



Effect of MS Medium Strength and Growth Hormones on Shoot Proliferation of Yeheb (*Cordeauxia edulis*, Hemsl.): A review

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Abstract

Cordeauxia edulis Hemsley, locally called yeheb, is a small evergreen multi-stemmed tree or shrub of the legume subfamily Caesalpinioideae, endemic to Eastern Ethiopia and central Somalia. It is drought hardy and one of the major sources of food for both animals and humans. Even though it is a multipurpose plant and being encouraged by most people in the region, it becomes endangered due to low seed viability and over exploitation. Due to low seed viability, propagation via seed has limitations. Vegetative propagation via in vitro propagation of *C. edulis* has found advantages to address low seed viability and over exploitation problems. Shoot proliferation of once plant affect by various factors including type and strength of culture medium, growth hormones and the physical conditions. Therefore, the objective of this paper is to highlight the effect of MS medium strength and growth hormones on shoot proliferation of *C. edulis*. The study of various scholars over *C. edulis* confirmed that BAP showed the highest shoot proliferation at its 2 mg/l on full strength MS medium at 5.8 PH from shoot tip. On the other hand, BAP (2 mg/l) in combination with GA3 (6 mg/l) produced higher amount of shoot from cotyledonary node. The increasing and decreasing of BAP concentration alone or in combination with auxins reduce shoot multiplication. Half and one third MS medium strength produced stunted and fragile shoots. Each plant species and explant type is specific and performs variably for growth hormones. Therefore, it is preferable to use the growth regulators in accordance to the types of explant.

Key words: Explant, Growth hormones, Medium, Propagation, Shoot Proliferation, Yeheb,

Introduction

Yeheb (*Cordeauxia edulis*) belong to the class Magnoliopsida, order Fabales, family Fabaceae, and genus *Cordeauxia* and it is the only species in the genus *Cordeauxia* with multipurpose usage (Bally, 1966 cited in Liew, 2003). It is an evergreen shrub in the bean family that produces seeds called yeheb nut (Katz, 2008). Vivero et al. (2005) stated that *C. edulis* is leguminous shrub in semi-arid bush land on

sandy soils of Somalia and Ethiopia. As it is indicated in Ethiopia's 4th Country Report to CBD (2009), *C. edulis* is grouped under pulse species and is found in the bush lands of the Ogaden area of Somali Regional State.

Cordeauxia edulis is an advantageous plant with different local names. For instance, it is called yicib in Amharic and yeheb nut in Somali, English and its trade name in Somali (www.worldagroforestry.org). Bally (1966) cited in Liew (2003) reported the following spellings ghieheb, giaeb, giaheb, gieheb, ieebb, ice-ep, ieheb, iieb, jeebb, jieheb, ye'eb, yee-ep, yeheb, yehib, but phonetically they state very much the same sound. Ali (1988) also reported as *C. edulis*, locally named Yeheb by Somalis, is well known for its seed that are locally called yeeb, yebb, yeheb or jeheeb, which all mean a seed of *C. edulis* and the plant itself is called Gud by local travelers.

Cordeauxia edulis is a multi-purpose plant where most parts of the plant are used. The seeds are edible and eaten fresh, roasted, boiled or dried. They are usually roasted to provide keeping qualities. Compared with other leguminous seeds, *C. edulis* seeds are rich in fats (10-13%), sugars (12-25 %) and carbohydrate (31-41%), but contain lower protein (11-16%) levels. However, protein quality is good since it is rich in most essential amino acids (Booth and Wickens, 1988), particularly good source of lysine and arginine and therefore, the seeds have very favorable amino acid balance (Grivetti, 1980). On the other hand, the corresponding values reported by FAO (1988) are 11, 24, 13 and 37 percent for fat, sugars, protein and carbohydrates, respectively. Moreover, the nut is sold on the market and even exported to the coastal cities and generating a significant household income for the local people (Jacques and Miede, 1977). It has high demand by the urban people among other wild fruit plants (Johansson, 2006).

The seed yield of each *C. edulis* shrubs can be 5-8 kg per year or none in drought years (Brink, 2006). Somalis drink the sweet water left over from boiled seeds and leaves are used for tea preparation. In addition, seeds may be used as a coffee replacement (Booth and Wickens, 1988). The seeds are free of the toxic phytohemagglutinins, lectins, alkaloids and glucosides. A substance involving liquid oil and fat mined from *C. edulis* and it is less pleasant than olive oil, but helpful for soap making (Demel Teketay and Abeje Eshete, 2004). The plant is also used as medicine by the local people (Mussa Yusuf et al., 2012).

Plants may provide up to half of the biomass of the area and use by camels and goats as vital dry season browse. The expected average forage production is 325-450 kg/ha (1.4-2 kg/plant) (Gutale, 1983; Brink, 2006; MussaYusuf et al., 2012). Fodder value of the leaves is as good as to other tropical tree legumes, but some mineral intensity (P, Mg, Mn and partly Zn) would not assure the demands of animals if *C. edulis* were the only supply of fodder (Drechsel and Zech, 1988). On the other hand, *C. edulis* is categorized along with major fodder trees and shrubs (Dicko and Sikena, 1992). Like other tree and shrub species, *C. edulis* is critical to progress the forest and rangelands and afford further income for pastoral communities (Zanini, 2006).

According to the report of Booth and Wickens (1988), leaves of *C. edulis* have used to stain cloths, calico and wool since the cordeauxiaquinone forms intensely colored and unsolvable combinations with various metals. As indicated in PROTA (2007), several species are potential sources of natural dyes for coloring food or textiles. In inspection of the shift towards natural food additives,

Kalahari white baobab (*Bauhinia petersiana*) and *C. edulis* used for dyes, but their development and sustainable harvesting still have problems that make it complicated to assess their potential. As Liew (2003) and Mussa Yusuf (2010) reported, *C. edulis* is known to be termite resistant and therefore used by local people to build houses. It is used for various purposes including firewood, bee forage, mulch, soil conservation, nitrogen fixation, live fence and tannins (Mbuya et al., 1994).

As it indicated by various scholars, *C. edulis* is one of a hopeful species that has been endangered because of over use in times of crop scarcity in Eastern Ethiopia (Asfaw and Tadesse, 2001). This species included among minor crops that are in threat of economic loss and is mentioned as endangered in 1997 by IUCN (Holsinger, 2005).

Cordeauxia edulis produces seeds. However, it grows slowly especially in the seedling stage while it is establishing its huge root system. The seed germination states of *C. edulis* is obtained better (Mussa Yusuf et al., 2013), but the storage is questionable due to lower seed viability (Johanson, 2006). As it stated on study of Chanie and Tileye (2015), the populations of *C. edulis* have declined due to deterioration of the vegetation caused by widespread human intervention, natural states of the plant including biological factors and climate changes that always accompanied the process of deforestation. Furthermore, only some research efforts on *C. edulis*; in situ conservation, management and utilization, biochemical analysis, preliminary assessment of the phenology as well as micropropagation and regeneration were conducted by various scholars.

Micropropagation is a technique which is important to produce large amount of plantlets within small area under aseptic conditions. James et al. (2000) stated that plant tissue culture is based on the promise that plants can be separated into their component part, manipulated in vitro and grown to complete plants. Concomitantly, another feature of plant tissue culture greatly served the increased research activity in plant in the near future. For example, Olembo (2002) stated that quick elimination of once native forests and the severity of the encroaching desert conditions over the arid and semi-arid areas have encouraged someone to take measure to uphold the reforestation in all parts of the country. In vitro propagation technology being applied for these seeds either inaccessible (eg. *Ocotea usambarensis*), have low viability (eg. *Grevillea robusta*) or difficult to propagate by conventional methods (eg. *Cordeauxia edulis* and *Chlorophora axcelisa*). Olembo (2002) grouped *C. edulis* with other tree species such as *O. usambarensis* and *Eucalyptus granolis* which can be propagated through micropropagation.

Therefore, it is indispensable to publicize research efforts, protocols and techniques with respect to factors for the producers /end users/ for the contribution of further utilization. Thus, the aim of this review paper was to highlight the effect of MS medium strength and growth hormones on shoot proliferation of *C. edulis* as an alternative means to develop profit from it.

Plant Propagation

The method of plant propagation work depends on several consistent variables including genetic consideration, the propagation characteristics of a specified species, site and environmental factors, economic and procurement considerations and construction programs and purposes (Joseph et al., 1999). Micropropagation has advantage over conventional means of propagation because of high multiplication rate and preserving the species, which are difficult to

restore through conventional means and keep from loss (Yadav et al., 2012). According to Napolione (1985) prior to propagation with tissue culture can be commercially viable and cost effective, it will be compulsory to realize a high rate of multiplication and continued existence rate for the resulting plantlets in typical soil conditions.

Vegetative Propagation

Sexual reproduction is accountable for the extensive genetic assortment and it is observed in numerous tropical tree species due to stable recombination of genes, but vegetative propagation intends at the same duplicate of plants with advantageous features such as high output, better quality and high tolerance to various factors. Therefore, it plays vital role in continuing favored characters over generations (Tchoundjeu et al., 2006). As Brink, (2006) pointed out, *C. edulis* is usually reproduced by seed, but propagation via vegetative means using stem cuttings is also promising.

Propagation by Seeds

Seed germination is significantly chief occasion in the plant life cycle and the capability of seed imbibitions to start germination can be considered as a vital regulatory step in plant development (Johnson, 2000). Seed is the best source of multiplication of large amount of plant species. There are diverse varieties of seeds some of which germinate easily, uniformly; some are difficult and erratic in germination in normal situation (Singh, 2003).

Cordeauxia edulis is self-reseeding and the seeds are relatively big and heavy. According to Demel Teketay and Abebe Eshete (2004), nursery seedling production from seeds is problematical and hard since tap root is fast developing and the plant dies if it breaks away. The plant does not stand water logging and seedlings are fragile and require care during transportation to protect from damage (IBPGR, 1984; Booth and Wickens, 1988).

Germination *C. edulis* is epigeous where sprouts with thick cotyledons produce leaves with numerous leaflets varying in number from one individual to the other and also on the same plant. The leaves are alternate; occasionally the first two are opposite (Jacques and Miede, 1977). Jacques and Miede (1977) pointed out that the *C. edulis* has the ability of growing in sub desert climatic conditions allied with the slow growth of its first developing phases. The small proportion of reserve proteins may be the reason for this slow shoot development. The poor growth of the aerial parts, especially in their early stages, balanced by a speedy and profuse growth of the root system, as detected in other species of desert areas. A tap root is formed which drives deeply into the ground, utilizing the water reserves as much as possible.

Plant Tissue Culture

Micropropagation is an in vitro vegetative propagation technique believed important in agriculture and forestry for generating pathogen free stock plants, genetically better quality clones that cannot be propagated by seeds and whose propagation efficiency is low in conventional vegetative propagation (Kubota, 2002). It is the development of new plantlets in tissue culture (Geneve, 2006). Plant tissue culture is a technique of separating plant cells, tissues and organs from the donor plant and rising on synthetic media under aseptic conditions (Erica, 2000; Ahloowalia et al., 2004; Tchoundjeu et al., 2006; George, 2008). It includes asexual progression and gives true-to-type regenerants. However, epigenetic alteration and

somaclonal variation are common events in plant tissue culture (Bairu and Kane, 2011).

Plant cell and tissue culture is used for clonal propagation, production of disease free plants, germplasm preservation, genetic transformation, haploid and triploid production, in vitro pollination and fertilization (Erica, 2000; Hartmann et al., 2002; Tileye Feyissa, 2006). Principally, useful in low germination potential plant species that have recalcitrant seeds as well as in dying out species (Minocha and Jain, 2000; Pasqual et al., 2012). It played significant function for the reintroduction of endangered species (Narayan et al., 2010). In general, plant tissue culture is a sun rise technology that has a great impact on both agriculture and industry in the course of providing plants to meet the need of ever increasing world population (Omar and Aouine, 2007).

Complete plants can be obtained from various explants via direct or indirect morphogenesis and somatic embryogenesis. Direct morphogenesis is the production of shoots from explants without passing through callus (unorganized tissue) phase known as organ culture, which includes meristem cultures, shoot cultures, embryo cultures and isolated root cultures. Indirect morphogenesis refers to generation of shoots through callus phase, which includes callus cultures, suspension or cell cultures or other cultures. The direct approach is considered that it does not cause genetic instability (George, 1993).

Effect of MS Media and Growth Regulators on Shoot Proliferation of *C. edulis*

Nutrient media composition, growth regulators and physical conditions at each stage, incubation conditions (temperature, light intensity, duration of light, darkness and humidity), hardening conditions and transfer to soil are the major factors that affect plants in culture (Omar and Aouine, 2007). Only some cells are capable to divide and give rise to full plant in tissue culture. Even within a species, some cultivars respond better than others and are appropriate to select for micropropagation (Panhwar, 2005). The type and concentration of PGRs greatly affected the number and length of shoots and there was a considerable relation with the PGRs themselves and explants. Diaz et al. (2012) supported this idea as the most noticeable group of plant hormones (auxins and cytokinins) regulating cell division, elongation and morphogenesis.

Effect of MS Medium Strength

Although numerous media have been developed, the medium of Murashige and Skoog (1962) is widely used in diverse types of tissue culture methods. MS media showed the highest shoot multiplication in other culture media (Roy et al., 2016). As it indicated on the study of Chanie and Tileye (2015), *C. edulis* showed better shoot proliferation in full strength MS media with 5.8 PH, but in half and one third strength MS media the shoots become stunted and fragile. They added that *C. edulis* began to grow Shoots after ten days of culture on full strength MS media.

Effect of BAP and IBA

Like other leguminous tree species, *Prosopis laevigata* (Gonzalez et al., 2007), *Cicer arietinum* (Ugandhar et al., 2012), BAP was found best for shoot multiplication from shoot tip culture of *C. edulis* (Chanie and Tileye, 2015). BAP is the most efficient growth hormone for the shoot proliferation (). As Chanie and Tileye (2015) observed

the maximum number of shoots (4.2 ± 0.85) produced in full strength MS medium containing 2.0 mg/l BAP within five weeks of culture from in vitro germinated shoot tips. On the other hand, Yohannes Seyoum and Firew Mekbib (2014) reported that the maximum mean number of shoots (3.00 ± 0.33) was obtained on MS medium supplemented with 2 mg/l BAP from cotyledonary nodal explants and the higher and lower concentration of BAP reduces shoot multiplication. Consequently, the variation might rise due to the difference in explant source which were from shoot tip and cotyledonary node respectively. Siddique and Anis (2008); Ahmed et al. (2010) confirmed that the relevance of PGRs for every explants of every species is different and Tileye et al. (2005) verified that the culture media used for multiplication may not be highly genotype or explant source specific while they conducted micropropagation of *Hagenia abyssinica*.

As it reported by Chanie and Tileye (2015), higher concentration of BAP alone (towards 3 mg/l) produced lower number of shoots than lower and moderate concentrations of it. Similarly, the finding of Kumar et al. (2005); Husain and Anis (2009), shoot proliferation in woody plant, *Melia dubia*, was hampered when the concentration of BAP became higher.

The sativus did not respond to the BAP at higher concentration because there are level of concentrations that plant could tolerate the substance before it could become toxic (Muhammad et al., 2017). Besides, Reddy and Saritha (2012) explained that the percentage of shoot sprouting decreases with increasing concentration of cytokinins.

When the concentration of BAP in combination with IBA increased above 3.0 mg/l BAP and 0.5 mg/l IBA, the rate of shoot multiplication of *C. edulis* was reduced and formed callus like appearance at the bases of the explants which might due to inhibitory effect of IBA. In contrast, relatively higher amount of *C. edulis* shoots were obtained in the medium supplemented with BAP alone than combination of it with IBA (Chanie and Tileye, 2015). The report by Gurel and Gulsen (1998) showed that when IBA excluded from the culture medium, the increasing BAP concentrations seemed to broadly promote shoot formation from *Amygdalus communis* explants. In addition, Rajana et al. (2011) noted that inclusion of auxin produced excessive callusing and reducing shoot number of a legume tree, *Bauhinia racemosa*.

Effect of BAP and GA3

Shoot proliferation of *C. edulis* was influenced by the combination of BAP with GA3 than individually. As it showed in the study of Yohannes Seyoum and Firew Mekbib (2014), *C. edulis* produced the maximum number of shoots (4.56 ± 0.20) on MS medium supplemented with 2 mg/l BAP in combination with 6 mg/l GA3. They added that shoot number increased when the BAP concentration increased along with increased of GA3 concentration in media. Similarly, Gibberellic acid in combination with BAP had been shown to improve shoot multiplication and elongation in cultures of *Melia azedarach* (Vila et al., 2002; Scocchi and Mroginski, 2004) and *Dalbergia latifolia* (Anitha et al., 2012).

Effect of TDZ and NAA

Cordeauxia edulis produced higher amount of shoots when the concentration of TDZ reduced (towards 0.5 mg/l) than its higher concentration (towards 1.5 mg/l). In addition, the combination of TDZ (5 mg/l) and NAA (0.5 mg/l) produced lower shoots (1.17 ± 0.38) and most of the explants didn't produce shoot and formed calli like

appearance around the explants (Chanie and Tileye, 2015). Likewise, Donna and Preece (2004) stated that woody plants have produced maximum number of shoots at lower concentration of TDZ. In contrast, *Myrica rubra*, produced fewer shoots at lower concentration of TDZ from shoot tip explants (Asghari et al., 2013) and TDZ promoted the highest multiplication rates for *Cassia angustifolia* nodal segments, both alone and in combination with NAA (Siddique and Anis, 2007).

Conclusion and Recommendation

Cordeauxia edulis is a multi-purpose plant where most parts of the plant are useable. This plant is mainly propagated via seed. However, due to low seed viability, propagation using seed is poor. So in vitro propagation is enhanced using various explants. According to the study of various scholars, *C. edulis* produced maximum number of shoots from shoot tip explants in full strength MS medium containing 2.0 mg/l BAP. On the other hand, the combination of BAP (2 mg/l) and GA3 (6 mg/l) produced higher amount of shoots from cotyledonary nodes. Therefore, each plant species and explant is media strength and growth regulator specific. So, producers should select the protocol of in vitro propagation accordingly.

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