg $KNO_3$) and M11(MS+300 ml/l CW+250 mg $KNO_3$). The lowest Shoot emergence (79.17 %) was observed in explants grown on medium M12 (MS+300 ml/l CW+1000 mg $KNO_3$).

### 4.1.2. Plantlets height and internodes length

Results from data analysis on plantlet height and internodes length 30 days after propagating are presented in table 4.1, there were significant differences ($P<0.01$) in plant height and internodes lengths between the different culture media means. The largest plantlet height was observed on the culture medium M7 (16 cm) with an internode length of 1.4 cm. It was followed by the culture medium M8 with 13.5 cm and 1.4 cm internode length then media M4 and M2 respectively (12.8 cm of shoot length and 1.4 cm internode length and 12.3 cm shoot length and 1.4 cm internode length). The shortest plant height of 4.8 cm was observed in M12 medium with an internode length of 0.9 cm.

**Table 4.1: Media effect on shoot emergence after 10 days, plant height and internode length after 30 days *in vitro* culturing.**

| Culture media | Shoot emergence % | Plant height (cm) | Internode length (cm) |
|---|---|---|---|
| **M1:** MS (control) | 83.33 $^{bc}$ | 10.00 $^{d}$ | 1.43 $^{b}$ |
| **M2:** MS+250 mg $KNO_3$ | 91.67 $^{abc}$ | 12.30 $^{c}$ | 1.54 $^{a}$ |
| **M3:** MS+1000 mg $KNO_3$ | 95.83 $^{ab}$ | 9.90 $^{d}$ | 1.27 $^{c}$ |
| **M4:** MS +40 ml/l Coconut Water | 83.33 $^{bc}$ | 12.78 $^{c}$ | 1.42 $^{b}$ |
| **M5:** MS+100 ml/l CW | 95.83 $^{ab}$ | 8.68 $^{e}$ | 1.09 $^{de}$ |
| **M6:** MS+300 ml/l CW | 95.83 $^{ab}$ | 6.93 $^{g}$ | 0.99 $^{ef}$ |
| **M7:** MS +40 ml/l CW+250 mg $KNO_3$ | 100 $^{a}$ | 16.00 $^{a}$ | 1.40 $^{b}$ |
| **M8:** MS +40 ml/l CW+1000 mg $KNO_3$ | 100 $^{a}$ | 13.53 $^{b}$ | 1.40 $^{b}$ |
| **M9:** MS+100 ml/l CW+250 mg $KNO_3$ | 91.67 $^{abc}$ | 8.95 $^{e}$ | 1.18 $^{cd}$ |
| **M10:** MS+100 ml/l CW+1000 mg $KNO_3$ | 100 $^{a}$ | 7.60 $^{f}$ | 1.18 $^{cd}$ |
| **M11:** MS+300 ml/l CW+250 mg $KNO_3$ | 100 $^{a}$ | 5.45 $^{h}$ | 0.92 $^{f}$ |
| **M12:** MS+300 ml/l CW+1000 mg $KNO_3$ | 79.17 $^{c}$ | 4.80 $^{i}$ | 0.90 $^{f}$ |
| **p-value** | 0.039 | <.001 | <.001 |

Letters represent significant differences among culture media according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.1.3. Number of Leaves

The data recorded on number of leaves at 30 days after propagating *in vitro* are presented in table.4.2, Highly significant differences ($p<0.01$) were observed in mean leaves numbers on the various culture media. However, the culture medium M7 plantlets showed the highest mean number of leaves (13.45). It was followed by M8 with a mean of 11.7 leaves, then the medium MS+40 ml/l coconut water (11 leaves). No significant difference was observed in mean leaves number between the culture media M2: MS+250 mg /l of $KNO_3$, M5: MS+100 ml/l of coconut water and M3: MS+1000 mg $KNO_3$ with the respective leaf means of 10, 10 and 9.85. The least number of leaves was produced on the culture medium M12: MS+300 ml/l CW+1000 mg $KNO_3$ with 7.35 leaves.

### 4.1.4. Number of Roots

Statistical analysis from data on plant roots numbers after 30 days *in vitro* propagation are presented in table 4.2, this shows highly significant differences ($p<0.01$) between the 12-culture media. The highest roots number per plant was observed in culture media M7 and M8 with nine roots each. The medium M3 recorded the least root number (1.2).

### 4.1.5. Number of nodes

Analysis of the data on plant nodes number after 30 days *in vitro* culture are presented in table 4.2. There were significant differences ($p<0.01$) between the culture media means. The culture medium M7 showed the highest mean number of nodes (11.45) per plant. It was followed by M8 with a mean of 9.65 nodes and M4: MS+40 ml/l Coconut Water (9 nodes). The lowest number of nodes occurred on the culture medium M12 (5.35).

**Table 4. 2: Comparison of the effect of different media on No. of leaves, roots and nodes of *in vitro* grown potato plantlets after 30 days.**

| Culture media | No. of Leaves | No. of Roots | No. of nodes |
|---|---|---|---|
| M1: MS (control) | 9.00 $^{ef}$ | 5.10 $^{d}$ | 7.00 $^{ef}$ |
| M2: MS+250mg $KNO_3$ | 10.00 $^{d}$ | 6.35 $^{c}$ | 8.00 $^{d}$ |
| M3: MS+1000mg $KNO_3$ | 9.85 $^{d}$ | 6.95 $^{b}$ | 7.85 $^{d}$ |
| M4: MS +40ml/l Coconut Water | 11.00 $^{c}$ | 6.80 $^{b}$ | 9.00 $^{c}$ |
| M5: MS+100ml/l CW | 10.00 $^{d}$ | 4.00 $^{f}$ | 8.00 $^{d}$ |
| M6: MS +40ml/l CW+1000mg $KNO_3$ | 9.00 $^{ef}$ | 1.60 $^{h}$ | 7.00 $^{ef}$ |
| M7: MS +40ml/l CW+250mg $KNO_3$ | 13.45 $^{a}$ | 9.00 $^{a}$ | 11.45 $^{a}$ |
| M8: MS +40ml/l CW+1000mg $KNO_3$ | 11.65 $^{b}$ | 9.00 $^{a}$ | 9.65 $^{b}$ |
| M9: MS+100ml/l CW+250mg $KNO_3$ | 9.60 $^{de}$ | 4.00 $^{f}$ | 7.60 $^{de}$ |
| M10: MS+100ml/l CW+1000mg $KNO_3$ | 8.45 $^{fg}$ | 4.50 $^{e}$ | 6.45 $^{fg}$ |
| M11: MS+300ml/l CW+250mg $KNO_3$ | 7.95 $^{gh}$ | 2.20 $^{g}$ | 5.95 $^{gh}$ |
| M12: MS+300ml/l CW+1000mg $KNO_3$ | 7.35 $^{h}$ | 1.20 $^{i}$ | 5.35 $^{h}$ |
| **p-value** | <.001 | <.001 | <.001 |

Letters represent significant differences among media according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.1.6. Plant fresh and dry weight

The data recorded at 30 days after propagating *in vitro* revealed significant differences ($p<0.01$) in mean plant fresh and dry weights between the different culture media.

The highest plant fresh and dry weights (respectively 545.50 and 36.40 mg) were observed on culture medium M7 (MS+40 ml/l of coconut water+250 mg of potassium nitrate). It was followed by M2 (MS+250 mg/liter of potassium nitrate) with the means of 364.50 mg for the fresh weight and 23.15 mg for the dry weight. The lowest plant fresh and dry weights were recorded on the culture medium M12: MS+300 ml/l of coconut water+1000 mg/liter of potassium nitrate with 99.20 mg for mean fresh and 13.20 mg for mean dry weight of plant (Fig. 4.1).

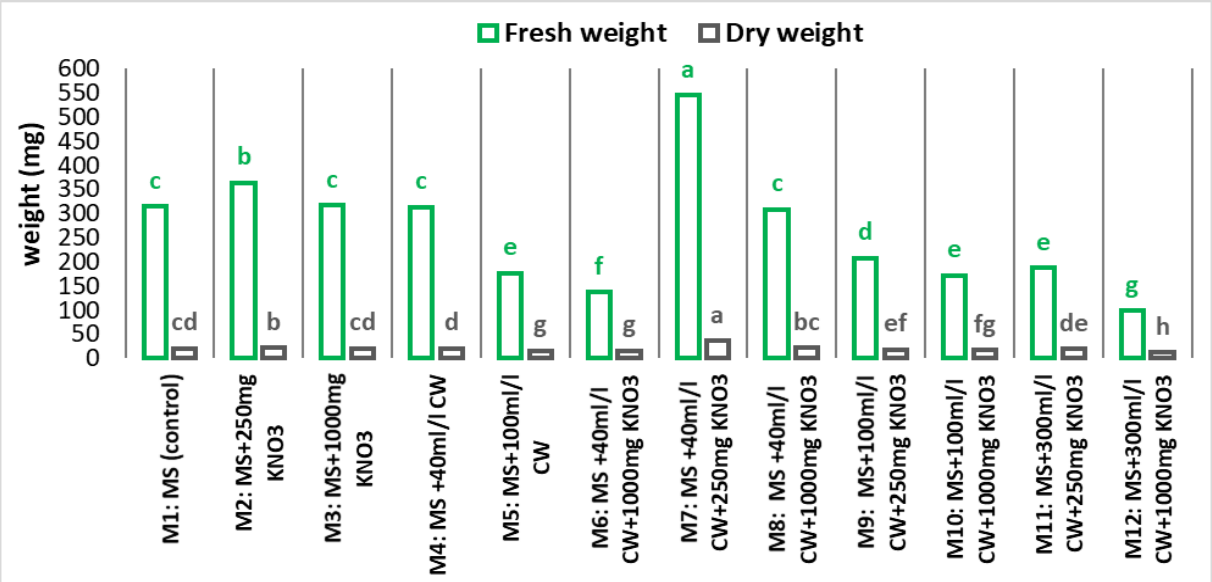


**Figure 4. 1: Effect of media on plantlet fresh and dry weights 30 days after culturing *in vitro*.**

Letters represent significant differences among media according to Fisher's LSD test at 5% significance.


**Figure 4.2: Development of *in vitro* plantlets 30 days after culture on media M1, M2, M3 and M4.**


**Figure 4.3: Development of *in vitro* plantlets 30 days after culture on media M5, M6, M7 and M8.**

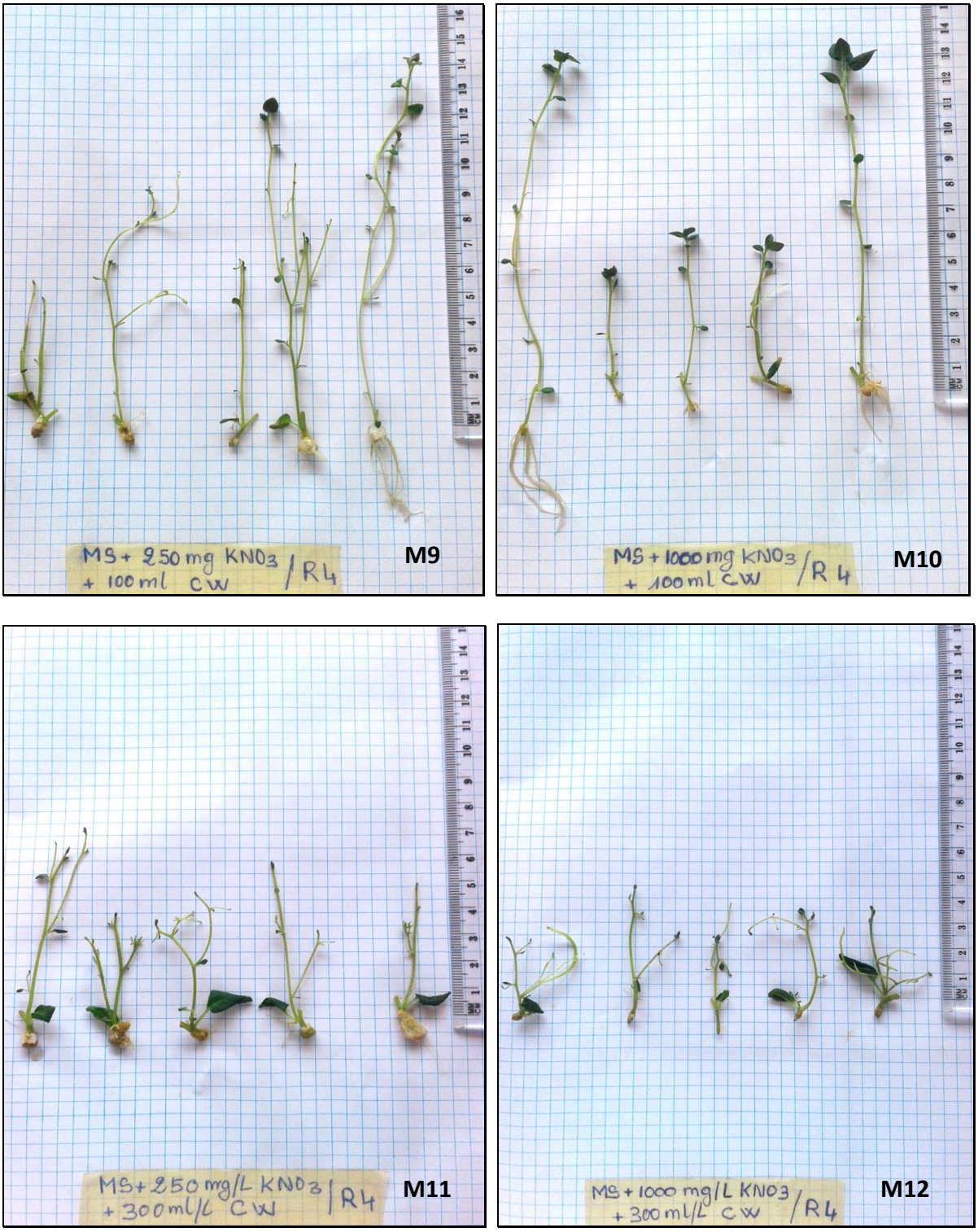


**Figure 4. 4: Development of *in vitro* plantlets 30 days after culture on media M9, M10, M11 and M12.**

## 4.2. Plantlets weaning age and substrate composition effects on mini tubers production

### 4.2.1. Properties of soil, cow dung and the three substrate mixtures

#### 4.2.1.1. Soil properties

The results of the laboratory analysis of physical and chemical properties of the soil used as the basic substrate in the experiment show that the soil is sandy loam texture and pH is 7.68 (Table.4.3).

**Table 4. 3: Soil properties before substrate mixture**

| Soil properties | Amount |
|---|---|
| Texture: Sand (%) | 72.00 |
| Silt (%) | 26.00 |
| Clay (%) | 2.00 |
| pH in water | 7.68 |
| pH in KCl | 6.96 |
| Organic carbon (%) | 0.65 |
| Nitrogen total (%) | 0.05 |
| Phosphorus assimilable (ppm/100g) | 78.32 |
| Potassium assimilable (mg/100g) | 10.64 |

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#### 4.2.1.2. Cow dung properties

The results of the laboratory analysis of the chemical properties of the cow dung used in the substrate composition for the experiment show that the pH is 7.26 with the presence of significant amount of NPK (Table 4.4).

**Table 4. 4: Chemical properties of the cow dung used**

| Cow dung properties | Amount |
|---|---|
| pH in water | 7.26 |
| pH in KCl | 6.63 |
| Nitrogen (N %) | 0.81 |
| Phosphorus ($P_2O_5$ %) | 0.89 |
| Potassium ($K_2O$ %) | 0.25 |

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#### 4.2.1.3. Chemical properties of spent substrates

The results of the laboratory analysis of physical and chemical properties of the three substrates used in the experiment show that the pH of the three substrates tested were reduced compared to the initial state of the soil before mixture of substrates and planting (Table 4.5).

**Table 4. 5: The three Substrates properties after growing**

| Substrate | pH in water | pH in KCl | Organic carbon (%) | Nitrogen total (%) | Phosphorus assimilable (ppm/100 g) | Potassium assimilable (mg/100 g) |
|---|---|---|---|---|---|---|
| S1 | 7.24 | 6.94 | 0.56 | 0.01 | 122.06 | 45.13 |
| S2 | 7.38 | 6.94 | 1.12 | 0.04 | 142.16 | 62.35 |
| S3 | 7.53 | 7.23 | 1.39 | 0.05 | 157.65 | 84.05 |

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### 4.2.2. Plant re-establishment

The Statistical analysis from data on plant re-establishment at 20 days after transplanting are presented in table 4.6, indicated that there was significant difference ($p<0.01$) among plantlet weaning age. Weaning at 25 days produced greatest percentage of plant re-establishment after 20 days of screen house culturing.

Significant differences ($p<0.01$) were observed between substrate mixtures. The substrate S1 (soil and cow dung 1:0) recorded the highest percentage of plant survival while substrate S3 (soil and cow dung 1:1) showed the lowest survival percentage.

The age and substrate interaction was also significant ($p<0.01$). The Treatment T1 (Seedlings ages 25 days and cultured on Soil and Cow dung 1:0) and T2 (age 25 days and cultured on Soil and Cow dung 2:1) recorded the highest plant re-establishment percentage of 98.7 % and 97.8 % respectively. They were followed by T3 (Seedlings ages 25 days: on Soil and Cow dung 1:1) with 91.5 % re-establishment. The lowest percentage plant re-establishment

occurred on T6 (Seedlings ages 45 days: on Soil and Cow dung 1:1) with 59.8 % re-establishment after 20 days of culturing.

**Table 4. 6: Effect of weaning age of *in vitro* plantlet and substrate mixture on plant re-establishment percentage at 20 days after transplanting**

| Age | Substrate (Soil and Cow dung) | | | Mean |
|---|---|---|---|---|
| | 1:0 | 2:1 | 1:1 | (Age) |
| 25 days | 98.66 [a] | 97.77 [a] | 91.52 [b] | *95.98 a* |
| 45 days | 83.93 [c] | 74.11 [d] | 59.82 [e] | *72.62 b* |
| Mean (Substrate) | *91.29 a* | *85.94 b* | *75.67 c* | |
| | Age | Substrate | Age*Substrate | |
| p-value | <.001 | <.001 | <.001 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.3. Plant height

The data collected on plant height after 15, 40 and 60 days of culturing *in vivo* indicated significant differences among both plantlet age and various substrates. Plantlet age and substrate interaction also were significant (Table.4.7).

From 15 days to 40 days of culturing T1 plants showed the greatest plant height with 7.7 cm and 28.3 cm respectively, but at 60 days of culturing T2 recorded the highest plant height (49 cm). The lowest plant heights were observed in T3 and T4 at 15 days, T4, T5 and T6 at 40 days and T3, T4 and T6 at 60 days of culturing *in vivo*.

**Table 4. 7: Plant height**

| Treatments | | Plant height (cm) | | |
|---|---|---|---|---|
| Age | Substrate | at 15 days | at 40 days | at 60 days |
| 25 days weaning | Soil: Cow dung 1:0 = T1 | 7.69 [a] | 28.25 [a] | 48.98 [b] |
| | 2:1 = T2 | 6.67 [b] | 21.90 [b] | 53.93 [a] |
| | 1:1 = T3 | 6.04 [c] | 22.68 [b] | 40.15 [c] |
| 45 days weaning | 1:0 = T4 | 6.10 [c] | 15.65 [c] | 39.10 [c] |
| | 2:1 = T5 | 6.67 [b] | 14.88 [c] | 46.03 [b] |
| | 1:1 = T6 | 6.57 [b] | 15.88 [c] | 41.50 [c] |
| p-value | | 0.009 | <.001 | 0.001 |

Letters represent significant differences among treatments according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.4. Stem Diameter

Plant stem diameters recorded after 15, 40 and 60 days of culturing are presented in Table.4.8. Significant differences ($p<0.01$) were observed in stem diameters at plantlet weaning age at 15 days and 40 days after transplanting. The largest plant stem diameters were formed in plantlets weaned at age 25 days both at 15 days and 40 days after transplanting. But there was no significant difference ($p>0.05$) at 60 days after transplanting. The substrate showed no significant difference ($p>0.05$) at 15 days but there were highly significant differences ($p<0.01$) for the substrate mixture at 40 days and 60 days after transplanting. The substrate S3 recorded largest stem diameters at 40 days and 60 days of culturing, it is joined by the substrate S2 at 60 days of culturing. The smallest plant stem diameters were formed in substrates S2 and S1 at 40 days and in S1 at 60 days of transplanting.

There were significant differences ($p<0.05$) at 15 days and ($p<0.01$) at 40 days and 60 days of culturing with interactions between weaning age and substrate mixture. The greatest stem diameters were observed in T1 at 15 days (1.5 mm), in T3 (5.2 mm) and T1 (5.2 mm) at 40

days and in T6, T5, T1 and T3 at 60 days of culturing respectively with 5.6 mm, 5.6 mm, 4.5 mm and 5.5 mm. The lowest diameters were showed by T4 at 15, 40 and 60 days respectively with 1.1 mm, 3.2 mm and 4.6 mm; and treatment T5 at 40 days with 3.3 mm.

**Table 4. 8: Plant stem diameter after 15, 40 and 60 days of culturing *in vivo*.**

| Treatments | | Stem diameter (mm) | | |
|---|---|---|---|---|
| Age | Substrate | at 15 days | at 40 days | at 60 days |
| 25 days weaning | Soil: Cow dung 1:0 = T1 | 1.472 $^{a}$ | 5.18 $^{ab}$ | 5.46 $^{ab}$ |
| | 2:1 = T2 | 1.277 $^{b}$ | 4.99 $^{b}$ | 5.18 $^{b}$ |
| | 1:1 = T3 | 1.20 $^{bc}$ | 5.24 $^{a}$ | 5.45 $^{ab}$ |
| 45 days weaning | 1:0 = T4 | 1.06 $^{bc}$ | 3.15 $^{d}$ | 4.55 $^{c}$ |
| | 2:1 = T5 | 1.11 $^{bc}$ | 3.33 $^{d}$ | 5.56 $^{a}$ |
| | 1:1 = T6 | 1.13 $^{c}$ | 3.93 $^{c}$ | 5.57 $^{a}$ |
| p-value | | 0.026 | <.001 | <.001 |

Letters represent significant differences among treatments according to Fisher's LSD test at 5% significance.
p-value= probability value

### 4.2.5. Number of leaves at 40 days of culturing

Statistical analysis of the data on plant leaves numbers at 40 days of culturing are presented in (Table.4.9). There was no significant difference (P>0.05) among *in vitro* plantlet weaning age means. Highly significant differences (P<0.01) were indicated between substrate means. The substrate S2 (Soil and Cow dung 2:1) showed the highest number of leaves (9.74). The lowest leaves number was observed in substrate S1 (Soil and Cow dung 1:0) with 8.41 leaves. No significant differences (P>0.05) were observed in the interactions between weaning age and substrate mixture means.

**Table 4. 9: Mean number of leaves of different treatments at 40 days of culturing**

| **Age** | **Substrate (Soil and Cow dung)** | | | ***Mean*** |
|---|---|---|---|---|
| | 1:0 (S1) | 2:1 (S2) | 1:1 (S3) | ***(Age)*** |
| 25 days | 8.43 | 9.73 | 9.35 | 9.17 |
| 45 days | 8.40 | 9.75 | 9.00 | 9.05 |
| **Mean (Substrate)** | 8.41 c | 9.74 a | 9.18 b | |
| | Age | Substrate | Age*Substrate | |
| p- value | 0.410 | <.001 | 0.498 | |

Letters represent significant differences among substrates according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.6. Plant fresh and dry weights at harvest

Results from data analysis of plant fresh and dry weights at 75 days of culturing are presented in Table 4.10. No significant difference ($p>0.05$) was showed among plantlet weaning age in the plant fresh weight but there was highly significant difference ($p<0.01$) between the plant dry weight means. The greatest plant dry weight was recorded by the weaning age 25 days. Highly significant differences ($p<0.01$) were observed in the substrate mixture means both for plant fresh and dry weights. The greatest plant fresh and dry weights were both recorded in substrate S2 (Soil and Cow dung 2:1). The lowest plant fresh and dry weights were observed in substrate S1 (Soil and Cow dung 1:0). There were Highly significant differences ($p<0.01$) in the interactions between weaning age and substrate mixture. The highest plant fresh weight was observed in T6 with a mean of 42 g. It was followed by T2 (37.9 mg) and T5 (37.8 mg). However, the greatest plant dry weights were obtained in T2 (2.6 g), T1 (2.6 g), T6 (2.5 g) and T5 with a mean of 2.5 g. They are followed by T3 (2 g). The lowest plant fresh and dry weights were produced by T4 with 16.7 g and 1.4 g respectively.

**Table 4. 10: Plant fresh and dry weights at 75 days of culturing.**

| Age | Substrate | Plant fresh weight (g) | Plant fresh weight (g) |
|---|---|---|---|
| 25 days weaning | Soil: Cow dung 1:0 = T1 | 33.43 [c] | 2.55 [a] |
| | 2:1 = T2 | 37.88 [b] | 2.63 [a] |
| | 1:1 = T3 | 25.75 [d] | 1.99 [b] |
| 45 days weaning | 1:0 = T4 | 16.70 [e] | 1.38 [c] |
| | 2:1 = T5 | 37.78 [b] | 2.45 [a] |
| | 1:1 = T6 | 41.98 [a] | 2.48 [a] |
| p-value | | <.001 | <.001 |

Letters represent significant differences among treatments according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.7. Yield in tubers

Table.4.11 shows the effect of weaning age of *in vitro* plantlet and substrate for transplantation on tuber yield. Significant difference ($p<0.01$) was observed for plantlet weaning age. The 25 days weaning recorded the highest tuber yield. There was highly significant difference ($p<0.01$) in substrate mixture effect. The substrate S2 (Soil and Cow dung 2:1) yielded more in tuber than the two others substrates. Highly significant differences ($p<0.01$) were also observed in the interactions between weaning age and substrate mixture. The greatest yield of tubers was obtained in T2 (59.3 t/ha) and T1 (58.1 t/ha) followed by T5 (47.1 t/ha) and T6 with 46 t/ha of tubers. The lowest yield was observed in T4 with 33.1 tons per hectare of tubers.

**Table 4. 11: Yield of tubers in ton per hectare**

| | **Substrate (Soil and Cow dung)** | | | ***Mean*** |
|---|---|---|---|---|
| **Age** | 1:0 (S1) | 2:1 (S2) | 1:1(S3) | ***(Age)*** |
| 25 days weaning | 58.12 [a] | 59.28 [a] | 44.03 [c] | *58.81 a* |
| 45 days weaning | 33.08 [d] | 47.08 [b] | 46.03 [bc] | *42.06 b* |
| ***Mean (Substrate)*** | *45.60 b* | *58.18 a* | *45.03 b* | |
| | Age | Substrate | Age*Substrate | |
| p-value | <.001 | <.001 | <.001 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.8. Mean weight of tuber (g)

Analysis of the data on the average weight (gram) of one tuber are presented in (Table 4.12). Highly significant difference ($p<0.01$) occurred in weaning age effect of *in vitro* plantlet. The 25 days at weaning produced the greatest average weight of a tuber. There was significant difference ($p<0.01$) among substrate mixture means. The substrate S2 (Soil and Cow dung 2:1) produced higher mean tuber weight than the substrate mixtures S1 and S3. Highly significant difference ($p<0.01$) also occurred in the interactions between age at weaning and substrate mixture. The T1 showed the highest mean weight (12 g per tuber) followed by T2 with a mean of 7.9 g per tuber, then by the treatment T3 with 7.3 g per tuber. The lowest weights were shown in the treatments T4 (5.6 g per tuber) and T5 with a mean tuber weight of 5.5 g.

**Table 4. 12: Average weight of one tuber**

| | **Substrate (Soil: Cow dung)** | | | ***Mean*** |
|---|---|---|---|---|
| **Age** | 1:0 (S1) | 2:1 (S2) | 1:1(S3) | ***(Age)*** |
| 25 days weaning | 11.98 [a] | 7.83 [b] | 7.25 [c] | *9.02 a* |
| 45 days weaning | 5.56 [e] | 5.54 [e] | 6.14 [d] | *5.75 b* |
| ***Mean (Substrate)*** | *8.77 a* | *6.69 b* | *6.69 b* | |
| | Age | Substrate | Age*Substrate | |
| p-value | <.001 | <.001 | <.001 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.9. Tubers numbers per plant

The Statistical analysis of data on mini tuber numbers per plant are presented in (Table.4.13) there was significant difference ($p< 0.01$) among plantlet weaning age. The 45 days weaning produced the highest number of tubers per plant. Significant difference ($p< 0.01$) was also showed in substrate mixture. The substrate S2 (Soil and Cow dung 2:1) significantly affected

the number tubers. The lowest number of tubers was recorded in substrate S1 (Soil and Cow dung 1:0) conditions. There were significant differences between plantlet weaning age and substrate mixture interactions ($p<0.05$). The treatment T5 showed the highest mean number of tubers per plant (8.5) it was followed by treatments T2 and T6 with 7.6 and 7.5 tubers respectively. The lowest number of mini tubers per plant was obtained in treatment T1 with 4.9 tubers.

**Table 4. 13: Number of tuber per plant at harvest**

| | Substrate (Soil: Cow dung) | | | ***Mean*** |
|---|---|---|---|---|
| Age | 1:0 (S1) | 2:1 (S2) | 1:1 (S3) | ***(Age)*** |
| 25 days weaning | 4.85 [d] | 7.58 [b] | 6.08 [c] | *6.17 b* |
| 45 days weaning | 5.95 [c] | 8.50 [a] | 7.50 [b] | *7.32 a* |
| ***Mean (Substrate)*** | *5.40 c* | *8.04 a* | *6.79 b* | |
| | Age | Substrate | Age*Substrate | |
| p-value | <.001 | <.001 | 0.009 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance.

p-value= probability value

### 4.2.10. Tuber size grading quality

Analysis of the data on tuber size grading (A: tubers whose diameter is between 28 and 45 mm, B: tubers with a diameter of less than 28 mm and C: tubers with a diameter greater than 45 mm) are presented in Tables 4.14 and 4.15; and Fig. 4.8. There were significant differences ($p<0.01$) between the weaning ages of *in vitro* plantlets for A, B and C size grading. Seedlings weaned for 25 days produced the highest percentages of tubers in grade A and C sizes, compared to those weaned for 45 days, which recorded highest percentage in grade B size. Significant differences ($p<0.05$) were observed in substrate for grades A, B and C. The substrate S1 (Soil and Cow dung 1:0) and S3 (Soil and Cow dung 1:1) produced the highest percentages of grade A tubers. The substrate S2 (Soil and Cow dung 2:1) produced

the highest percentage of grade B tubers. The substrate S1 produced more tubers of grade C size than the others substrates. There were significant differences in the interactions between age and substrate ($p<0.01$) for grade A and B and significant differences ($p<0.05$) for grade C sizes. The treatment T1 recorded the highest percentage of tuber grade A. The treatments T4, T5 and T6 produced more grade B tubers than the others. In grade C, the treatment T1 mean was significantly higher.

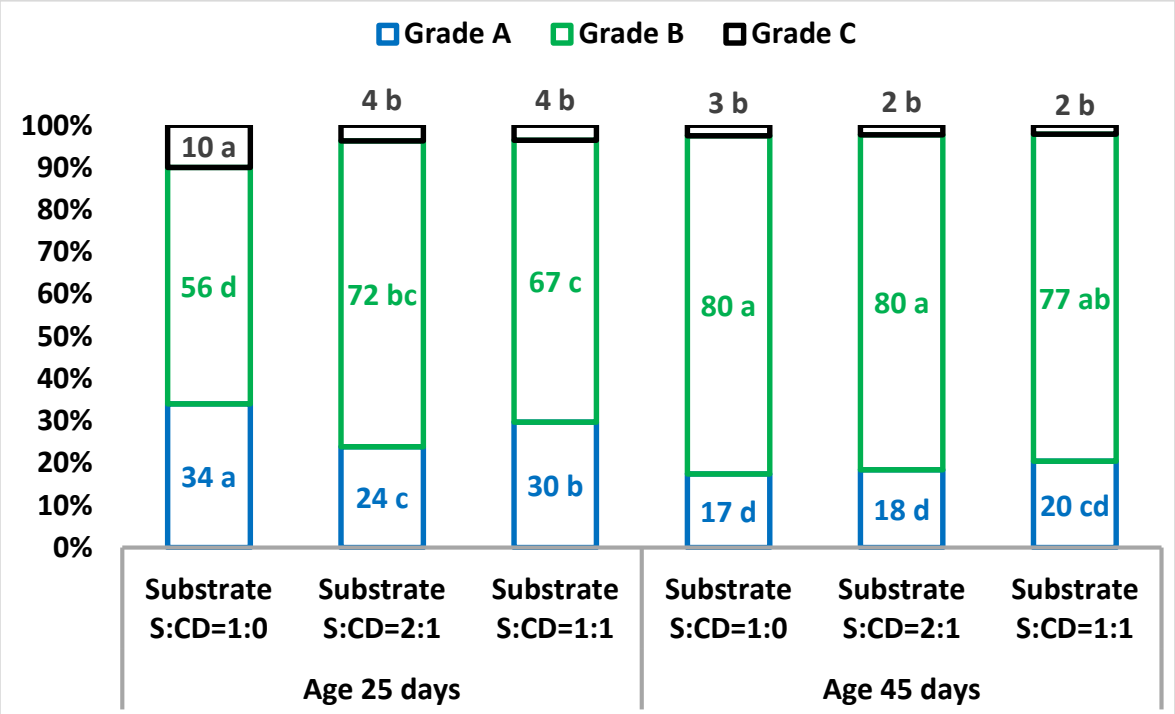


**Figure 4. 5: Tuber size grading.**

Letters represent significant differences among means according to Fisher's LSD test at 5% significance. Grade A (p-value: <.001), Grade B (p-value: 0.001), Grade C (p-value: 0.043).

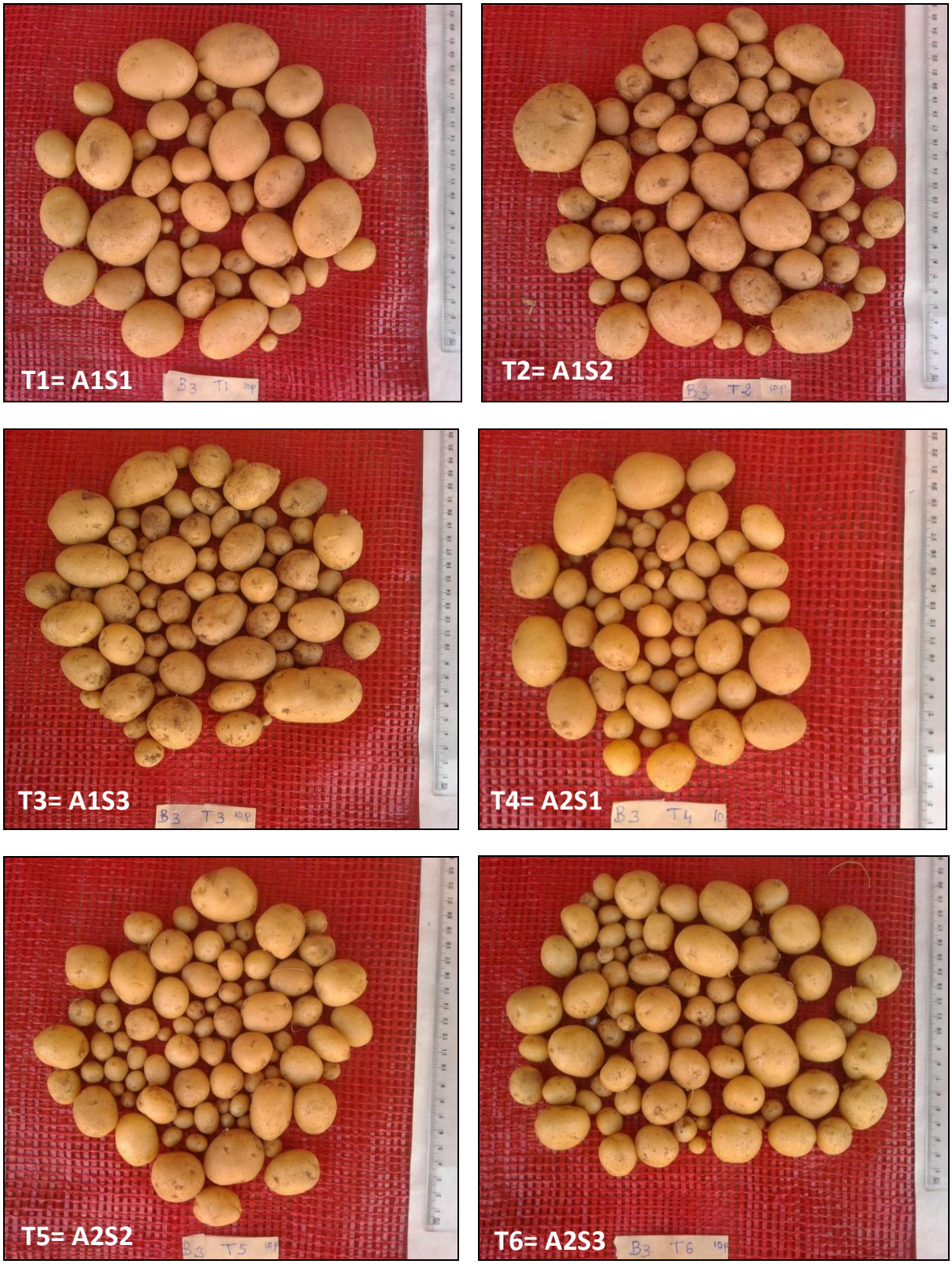


**Figure 4.6: Tubers produced at harvest by 10 plants under the 6 treatment conditions**

A1= plantlet weaning age 25 days; A2= plantlet weaning age 45 days.
S1= soil and cow dung 1:0; S2= soil and cow dung 2:1 and S3= soil and cow dung 1:1.

### 4.2.11. Tubers weight loss in storage

Data on weight loss (%) recorded on tubers stored for 8 weeks showed no significant difference ($p>0.05$) for age at 2, 4, 6 and 8 weeks of storage. Significant differences occurred for substrate at 2, 6 and 8 weeks of storage ($p< 0.05$). But there was no significant difference for substrate at 4 weeks ($p>0.05$). The interactions (age*substrate) were significant ($p<0.01$) from 2 to 8 weeks of storage. The lowest percentages of weight loss in stored tubers were obtained in substrate S1. The highest percentages of weight loss of stored tubers were obtained in substrates S2 and S3. The lowest percentages of weight loss were observed in the tubers produced in treatments T1, T2, T6 and T4 at 2 weeks; in T1 and T6 at 4 weeks, in T1 at 6 weeks and in T1 and T6 conditions at 8 weeks of storage. The highest percentages of weight loss were showed by treatments T3 at 2 weeks, by T4 and T5 at 4 weeks, by T5 at 6 weeks and by T2 tubers at 8 weeks of storage (Table 4.14).

**Table 4. 14: Tubers percentage weight loss from harvest to 8 weeks in storage**

| Treatments | | Tubers weight loss (%) | | | |
|---|---|---|---|---|---|
| Age | Substrate | at 2 WIS | at 4 WIS | at 6 WIS | at 8 WIS |
| 25 days weaning | Soil: Cow dung 1:0 = T1 | 4.46$^{a}$ | 6.76$^{a}$ | 9.37$^{a}$ | 12.50$^{a}$ |
| | 2:1 = T2 | 4.84$^{a}$ | 7.46$^{b}$ | 10.87$^{bc}$ | 18.48$^{d}$ |
| | 1:1 = T3 | 6.50$^{c}$ | 8.50$^{c}$ | 12.54$^{d}$ | 17.75$^{cd}$ |
| 45 days weaning | 1:0 = T4 | 5.05$^{ab}$ | 8.26 $^{c}$ | 10.73$^{bc}$ | 15.08$^{b}$ |
| | 2:1 = T5 | 5.53$^{b}$ | 8.27$^{c}$ | 11.58$^{c}$ | 15.86$^{bc}$ |
| | 1:1 = T6 | 4.43$^{a}$ | 7.07$^{ab}$ | 10.31$^{b}$ | 13.60$^{ab}$ |
| p-value | | <.001 | <.001 | <.001 | 0.003 |

Letters represent significant differences among treatments according to Fisher's LSD test at 5% significance. Comparisons direction is ascending. WIS: week in storage. p-value= probability value

### 4.2.12. Tuber sprout numbers after 3 months in storage

The results of potato tuber sprout number after three (3) months in storage are presented in Table 4.15. Significant differences ($p< 0.01$) occurred for weaning age of *in vitro* plantlet.

The plantlets weaned for 45 days had the lowest tuber sprout number whilst plantlets weaned 25 days recorded the highest number of sprouts after three months of storage.

Significant differences ($p<0.01$) were observed for the substrate used for transplantation. The substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) significantly reduced the number of sprouts on a tuber compared to the substrate S1 (soil and cow dung 1:0) condition which produced the highest number of sprouts on a tuber after three months of storage. No significant difference ($p>0.05$) was observed in Age and Substrate interaction.

**Table 4. 15: Tuber sprout number after 3 months in storage**

| | Substrate (Soil and Cow dung) | | | Mean |
|---|---|---|---|---|
| Age | S1 = 1:0 | S2 = 2:1 | S 3= 1:1 | (Age) |
| 25 days weaning | 9.45 | 7.72 | 7.40 | *8.19 b* |
| 45 days weaning | 6.92 | 6.50 | 5.75 | *6.39 a* |
| Mean (Substrate) | *8.19 b* | *7.11 a* | *6.58 a* | |
| | Age | Substrate | Age*Substrate | |
| p-value | <.001 | 0.004 | 0.287 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance. Comparisons direction is ascending. p-value= probability value

### 4.2.13. Sprout number per eye after 3 months storage

Table 4.16. shows the effect of weaning age of *in vitro* plantlet and substrate for transplanting on sprout numbers per eye after 3 months of storage. There was significant difference ($P<0.05$) among the means for weaning age. The lowest number of sprouts per eye was observed on tubers which were grown at a weaning age of 45 days. The highest number of sprouts per eye was obtained in tubers from the weaning age 25 days.

Significant differences ($p< 0.05$) were observed depending on the substrate used for transplanting. The substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) produced tubers with the lowest number of sprouts per eye. The highest number of sprouts per eye was

recorded in tubers which were produced on substrate S1. Also, there were significant differences ($p<0.05$) for age and substrate interaction. The lowest number of sprouts per eye was showed by treatments T2, T3, T4, T5 and T6. T1 was the only treatment in which tubers showed the highest number of sprouts per eye.

**Table 4. 16: Sprout numbers per eye after 3 months storage**

| | Substrate (Soil and Cow dung) | | | Mean |
|---|---|---|---|---|
| Age | S1 = 1:0 | S2 = 2:1 | S 3= 1:1 | (Age) |
| 25 days weaning | 1.90 [b] | 1.42 [a] | 1.35 [a] | *1.56 b* |
| 45 days weaning | 1.31 [a] | 1.20 [a] | 1.25 [a] | *1.25 a* |
| Mean (Substrate) | *1.60 b* | *1.31 a* | *1.30 a* | |
| | Age | Substrate | Age*Substrate | |
| p-value | 0.001 | 0.008 | 0.047 | |

Letters represent significant differences among means according to Fisher's LSD test at 5% significance. Comparisons direction is ascending. p-value= probability value

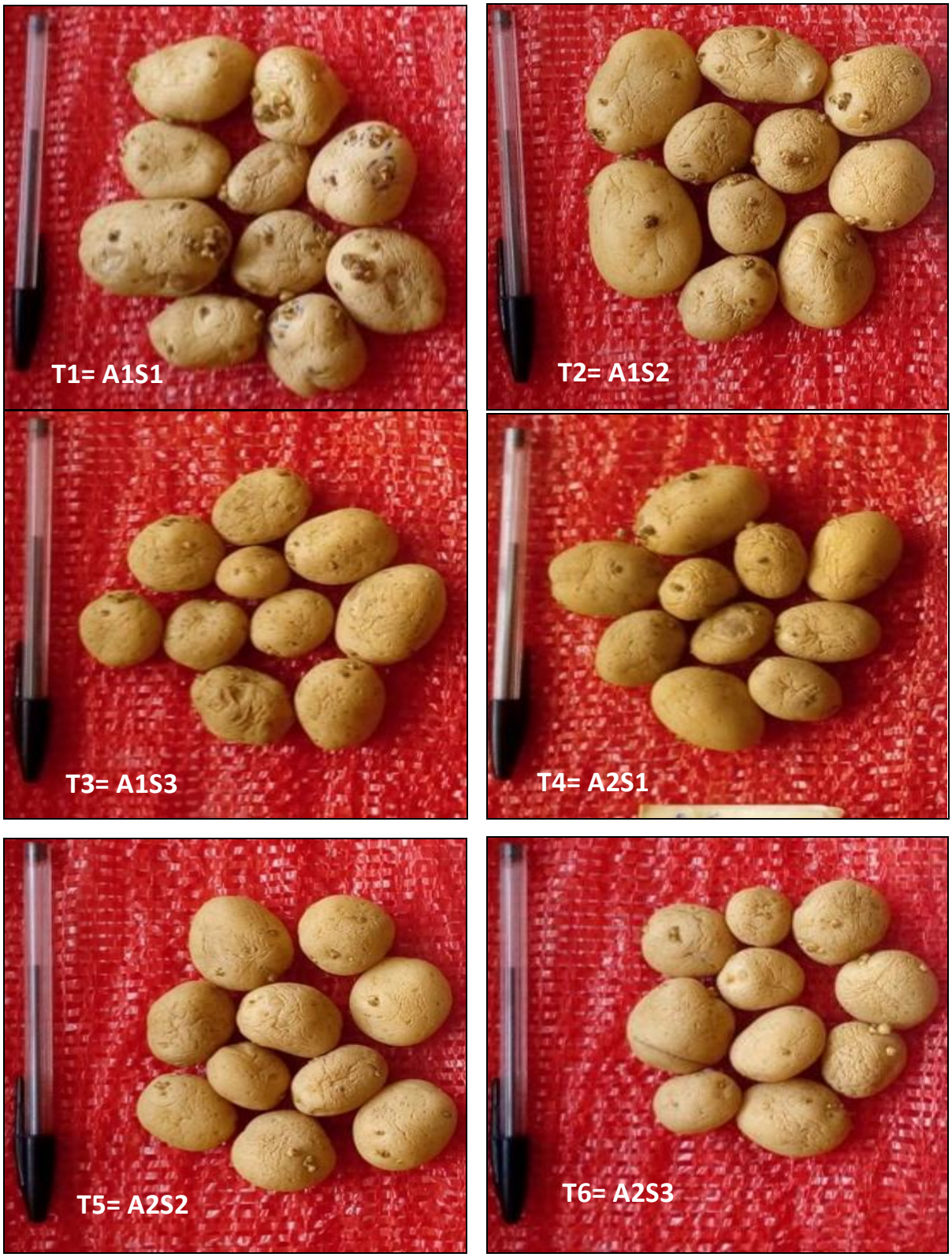


**Figure 4.7: Tubers produced under the 6 treatment conditions 3 months after storage**

A1= plantlet weaning age 25 days; A2= plantlet weaning age 45 days.

S1= soil and cow dung 1:0; S2= soil and cow dung 2:1 and S3= soil and cow dung 1:1

# CHAPTER FIVE

# 5.0. DISCUSSION

## 5.1. effects of culture media on plants growth *in vitro*

The results of the study showed that all growth and development parameters after 30 days of *in vitro* culturing stimulated by the culture medium M7 where 40 ml of coconut water and 250 mg of potassium nitrate per liter of MS medium was used. This stimulation maybe due to the presence of adequate quantities of coconut water and potassium nitrate. The coconut water contains several organic compounds such as sucrose; phytohormones: auxin, cytokinins, gibberellins and abscisic acid; vitamins of B group: nicotinic acid, biotin, folic acid and mineral nutrients: potassium, sodium, calcium, phosphorous (Reddy and Lakshmi, 2014; Yong *et al*., 2009 ; Mullukattil, 2013). The growth of *in vitro* explants could be stimulated on a medium supplemented with 5% to 15% of coconut water (Prades *et al*., 2011 ; CIDES, 1999). This quantity (5%) is close to the concentration of 40 ml of coconut water per liter medium (4% of culture medium volume). The 250 mg of potassium nitrate combined with coconut water at 40 ml per liter MS medium played a role in the plant growth stimulation observed.

The media M7 (MS + 40 ml/l of coconut water + 250 mg of potassium nitrate), M8 (MS + 40 ml/l of coconut water +1000 mg of potassium nitrate), M10 (MS+100 ml/l of coconut water + 1000 mg of potassium nitrate) and M11 (MS+300 ml/l of coconut water +250 mg of potassium nitrate) promoted faster shoots emergence than the other media resulting in 100 % emergence, compared to M1 (MS medium) control which recorded 83 % shoot emergence.

The greatest *in vitro* plantlet height (16 cm) was shown by the culture medium M7 (MS + 40 ml /l of coconut water + 250 mg of potassium nitrate) after 30 days of culturing compared to the MS medium used as control which recorded a maximum of 10 cm shoot length. This maybe explained as the presence of the adequate quantity of growth promoting substances in coconut water such as $N_6$-Furfuryladenine (Kinetin), which promotes cell division in plants

(Yong *et al*., 2009) as well as the effect of the nitrogen contained in potassium nitrate on the cultured explants. Nitrogen is an essential element for cell formation and photosynthesis (chlorophyll). It is the main factor of plant growth and a quality factor that influences the protein content of plants (UNIFA, 2005).

The highest number of nodes (11.5) after 30 days of *in vitro* culturing was observed in culture medium M7 containing 40 ml of coconut water and 250 mg of potassium nitrate per liter as supplement components to MS medium. However, the medium M1 (MS) which is the control medium recorded only 7 nodes. The number of node per plant refers to the plant multiplication rate. The highest mean number of leaves (13.5) was recorded by the culture medium M7. But the control, the culture medium M1 (MS) recorded fewer leaves than medium M7 containing 40ml/l of coconut water and 250 mg /L of potassium nitrate were used as supplements to MS medium. (Afshin *et al*., 2011) reported that cytokinin (kinetin) is known to promote bud formation in many *in vitro* cultured organs such as leaves and node numbers.

The roots number per plant was greatest in culture media M7 and M8 (9 roots) versus medium M1 (MS) which recorded 5.1 roots. This result maybe due to the presence of indole-3-acetic acid (auxin) in the coconut water. The IAA plays a significant role in plant root emergence and growth regulation (Muhammad *et al*., 2015). However, in this study it was realized that media M6, M11 and M12 where 300 ml of coconut water per liter were used, recorded the least root numbers per plant 1.6, 2.2 and 1.2 roots respectively. This can be explained by the higher concentration of cytokinin contained in 300 ml of coconut water per liter of medium. CIDES (1999) reported that cytokinin at higher concentrations in culture medium is responsible for multiple shoots and callus formation.

The highest plantlet fresh and dry weights (respectively 545.5 and 36.4 mg) were observed in culture medium M7 (MS + 40 ml/l of coconut water and 250 mg of potassium nitrate) after 30 days after propagating *in vitro*. In *Calanthe* hybrids 50 ml/l of coconut water in culture medium increased plant fresh and dry weights (Abdullahil *et al*., 2011). In this study, the maximum potato plantlets fresh and dry weights were observed in culture medium M7 where MS was supplemented with 40 ml/l of coconut water and 250 mg of potassium nitrate. This concentration of coconut water is close to the 50 ml/l cited above.

The interaction of lower concentrations of coconut water (40 ml) and potassium nitrate (250 mg) per liter of MS medium affected all the *in vitro* growth parameters significantly after 30 days of culturing. The higher concentrations of coconut water (300 ml per liter of MS medium) blocked plant growth in height and roots emergence.

### 5.2. Physiological age and substrate effects on mini tubers production *in vivo*

The pH of the three substrates used in the experiment were reduced compared to the initial state of the soil before mixture of substrates and planting. This decrease in pH level for all substrates could be due to the application of cow dung or mineral fertilizer (NPK) which was applied to all the three substrates. This result is consistent with those obtained by Suh *et al.*,(2015) and Monirul *et al*., (2013). The reduction was more marked with the substrate S1 (soil and cow dung 1:0) which received no cow dung but had the application of mineral fertilizer (1000 kg /ha of NPK 17:17:17). In this substrate a reduction of 5.7 % of the initial pH was observed (7.68).

The maximum plant re-establishment percentage recorded in treatments T1 (age 25 days and substrate only soil) and T2 (age 25 days and substrate 2:1 Soil and Cow dung).

The *in vivo* survival and growth of plantlets may be affected by the growing substrate pH (Conner and Thomas, 1982) . The laboratory analysis of substrates showed that the lower pH is in substrate S1 (7.24), where the maximum plant survival percentage was observed. The substrate S2 (soil and cow dung 2:1) condition promoted significantly higher plant stem length, stem diameter as well as plant fresh and dry biomass formation. The effect of plantlet weaning age was not significant for plant stem diameter and plant fresh and dry weight. The treatments T2 (59.28 t/ha) and T1 (58.12 t/ha) recorded highest yield of tubers. This could be due to the level of organic carbon and the pH in S2. However, S3 presented higher amount of organic carbon than S2 but showed a higher alkaline pH level than others substrates, which could be a disadvantage for plant growth and tuber formation. According to Mimouni (2011) the alkaline pH of soil can block the absorption of phosphorus, boron, copper, iron, manganese and zinc.

The maximum number of tubers per plant occurred in the treatment T5 (weaning age 45 days and substrate 2:1 Soil and Cow dung). This may be due to the high nutrient level of the two substrates and the ability to increase substrate nutrient availability through high biological activity (Pengthamkeeratia, 2011).

Plantlets with weaning age 25 days produced significant numbers of grade A. The substrates S1 and S3 (soil and cow dung 1:1) means were significant in grade A.

The composition of the substrate positively affected weight loss (%) of stored tubers after 8 weeks. The lowest percentages weight loss of stored tubers were obtained in substrates without cow dung. The Substrate S1 (only soil) showed the same trend of low weight loss of tubers from the 2$^{nd}$ week to the 8$^{th}$ week of storage. The highest percentages of weight loss in stored tubers were obtained in Substrates S2 (Soil and Cow dung 2:1) and S3 (soil and cow dung 1:1) where the substrate is contained cow dung. The highest percentages of weight loss

can be due to the lower immaturity of tubers' skin in Substrates S2 (Soil and Cow dung 2:1) and S3 (soil and cow dung 1:1). At harvest it was observed that in Substrate S1 (only soil) plots, all the plants had reached senescence stage (physiological maturity) which generally resulting in the progressive yellowing of the leaves from the base to the top of the plant with subsequent drying out. However, in substrates S2 (Soil and Cow dung 2:1) and S3 (soil and cow dung 1:1), plants in all plots were still growing. This can lead to the presence of immature tubers among the tubers harvested from these treatment plots. A study carried out by ARVALIS in collaboration with Paris IV University showed that water loss in tubers was proportional to Vapor Pressure Deficit (VPD) between the tuber and the ambient air. it also showed that water loss was significantly higher in case of immaturity of the skin or injury of tubers (Martin, 2006).

The number of sprouts per tuber and sprouts number per eye on tubers were minimized significantly for weaning age 45 days and the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) as compared to the weaning age 25 days, and substrate S1 (soil and cow dung 1:0) which promoted early emergence and higher sprouts number on tuber after three months of storage. Tuber sprouting is one of the most important factors in the deterioration of tuber quality during storage (ARVALIS /Institut-du-végétal, 2013). Reust (1982) reported that the tuber dormancy time for the small size grade is longer than that of larger sizes. In the present study it was in weaning age 45 days and the substrates S2 (soil and cow dung 2:1) conditions that maximum quantities of small size grade tubers were produced.

# CHAPTER SIX

# 6.0. CONCLUSIONS AND RECOMMENDATIONS

## 6.1. Conclusions

The results of the study showed that the culture medium M7 (MS+40 ml/l of coconut water +250 mg of potassium nitrate) had the highest plantlets multiplication rate with an average number of 11.45 nodes per plant after 30 days of *in vitro* culture. The interaction between lower concentrations of coconut water (40 ml) and potassium nitrate (250 mg) per liter of MS medium has significant and positive effects on all the *in vitro* growth parameters. Also, higher concentrations of coconut water (300 ml) potassium nitrate (1000 mg) per liter of MS medium adversely affects plant growth parameters during 30 days of culturing. Plantlet weaning age of 25 days and the post-flask culture substrate S1 (only soil) appears to give the best plant survival percentage at 20 days after transplanting *in vivo*. The substrate S2 (soil and cow dung 2:1) positively affects all plant *in vivo* growth and tuber yield parameters. The weaning age 45 days and the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) conditions produced significantly reduced mini tubers quality loss in storage 3 months after harvest.

## 6.2. Recommendations

It is recommended to undertake further research work on coconut water concentrations for *in vitro* plantlets multiplication and substrate compositions studies for *in vivo* culture on other potato varieties.

Also, from the results obtained in this study it is recommended that the coconut water concentration used as supplement to MS medium for potato *in vitro* culture should be 40 ml per liter of medium for better and more rapid growth of plantlets.

It is also recommended that the proportion of cow dung used in substrate composition should not exceed the soil and cow dung 2:1 mixture for maximum post-transplanting plant re-establishment rate *in vivo* and for rapid maturity of mini-tubers.

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# APPENDICES

**Appendix 1: ANOVA results performed for the media evaluation *in vitro***

Combined ANOVA for plantlets shoot emergence

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 2407.4 | 218.9 | 2.18 | 0.039 |
| Residual | 36 | 3611.1 | 100.3 | | |
| Total | 47 | 6018.5 | | | |
| Least significant differences of means (5% level) | | | | 14.36 | |
| s.e. | | | | 10.02 | |
| cv% | | | | 10.80 | |

Combined ANOVA for plant height *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 505.7667 | 45.9788 | 281.98 | <.001 |
| Residual | 36 | 5.8700 | 0.1631 | | |
| Total | 47 | 511.6367 | | | |
| Least significant differences of means (5% level) | | | | 0.58 | |
| s.e. | | | | 0.40 | |
| cv% | | | | 4.10 | |

Combined ANOVA for number of leaves *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 123.3100 | 11.2100 | 61.33 | <.001 |
| Residual | 36 | 6.5800 | 0.1828 | | |
| Total | 47 | 129.8900 | | | |
| Least significant differences of means (5% level) | | | | 0.61 | |
| s.e. | | | | 0.43 | |
| cv% | | | | 4.40 | |

Combined ANOVA for number of roots *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 307.69667 | 27.97242 | 437.83 | <.001 |
| Residual | 36 | 2.30000 | 0.06389 | | |
| Total | 47 | 309.99667 | | | |
| Least significant differences of means (5% level) | | | | 0.36 | |
| s.e. | | | | 0.25 | |
| cv% | | | | 5.00 | |

Combined ANOVA for number of nodes *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 123.3100 | 11.2100 | 61.33 | <.001 |
| Residual | 36 | 6.5800 | 0.1828 | | |
| Total | 47 | 129.8900 | | | |
| Least significant differences of means (5% level) | | | | 0.61 | |
| s.e. | | | | 0.43 | |
| cv% | | | | 5.50 | |

Combined ANOVA for internodes length *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 2.097323 | 0.190666 | 39.31 | <.001 |
| Residual | 36 | 0.174625 | 0.004851 | | |
| Total | 47 | 2.271948 | | | |
| Least significant differences of means (5% level) | | | | 0.10 | |
| s.e. | | | | 0.07 | |
| cv% | | | | 5.70 | |

Combined ANOVA for plant fresh weight *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 668615.8 | 60783.3 | 418.34 | <.001 |
| Residual | 36 | 5230.7 | 145.3 | | |
| Total | 47 | 673846.4 | | | |
| Least significant differences of means (5% level) | | | | 17.29 | |
| s.e. | | | | 12.05 | |
| cv% | | | | 4.60 | |

Combined ANOVA for plant dry weight *in vitro*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Media | 11 | 1448.197 | 131.654 | 112.52 | <.001 |
| Residual | 36 | 42.120 | 1.170 | | |
| Total | 47 | 1490.317 | | | |
| Least significant differences of means (5% level) | | | | 1.55 | |
| s.e. | | | | 1.08 | |
| cv% | | | | 5.20 | |

**Appendix 2: ANOVA results performed for the age and substrate evaluation *in vivo***

Combined ANOVA for plant re-establishment *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 23.783 | 7.928 | 0.8 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 3275.005 | 3275.005 | 329.24 | <.001 |
| Substrate | 2 | 1008.716 | 504.358 | 50.7 | <.001 |
| Age.Substrate | 2 | 288.053 | 144.026 | 14.48 | <.001 |
| Residual | 15 | 149.208 | 9.947 | | |
| Total | 23 | 4744.765 | | | |
| Least significant differences of means (5% level) | | Age 2.74 | Substrate 3.36 | Age* Substrate 4.75 | |
| s.e. | | | | | 3.15 |
| cv% | | | | | 3.70 |

Combined ANOVA for plant height at 15 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.58645 | 0.19548 | 2.37 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.75260 | 0.75260 | 9.14 | 0.009 |
| Substrate | 2 | 1.40676 | 0.70338 | 8.54 | 0.003 |
| Age.Substrate | 2 | 4.86541 | 2.43270 | 29.55 | <.001 |
| Residual | 15 | 1.23508 | 0.08234 | | |
| Total | 23 | 8.84630 | | | |
| Least significant differences of means (5% level) | | Age 0.2497 | Substrate 0.3058 | Age* Substrate 0.4325 | |
| s.e. | | | | | 0.2869 |
| cv% | | | | | 4.3 |

Combined ANOVA for plant height at 40 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 1.078 | 0.359 | 0.35 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 465.520 | 465.520 | 449.79 | <.001 |
| Substrate | 2 | 55.026 | 27.513 | 26.58 | <.001 |
| Age.Substrate | 2 | 43.181 | 21.590 | 20.86 | <.001 |
| Residual | 15 | 15.525 | 1.035 | | |
| Total | 23 | 580.33 | | | |

| Least significant differences of means (5% level) | Age | Substrate | Age* Substrate |
|---|---|---|---|
| | 0.89 | 1.08 | 1.53 |

| | |
|---|---|
| s.e. | 1.02 |
| cv% | 5.10 |

Combined ANOVA for plant height at 60 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 56.145 | 18.715 | 2.79 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 179.854 | 179.854 | 26.79 | <.001 |
| Substrate | 2 | 344.791 | 172.395 | 25.68 | <.001 |
| Age.Substrate | 2 | 143.643 | 71.821 | 10.70 | 0.001 |
| Residual | 15 | 100.688 | 6.713 | | |
| Total | 23 | 825.120 | | | |

| Least significant differences of means (5% level) | Age | Substrate | Age* Substrate |
|---|---|---|---|
| | 2.254 | 2.761 | 3.905 |

| | |
|---|---|
| s.e. | 2.59 |
| cv% | 5.8 |

Combined ANOVA for plant stem diameter at 15 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.12753 | 0.04251 | 3.31 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.28167 | 0.28167 | 21.91 | <.001 |
| Substrate | 2 | 0.04623 | 0.02312 | 1.80 | 0.200 |
| Age.Substrate | 2 | 0.12023 | 0.06012 | 4.68 | 0.026 |
| Residual | 15 | 0.19287 | 0.01286 | | |
| Total | 23 | 0.76853 | | | |

| Least significant differences of means (5% level) | Age | Substrate | Age* Substrate |
|---|---|---|---|
| | 0.0987 | 0.1208 | 0.1709 |

| | |
|---|---|
| s.e. | 0.1134 |
| cv% | 9.4 |

Combined ANOVA for plant stem diameter at 40 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.01865 | 0.00622 | 0.35 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 16.68334 | 16.68334 | 936.99 | <.001 |
| Substrate | 2 | 0.93543 | 0.46772 | 26.27 | <.001 |
| Age.Substrate | 2 | 0.51870 | 0.25935 | 14.57 | <.001 |
| Residual | 15 | 0.26708 | 0.01781 | | |
| Total | 23 | 18.42320 | | | |
| Least significant differences of means (5% level) | | Age 0.1161 | Substrate 0.1422 | Age* Substrate 0.2011 | |
| s.e. | | | | 0.1334 | |
| cv% | | | | 3.1 | |

Combined ANOVA for plant stem diameter at 60 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 1.34255 | 0.44752 | 10.14 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.11207 | 0.11207 | 2.54 | 0.132 |
| Substrate | 2 | 1.08331 | 0.54165 | 12.28 | <.001 |
| Age.Substrate | 2 | 1.87466 | 0.93733 | 21.24 | <.001 |
| Residual | 15 | 0.66180 | 0.04412 | | |
| Total | 23 | 5.07438 | | | |
| Least significant differences of means (5% level) | | Age 0.1828 | Substrate 0.2239 | Age* Substrate 0.3166 | |
| s.e. | | | | 0.21 | |
| cv% | | | | 4.0 | |

Combined ANOVA for plant number of leaves at 40 days *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.7517 | 0.2506 | 2.21 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.0817 | 0.0817 | 0.72 | 0.410 |
| Substrat | 2 | 7.0758 | 3.5379 | 31.16 | <.001 |
| Age.Substrat | 2 | 0.1658 | 0.0829 | 0.73 | 0.498 |
| Residual | 15 | 1.7033 | 0.1136 | | |
| Total | 23 | 9.7783 | | | |
| Least significant differences of means (5% level) | | Age 0.2932 | Substrate 0.3591 | Age* Substrate 0.5079 | |
| s.e. | | | | 0.34 | |
| cv% | | | | 3.7 | |

Combined ANOVA for plant fresh weight *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 11.630 | 3.877 | 3.31 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.240 | 0.240 | 0.20 | 0.657 |
| Substrate | 2 | 682.727 | 341.364 | 291.43 | <.001 |
| Age.Substrate | 2 | 1085.732 | 542.866 | 463.46 | <.001 |
| Residual | 15 | 17.570 | 1.171 | | |
| Total | 23 | 1797.900 | | | |
| Least significant differences of means (5% level) | | Age 0.942 | Substrate 1.153 | Age* Substrate 1.631 | |
| s.e. | | | | 1.08 | |
| cv% | | | | 3.4 | |

Combined ANOVA for plant dry weight *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.35615 | 0.11872 | 3.03 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.49594 | 0.49594 | 12.65 | 0.003 |
| Substrate | 2 | 1.32438 | 0.66219 | 16.89 | <.001 |
| Age.Substrate | 2 | 2.80187 | 1.40094 | 35.72 | <.001 |
| Residual | 15 | 0.58823 | 0.03922 | | |
| Total | 23 | 5.56656 | | | |
| Least significant differences of means (5% level) | | Age 0.1723 | Substrate 0.2110 | Age* Substrate 0.2985 | |
| s.e. | | | | 0.20 | |
| cv% | | | | 8.8 | |

Combined ANOVA for Tuber yield t/ha *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 7.463 | 2.488 | 1.02 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 828.375 | 828.375 | 339.30 | <.001 |
| Substrate | 2 | 331.023 | 165.512 | 67.79 | <.001 |
| Age.Substrate | 2 | 732.310 | 366.155 | 149.97 | <.001 |
| Residual | 15 | 36.622 | 2.441 | | |
| Total | 23 | 1935.793 | | | |
| Least significant differences of means (5% level) | | Age 1.360 | Substrate 1.665 | Age* Substrate 2.355 | |
| s.e. | | | | 1.56 | |
| cv% | | | | 3.3 | |

Combined ANOVA for mean weight tuber *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.13991 | 0.04664 | 0.61 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 64.33067 | 64.33067 | 839.46 | <.001 |
| Substrate | 2 | 23.16560 | 11.58280 | 151.15 | <.001 |
| Age.Substrate | 2 | 31.08231 | 15.54116 | 202.80 | <.001 |
| Residual | 15 | 1.14949 | 0.07663 | | |
| Total | 23 | 119.86798 | | | |
| Least significant differences of means (5% level) | | Age 0.2409 | Substrate 0.2950 | Age* Substrate 0.4172 | |
| s.e. | | | | 0.28 | |
| cv% | | | | 3.7 | |

Combined ANOVA for number of tubers per plant *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.05833 | 0.01944 | 0.98 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 7.93500 | 7.93500 | 401.21 | <.001 |
| Substrate | 2 | 27.85083 | 13.92542 | 704.09 | <.001 |
| Age.Substrate | 2 | 0.25750 | 0.12875 | 6.51 | 0.009 |
| Residual | 15 | 0.29667 | 0.01978 | | |
| Total | 23 | 36.39833 | | | |
| Least significant differences of means (5% level) | | Age 0.1224 | Substrate 0.1499 | Age* Substrate 0.2120 | |
| s.e. | | | | 0.14 | |
| cv% | | | | 2.1 | |

Combined ANOVA for tuber size grade A *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 21.376 | 7.125 | 1.29 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 652.636 | 652.636 | 117.89 | <.001 |
| Substrate | 2 | 99.785 | 49.893 | 9.01 | 0.003 |
| Age.Substrate | 2 | 126.426 | 63.213 | 11.42 | <.001 |
| Residual | 15 | 83.041 | 5.536 | | |
| Total | 23 | 983.263 | | | |
| Least significant differences of means (5% level) | | Age 2.047 | Substrate 2.508 | Age* Substrate 3.546 | |
| s.e. | | | | 2.353 | |
| cv% | | | | 9.8 | |

Combined ANOVA for tuber size grade B *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 20.80 | 6.93 | 0.48 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 1158.71 | 1158.71 | 79.68 | <.001 |
| Substrate | 2 | 252.60 | 126.30 | 8.68 | 0.003 |
| Age.Substrate | 2 | 320.14 | 160.07 | 11.01 | 0.001 |
| Residual | 15 | 218.14 | 14.54 | | |
| Total | 23 | 1970.39 | | | |
| Least significant differences of means (5% level) | | Age 3.318 | Substrate 4.064 | Age* Substrate 5.748 | |
| s.e. | | | | 3.81 | |
| cv% | | | | 5.3 | |

Combined ANOVA for tuber size grade C *in vivo*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 2.301 | 0.767 | 0.12 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 72.133 | 72.133 | 11.63 | 0.004 |
| Substrate | 2 | 59.459 | 29.729 | 4.79 | 0.025 |
| Age.Substrate | 2 | 48.379 | 24.189 | 3.90 | 0.043 |
| Residual | 15 | 93.059 | 6.204 | | |
| Total | 23 | 275.331 | | | |
| Least significant differences of means (5% level) | | Age 2.167 | Substrate 2.654 | Age* Substrate 3.754 | |
| s.e. | | | | 2.49 | |
| cv% | | | | 62.1 | |

Combined ANOVA for tuber weight loss at 2 weeks in storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 1.8736 | 0.6245 | 2.72 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.4076 | 0.4076 | 1.77 | 0.203 |
| Substrate | 2 | 2.0543 | 1.0272 | 4.47 | 0.030 |
| Age.Substrate | 2 | 9.7454 | 4.8727 | 21.21 | <.001 |
| Residual | 15 | 3.4464 | 0.2298 | | |
| Total | 23 | 17.5273 | | | |
| Least significant differences of means (5% level) | | Age 0.4171 | Substrate 0.5108 | Age* Substrate 0.7224 | |
| s.e. | | | | 0.48 | |
| cv% | | | | 9.3 | |

Combined ANOVA for tuber weight loss at 4 weeks in storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.9967 | 0.3322 | 2.70 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.5034 | 0.5034 | 4.09 | 0.061 |
| Substrate | 2 | 0.5535 | 0.2768 | 2.25 | 0.140 |
| Age.Substrate | 2 | 9.3814 | 4.6907 | 38.13 | <.001 |
| Residual | 15 | 1.8453 | 0.1230 | | |
| Total | 23 | 13.2804 | | | |
| Least significant differences of means (5% level) | | Age 0.3052 | Substrate 0.3738 | Age* Substrate 0.5286 | |
| s.e. | | | | 0.35 | |
| cv% | | | | 4.5 | |

Combined ANOVA for tuber weight loss at 6 weeks in storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 4.2044 | 1.4015 | 4.10 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.0204 | 0.0204 | 0.06 | 0.810 |
| Substrate | 2 | 8.8463 | 4.4232 | 12.95 | <.001 |
| Age.Substrate | 2 | 14.5728 | 7.2864 | 21.34 | <.001 |
| Residual | 15 | 5.1223 | 0.3415 | | |
| Total | 23 | 32.7663 | | | |
| Least significant differences of means (5% level) | | Age 0.508 | Substrate 0.623 | Age* Substrate 0.881 | |
| s.e. | | | | 0.58 | |
| cv% | | | | 5.4 | |

Combined ANOVA for tuber weight loss at 8 weeks in storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 17.645 | 5.882 | 2.11 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 11.701 | 11.701 | 4.19 | 0.058 |
| Substrate | 2 | 46.094 | 23.047 | 8.26 | 0.004 |
| Age.Substrate | 2 | 49.778 | 24.889 | 8.92 | 0.003 |
| Residual | 15 | 41.843 | 2.790 | | |
| Total | 23 | 167.062 | | | |
| Least significant differences of means (5% level) | | Age 1.453 | Substrate 1.780 | Age* Substrate 2.517 | |
| s.e. | | | | 1.67 | |
| cv% | | | | 10.7 | |

Combined ANOVA for number of sprouts on tuber after 3 months storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 1.0483 | 0.3494 | 0.54 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 19.4400 | 19.4400 | 30.04 | <.001 |
| Substrate | 2 | 10.7858 | 5.3929 | 8.33 | 0.004 |
| Age.Substrate | 2 | 1.7575 | 0.8788 | 1.36 | 0.287 |
| Residual | 15 | 9.7067 | 0.6471 | | |
| Total | 23 | 42.7383 | | | |

| Least significant differences of means (5% level) | Age | Substrate | Age* Substrate |
|---|---|---|---|
| | 0.700 | 0.857 | 1.212 |
| s.e. | | | 0.80 |
| cv% | | | 11.0 |

Combined ANOVA for number of sprouts per eye on tuber after 3 months storage

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block stratum | 3 | 0.05853 | 0.01951 | 0.56 | |
| Block.*Units* stratum | | | | | |
| Age | 1 | 0.54152 | 0.54152 | 15.59 | 0.001 |
| Substrate | 2 | 0.47911 | 0.23956 | 6.90 | 0.008 |
| Age.Substrate | 2 | 0.26306 | 0.13153 | 3.79 | 0.047 |
| Residual | 15 | 0.52100 | 0.03473 | | |
| Total | 23 | 1.86322 | | | |

| Least significant differences of means (5% level) | Age | Substrate | Age* Substrate |
|---|---|---|---|
| | 0.1622 | 0.1986 | 0.2809 |
| s.e. | | | 0.19 |
| cv% | | | 13.3 |