# *IN VITRO* REGENERATION OF CASTOR PLANT (*Ricinus communis L.*) UNDER THE INFLUENCE OF SUCROSE OSMOTIC STRESS

**OLUWOLE LOLA VICTORIA**

**MATRIC NUMBER:**

**BTH/11/0261**

**A FINAL PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCE AND BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE(B.Sc.)DEGREE IN PLANT SCIENCE AND BIOTECHNOLOGY OF THE FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE.NIGERIA**

**OCTOBER, 2015**

CHAPTER THREE



CHAPTER FOUR

## LIST OF FIGURES

**FIGURES** **PAGE**



## LIST OF PLATES

**PLATES** **PAGE**

## LIST OF TABLES

**TABLE** **PAGE**

## ABSTRACT

Environmental stress results in water deficiency for plant, thus impairing its numerous biological roles. In vitro screening for stress tolerance will have its significance in identifying accessions with optimal stress tolerance and productivity. Studies on castor plant (*Ricinus communis*) could improve our understanding of the mechanism of plant resistance to drought stress. This investigation therefore was aimed at examining the effect of osmotic stress generated by sucrose on shoot length, fresh weight shoot, fresh weight leaves, plant regeneration and chlorophyll content related to drought stress. Nodes of three *Ricinus* accessions (accessions 004, 005, 006) were cultured on MS (MS macro and microelements) medium with addition of 1.6 mg BAP (6-benzylaminopurine) and 0.08 mg of IAA (Indole acetic acid). Different levels of osmotic stress were induced by using two concentration of sucrose (0% and 6%) in MS medium. Accession 004 is resistance to drought stress than accession 005 and 006. Accession 004 under control and sucrose levels has the highest growth parameters such as shoot fresh weight, plant regeneration percentage, plant height and leaves fresh weight than other accessions 005 and 006 but there was no significant difference in leaves numbers among the accessions. In turn accession 004 has the highest chlorophyll a and carotene than the other accessions. Accession 006 showed reduction in chlorophyll a content and physiological growth parameters than other accessions raised under 6% sucrose. Therefore, accession 004 is resistance to drought stress whereas; accession 006 is a drought sensitive. Farmers especially those in semi-arid and arid region of Nigeria are therefore encourage to cultivate castor accession 004 in order to obtain optimal castor yield.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the study

The castor plant (*Ricinus communis*) is a species of flowering plant belonging to the family of Euphorbiaceae and is one of the medicinally important oil seed crop (Kumari *et al.,* 2008). Castor plants are also referred to as castor oil plant, castor bean plant, wonderboom, Dautra, Eranda, plama Christi (Armstrong, 1982). Castor is a large perennials often developing into small trees, and short-internodes types are commonly referred to as giant and dwarf castor types respectively (Weiss, 1983). The plant may grow up to a height of 6 to 15 feet and can leave for many years and the large, palmately lobed leaves may be over 20 inches (Khahagi, 2007). However, castor grows at an amazingly fast rate, if they are situated in full sun and provided with ample fertilizer and water.

Castor oil can be used to treat infestation of intestinal worms. Infusion of leaves can be used as a remedy for rash, itch and eye inflammation. An alcoholic extract of the leaf was used to protect the liver from damage from certain poison (Joshi *et al.,* 2004). Methanolic extracts of the leaves of *Ricinus communis* were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antibacterial properties. The extract was not toxic (Oyewole *et al.,* 2003). The decoction of leaves was used for skin diseases, diarrhea and kidney, urinary bladder infections (Boulos, 1983). Water extract of the root bark showed analgesic activity in rats (Williamson, 2002). Antihistamine and ant-inflammatory properties were found in ethanolic extract of *Ricinus communis* (Lomash *et al.,* 2010). Extract of *Ricinus communis* exhibited acaridal and insecticidal activities against the adult of *Haemaphysalis bispinosa* Neumann (Acarina: Ixodidae and hematophagus fly *Hippobosca masculata* Leach (Diptera: Hippobosciidae (Bagavan *et al.,* 2010). Castor seed contains ricinoleic acid which is

the main component of castor oil, and exerts anti-inflammatory effects (Viera *et al.,* 2000). It is believed that the antibacterial properties of the castor oil are mainly due to it high ricinoleic acid content (Oduvaido, 2007).

Castor seeds contain approximately 46-60% oil and are the only commercial source of ricinoleic acid that is used as industrial lubricants, paints, coating and plastics (Caupin, 1997, Ogunniya, 2006). The oil has great promises in being used as a source of biodiesel, the oil can also be used for manufacturing candles, soaps and cosmetics (Deore and Johnson, 2008). The oil has also been used as a lubricant in the internal combustion engines in Aeroplanes (Alam*et al.,* 2010). In the tropical region, India is the largest producer of castor oil, representing 60% of the global production followed by China and Brazil (FAO, 2006).

Plant regeneration by tissue culture techniques is the method used to improve production of high quality plants which are free of any disease and pest ensuring the maximum production potential of varieties that are genetically identical to parent as well as to one another (Raven *et al.,* 1999). This can be achieved by the process of either somatic embryogenesis or organogenesis.

Castor plant has long been recognized to have a drought hardy nature and exhibits a high developmental and physiological plasticity to drought stress (Vijaya Kumar *et al.,* 1996; Heckenberger et al. 1998; Schurret*et al.,* 2000). With regard to photosynthetic responses, Dai *et al.,* (1992) reported that under increased vapor pressure deficits, stomatal limitation may be responsible for the inhibitory effect of drought on photosynthesis in castor plants. Sausen and Rosa (2010) suggested that drought resistance of the castor bean could be related to a pronounced early growth response, an efficient stomatal control, and a capacity to keep high net $CO_2$ fixation. However, little is known about the physiological responses of castor plant. These responses are important in assessing its ability to adapt

to varying low-water conditions. Plastic responses include both inevitable effects of environmental limits on growth and physiology and adaptive adjustments that enable a given genetic individual to withstand sudden environmental changes (Sultan 2000). Plants respond to drought stress by altering a range of leaf characteristics, such as stomatal closure, leaf area reduction, and osmotic adjustment (Marron*et al.,* 2002). The stomatal closure and the decreased leaf area limit water loss but inevitably inhibit photosynthesis. These responses may not be considered as adaptive plasticity for plants. Therefore, the question arises whether there is some compensatory mechanism in drought-tolerant plants that enables them to maintain a high photosynthesis while limiting transpiration.

## 1.2 AIMS AND OBJECTIVES

- Identification of drought tolerant castor varieties
- In vitro production of castor plant under drought stress.
- Determination of growth and chlorophyll contents of castor plant under drought stress.

## 1.3 PROBLEM STATEMENT

Castor plant produces oil which is one of the world most useful and economically important natural plant oils. Despite its great economic value, an increase in drought stress poses threats to the availability of this plant. Hence, in vitro regeneration of castor plant under drought stress.

## 1.4 LITERATURE REVIEW

## 1.5 ORIGIN, DESCRIPTION AND DISTRIBUTION OF CASTOR PLANT

The castor plant (*Ricinus communis*) is a species of flowering plant belonging to the family of Euphorbiaceae and is one of the medicinally important oil seed crop (Kumari *et al.,* 2008). Castor plants are also referred to as castor oil plant, castor bean plant, wonderboom, Dautra, Eranda, plama Christi (Armstrong, 1982). Castor is the generic name of the North American beaver (Castor canadensis) and one of the brightest double stars in constellation Gemini. In Greek and Roman legend, castor was one of the twin sons of Jupiter and Leda (Weiss, 1971). Castor was apparently coined by English traders who confused it with the oil of another shrub, Vitex angus-castus, which the Spanish and Portuguese in Jamaica called "agno-castor (Armstrong, 1982). Castor plant varies greatly in its growth and appearance. It varies in growth habit, stems, colour of foliage, seed size and colour, and oil content, so that varieties often bear little resemblance to one another. Castor may be large perennials often developing into small trees, and short-internodes types are commonly referred to as giant and dwarf castor types respectively (Weiss, 1983). The pant may grow upto a height of 6 to 15 feet and can leave for many years and the large, palmately lobed leaves may be over 20 inches (Khahagi, 2007). However, castor grows at an amazingly fast rate, if they are situated in full sun and provided with ample fertilizer and water. The castor plant is considered by most authorities to be native of the Tropical Africa, and may have originated in Abyssinia, Ethopia (Weiss, 1971). Castor is indigenous to the southeastern Meditteranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (Philips, 1999).

## 1.6 MEDICINAL AND ECONOMIC USES OF CASTOR PLANT

Castor oil can be used to treat infestation of intestinal worms. Infusion of leaves can be used as a remedy for rash, itch and eye inflammation. An alcoholic extract of the leaf is used to protect the liver from damage from certain poison (Joshi*et al.,* 2004). Methanolic extracts of the leaves of *Ricinus communis* were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The extract was not toxic (Oyewole *et al.,* 2003). The decoction of leaves is used for skin diseases, diarrhea and kidney, urinary bladder infections (Boulos, 1983). Water extract of the root bark showed analgesic activity in rats (Williamson, 2002). Antihistamine and ant-inflammatory properties were found in ethanolic extract of *Ricinus communis* (Lomash *et al.,* 2010). Extract of *Ricinus communis* exhibited acaridal and insecticidal activities against the adult of *Haemaphysali sbispinosa* Neumann (Acarina: Ixodidae and hematophagus fly *Hippobosca masculata* Leach (Diptera: Hippobosciidae (Bagavan *et al.,* 2010). Castor seeds contains ricinoleic acid which is the main component of castor oil, and exerts anti-inflammatory effects (Viera*et al.,* 2000). It is believed that the antibacterial properties of the castor oil are mainly due to it high ricinoleic acid content (Oduvaido, 2007).

Castor seeds contain approximately 46-60% oil and are the only commercial source of ricinoleic acid that is used as industrial lubricants, paints, coating and plastics (Caupin, 1997, Ogunniya, 2006). The oil has great promises in being used as a source of biodiesel, the oil can also be used for manufacturing candles, soaps and cosmetics (Deore and Johnson, 2008). The oil has also been used as a lubricant in the internal combustion engines in Aeroplanes (Alam *et al.,* 2010).

In the tropical region, India is the largest producer of castor oil, representing 60% of the global production followed by China and Brazil (FAO, 2006).

## 1.7 ROLE OF REGENERATION OF PLANT IN VITRO

Plant regeneration by tissue culture techniques is the method of improving the quality and production of high quality plants which are free of any disease and pest ensuring the maximum production potential of varieties that are genetically identical to parent as well as to one another (Raven *et al.,* 1999). Regeneration of plant regeneration is achieved by the process of either somatic embryogenesis or organogenesis. Regeneration in plant tissue culture will be successful by maintaining various factors involved, including media factors and environmental factors. The media factors include media constituents, macronutrients, micronutrients, vitamins amino acids, carbon source, complex nutritive mixtures, gelling agents, activated charcoal, plant growth regulators and pH of the medium. Environmental factors on the other hand are the culture conditions under which explants are maintained. The environmental factors involved include the temperature and illumination of the culture room, agitation process and incubation period of the culture (Ahmed *et al.,* 2011). For the initiation of callus culture, the following factors are important: the origin of explants used for the establishment of callus culture, the cellular/tissue differentiation status, external plant growth regulators, culture media and culture conditions (Yeoman *et al.,* 1996). Cellular competence to plant hormones is understood as the status in which a cell must possess the ability to perceive a transducer and respond to a signal (Osborne *et al.,* 2005).

### 1.7.1 ORGANOGENESIS

Organogenesis refers to the formation of shoot/root. The callus may remain in a differentiated condition regardless of the hormones and nutrients to which it is exposed the secondary metabolites and these metabolites have biological activity (Ahmed *et al.,* 2008).

Organ formation generally follows cessation of unlimited proliferation of callus. Individual cells or group of cells of smaller dimensions may form small nets of cells scattered throughout the callus tissue, the so-called meristemoids. These meristemoids become transformed into cyclic nodules from which shoot bud or root primordial may grow as shoots/roots. Shoot bud formation may decrease with age and subculture duration of the callus tissue but the capacity of rooting may persist for longer period. In some calli, rooting occur more often than in other form of organogenesis. During organogenesis, if the roots are first formed, then it is very difficult to induce adventitious shoot formation from the same callus tissue. If the shoots are first formed, it may form roots later on or may remain rootless condition unless and until the shoots are transformed to another medium or hormones less medium or condition that induce root formation.

### 1.7.2 SOMATIC EMBRYOGENESIS

Somatic embryogenesis is the process by which a non-zygotic embryo is produced from plant tissue or cell which can develop from new plant. Formation of somatic embryos occurs in two step first callus is cultured onto auxin rich medium (2,4-D commonly used) forming embryogenic clumps, these clumps are then transferred into medium without auxins resulting in the formation of mature embryos. Growth and formation of mature embryos depends on auxins and nitrogen levels in the medium. Successful plant regeneration has been achieved in aloe vera by somatic embryogenesis.

In other words, groups of somatic cells/ tissues lead to the formation of somatic embryos which resemble the zygotic embryos of intact seeds and grow into seedlings on suitable medium. The primary somatic embryos are also capable of producing more embryos through secondary somatic embryogenesis. Plant regeneration via somatic embryogenesis produce an embryo and then a complete plants, has been demonstrated in many medicinal plant species (Tripathi and Tripathi, 2003).

Arumugam and Bhojwani (1990) noted the development of somatic embryos from zygotic embryos of *Podophyllum hexaandrum* on Msmedium containing BAP and IAA. Efficient development and germination of somatic embryos are prerequisites for commercial plantlet production.

Chand and Sahrawat (2002) reported the somatic embryogenesis of *Psoralea Corylifolia L.* from root explant on medium supplemented with NAA and BAP. Rooting shoot was best achieved using different concentrations of auxins.

In *A. maemelos* MS half strength medium supplemented with IAA proved better (Yadav and Singh, 2011). In *P.cineraria* ,rooting was achieved on half strength MS medium supplemented with 3.0mg/IBA (Kumar and Singh, 2009), while in *L. leucocephala*, NAA resulted in better root formation (Singh and Lal, 2007).Danso *et al* (2011) who indicated successful regeneration of plantlets from shoot tips explant for future genetic transformation of the plant. The callus formation including regeneration confirm the finding of Kumari *et al* (2008) who demonstrated the effect of growth regulator on multiples shoot formation from callus of *Ricinus communis*. In vitro propagation of *Ricinus communis* was achieved from shoot tip explants of 4 months old castor oil plant. Propagation from shoot tip was evaluated on a range of concentrations of Benzyladenine (BA) with indole-3-butyric acid (IBA) as 2.22 µM/L BA and 4.9 µM/L IBA, 1.11 µM/M BA and 0.48 µM/L IBA, 0.42 µM/L BA and 0.46 µM/L IBA and 0.44 µM/L BA and0.44 µM/L IBA. Higher regeneration potential that direct adventitious shoot induction was recorded highest on MS medium with 1.11 µM/L BA and 0.48 µM/L IBA(Nahar and Borna, 2012).

## 1.8 EFFECT OF DROUGHT STRESS ON AGRICULTURE SYSTEM

Globally, crop yield decreased by biotic or abiotic stresses. Drought, flooding, heat, wind and cold are the abiotic stresses. Agricultural scientists are facing the challenge of drought in the current situation of water shortage which may affect negatively the arable area. Drought is a meteorological term and take place when there is more moisture loss from soil surface and fewer water supplies to soil in the form of rainfall or other sources of precipitation. Drought is a serious threat for crop production and food security. During the drought conditions water potential and turgor are decreased and it situation disturbs the normal functioning of plant body (Hsiao, 1973).

Drought is a worldwide problem and dangerous for arable field crops growth and subsequently for food security (Jaleel *et al.,* 2009). Currently selection as criteria are applied for good variety selection as compare to breeding techniques which are time consuming (Zhu, 2002). Drought stress is globally renowned feature of climate, also an alarming threat to our unavoidable. Kramer (1980) studied that one third part of arable land of the world faces the water shortage which also disturb the crop production. Water is an integral part of plant body plays an important role in growth initiation, maintenance of developmental process of plant life and hence has pivotal function in crop production.

The irregular occurrence of drought periods accompanied by high temperature due to climatic changes influences the growth and yields of cultivars grown in agricultural fields. Many papers have reported the morphological and physiological response of plants to drought (Costa-Franca *et al.,* 2000; Lin & Markhart, 1996; Lizana *et al.,* 2006; Martinez *et al.,* 2007; Sariyeva *et al.,* 2009), but the effects of water supply on the physiological processes during different stages of crop development under field conditions have not been adequately studied. At field scale, drought and high (air/soil) temperature stress occur concurrently and not easily separable. Reduced evapotranspiration from a field due to

drought is associated with increases in the temperature of the soil surface and that of the plant. In well watered plants, high temperature increases the water vapour pressure within the leaf and hence the rate of transpiration increases with the consequence of lower leaf water potential (Hsiao, 1994). Water stress may arise from either excess or deficit. Some days of waterlogging induce a rapid increase in the stomatal resistance which in turn reduces transpiration and net photosynthesis as a result of stomatal closure. These symptoms are similar to the responses to water deficit stress (Takele & McDavid, 1995). The flooding resulted in stomatal closure due to the leaf dehydration caused by increased resistance to water uptake as a result of the lowered permeability of roots (Amico *et al.,* 2001). However, the more common water-related stress is the water deficit.

Plant responses to water stress differ significantly depending on (i) the intensity and duration of stress, on (ii) the plant species, and (iii) its stage of development. The most common field species can be categorized into three groups based on their sensitivity of drought. The tolerant group includes wheat, barley, sorghum, oat and alfalfa; the group with moderate sensitivity includes species as corn, sunflower, soybean, beans, peas; while potato, tomato and rice are belong to the extremely drought sensitive group (Heszky, 2007).The species ranged as tolerant are able to escape drought while those with moderate sensitivity can prevent the water deficit in their cells and tissues.

Various defence mechanisms have been developed in legumes in order to tolerate drought. The soil water content, high air/soil temperature and humidity adversely affect the shoot growth and yield of plants. Water deficit in the soil (-14.85 kPa) hampers the development and operation of root nodules of legumes. The high temperature (33°C) in the upper soil layer increases the number of nodules but decreases their size and the growth of the plants (Piha & Munns, 1987). Under severe dry conditions (-0.03- -0.5 MPa rooting medium water potential), the nitrogen-binding activity of root nodules decreases (Smith *et al.,* 1988) resulting in the decrease of leaf area and leaf weight of the plants. Root

characteristics, especially root length, root length density, and the number of thick roots, are important for plants to develop their aboveground parts by exploiting the soil available water. Expansive growth of leaves is also sensitive to water stress (Hsiao, 1994) and a short period (7 days) mild water stress (soil field capacity SFC 40%) already reduces the rate of leaf area development and leaf mass (Nemeskéri, 2001, Nemeskéri *et al.,* 2010). The changes in the morphology and anatomy of leaves as induced by drought are similar in many crops due to the same defence mechanisms (e.g. leaf movements). Most responses of plants to climatic factors such as water deficiency and high temperature under the field conditions are often different from that of plants grown in greenhouses (Nemeskéri *et al.,* 2010). The cropping system (outdoor or greenhouse) can affect plant response to drought, this creates further complexity as concern the appraisal of on-set of stress. Therefore, it is important to examine plant traits variation in response to drought in order to create a sort of stress marker which can be used for the irrigation schedules and the modeling of crop transpiration. The determination of drought-stress markers is important in vegetables to maintain their yield production and food quality. In this chapter the defensive strategies of some annual crops (i.e. green bean and green pea) against drought and the relationship markers as measured in various developmental stages and yield level will be presented.

## 1.9 GROWTH AND PRODUCTIVITY OF CASTOR PLANT UNDER DROUGHT SRESS

Drought is a major environmental factor that determines the growth, productivity and distribution of plants. It is the most serious and worldwide yield reducing stress in agriculture (Ober, 2008). Drought affects more than 10% of arable soil (Bray *et al.,* 2000; Zidenga, 2006) and this drought condition is continually distressed by the explosive increase of world population, continuous deterioration of arable land, shortage of fresh water, and the current climate change. The increase in drought stress threatens the global agriculture production and food availability. It has been estimated

that two thirds of the yield potential of major crops are routinely lost due to drought stress (Bray *et al.,* 2000; Lafitte *et al.,* 2004; Zidenga, 2006; Magombeyi and Taigbenu, 2008). Therefore, the sustainability of production will depend on the identification and development of new drought tolerant varieties (Cochard *et al.,* 2008). Studies on tolerance to drought stresses have been done on various crops such as rice, maize, potato, cassava, bean and others (Bartels and Sunkar, 2005; Hirasawa *et al.,* 2006).

Water availability is one of the most limiting environmental factors affecting crop productivity stress imposed during these period drastically affect crop growth ultimately leading to a massive loss in yield and quality (Pirzad *et al.,* 2011). Water stress usually induces the accumulation of Reactive Oxygen Species (ROS) (Sorial *et al.,* 2010). Reactive Oxygen Species such as $O_2$ and $H_2O_2$ are produced during photosynthesis,photorespiration, respiration, flowering and other reaction of cellular metabolism, plant possess a protective system composed of antioxidant such as peroxidase and catalase.

Catalase is primary $H_2O_2$ scavenger in the peroxisomes and the mitochondria. An increase in peroxidase activity has been reported as an early response to different stresses and may provide cell with resistance against formation of $H_2O_2$ which is formed when plants are exposed to stress factor (Zolfaghari *et al* ., 2010). Osmotic regulation will help in cell development and plant growth in water stress so plant reduce their osmotic and plant growth in water so plant reduce their osmotic potential for water absorption by congestion of proline (Keyvan, 2010).

Proline plays a role in stabilizing membrane and proteins. It can also act as an antioxidant and regulates the cytosolic acidity (Akhkha *et al.,* 2011).

A decrease in osmotic potential in response to water stress is a well-known mechanism by which many plants can cope with drought conditions, Most organisms increase the cellular concentration of osmotically active compounds, termed "compatible solutes", under desiccation by drought or lowering of osmotic potential Proline and soluble sugar are important solutes for adaptation to low water potential (Saglam *et al.,* 2010). Proline is one amongst the most important cytosolutes and accumulates in plants during the adaptation to various types of environmental stress, such as drought, salinity, high temperature, nutrient deficiency, and exposure to heavy metals and high acidity (Nazarli *et al.,* 2011). Free proline and sugar contents significantly increased in *Vigna radiata*. Nodules under drought, but nodules had more proline than leaves (Ashraf and Iram, 2005).

Osmotic adjustment has been reported also in chickpea under water deficit conditions (Najaphy *et al.,* 2010). Proteins are compounds of fundamental importance for all functions in the cell, It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions, under conditions of water deficit (dehydration) numerous processes are modified or impaired, Water stress affects the protein levels of plants but the results of different authors are contradictory. Some authors show decreased protein levels under water stress (Bakalova*et al.,* 2008).

Castor plants has long been recognized to have a drought hardy nature and exhibits a high developmental and physiological plasticity to drought stress (Vijaya Kumar *et al.,* 1996; Heckenberger *et al*., 1998; Schurret *et al.,* 2000). With regard to photosynthetic responses, Dai *et al.* (1992) reported that under increased vapor pressure deficits, stomatal limitation may be responsible for the inhibitory effect of drought on photosynthesis in castor plants. Sausen and Rosa (2010) suggested that drought resistance of the castor bean could be related to a pronounced early growth response, an efficient

stomatal control, and a capacity to keep high net $CO_2$ fixation. However, little is known about the physiological responses of castor plant. These responses are important in assessing its ability to adapt to varying low-water conditions. Plastic responses include both inevitable effects of environmental limits on growth and physiology and adaptive adjustments that enable a given genetic individual to withstand sudden environmental changes (Sultan 2000). Plants respond to drought stress by altering a range of leaf characteristics, such as stomatal closure, leaf area reduction, and osmotic adjustment (Marron *et al*., 2002). The stomatal closure and the decreased leaf area limit water loss but inevitably inhibit photosynthesis. These responses may not be considered as adaptive plasticity for plants. Therefore, the question arises whether there is some compensatory mechanism in drought-tolerant plants that enables them to maintain a high photosynthesis while limiting transpiration.

# CHAPTER TWO

## 2.0 MATERIALS AND METHODS

### 2.1COLLECTION OF PLANT MATERIALS

Castor seed was gotten from National Cereal Research Institute, Badeggi, Niger state and explant was collected from castor seedling from Federal University Oye- Ekiti which was planted in a loamy soil mixed with compost manure. The experiment was carried out in the Department of Plant Science and Biotechnology. Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria.

### 2.2PROPAGATION OF CASTOR PLANT

#### 2.2.1 BREAKING OF SEED DORMANCY

The accessions (seeds) were surface sterilized with 10% sodium hypochlorite for 15 minutes. It was rinsed thoroughly with distilled water and later soaked inside warm water for 24 hours. This is done so as to break seed dormancy.

#### 2.2.2 PREPARATION OF SOIL FOR PLANTING

Air dried loamy soil was mixed with compost manure inside a perforated container for different accessions. Holes were bored with a tip of finger, and soil was used to cover the seeds. The seed were watered adequately.

## 2.3 IN VITRO REGENERATION OF CASTOR PLANT

### 2.3.1 STERILIZATION OF GLASS WARE

Glass wares such as bottles, petri dishes, beaker, test tubes and equipment (dissecting tools ) such as forcep, blade holder were soaked for 2 hours inside a basin containing water, sodium hypochlorite and tween 20 ( liquid soap). After 2 hours, the glass wares were scrubbed with brush and rinsed with water containing sodium hypochorites and again rinsed three times with running water so as to remove component of the liquid soap. The washed glass wares were placed in the oven for 15 minutes at 25°C along with forcep and scalpel. After oven dry, the tools such as (forcep and scalpel were wrapped with aluminium foil) and placed inside the autoclave for 30 minutes at 121°C temperature and pressure (0.15 pascal).

### 2.3.2 EX-PLANT COLLECTION

Sterilized clean collection bottles were used in excised nodes. Sterilized blade were used to excised ex-plant (node) from mother plant and placed inside the bottles containing tap water of different accessions. The collection bottles were labeled appropriately for proper identification. Labeling was done with pen for easy identification.

### 2.3.3 MEDIA PREPARATION

Measure 200ml of tissue culture grade water (Double distilled deionized water) into a 1000ml beaker. While stirring the water, add 20 ml of Macronutrient solution (Stock 1). Continue stirring the mixture while adding 2 ml of Micronutrient Solution (Stock 2). Add 0.01492g EDTA and 0.0112g $FeSO_4$ followed by 0.04g inositol is added while stirring. Note EDTA was added before FeSO4. Then followed with the addition of 2 ml of Vitamins (Stock 4). Add 1.6 mg of 6-Benzylaminopurine and

0.08 mg of Indole acetic acid with a pipette while stirring. The culture mixture was made up to 400ml using distilled water. The culture mixture was divided into 8 bottles. The bottles were labeled 0% and 6%. While stirring, 0% and 6% of sucrose were added. The PH of the medium was adjusted to 5.7. Heat on the magnetic stirrer until the sucrose is cleared. 0.7g of agar was added. Five millimeter (5 ml) of the medium was dispensed into the culture test tubes before autoclaving. The medium were sterilized in an autoclave of 15 psi at 121°C for 30 minutes.

### 2.3.4 DISINFECTION OF EX-PLANT

Ex-plants (nodal) were rinsed thoroughly under running water. Liquid soap was added to eliminate surface dirt. The ex-plants were rinsed three times with distilled water. It was further sterilized with 70% v/v ethanol for 5 minutes and decant. Therefore, 10% v/v of sodium hypochlorite was used for 20 minutes. Sterilized water was used to rinse the explants thoroughly.

### 2.3.5 CULTURE PROCEDURE

Laminar flow hood switched on for 15 minutes before the inoculaton. The flow hold was surface sterilized with 70% ethanol using cotton wool. Rolled in the trolley to pack culture materials, medium, petri dishes from growth room. Disinfect hand with 70% ethanol. The spirit lamp was filled with absolute ethanol and culture tools were placed inside alcohol. Nodal ex-plant were excised sterilized petri dishes, forcep and blade holder. The ex-plants were inoculated into culture tubes. The culture tubes were corked and labeled. After wards, they were placed in the growth room.

### 2.3.6 AUTOCLAVED MATERIALS

MS medium dispense into a test-tubes cork with cotton wool (wrapped with aluminium foil), Forceps and scalpel (wrapped with aluminium foil), beaker containing sterilized water corked with

cotton wool (wrapped with aluminium foil), Flask containing 70 % v/v ethanol and 10% v/v sodium hypochlorite cork with cotton wool (wrapped with aluminium foil), Petri-dishes (wrapped with aluminium foil) and one empty beaker.

## 2.4 PLANT PHYSIOLOGICAL GROWTH (GROWTH PARAMETERS)

After 4 weeks of inoculation, shoot length, number of leaves, fresh weight shoot, fresh weight leaves were measured. The percentage of plant regeneration were estimated using the formula, Plant regeneration (%) = (Number of plant survive/Number of nodal inoculated) X 100.

## 2.5 BIOCHEMICAL EXTRACTION

### 2.5.1 CHLOROPHYLL EXTRACTION

After 4 weeks, fresh leaves cultivated in vitro under drought stress were collected and freeze. Leaves of 0.20g were macerated in 10 ml of 80% acetone using mortar. The extracts were centrifuged at 3000 rpm for 5 minutes. Upper phase were read at absorbance 663nm, 646nm and 470nm with 80% as blank using spectrophotometer.

Chlorophyll a (Ca) = 11.75 A662 - 2.350 A645
Chlorophyll b (Cb) = 18.61 A645 - 3.960 A662
Total Carotene = 1000 A470 - 2.270 Ca - 81.4 Cb/227

According to the formulas of Lichtentaler and Wellburn (1985).

# CHAPTER THREE

## 3.0 RESULT

### 3.1 Physiological Growth of Castor Plant under drought stress:

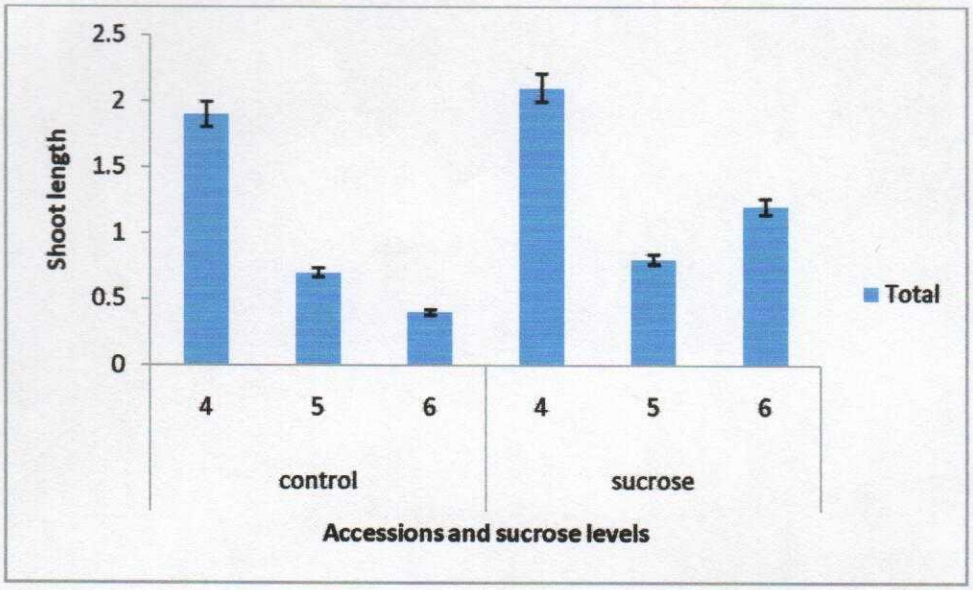


**Figure 1:** Shoot length (cm) of Castor plant under drought stress

The result above shows the significant difference ($p>0.05$) between the accessions under control and sucrose levels. Accession 004 has the highest shoot length (Plate 1) under control and sucrose levels than the other treatments levels.

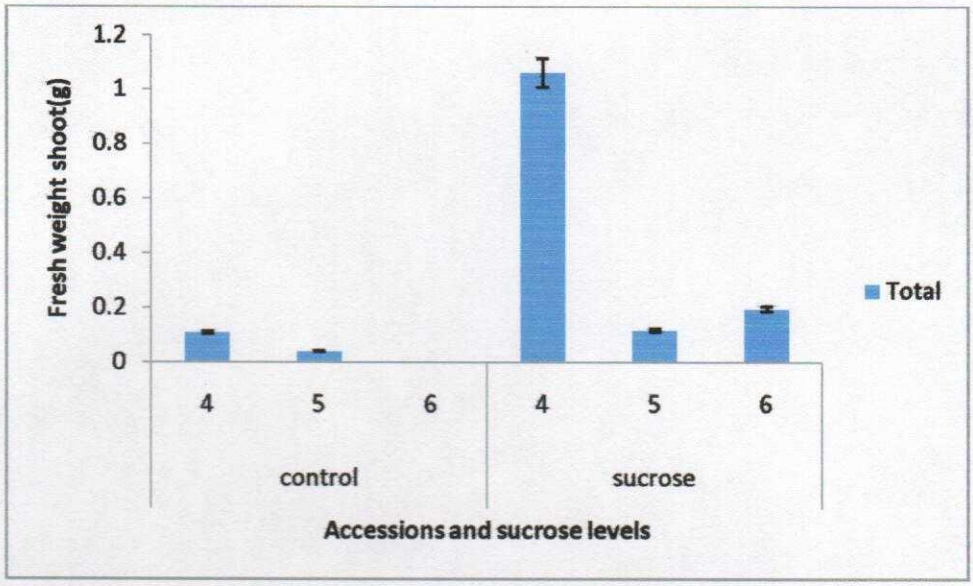


**Figure 2:** Fresh Weight Shoot (g) of Castor plant under drought stress

The result above shows (Figure 2) the significant difference between the cultivars under control and sucrose levels. Accession 004 under sucrose level produced shoot weight that were significantly ($p>0.05$) higher than that of the control (Figure Plate 1). Accession 005 under sucrose level was also higher than that of control (Plate 2). While accession 006under control showed no growth due to contamination (Plate 3).

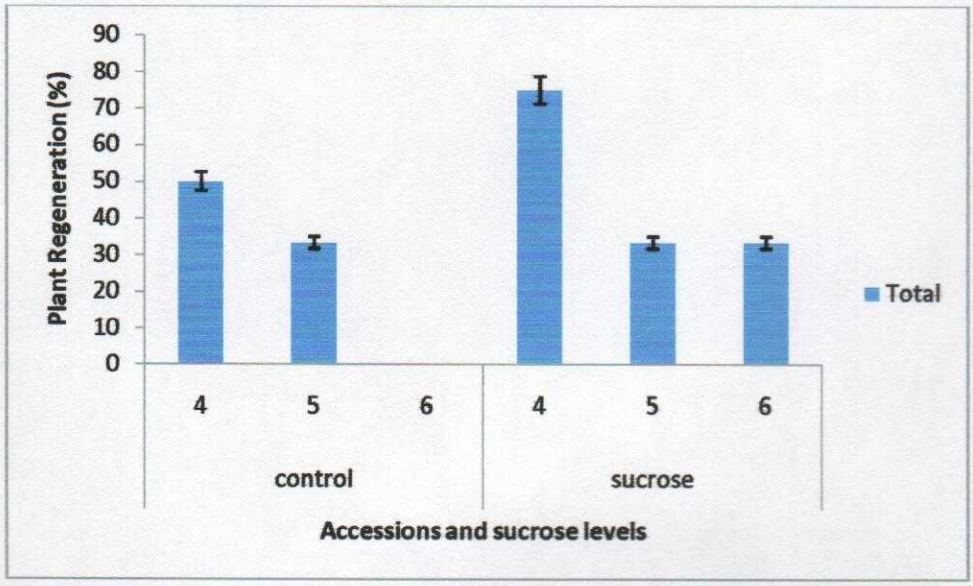

**Figure 3**: Plant Regeneration (%) of Castor plant under drought stress

The result above shows (figure 3) the significant difference between the accessions under control and sucrose levels. Accession 004 inoculated under drought stress has the highest percentage of plant regeneration of above 70% thanother accessions used even when compared to control.

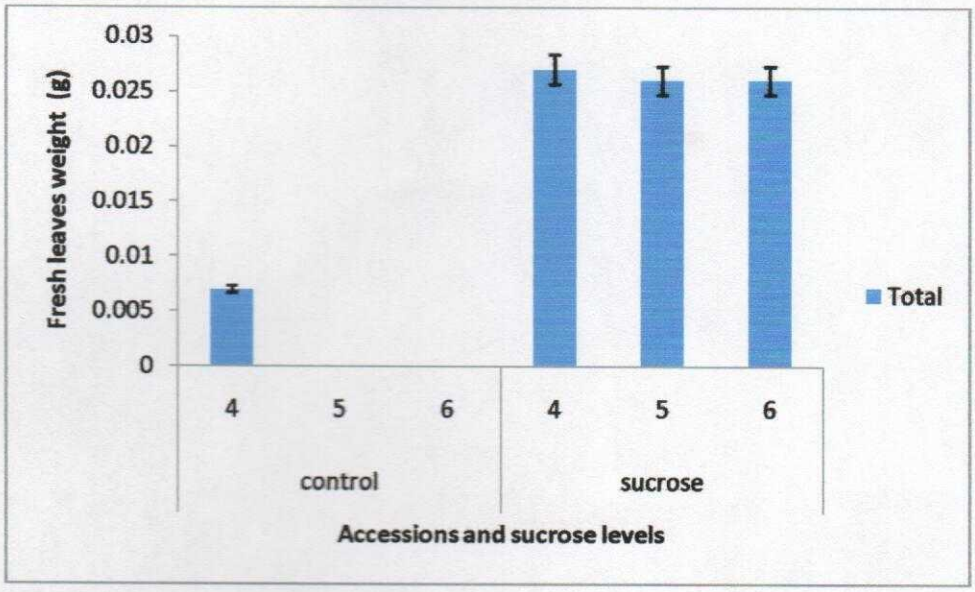


**Figure 4:** Fresh Leaves Weight (g) of Castor plant under drought stress

The result above shows (figure 4) the significant difference between the cultivars under control and sucrose levels. Accession 004 (Plate 1) has the highest fresh leaves than cultivars 005 and 006. Growth of accessions 005 and 006 was not determined due to contamination.

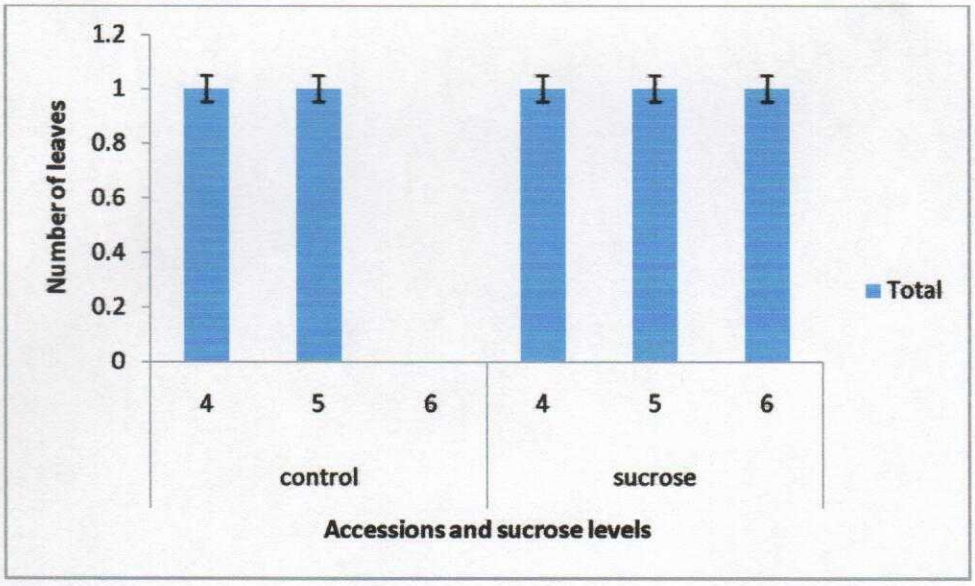


**Figure 5**: Number of Leaves of Castor plant under drought stress

The result above shows (figure 5) that there was no significant difference between the accessions under control and sucrose levels.

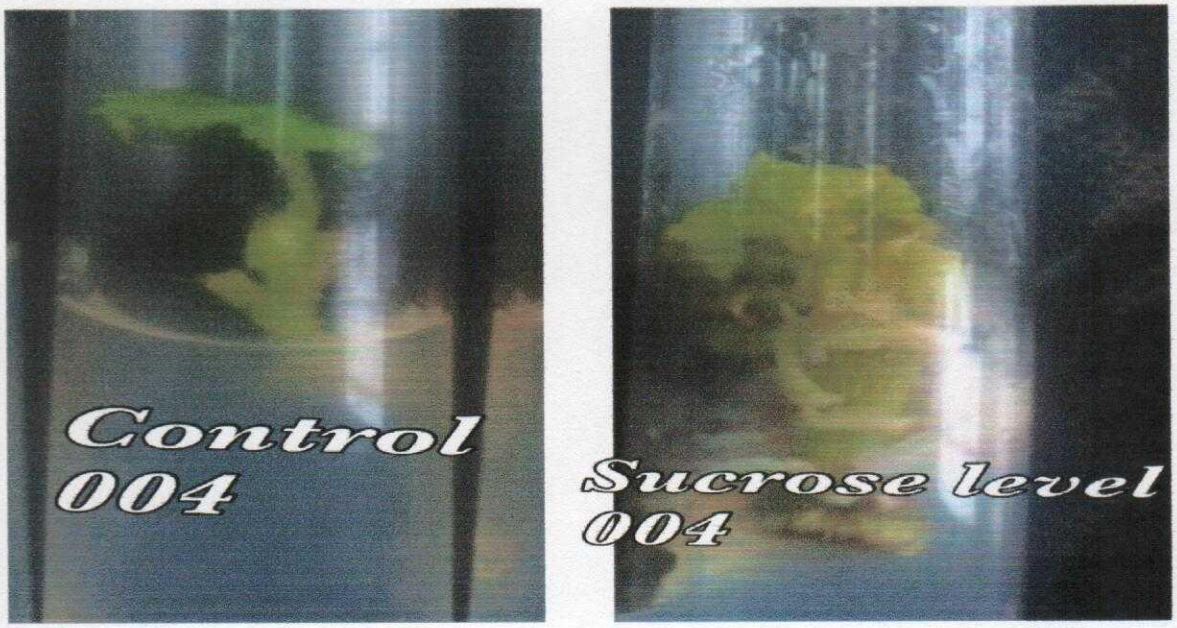


Plate 1: Castor Plant Under drought stress levels for Accession 004

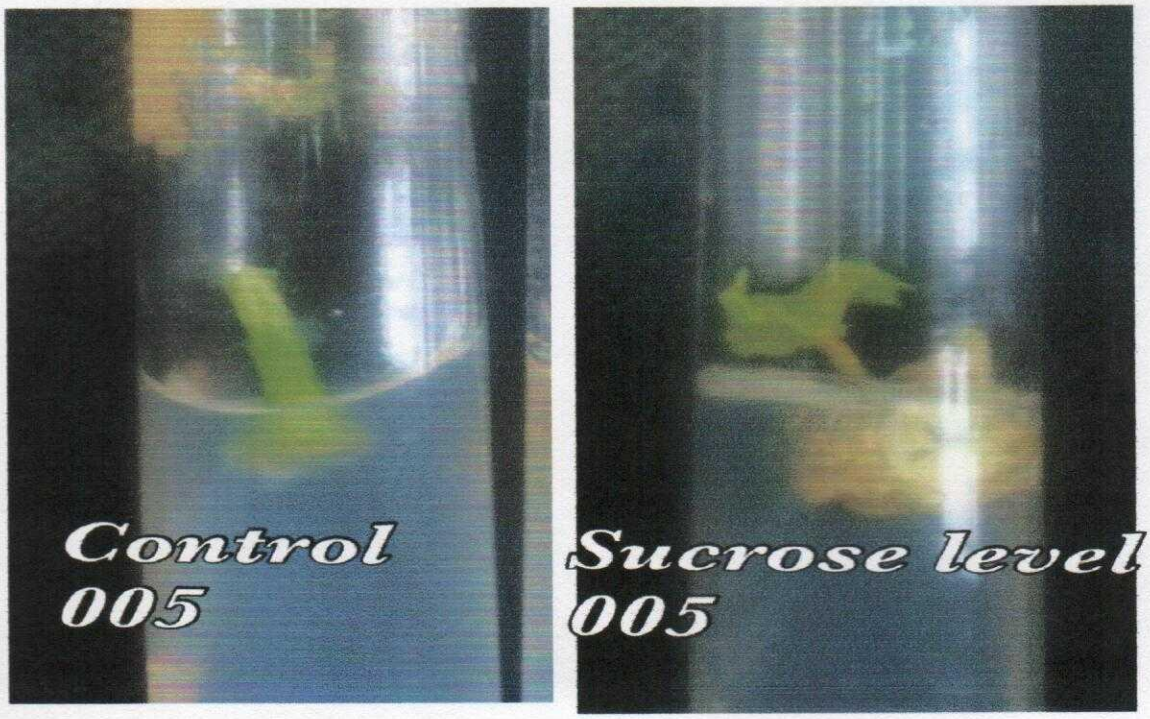


Plate 2: Castor Plant Under drought stress levels for Accession 005


Plate 3: Castor Plant Under drought stress levels for Accession 006

**Table 1:** Chlorophyll a, b and carotene contents of Castor plant of different cultivars.

| Castor Accessions | Treatments | Chlorophyll Contents | | |
|---|---|---|---|---|
| | | A | B | total Carotene |
| **004** | 0% | 11.85b | 10.31b | 881.15b |
| | 6% | 14.32a | 10.74b | 924.64a |
| **005** | 0% | ND | ND | ND |
| | 6% | 11.80b | 9.97c | 924.63a |
| **006** | 0% | ND | ND | ND |
| | 6% | 7.74c | 11.1a | 923.41a |

Where ND is not determined

Chlorophyll a (Ca) = 11.75 A662 - 2.350 A645

Chlorophyll b (Cb) = 18.61 A645 - 3.960 A662

Total Carotene = 1000 A470 - 2.270 Ca - 81.4 Cb/227

According to the formulas of Lichtentaler and Wellburn (1985).

Table1 showed that accession 006 at 6% of sucrose has the lowest amount of chlorophyll a content and also has the highest amount of chlorophyll b content. At 6% of sucrose, accession 004 has the highest amount of chlorophyll a. Whereas, accessions 004, 005 and 006 at 6% sucrose level has the highest amount of total carotene.

# CHAPTER FOUR

## 4.1 DISCUSSION

Sucrose as a carbon source support growth of plant cells in culture (Gamborg and Phillips, 1995). A sucrose concentration of 1-5% is generally used for in vitro tissue culture, since it is also synthesized naturally by the tissue (Pierik, 1987). Sucrose is also used to induce drought stress. In this experiment, accession 004 inoculated *in vitro*under 6 % sucrose level recorded highest shoot length, fresh weight shoot, plant regeneration and fresh weight leaves.Whereas, accessions 006 showed lowest growth regeneration under 6 % sucrose. There was a significant variation within the accession of castor plants subjected to drought stress. Julkunen-Titto (1996) reported willow plantlets cultured in vitro grew well at 6% sucrose concentration than other levels.Oxidative stress generated in plant cells is as a result of extended drought causes reduction in chlorophyll and carotenoid contents in many species (Smirnoff 1993, kiani *et al;* 2008). Accession 004 further showed no damage to chlorophyll a and level of total carotenoid show no significant different with other accessions under 6% sucrose.

Higher level of carotenoid in accession 006 implies that these plants were currently undergoing oxidation stress due to drought stress. Accession 006 showed reductions in content of chlorophyll a and higher amount carotenoid and chlorophyll b which directly showed that the plants were adversely affected by the drought stress. This further showed damage in the photolytic activities (photosystem I and II) of the plant. It was observed that cultivar 004 has the highest growth regeneration in vitro under drought stress and showed no damage to chlorophyll-a thus, accessions 004 could be a drought resistanceaccession while accession 006 could be a drought

sensitive accession. Jaleel *et al.* (2009) reported carotenoid as a part of the plant antioxidant defense system whichplays an additional role in plant resisting to drought stress.

## 4.2 CONCLUSION

The present study contributed to determine the concentration of sucrose in culture media for the development of nodal culture of *Ricinus communuis*. Efficient in vitro regeneration of accession 004 of sucrose level (6%) was determined which yielded a positive result thereby having the highest physiological growth and resistant to drought than accession 005 and 006.

## 4.3 RECOMMENDATION

Therefore, cultivation of castor accession 004 can be recommended to farmers due its resistivity to drought stress in a semi-arid and arid region of the Nigeria.

## REFERNCES


Ahmed A.B.A, Rao A.S, Rao M.V, Taha R.M. Effect picloram, additives and plant growth regulators on somatic embryogenesis of *Phyla nodiflora*, *Brazilan Archives of Biology and Technology* 2011b, 54 (1), 7-13.

Akhka, A., T Boutraa and A. Alhejely, 2011.The rates of photosynthesis, chlorophyll content, dark respiration, proline andabscicic acid (ABA) in wheat (*Triticum durum)* under water deficit condition.*Int. J. Agric. Biol.,* 13: 215- 221.

Alam, I., S.A.Sharing, S.C. Mondal, J. Alarm,M, Khalekuzzaman, Anisuzzaman and M.F. Alam, 2010. In vitro micro propagation through cotyledonary node culture bean (*Ricinus communis L.*) *Aust. J. Crop Sci,* 4:81-84.

Armstrong WP (1982). "Not Beavers, Stars or Sons of Jupiter. "Environment Southwest., 496: 4-7.

Bartels D, Sunkar R (2005). Drought and salt tolerance in plants. *Crit. Rev. Plant Sci.* 24:23-58.

Boulos, L. 1983. Medicinal plants of North Africa. Algonoc, Michigan Reference Publications, Inc USA.

Bray EA, Bailey-Serres J, Weretilnyk E (2000). Responses to abiotic stresses. In: Gruissem, W, Buchnnan B, Jones R, editors, Biochemistry and molecular biology of plants. Am. Soc. Plant Physiol. Rockviile, MD. pp. 1158-1203.

Caupin H.J (1997) Products from castor oil: Past, present, and future. In:Gunstone FD and Padley F.B (eds.) Lipid technologies and applications. Marcel Dekker, New York, pp. 787-795.

Cochard H, Barigah S, Kleinhentz M, Eshel A (2008). Is xylem cavitation resistance a relevant criterion for screening drought resistance among Prunus species? *J. Plant Physiol.* 165:976-982.

Costa-Franca, M.G., Pham, Thi, A.T., Pigmental, C., Rossiello, R.O.P., Zuily-Fodil, Y., and Laffray, D. (2000). Differences in growth and water relation among *Phaseolus vulgaris* cultivars in response to induced drought stress, *Environ Exp Bot.* 43: 227-237

Dai Z, Edwards GE, Ku MSB. 1992. Control of photosynthesis and stomatal conductance in *Ricinus communis L.* (castorbean) by leaf to air vapor pressure deficit. Plant Physiol.99:1426–1434.

Deore A.J, Johnson TS (2008) High frequency plant regeneration from leaf-disc cultures of *Jatropha curcas L.*:an important biodiesel crop. *Plant Bio technol* Rep 2: 7-11.

FAO (Food and Agriculture organization) online http://faostat.fao.org 2006 (Accessed on 06 November 2007).

Gamborg, O.L and G. Philip. 1995. Media Preparation and handling. In O.L. Gamborg and G.C Philips (Eds). Plant cell, tissue and organ culture: Fundamental methods, springer, Germany, 21-34pp.

Heckenberger U, Roggatz U, Schurr U. 1998. Effect of drought stress on the cytological status in *Ricinus communis*.J ExpBot. 49:181–189.

Heszky, L. (2007). Szarazsagesa noveny kapcsolata, *Agroforum* Vol.18. (11/M): 37-41. (In Hungarian)

Hirasawa T, Ashitani K, Yoshikawa K, Nagahisa K, Furusawa C, Katakura Y, Shimizu H, Shioya S (2006). Comparison of transcriptional responses to osmotic stresses induced by NaCl and sorbitol additions in Saccharomyces cerevisiae using DNA microarray. *J. Biosci. Bio eng.* 102:568-571.

Hsiao T.C., 1973 – Plant response to water stress. Ann. Rev. Plant Physiol., 24: 519-570.

Hsiao,T. (1994). Crop productivity and future world of elevated CO2 and changed climate, In: Third Congress of the European Society for Agronomy Proceedings Padova University, (eds. M. Borin and M. Sattin) France, Colmar Cedex. Pp 6-17.

Jaleel C.A., P. Manivannan, A. Wahid, M. Farooq, R. Soma sundaram, R. Paneeeselvam, 2009 – Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.,* 11: 100-105.

Joshi M., Waghmare S., Chougule P., Kanase A.(2004). "Extract of *Ricnus communis* leaves mediated alterations in liver and kidney functions against single dose of $CCl_4$ induced liver necrosis in albino rats."*Journal of Ecophysiology and Occupational Health 4 (3-4): 169-173.*

Kafagi, I. K. 2007. Variation of callus induction and active metabolic accumulation in callus culture of two varieties of (*Ricinus cummunis L.)* Biotechnology 6: 193-201.

KamrunNahar and Rita Sarah Borna.(2012).In vitro propagation from Shoot tip Explants of Castor oil plant (*Ricinus communis L*).*Canadian Journal on Scientific and Industrial Research* (5).

Keyvan, S.H., 2010. The effects of drought stress on yield, relative water Content, proline, soluble carbohydrate and chlorophyll of bread wheat Cultivar. *J. Anim. Plant Sci.*,8: 1051- 1060.

Kramer P.J., 1980 – Drought stress and origin of adaptation. In N.C. Turner, and P.J. Kramar (ed.) Adaptation of plant to water and High Temperature stress. John Wiley and Sons, New York. P. 7-19.

Kumari K. G, Ganesan M, Jayabalan N (2008) Somatic organogenesis and plant regeneration in *Ricinus communis*. *Biol Plantarum* 52: 17-25.

Lafitte H, Price A, Courtois B (2004). Yield response to water deficit in an upland rice mapping population: Associations among traits and genetic markers. *Theor.Appl. Genet.* 109:1237-1246.

Lomash V, PariharSk, Jain NK, Katiyar AK (2010). "Effect of *Solanum nigrum* and *Ricinus Communis* extracts on histamine and carrageenan-induced inflammation in the chicken skin". *Cell.Mol. Biol.* (*Noisy-le-grand*) 56 (Suppl):OL1239-51.

Magombeyi MS, Taigbenu AE (2008). Crop yield risk analysis and mitigation of smallholder farmers at quaternary catchment level: Case study of B72A in Olifants river basin, *South Africa. Phys. Chem. Earth* 33:744-756.

Marron N, Delay D, Petit J, Dreyer E, Kahlem G, DelmotteFM, Brignolas F. 2002. Physiological traits of two Populus × euramericana clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. Tree Physiol. 22:849–858.

Nemeskeri, E. (2001). Water deficiency resistance study on soya and bean cultivars, *Acta Agronomica Hungarica* Vol. 49 (No.1): 83-93.

Nemeskeri, E., Sardi, E., Remenyik, J., Koszegi, B., & Nagy, P. (2010).Study of defensive mechanisms against drought of French bean (*Phaseolus vulgaris L.)* varieties, Acta*Physiol.Plant.* Vol. 32 (No.6): 1125-1134. DOI:10.1007/s11738-010-0504-z

Ober E (2008).Breeding for improved drought tolerance and water use efficiency. Arable cropping in a changing climate, Rotham sted Research, Broom's Barn Research Centre, Higham, Bury St Edmunds, IP28NP. 23 -24, Jan. pp. 28-37.

Oduvaldo CMPJ. (2007). "Comparison Between Polyurethanes Containing Castor Oil (Soft Segment and Cancellous Bone Autograft in the Treatment of Segmental Bone Defect Induced in Rabbits". *J. Bio mater. Appl.,* 21(3): 283-297.

Osborne D.J, McManus M.T. Hormones, Signals and target cells in plant development. Cambridge University Press 2005, Pp. 252.

Oyewole O.I., Owoseni A.A., Faboro E.O. (2010). "studies on medicinal and toxicological properties of *Cajanus cajan, Ricinus cummunis and Thymus vulgris leaf extracts". Journal of Medicinal plant Research 4 (19): 2004-8.*

Piha, M.I., and Munns, D.N. (1987). Sensitivity of the common bean (*Phaseolus vulgaris L)* symbiosis to high soil temperature, *Plant Soil* Vol.98 183-194.

Pirzad, A., M.R. Shakiba, Z.S. Salamasi, A.S. Mohammadi, K. darvishzade and A. Samadi, 2011. Effect of water stress on leaf water content, chlorophyll, proline and soluble carbohydrate in *Matricariachamomilla* L.J. Med. *Plants Res.,* 5: 2483-2488.

Pierik, R.L.M. 1987. *In vitro* culture of higher plants martinusNighoff Publishers, Dordrecht, Nether Lands.

Raven P.H., R.F, Evert and S.E Eichorn.1999.Biology of plant.6th edition. New York: W.H Freeman and Company, 1:11-15.

Sausen TL, Rosa LMG.2010. Growth and limitations to carbon assimilation in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions. *Acta Bot Brasilica.*24:648–654.

Schurr U, Heckenberger U, Herdel K, Walter A, Feil R. 2000. Leaf development in *Ricinus communis* during drought stress: dynamics of growth processes, of cellular structure and of sink-source transition. *J Exp Bot.* 51:1515–1529.

Smith, D.L., Dijak, M., & Hume, D.J. (1988). The effect of water deficit on N2 (C2H2) fixation by white bean and soybean, *Canadian J plant Sci.* (Ottawa), Vol. 68 (No. 4):957-967.

Sorial, M.,S. Gamal and A. Gendy,2010. Response of sweet basil to jasmonic acid application in relation to different water supplies.*Bio.Sci. Res.,* 7: 39-47.

Sultan SE. 2000.Phenotypic plasticity for plant development, function and life history.*Trends Plant Sci.* 5:537–542.

Takele, A. and McDavid, C.R. (1995). The response of pigeonpea cultivars to short durations of waterlogging, *African Crops Science Journal* Vol.3 (No.1): 51-58.

Vieira C, Evangelista S, Cirillo R, Lippi A, Maggi CA, Manzini S (2000). "Effect of ricinoleic acid in acute and subchronic experimental models of inflammation".*Mediators Inflamm.*9 (5): 223-8.

Vijaya Kumar P, Srivastava NN, Victor US, GangadharRao D, Subba Rao AVM, Ramakrishna YS, RamanaRao BV. 1996. Radiation and water use efficiencies of rainfedcastor beans (*Ricinus communis L.)* in relation to differentweather parameters. Agric For Meteor. 81:241–253. Vilagrosa A, Morales F, Abadía A, Bellot J, Cochard H, Gil.

Weiss EA (1971). Castor, Sesame and Sunflower. Leonard Hill, London, pp. 235.

Weiss, E.A. (1983). Oilseed crops. Longman Group Ltd. Pp. 31-99.

William E. M. (ed) "Major Herbs of Ayurveda", Churchill Livingstone 2002.

Yeoman M.M, Yeoman C.L. (1996). Manipulating secondary metabolism in cultured plant cells.*New Phytologist*, 134, 553-569.

Zahir AA, Rahuman AA, Bagavan A; et al (August 2010). "Evalution of botanical extracts against *Haemaphysalis bispinosa* Neumann and *Hippobosca maculate* Leach". *Parasitol. Res.* **107** (3): 585-92.

Zhu J.k., 2002 – Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol.*, 53: 247-257.

Zidenga T (2006). Progress in Molecular Approaches to Drought Tolerance in Crop Plants.Informationsystems for biotechnology.NewsRepport. http://www.isb.vt.edu/news/2006/news06.mar.htm (accessed 18 June 2012).

Zolfaghari, R., S. Hosseini and S. Korori, 2010. Relationship btw peroxidase and catalase with metabolism and environment factors in Beech (*Fagusorientalis Lipsky*) in three different elevations. *Int.J. Environ. Sci.*, 1: 243-252.