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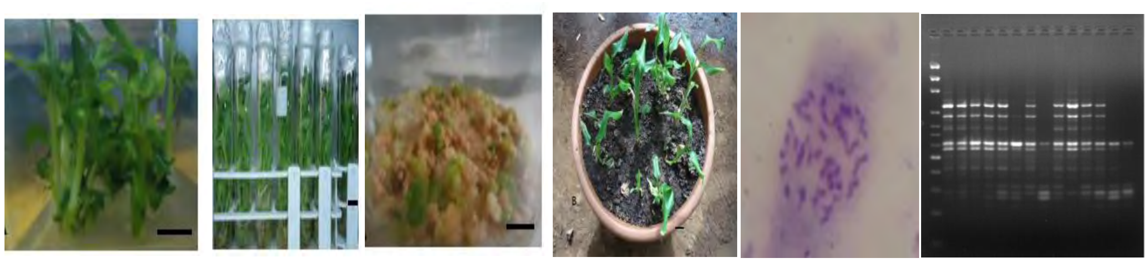
In Vitro Propagation and Genetic Diversity Study ofKorarima (*Aframomum corrorima* (Braun) P.C.M. Jansen)from Southern and Northwestern Ethiopia



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List of Abbreviations

2,4-D ،	2,4-Dichlorophenoxyacetic acid
AFLP	Amlified Fragment Length Polymorphism
AMOV	Analysis of molecular variance
ANOVA	Analysis of variance
BAP	6-Benzyl Amino Purine
CTAB	Cetyltrimethyl Ammonium Bromide
EDTA	Ethylene Di-Amine Tetra acetic Acid
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agricultural Organisation
IAA	Indole-3- Acetic Acid
IBA	Indole-3-Butyric Acid
ISSRInter	Simple Sequence Repeat
KN	Kinetin
MS	Murashige and Skoog (1962)
NAA	Naphthalene acetic acid
NJ	Neighbor Joining analysis
NPL	Number of polymorphic loci
PCoA	Principal Coordinate Analysis
PGRs	Plant Growth Regulators
PP	Percent Polymorphism
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Polymorphism
SNNPRs	Southern Nations, Nationalities and Peoples‟Region
TDZ	Thidiazuron
UPGMA	Un-weighted pair group method with arithmetic mean

Abstract

Korarima [*Aframomum corrorima* (Braun) P.C.M. Jansen]is perennial rhizomatous plant which is indigenous to Ethiopia and widely used for culinary and medicinal purposes.Traditionally, korarima is propagated by stem cutting which is labor intensive and time consuming process. In vitro propagation of plant has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants.Surface sterilization of seeds in 25% Clorox for 30 minutes followed by dipping in 70% ethanol for one minute showed the maximum aseptic cultures and high percentage of germination (70%).The maximum shoot initiation percentage (90%) was obtained on MS medium supplemented with 1.0 mg/l BAP in combination with 0.1mg/l NAA.Shoot multiplication using 1.5 mg/l BAP in combination with 2.0 mg/l KNresulted in the highest mean shoot number per explants (5.13 ± 0.64) within four weeks time. Table sugar and laboratory grade sucrose have been found to play substantial roles in micropropagation of A. corrorima. The highest shoot number (6.67 ± 0.55) per explant was observed in explants cultured on MS medium supplemented with 3% laboratory grade sucrose followed by 4.10 ± 0.44 shoot number per explant using 3% table sugar.Full strength MS medium resulted in the highest number of shoots per explant (5.27 ± 0.53) and mean shoot length (3.08 ± 0.20 cm). The highest number of shoot per explant observed on MS liquid and solid media was 4.41 ± 0.65 and 3.00 ± 0.25 respectively. The maximum callus induction frequency (80 ± 5.77%) was obtained on MS medium containing 0.5 mg/l 2,4-D alone and 5.0 mg/l 2,4-D in combination with 0.25 mg/l BAP. The highest frequency of shoot regeneration (25.33 ± 1.99%) was obtained upon transfer of calli onto regeneration medium containing 0.5 mg/l BAP combined with 0.25 mg/l IAA. Healthy shoots were rooted on half strength MS medium containing different concentration of auxin with highest root number per shoot (9.77 ± 0.78 and 18.50 ± 1.15) obtained using solid and liquid MS media respectively. The plantlet with well developed shoot and root system were transferred to greenhouse and 78% and above 86% rate of survival has been recorded in liquid and solid media respectively.The somatic chromosome number of A. corrorima was found to be 2n = 48. The amplification of genomic DNA with six ISSR primersamong 102 individuals from 11 populations of A. corrorimayielded 83 scorable loci of which 100% were found to be polymorphic. High levels of genetic diversity and significantly high genetic differentiation (P < 0.001) involving within and among populations were revealed by this marker in all studied populations. Nei‟s gene diversity and Shannon‟s information index were 0.3041 and 1.1441 respectively. The analysis of molecular variance showed that the maximum value of genetic variation was found within populations (76%), whereas low value of genetic variance was observed among populations (24%). Both PCoA and UPGMA cluster analysis showed the clustering of all 11 populations into three groups, corresponding to the geographic distribution patterns of the population. Proper identification and characterization of the plant‟s germplasm is central in genetic improvement of the plant and for developing appropriate conservation strategies.

Key Words/ Phrases: *Aframomum corrorima*, callus induction, genetic diversity, in vitro regeneration, ISSR marker, micropropagation, MS medium, Plant Growth Regulator

1. Introduction

Ethiopia is home to different plant, animal and microbial species that live on its highlands and rift valley due to its varied agroecology. There is abundant growth of several indigenous and exotic spices, herbs, medicinal and other essential oil bearing plants. The country is suitable for growing 60–100 species of spices, for example korarima, long pepper, black cumin, white cumin, coriander and ginger. The average land covered by spices is approximately 222,700 hawith the production of 244,000 ton/annum. Spices in Ethiopia are mainly used as a major raw material in local food preparations and cashcrop of many smallholders**.**Considering all the potentials, opportunities and its long history of cultivation**,** use of the spice sub-sector has increase from year to yearall over the world(EIAR, 2010).

Spices can be defined as vegetable products used for flavoring, seasoning and imparting aromain foods (FAO, 2005). Herbs are leafy spices and some like dill and coriander can provide both spice seeds and leafy herbs. Many spice and culinary herb plants are widely regarded as having medicinal properties and there is therefore some overlap between them and medicinal aromatic plants. Spices and herbs are used throughout theworld to flavor food and create the uniquecharacteristic tastes of the different dishes.The delightful flavor and pungency of spices makesthem very important in the preparation of palatabledishes. In addition, they are considered to possess severalmedicinal and pharmacological properties and hence findtheir place in the preparation of a number of medicines(Risch, 1997). Around fifty spice and herb plants have global trade importance, but many other spice and herb crops are used in traditional cooking, healthcare or other applications in particular regions and traded locally. Spices and herbs are grown as trees, shrubs, perennials, annuals, wild and cultivated. Spice and herb plants provide seeds and fruits, leaves and stems, flowers and buds, roots and rhizomes, bark and resins that can all be commercialized in various forms: sold fresh, frozen, dried, whole or ground, distilled into oils or solvent extracted into oleoresins. Their aroma results from complex mixtures of volatilecompounds, e.g. monoterpenes and sesquiterpenesand their oxygenated derivatives, which usually occurin low concentrations (Umar and Salihu, 2014; Etonihu*et al*., 2013).

Somespices like turmeric are widely used in Ethiopiaand elsewhere in the world for food coloring (Purseglove*et al*., 1981; Pruthi, 1998).The coloring power of turmericis also used for industrial purpose such as in garmentand coloring of pharmaceutical products. Uses ofhighland seed spices such as black cumin, coriander andfenugreek in the Ethiopian community has also been sodiverse. A drop of black cumin fatty-oil or smell of 10 to15 black seeds knotted in a small clean cloth is verycommon curative for headache. Fruits/capsules and branches/leaves ofcoriander are very common medicinal plant used as freshvegetable. Vegetative part of fenugreek and flour of the fenugreek seeds areused as food (protein source) and as food flavoring.Almost all of these spices are commonly used for foodand/or beverages flavoring in Ethiopia (Jansen, 1981). Inaddition to such uses, the extract products of mostspice crops have high potential for generatingalternative export products to the country. Ginger, turmeric, pepper, black pepper, korarima, black cumin, coriander and fenugreek are exportedeither in their dried forms and/or as oleoresin or essentialoils extract(Roukens *et al*., 2005; Masresha Yimer, 2010).

Korarima (*Aframomum corrorima*) also called Ethiopian cardamom or false cardamom is an indigenous and endangered species to Ethiopia which is used as spice and medicine (Jansen, 1981; Sebsebe Demissew, 1993). The crop has a relatively broader adaptation, higher productivity and also better tolerance to moisture stress than its close relative, Indian cardamom (*Elettaria cardamomum*) (EIAR, 2010) and is less pungent than seeds of West African species, such as *Aframomum melegueta*(Grains of Paradise). *A. corrorima*occurs as a cultivated crop only in Ethiopia.The seed of korarima is mainly used as sources of spicesin traditional Ethiopian dishes. It is a source of income forgrowers as its seeds fetch high prices in local andexport markets. Korarima isalso important plant for soil conservation as the rhizomes andleaves spread on the ground covering and protecting thesoil from erosion in hilly areas throughout a year (Solomon Eyob *etal*., 2008).

Quality seeds of any preferred varieties of spices, aromatic and medicinal plants are the basis of improved productivity since they answer to farmers needs in terms of value of production and household benefit (Pelmer, 2005). In addition to appropriate agronomic practices, improved variety is one of the major factors that influence yield of any cultivated crop (Purseglove *et al*., 1981).The major production constraints in korarima are lack of high yielding improved varieties with improved agronomic practices like propagation methods (Jansen, 2002). The slow seed germination and growth of the subsequent seedlings were concerns of korarima farmers. There might be some kind of dormancy, possibly associated with the hard seed coat (Solomon Eyob *et al*., 2009). Although korarima is mainly propagated by vegetative method using one year old rhizomes, the need for mass of rhizome as planting materials and slow multiplication rate of rhizomes are also critical problems. The destructive harvestings of the rhizomes for vegetative propagation seems not to be feasible because there is always the possibility of losing the mother plant during this process. The cultivators retain bulk of annual production for raising the following season crop and this requires much attention, space and transportation cost. Rhizomes are prone to damages due to different factors during transportation. Susceptibility to unknown diseases is another major problem faced by korarima growers in Ethiopia (Solomon Eyob, 2009).

Seed propagation of korarima is carried out to cover large areas of land retaining the mother productive stand undamaged; however, it is essential to give the maximum care while preparing the seeds. Therefore, well ripened korarima capsules are selected and collected during the peak harvest season and its seeds are removed and rubbed with ash to facilitate their drying and protect fungal development, as well as to ease their handling during sowing. In the course of drying, the seeds are kept under shade for four to five but not more than 10 days, prior to their sowing in polyethylene bags filled with forest soil and compost or directly on seed beds with soil rich in compost. Germination and seedling emergence takes from one to two months. To increase productivity of korarima the seedlings are transplanted to their permanent field at a spacing of 2 x 2 m after eight to nine months of sowing at the beginning of the main rainy season (Oromia Bureau of Agriculture, 2000).

Although korarima is important cash crop having a good export potential, research conducted on this crop has been limited to very few activities **(**Fissiha Gebreyesus and Zemed Mizan, 2015). Onlyinadequate efforts have been made to improve this crop usingconventional breeding and modern biotechnological approaches. Development of an in vitro regeneration system for any plant species has many advantages such as large-scale multiplication of important genotypes, season independent production of plants, production of disease free plants, and production of secondary metabolites, prerequisite for crop improvement through genetic transformation, conservation of rare, threatened or endangered medicinal plants, and facilitating easy exchange of germplasm (Singh*et al*., 2011).Toachieve such an improvement, proper agronomic andtissue culture procedures which assure successful andefficient large scale propagation protocolneed to be developed(Sahoo and Chand,1998; Prakash *et al*., 1999). There is increasing interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants (Sahoo *et al*., 1997).So far only very few studies have been conducted onmicropropagation methods (Wendyifraw Tefera and Wannakrairoj, 2004, 2006; Solomon Eyob, 2009; Rahel Hagos *et al*., 2011and Teferi Mekonnen and Tileye Feyissa, 2016), indigenous practices and farm based biodiversity (Solomon Eyob *et al.,* 2009), antioxidant and antimicrobial activities (Solomon Eyob *et al*., 2008). The essential oil yield and compositions from leaves, rhizomes, pods and seeds of korarima was done by Solomon Eyob *et al.* (2007, 2008) and the physical and biochemical propertiesof capsule and seed of korariamwas studied by Haimanot Mitiku *et al*. (2015). However, to the best of our knowledge, there are no reports on in vitro propagation using liquid medium and through somatic embryogenesis.To date, there has been only one report on the determination of the chromosome number of korarima (Wannakrairoj and Wondyifraw Tefera, 2013).There is also no previous studyon the degree and pattern of genetic diversity of *A. corrorima* in Ethiopia.Therefore, the present study was aimed to devedlop in vitro propagation and regeneration protocols,to study genetic diversity and to verify the chromosome number of *A. corrorima*.

2. Literature Review

2.1. The family Zingiberaceae

Zingiberaceae (ginger family) is generally regarded as one of the most highly evolved member of monocotyledonous family. The members are distributed mainly in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to central and south America. They are perennial rhizomatous herbs in shady habitats and are characterized bythe possession of a tuberous rhizome with an aerial shoot (pseudostem) often covered by sheathing leaf bases. The inflorescence is usually a spike or raceme. The flowers are bisexual, always epigynous and zygomorphic or asymmetric. Commercially the family is of great importance, since its members use in medicinal, perfumery, flavor and cosmetic industries. Quite a large number of plants from the family are being used as ornamentals too. The numbers of valid genera accepted today in zingiberaceae are 50 with about l000 species (Dahlgen *et al.,* 1985).

2.2. Origin, geographic distribution and cultivation of korarima

Korarima *(Aframomum corrorima)* grows in Keffa, Jimma, Sidama, Wollega, South Omo, Gamo Gofa,Illuababora, Awi and West Gojam, South Sudan, Uganda and Tanzania. Korarima is either cultivated in homegarden or directly collected from wild in the natural forest. In Gamo Gofa, South Omo and Kafa zones, korarima is cultivated in managed forest gardens similar to that of coffee. Korarima plants are purposely planted along with other perennial crop plants like enset, coffee, fruit trees and other multipurpose tree species (Jansen, 1981).Korarima is one of the different crop species that Ethiopia is known to be the center of origin and/or diversity **(**Girma Hailemichael *et al*., 2016).

In the semi-managed forest, farmers manage the naturally grown plants of korarima. Management practices include weeding of herbaceous plants and clearing shrubs and small trees in the lower forest strata to reduce competitions from other plants. Occasionally, farmers also plant korarima in wider spacing using young rhizomes taken from dense parts. The weeding is usually carried out once a year. Both in home-gardens and semi-managed forest where canopy opening is wide, korarima stand form large clusters through vegetative propagation (Lock, 1985). Under optimal light conditions, the plants even tend to be invasive. In unmanaged natural forest, korarima plants are sparsely distributed often single stems, and rarely forming small clusters, due to dense shade as well as competition from other shrubs and herbaceous plants. Farmers do not carry out any management practices other than harvesting when the fruits are ripe. The impact on the forest ecosystem is minimal, though it is not an economically attractive practice due to low productivity and low quality. The low quality is due to mixing of mature and immature fruits during harvest. Semi-managed forest and unmanaged forest based korarima production systems are common in Illuababora, Kafa, Jimma, Sheka and Wollega zones (Poulsen & Lock, 1997).

2.3. Taxonomy and botanical description

The name cardamom is used for herbs within two genera of the ginger family *Elettaria* (small cardamom) and *Amomum* (large cardamom). Both varieties take the form of a small seedpod, triangular in cross-section and spindle shaped, with a thin papery outer shell and small black seeds. *Elettaria*pods are light green in color, while *Amomum* pods are larger and dark brown. Small cardamom, *Elletaria cardamomum*, popularly known as the „Queen of spices,‟ is grown extensively in hilly regions of South India, but also in Sri Lanka, Papua New Guinea, Tanzania and Guatemala. It is typically 7 mm in size with green coloration and has a slightly sweeter fragrance than its larger cousin. Large cardamom, *Amomumsubulatum*, also known as Nepal cardamom, is a spice cultivated in eastern and northeastern India. It is typically 20 mm to 50 mm in size with black/brown coloration (USAID, 2011).

Aframomum corrorima or so called Ethiopian cardamom is herbaceous, perennial and aromatic crop. The genus *Aframomun* distinguished from other generaby the generally large size. It is closely related to *Amomum* from tropical Asia and was formerly included in it. *Aframomum zambesiacum* occurs in similar habitats as *Aframomum corrorima*. The seeds of the former species, however, are not used and in Ethiopia it is called monkey's korarima. Two major differences with the real korarima are that its leaves are less aromatic upon crushing, and its inflorescences bear 25–50 flowers (korarima only up to 5) **(**Jansen, 2002).

Korarima has leafy stems reaching 1–2 m tall; rhizome up to 1 cm in diameter, branched, red-brown, covered with thin, sub ovate scales and bearing thin, fibrous, pale brown roots; stem unbranched, mainly formed by the leaf sheaths, up to 1 cm in diameter but at base usually thickened up to 3 cm diameter. Leaves are alternate, simple and yellow-green with prominent veins and with ciliate margins. Inflorescence is a shortly stalked head arising from the rhizome near the base of a leafy stem, sometimes situated at the end of a rhizomatous runner, up to 5 flowered; peduncle up to 7 cm long, purplish-brown.The position of the stigma in the flower is below or against the base of the thecae of the anthers, hints at self-pollination, but there is neither observational nor experimental evidence. Occasionally cross-pollination by insects is possible due to the presence of large nectaries at the top of the ovaries.In many other *Aframomum* species, the stigma is situated at the top of the anther thecae and cross-pollination caused by insects is the rule, although the plants are self-fertile too. Animals such as monkeys eat the aril around the seeds and certainly contribute to natural dispersal of the seeds.Flowers are bisexual, white to pale violet and tubeup to 4.5 cm long. Fruit is an indehiscent, sub-conical berry usually, showing 3 longitudinal furrows but sometimes more, shiny green when immature, turning bright red at maturity, with 3 cells containing 45–65 seeds each. Seeds sub-globose in outline but usually somewhat angular, 2–5 mm in diameter, testa finely lined, glossy brown(Jansen, 1981).

2.4. Ecology

Korarima grows naturally at an altitude of 1350-2000 m in slightly shaded, more or less open sites in rain forest regions. It prefers a mean annual rainfall in the range 1400-

2000mm, but tolerates 1200-3000mm. There is no distinct dry season but usually most rain falls in June-August (50-60%). It grows best in areas where annual daytime temperatures are within the range of 16-24°C, but can tolerate 7-35°C. Korarima grows in Ethiopia in almost the same habitats (shade condition) as wild Arabica coffee (*Coffea arabica*). Korarima is a shade loving plant like *Elettaria cardamomum* under natural forest condition. It prefers some shade, but can succeed in a sunny condition. This plant succeeds in fertile, humus rich, and light to medium soil. Prefers pH in the range of 5.5-6.5but it can tolerate pH in the range of 4.5-7.5. Korarima performs well under moderate shade of around 50-60% canopy cover. Shade level management is one of the key agronomic practices in korarima production. Shade is very important for korarima production since it creates suitable microclimate and regulates moisture and temperature, which facilitates optimum growth and root development particularly when korarima rhizomes produce very shallow roots at each node (Jansen, 2002).

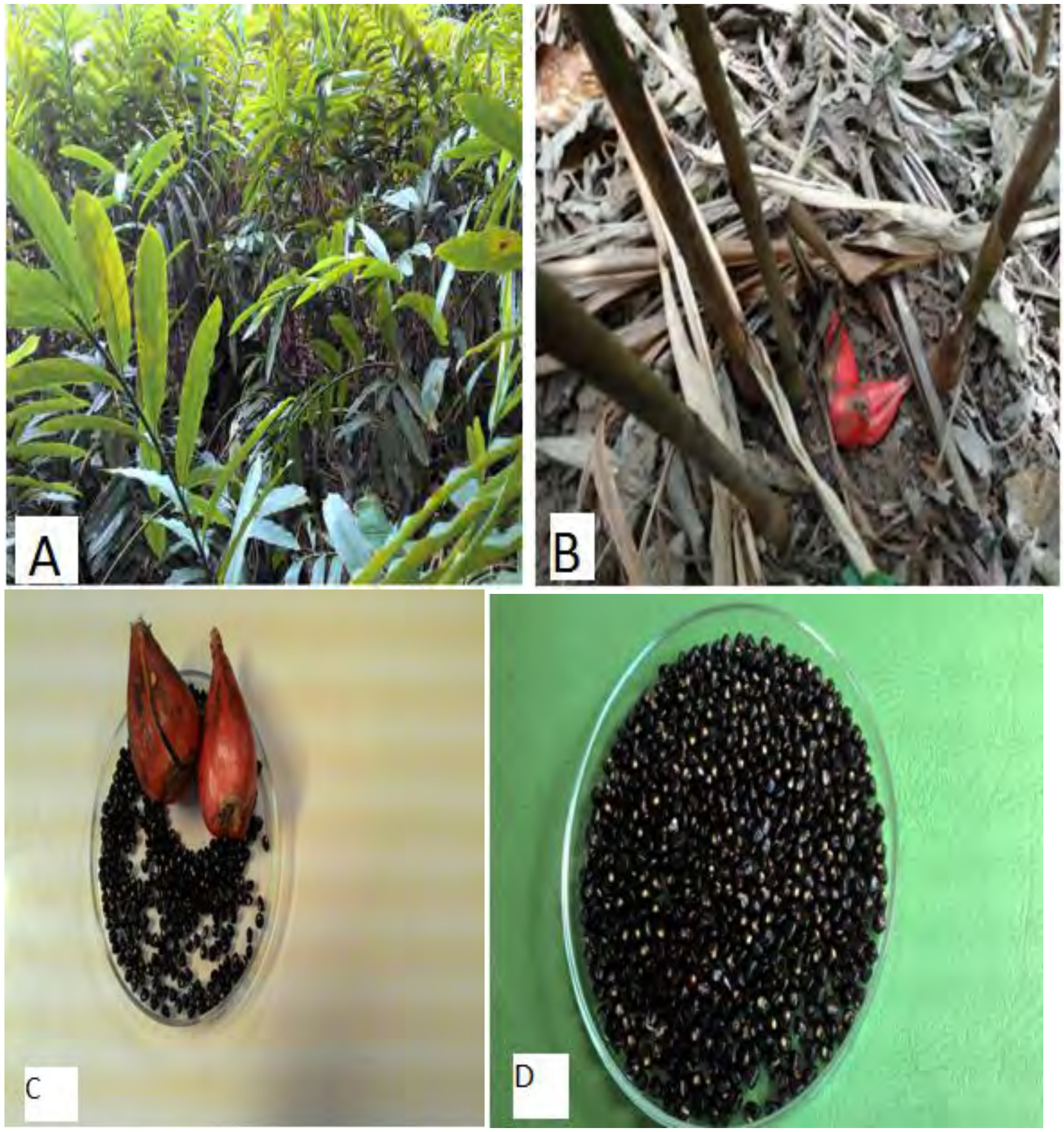

Figure 1. *Aframomum corrorima* (A)*A. corrorima* in Finote Selam, West Gojam(B) *A. corrorima* in fruiting stage(C) Red capsule of *A. corrorima* with seeds(D) *A. corrorima*seeds (Photo: Leweye Getie)

Korarima production can be integrated into any crop production system. It can be planted at the edge and between blocks of forest garden and managed forest systems. Since it tends to be invasive, the stand should be managed carefully to avoid competition with other crops of economic importance like coffee. Its demand for dense shade for optimal production makes it compatible with sustainable forest management. The diversity of shade tree species including endemic and rare shrubs and herbaceous species need due consideration to maintain korarima production under managed natural forest. Shrubs and herbaceous species of high conservation value that also compete with korarima should be retained in blocks of forest patches set aside for their conservation (Solomon Eyob *et al*., 2008).

2.5. Growth and harvesting

The seeds of*A. corrorima* are sown in the rainy season in the shade of other crops and transplanted the next rainy season for more spacing.The main flowering period is June-July, with fruiting in September-October. It is primarily harvested in October and November, but sometimes also bears fruits starting from June. Fruits mature about 2-3 months after flowering. At the early stage, the color of capsules is green but when it matures and ready for harvest, it turns to deep red color (Figure 1), but people usually start collecting still while it is green to get it prior to damage by baboons and rats(Girma Hailemichael *et al*., 2016).

Korarima flowers and red ripe fruits can be found at the same time in the field due to the irregular nature of flowering like that of *E. cardamomum*. It is mainly harvested from wildly grown plants in the forests of south, southwestern and western parts of Ethiopia. Korarima plants are propagated through asexual and sexual means and complete their juvenile phase and reach maturity after three to five years of planting to first harvest and have more than seven years of economical life (Solomon Eyob, 2009). To get quality product of korarima, the capsules should be red ripe and when the seeds are removed from the capsule, should be dark brown that have pungent and appreciable taste when crushed by teeth. There should be great care while harvesting the capsules of korarima not to create any opening on the capsules since through this opening important quality components (aroma and flavor) will be lost and it will serve as entrance for microorganisms(Mathewos Agize, 2016).

2.6. Uses of korarima

Many members of the family zingiberaceae are used therapeutically in traditional systems of medicine from time immemorial and some are well known spices. They have pharmacological, medicinal, economical and other industrial uses. Korarima has very widespread utilization in Ethiopia. The seeds are used to flavor all kinds of sauces, for which they are ground and usually mixed with other spices; occasionally they are also used to flavor coffee, tea, bread and butter (Zenebe Woldu, 2006). Strings of fruits are sometimes used as an ornament, or as rosaries (by the Arabs), and in the past the fruits had been used as currency in Ethiopia. Theseeds are darker and have a menthol-like taste. The oil composition of korarima isqualitatively similar to that of Indian cardamom, except for the reduced content of terpinyl acetate which is the majorcomponent of the later (Baser & Kürkçüoglu, 2001).

The use of korarima is little known outside Ethiopia. The dried pods are sold in almost every Ethiopian market and are quite expensive compared to other spices; fresh capsules are sold too in the production areas, rarely only the seeds, and are used daily by most families in rural areas(Kohl and Uhl, 2002). It is used to add unique characteristic to local food and is an ingredient of “berbere”, “mitmita”, “awaze” and other spice mixtures.Korarima seeds, pods, leaves, rhizomes and flowers are used in southern Ethiopia as traditional medicine for human and animal diseases caused by unknown agents; and particularly used to treat any part of the animal body upon swelling.In addition, korarima seeds are widely used medicinally as a tonic, laxative, carminative and purgative drug, and they are added to food for preservative purposes. It was effective against tonic convulsions, headache, stomach-ache and sore throat when taken orally (Solomon Eyob *et al*., 2008). Although korarima is consumed as a spice, it has also antioxidant and antifungal activity(Solomon Eyob, 2009; Hymete*et al*., 2006). Antioxidants have the ability to reduce oxidative damage by free radicals and prevent diseases like cancer, cardiovascular diseases, atherosclerosis, diabetes, asthma, hepatitis, liver injury, arthritis and ageing (Lee *et al*., 2000).

2.7. Yield and market

From an economic stand point, zingiberaceae form an important group with considerable economic potential with plants such as *Aframomum, Amomum,Curcuma, Elettaria, Zingiber and Kaempferia.* Ethiopia is situated on the ancient spice path from Asia, and the city of Axum was an important center in the early spice trade. Today Ethiopia is one of the largest consumers of spices in Africa. Ethiopia grows many spices, used not only to flavor bread, butter, meat, soups and vegetables, but also to produce medicines and perfumes (Boere *et al*., 2015). The majority of spices produced in Ethiopia (more than 80 %) are absorbed domestically. At the same time, export of spices is developing and brings increased foreign exchange. In 2009, spice exports reached 15,000 metric ton, equaling a value of 11 million USD (Spice Sector Strategy Coordinating Committee, 2010).

Estimate of world imports of spices is 5.25 million tons valued at 15 billion USD, with an annual growth rate of 4 percent. This is against a world production of 8.5 million tons valued at 25 billion USD. The difference between world production and import is domestic consumption of producing countries. As far as the product mix is concerned, the bulk of spices are exported in "whole" or "unground" form, while only 15-20 percent of spices are sold in ground form, as mixtures of ground spices and as essential oils and oleoresins. The substantive shift towards natural products in the West has stimulated the demand for spices in recent times. Added to this is the new demand wave for organic spices in Europe, USA and Japan. Though the size of this market is small (around 1 percent of the total market), the annual growth rate is to the tune of 25-30 percent. Hence, there is a large and growing world demand for unprocessed and ground spices as well as spice extracts such as essential oils and oleoresins (EIC, 2010). The export of ginger accounted for the lion share of 71% (worthing more than 38 million USD) of total spice export for the period 2005/06-2009/10, followed by turmeric (8.3%), and cumin (7.9%). However, fenugreek and coriander, that showed higher export volume share of total spice export (i.e., 3.4 and 3.3% respectively) higher than that of pepper (1.5%), korarima (0.03%) and other (2.04%).In value terms, ginger claimed a higher share of 62% of total spice export, followed by cumin seed (13.7%), turmeric (7.2%), pepper (4%), korarima (1.41%) and other (4.08%) (Masresha Yimer, 2010).

There is high potential for commercial production of korarima. For commercialization of korarima, appropriate agronomic practices, selection of high yielding and good quality planting stocks and mainstreaming of forest and agricultural development extension services are required. The economic returns obtained from korarima (yields per ha) were much higher than food cereals grown in the major korarima growing administrative zones as reported by Ethiopian Institute of Agricultural Research(EIAR, 2000). The great potential of this plant has, however, encountered different production problems. Yields, areas of production and biodiversity have declined both from farmers‟‟ field and natural forests of southern Ethiopia. Destruction of forest trees for expansion of arable and grazing land, new settlement and forest fire have resulted in low supply and high demand of korarima in local and export markets (Solomon Eyob *etal*., 2009).

The actual average yield of dried pods recorded in farmers field in the 1980s ranged from 700 to 950 kg per hectare when the korarima plants received filtered sunlight all day through permanent tree shades. Reduction in canopy cover and shade trees density has significantly affected productivity (Purseglove *et al*., 1981). The yield from a well-managed korarima stand is estimated to be 5.5 times that of the Indian cardamom. About 3–5 hour per day direct sunlight damages the plants. Currently the yield can be as much as 500 kg of dried fruits per ha **(**Mathewos Agize, 2016).

Dried capsule of *A. corrorima* has highly significant economic importance for local and as export commodity in addition to various uses. Previously, Ethiopia was well-known for its considerable exports of *A. corrorima* capsules to the world market, mainly as a substitute for the Indian cardamom (Wondyifraw Tefera and Surawit, 2004; Solomon Eyob, 2009). In 2012/13 farm gate prices of dried and locally processed *A. corrorima* capsules is 100 to 130Ethiopian birr per kg and when it reaches the central market, more than 40% price increase is very common. The current export price of some Ethiopian spices include black cardamom (US $7000-7500 / Ton), ginger US ($3500-6500/ Ton), cinnamon (US $5500-6000 / Ton) and black cumin (US $3000-3100 / Ton) (Alibaba Group, 2016).

As most of the spice is collected from inside the wild forest, the capsules are harvested mostly at immature and mature green stage because of competition among competitive spice collectors from the natural forest and wild animals (Apes, Monkeys, Squirrels)(Fissiha Gebreyesus *et al*., 2015). Some of the commercial productions in home-gardens in Gamo Gofa and South Omo area are declining to due to loss of shade trees and traditionally cultivated landraces. Korarima, on the other hand, is highly demanded on the local market, and even on international market. The leading cardamom (korarima) importer is Jordan with a 44% share value of cardamom export, followed by Saudi Arabia (18.5%), Israel (14.1%), Yemen (9.8%), India (4.5%) and US (3.8%)(Masresha Yimer, 2010).

2.8.Propagation and planting

Korarima can be propagated either by seed or by the division of rhizome(cutting of its clumps), the latter is the conventional vegetative propagation technique used in this plant. The vegetative method shortens the juvenile phase of the stand and enables propagation of true-to-type plants of a desired clone**(**Wondyifraw Tefera and Wannakrairoj, 2006).

During *in vivo* propagation of korarima, the suitable propagation technique is also by using seed and rhizome (Solomon Eyob, 2009).

2.8.1. Plant tissue culture

Tissue culture is the in vitroaseptic culture of cells, tissues, organs or whole plant undercontrolled nutritional and environmental conditions often to produce the clones ofplants (Thorpe, 2007).The controlledconditions provide the culture an environment conducive for their growth andmultiplication. These conditions include proper supply of nutrients, pH medium, adequatetemperature and proper gaseous and liquid environment.This technology is being widely used for large scale plant multiplication.Apart from their use as a tool of research, plant tissue culture techniques have industrial importance in the area of plant propagation, diseaseelimination, plant improvement and production of secondary metabolites. Small pieces oftissue (explants) can be used to produce hundreds and thousands of plants in acontinuous process. A single explant can be multiplied into several thousand plants inrelatively short time period and space under controlled conditions, irrespective of theseason and weather on a year round basis (Akin-Idowu*et al*., 2009). Endangered, threatened and rare species havesuccessfully been grown and conserved by micropropagation because of high efficiency ofmultiplication and small demands on number of initial plants and space.

In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of somaclonal and gametoclonal variants. Certain type of callus cultures give rise to clones that have inheritable characteristics different from those of parent plants due to the possibility of occurrence of somaclonal variability which leads to the development of commercially important improved varieties (George and Hall, 2008).The micropropagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted and high yielding genotypes with better disease resistance and stress tolerance capacities (Brown and Thorpe, 1995). Commercial production of plants through micropropagation techniques has several advantages over the traditional methods of propagation through seed, cutting, grafting and air-layering, etc. It is rapid propagation processes that can lead to the production of virus free plants (Garcia-Gonzales *et al*., 2010).

Callus induction and plant regeneration arekey tools in plant biotechnology that exploits the totipotentnature of plant cells. Systems of plant regeneration canbe categorized as direct and indirect (Mukherjee *et al*.,2011). Almost all types of explant tissues are now usedas regeneration systems through direct (direct generationfrom explants) and indirect methods (callus-mediatedshoot regeneration) (Varshney and Johnson, 2010). Shoots can be derived either throughdifferentiation of non-meristematic tissues known asadventitious shoot formation or through pre-existingmeristematic tissues known as axillary shoot formation. Asuccessful plant regeneration protocol requires appropriate choice of explant, age of the explant, definitemedia formulations, specific growth regulators, genotype,source of carbohydrate, gelling agent and other physicalfactors including light hour, temperature, humidity, etc(Deoreand Johnson, 2008).

2.8.1.1. Micropropagation via meristem culture or axillary bud/shoot tip culture

The direct shoot bud formation without any callus phase from appropriate explants is of great success for large scale clonal multiplication of desired clone all round the year to boost the commercial floriculture. Schaeffer (1990) defined micropropagation as the in vitro clonal propagation of plants from shoot tips or nodal explants, usually with an accelerated proliferation of shoots during subcultures. In vitro propagation through meristem culture is the best possible means of virus elimination and produces a large numbers of plants in a short span of time. It is a powerful tool for large scale propagation of horticultural crops. The term „meristem culture" specifically means that a meristem with no leaf primordia or at most 1–2 leaf primordial which are excised and cultured. Micropropagation is an alternative method of vegetative propagation, which is well suited for the multiplication of elite clones. It is accomplished by several means, i.e., multiplication of shoots from different explants such as shoot tips or axillary buds or direct formation of adventitious shoots or somatic embryos from tissues, organs or zygotic embryos.

Micropropagation generally involves five distinct stages: mother plant selection, establishment of aseptic culture, shoot multiplication, rooting of in vitro grown shoots, and acclimatization. Stage 1: culture initiation depends on explants type or the physiological stage of the donor plant at the time of excision. Explants from actively growing shoots are generally used for mass scale multiplication. Stage 2: shoot multiplication is crucial and achieved by using plant growth regulators i.e. auxin and cytokinin. Stage 3: the elongated shoots, derived from the multiplication stage, are subsequently rooted either ex vitro or in vitro. In some cases, thehighest root induction occurs from excised shoots in the liquid medium when compared with semi-solid medium. Stage 4: acclimatization of in vitro grown plants is an important step in micropropagation (Rout *et al*., 2006).

In vitro plants are exposed to invariably controlled growth conditions such as high amount of organic and inorganic nutrients, plant growth regulators, carbon source, and high humidity, low light and poor gaseous exchange. Although they may support rapid growth and multiplication, the controlled conditions induce structural and physiological changes in plants rendering them unfit to survive when transferred directly to the field. Thus, a gradual acclimatization from laboratory to field condition is necessary. The plants are gradually shifted from high humidity/low irradiance conditions to low humidity/high irradiance conditions, enabling them to survive under „adverse" conditions (Matysiak and Nowak, 2001).

2.8.1.2. Micropropagation via somatic embryogenesis

Somatic embryogenesis can be described as the process by which somatic cells develop into structures that resemble zygotic embryos through an orderly series of characteristic embryological stages without fusion of gametes (Raemakers *et al.*, 1995). It is an artificial process in which a plant or embryo is derived from a single somatic cell or group of somatic cells. No endosperm or seed coat is formed around a somatic embryo.

Somatic embryos, which are bipolar structures, arisefrom individual cells have no vascular connectionwith the maternal tissue of the explant (Haccius, 1978).Somatic embryos are induced from cultured callus cells by a relatively simple manipulation of the culturing condition. Applications of this process include: clonal propagation of genetically uniform plant material; elimination of viruses; provision of source tissue for genetic transformation; generation of whole plants from single cells called protoplasts; development of synthetic seed technology. Under controlledenvironmental conditions, somatic embryos germinatereadily, similar to their seedling counterpart. Thecommercial application of somatic embryogenesis willbe accomplished only when the germination rate ofsomatic embryos is high up to 80–85% (Jiménez, 2001). The major limitations are genotypic dependence of somatic embryo production and poor germination rate (Jain, 2002).

The mass propagation of plants through multiplication of embryogenic propagules is the most commercially attractive application of somatic embryogenesis (Merkle *et al.*, 1990). Somatic embryogenesis has many advantages over organogenesis in this respect: (a) it permits the culture of large numbers of „reproductive units, for example, 60,000 to 1.35 million somatic embryos per liter of medium with the presence of both root and shoot meristems in the same element; (b) themode of culture permits easy scale-up transfers with low labor inputs since embryos can be grown individually and freely floating in liquid medium; (c) unlike shoots, somatic embryos frequently originate from single cells and the embryogenic cultures can be arranged and purified so that one can deal with practically pure cultures of homogeneous material; and, (d) plants derived from somatic embryos are less variable than those derived by way of organogenesis (Osuga *et al.*, 1999).

Another application is in the production of plants with different levels of ploidy; i.e.,obtaining haploid embryos by cultivating anthers and raising triploids from endosperm have beensuggested and to a very limited extent exploited (Terzi and Loschiavo, 1990). Also, success in inducing dormancy and the accomplishment of long-term storage, together with the achievement of encapsulation of somatic embryos, has opened up the possibility for their use in the synthetic seed technology (Litz & Gray, 1995).One of the most important prerequisites for genetic manipulation of plants in vitrois the ability to grow somatic cells in sterile plant growth medium and to regenerate viable plants from these cultures. Somatic embryogenesis, therefore, is a more efficient pathway for studies involving production of genetically transformed plants (Vicient & Martínez, 1998).

2.8.1.3. Advantages of and disadvantages of somaclonal variation

Tissue culture induced somaclonal variation in fruit crops is similar to variations induced with chemical and physical mutagens, which proffers an opportunity to unearth natural variability for their potential utilization in crop improvement. Like any other technology, in vitroinduced somaclonal variation has its own intrinsic advantages and disadvantages.
The advantages of somaclonal variation include: cheaper than other methods of genetic manipulation, tissue culture systems are available for many plant species, not necessary to have identified the genetic basis of the trait, or indeed, in the case of transformation, to

have isolated and cloned it, novel variants have been reported among somaclones, variation may be generated from different locations of the genome than those, which are accessible to conventional and mutation breeding, no possibility of obtaining chimeric expression if somaclones are raised through cell culture. The dis advantages are: inability to predict the outcome as they are random and lack reproducibility, the variations are usually negative, positive changes are also altered in negative ways, sometimes, there are chances that the changes are not novel, the changes may not be stable after selfing or crossing, no in vitroselection methods exist for complicated traits such as yield, solids, sweetness, texture or shelf life (Krishna *et al*., 2008).

2.8.1.4. Micropropagation of Korarima

Plant tissue culture offers special advantages as it ensures a continuous supply of planting material in a short span of time and helps in multiplication, as well as, conserving wild germplasm. Micropropagation through rhizome explants (Solomon Eyob, 2009; Wondyifraw Tefera and Wannakrairoj, 2004; 2006) and shoot tips from in vitro germinated seeds (Teferi Mekonnen and Tileye Feyissa, 2016; Rahiel Hagos and Hailay Gebremdhin, 2015)could help the regeneration of large number of plants in a relatively short time.It is also possible to induce multiple shoot growth and complete plant development in highland korarima by using different concentrations of TDZ alone or in combination with BAP. Shoot tips obtained from in vitroseedlings showed better performance with respect to percent survival and shoot induction than rhizome buds obtained from crop grown under field condition. IBA at 1 mg/l was best to induce rooting from in vitroshoots of korarima (Solomon Eyob, 2009).

The combined use of TDZ with either of BAP or(imazalil) IMA resulted in a significant synergistic effect on shoot proliferation and growth of korarima.Considerable improvements in shoot multiplication were obtained when 0.5 mg/l TDZ was used together with 2 mg/l IMA. Culture medium supplemented with 3 mg/l BAP with this same level of TDZ (0.5 mg/l) gave about 14 shoots/explant that have better growth. In all cases, however, the longest shoots were obtained from the PGR-free medium(Wondyifraw Tefera and Wannakrairoj, 2006). The use of KN resulted in significantly lower shoot regeneration as compared to BAP. Increasing the concentration of BAP increased the number of regenerated shoots but decreased shoot length. The addition of BAPin the mediumstimulates the rate of shoot multiplicationcompared to KN and the maximummultiplication was found on media with 6.0 mg/l of BAP alone. Rahiel Hagos(2011)suggested that higher concentration of BAP could be used to obtain the maximum shoot multiplication on korarima

2.9. Chromosome cytology

Cytology has been widely utilized in tackling taxonomical problems in the past. It is believed as a dependable tool for solving taxonomic problems and for elucidating systematic relationships, phylogeny and evolution of related plant groups. The information like chromosome number, structure, morphology and behavior during mitotic and meiotic divisions has been of considerable value in understanding interrelationships and delimitation of taxa (Naruhashi and Iwatsubo,1991).

Out of about 1000 species so far, around ten percent of species are reported for their chromosome number. It is quite remarkable that little attention has so far been paid to the detailed cytological investigation and phytochemical screening of members of Zingiberaceae. Most of the cytological studies undertaken were concentrated mainly on the determination of chromosome number and have provided scarcely any data on the chromosome morphology and structure in detail. The detailed karyomorphological reports known are centered mainly on a few genera such as *Zingiber* and *Kaempfera*. In Zingiberaceae many of the members possess very small sized chromosome and hence detailed kanotype analysis is very difficult by routine procedures (Bhadra and Bandyopadhyay, 2016).

Plants of Zingiberaceae show a wide variation of somatic chromosome number ranging from 2n= 22 to 2n= 96. This variation in chromosome number is attributed primarily to intraspecific number variations (Ramachandran, 1969). The base chromosome number of the family is reported to vary from x=6 to x=25 (Joseph, 1998). It was suggested that such high base number (x=25) might not be the original basic number of the taxa, and recent reports indicate that the primary basic chromosome numbers were possibly x=6 to 9 (Joseph, 1998), from which other secondary basic numbers had evolved, following varied and complex lines of chromosomal mutation, and thus, evolution (Mahanty, 1970).

2.10. Genetic diversity

Understanding the molecular basis of the essential biological phenomena in plants is crucial for the effective conservation, management, and efficient utilization of plant genetic resources. In particular, an adequate knowledge of existing genetic diversity in plant population is necessary for the parental selection in order to maximize genetic improvement and for the efficient management of crop genetic resources. Genetic diversity is a study undertaken to classify an individual or population compared to other individuals or populations. This is a relative measure, as the distance between any pair of entries in the study is greater or lesser depending on all pairwise comparisons that can be made in the study. The improvement of crop genetic resources is dependent on continuous mixtures of wild relatives, traditional varieties and the use of modern breeding techniques. These processes all require an assessment of diversity at some level, to select resistant, highly productive varieties (Mondini *et al*., 2009).

There are three major types of markers that can be used to determine genetic diversity in plant species. These are morphological, protein-based and DNA-based markers.

2.10.1Morphological markers

Traditionally, diversity within and between populations were determined by assessing differences in morphology. These measures have the advantage of being readily available, do not require sophisticated equipment and are the most direct measure of phenotype, thus they are available for immediate use. However, morphological determinations need to be taken by an expert in the species. Morphological markers are routinely used for estimating genetic diversity of plants since they are cheap and fast. Morphological differences arise due to selection and/or genetic drift, and phenotypic variation (Serebryanaya and Shipunov, 2009). The commonly used traits for morphological characterization include phenotypic variability of plant organs such as flowers, leaves and stems.Morphological markers(also called "classical" or "visible" markers) which are phenotypic traits are influenced by environmental conditions. Thus, observations may not represent true genetic differences or similarities (Ikbal *et al*., 2010).They tend to be restricted to relatively few traits, display a low degree of polymorphism, are often environmentally variable in their manifestation, and can depend on the expression of several unlinked genes. Furthermore, some may affect plant viability or seed set, distorting gene frequencies in the progeny.

2.10.2. Biochemicalmarkers

Protein markers are usually named biochemical markers.Protein markers (seed storage proteins and isozymes) are generated through electrophoresis, taking advantage of the migration properties of proteins and enzymes, and revealed by histochemical stains specific to the enzymes being assayed. Detecting polymorphisms i.e. detectable differences at a given marker occurring among individuals in protein markers is a technique that shares some of the advantages of using morphological ones. However, protein markers are also limited by being influenced by the environment and changes in different developmental stages. Even so, isozymes are a strong complement to the simple morphometric analysis of variation they are still widely used asgenetic markers for reasons that include the following:they are inexpensive compared to DNA markers,the laboratory protocols are well-established innumerous plant species, they are products of structuralgenes whose roles in metabolism are known in mostcases and most importantly, their typical levels ofvariation makes them suitable markers for a numberof purposes (Farooq and Sayyed, 1999). Isozyme analysis is simple, fast and cheaper than DNA based methods; however, the limited number of loci available for study limits their usefulness (Jingura and Kamusoko, 2015).

2.10.3. Molecularmarkers

DNA polymorphisms can be detected in nuclear and organelle DNA, which is found in mitochondria and chloroplasts. Molecular markers concern the DNA molecule itself and they are considered to be objective measures of variation. DNA markers are the most widely used markers predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number (covering the entire genome) and are not affected by environmental factors and/ or the developmental stage of the plant (tests can be carried out at any time during plant development) (Winter and Kahl, 1995). Apart from the use of DNA markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within cultivars and fingerprinting the germplasms (Rahimi *et al*., 2012).

In general, molecular markers detect polymorphism by assaying subsets of the total amount of the DNA sequence variation in a genome. Different types of molecular markers are also different as to their potential to detect differences between individuals, their cost, facilities required, and consistency and replication of results (Bernardo, 2008). The choice of markers is objective dependent. The following techniques are those most used in genetic diversity studies. Polymorphisms detected by the RFLP assay reflect the variation of restriction fragment sites.

PCR-based polymorphisms result from DNA sequence variation at primer binding sites and from DNA length differences between primer binding sites. The Inter-simple sequence repeats (ISSR) are semiarbitrary markers amplified by polymerase chain reaction (PCR) in the presence of one primer complementary to a target microsatellite. SSR assay utilizes pairs of primers flanking each simple sequence repeat and polymorphisms differ for the number of repetitive di-, tri- or tetra-nucleotide units present at one locus. AFLP assay detects polymorphisms at multiple loci and involves the use of combination of primers specific for two distinct four-base and six-base long restriction sites flanking the target sequence unit. Although RFLP markers have provided useful estimates of the genetic diversity and relatedness in crop plants, there is some concern about their discriminatory power. Increasing the number of probe-enzyme combinations may improve the number of RFLP marker loci detectable, but the level of polymorphism that can be revealed by PCR-based markers still remains higher. In fact, owing to their own genetic nature, SSR markers usually detect multiple alleles at a given locus while AFLP assays mainly detect single alleles at multiple loci randomly distributed in the genome. A newly introduced method is represented by SNP markers based on the detection of single-nucleotide polymorphisms by direct DNA sequencing of target gene regions(Barcaccia, 2010).

2.10.3.1. Inter simple sequence repeat (ISSR) technique

In the assessment of genetic diversity, molecular markers based on DNA have many advantages. Among the polymerase chain reaction (PCR)-based marker techniques, inter-simple sequence repeats (ISSR) are one of the simplest and widely used markers (Vijayan, 2005). The ISSR marker belongs to a class of multi-locus, dominant genetic markers that also include the amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) markers, and their derivatives. Dominant markers do not allow clear distinction between homozygotes and heterozygotes. These markers, however, usually produce multiple DNA fragments (each of which is considered a locus) in a single reaction, allowing the generation of a large number of loci across the genome of any species without the need to first know the DNA sequences of the target regions (Serra *et al*., 2007).

ISSR markers use arbitrary primers represented by short repeated sequences. Allelic polymorphisms occur whenever the repeated sequence is missing or insertions/deletions modify the distance between repeats. ISSRs have the advantage of allowing analysis of multiple loci in a single reaction and to be more accessible to laboratories that lack sophisticated equipment and/or adequate technical expertise. ISSR gives better reproducibility of results, which can be explained by its use of longer primers (microsatellites) and higher annealing temperatures than RAPD.ISSR marker system can generate high resolution band patterns and high levels of polymorphism as high as 90%. ISSRs have been applied successfully in population genetic studies for a variety of plants (King *et al.* 2002, Wang *et al.* 2004).

For most genetic variation studies, a good genetic marker is defined by high genetic variability and the ability to generate multi-locus data from the genome under study (Anne, 2006). The generation of ISSR markers makes use of microsatellite sequences that are highly variable and ubiquitously distributed across the genome, at the same time achieving higher reproducibility compared to using RAPDs and costs less in terms of time and money compared to using AFLPs. ISSR marker involves amplification of genomic DNA by PCR using a primer typically attached at the 5“ or 3“ end with 1-4 arbitrary, often degenerate bases extended into the flanking sequences (Zietkiewicz *et al*., 1994). ISSR markers offer great potential for differentiating closely related cultivars and the generated bands are very repeatable on duplicate samples (Fang and Roose, 1997). ISSR markers have been used for characterization of germplasm, (Charters and Wilkinson, 2000) for assessment of genetic diversity and phylogenetic studies of different species (Joshi *et al*., 2000) for identification of DNA markers linked to agronomic traits (Ratnaparkhe *et al*., 1998) and for plant breeding (Redy, 2002).

3. Objectives

3.1. General objective

- To establish protocols for micropropagation and in vitro regeneration,to provide information on the chromosome number and to investigate genetic diversity of *Aframomum corrorima*using ISSR molecular marker.

3.2. Specific objectives

- To develop an efficient micropropagation protocol of *A. corrorima* from shoot tip explants
- To investigate the effect of different salt strength, sugar concentrationand to examine the effect of solid and liquid media on in vitro propagation of *A. corrorima* using shoot tip explant
- To establish callus induction and plant regeneration protocol of *A. corrorima*using rhizome explants
- To confirm previous results onchromosome number of*A. corrorima*
- To assess the extent of genetic variation within and among populations of *A. corrorima* found in Southern and Northwestern Ethiopia by using ISSR marker

4. Materials and Methods

All the laboratory activities and experiments were conducted in PlantTissue Culture and Molecular Biology Laboratory at Addis Ababa University.The greenhouse trial was also carried out in the greenhouse of AAU, College of Natural Sciences.

4.1. In vitro propagation of *A. corrorima*

4.1.1. MS Stock solution preparation

Murashige and Skoog (1962) basal medium was used throughout this research activity. Initially, full strength stock solutions of macronutrients, micronutrients, iron and vitamins were separately prepared. The appropriate amount of each nutrient wasweighted in grams per liter and dissolved in distilled water consecutively insuch a way that the next nutrient was added after the first one was completely dissolved. After allthe components were completely dissolved using magnetic stirrer, the solution was poured intoplastic bottles and stored at -20°C until used.

4. 1.2. Plant Growth Regulators Stock Solution Preparation

The plant growth regulators (PGRs) used for this study were 6-benzyl aminopurine (BAP), kinetin (KN) and tidiazuron (TDZ) and 2,4-Dichlorophenoxy acetic acid (2,4-D), α-naphthalene acetic acid (NAA), indol-3-butyric acid (IBA) and Indole acetic acid (IAA). Allgrowth regulator stock solutions were prepared by weighing and dissolving the powder in distilled water at the concentration of 1.0 mg/ml. To begin the dissolving process, 3-4 drops of 1N NaOH or1N HCl were added based on the requirement of the growth regulators (NaOHfor auxin and HCl for cytokinin). Then, the volume wasadjusted by adding distilled water. Finally, growth regulators stock solutions were storedin a refrigerator at a temperature of +4°C for a maximum of one month.

4. 1.3. Culture media preparation

A full strength MS basal medium was prepared using 50 ml/l MS macronutrients, 5 ml/l MS micronutrient, 5 ml/l MS vitamin, 5 ml/l Fe-Na-EDTA and FeSO4 mixture, 3 % sucrose. Appropriate type and amount of PGR was added and then the pH was adjusted to 5.7 using 1 N HCl and 1 N NaOH and 7.0 g agar was added and melted on a stirring hot plate. When the agar became clear solution, 50 ml medium was dispensed into Magenta GA-7 culture vessels for shoot initiation and multiplication and autoclaved ata temperature of 121°C and 105 KPa pressure for 15 minutes.

For callus induction and in vitro regeneration experiments, 25 ml of the medium was poured into each 90 mm diameter Petri dish in the laminar flow hood.MS basal mediumcontaining 3%, 2%, 1% sucrose and 3% table sugar were used separately to compare the effect of different sugar concentrations on shoot multiplication and elongation.Full strength MS basal liquid medium was preparedand 10 ml of it was dispensed in to test tubes in the laminar flow hood. The cultures were placed on an orbital shaker 120 rotations per minute for aeration.In order to test the effect of different salt strength on shoot multiplication the amount of macro nutrient, micro nutrient, Fe-EDTA and FeSO4 mixture and vitamin stock solutions used decreased by 1/2 and 1/3 in case of 1/2 and 1/3 salt strengths.

4.1.4. Plant material collection and surface sterilization

Capsules of *A. corrorima* were collected from Finote Selam Nursery site, West Gojam, Amhara Region during September to January of 2013. Capsules were cut open to release seeds and dried in shade for 3- 4days. The seeds were washed with detergent for 5-10 minutes and rinsed in running tap water for 3-5 minutes for each experiment. Then the seeds were sterilized in 70% alcohol for 1 min and rinsed three times with sterile distilled water and also further disinfected withdifferent concentrations of Clorox (NaOCl, 5.25% of available chlorine)(10%, 25%, 50% and 100% having 0.53%, 1.31%, 2.63% and 5.25% (v/v) available chlorine, respectively) for 30 minutes followed by five rinses with sterile distilled water. The surface sterilized seeds were cultured in culture jars containing 50 ml plant growth regulators free MS(Murashige and Skoog, 1962) medium. Ten seeds per culture vessels in ten replications were used. Seeds without any Clorox treatment were used as a control.

4. 1.5. Shoot initiation

Shoot tips of 1.0-2.0 cm long were excised from in vitro germinated seedlings and were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) in combination with NAA (0.1mg/l).For each treatment, five Magenta culture vessels (replications) each containing six explants was used, which is a total of 30 explants per treatment. The culture vessel with cultured explants were properly sealed with parafilm, labeled and placed in growth room chamber with 16 hours photoperiod and at a temperature of 25 ± 2°C. Percentage of initiation was computed and mean number of shoots, mean shoot length and mean number of leavesper explant were recordedafter 45 days.The stock solution composition, agar concentration and other physical conditions were the same for all thetreatments.PGR free medium was used as a control.

4. 1. 6. Shoot multiplication

After 45 days of growth on the culture initiation medium, young and healthy micro-shoots were cultured on shoot multiplication medium. The initiated cultures were aseptically cut off and cultured on shoot multiplication medium.The shoot multiplication medium is full strength MS medium containing different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) andKN(0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) separately, combination of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and KN (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), combination of BAP(0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and NAA (0.25 and 0.5 mg/l), combination of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and IBA (0.25 and 0.5 mg/l), combination of KN(0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and NAA (0.25 and 0.5 mg/l)and combination of KN (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l)and IBA (0.25 and 0.5 mg/l).To investigate the effect of different factors on shoot multiplication, young shoots obtained from shoot initiation medium were used as explants and cultured onto freshly prepared MS basal medium containing similar combination and concentration of growth factors. BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and TDZ (0.1 mg/l) combinations were used to compare the effect of different sugar concentration (3%, 2%, 1% sucrose and 3% common table sugar) for shoot multiplication. Similarly, to investigate the effect of different salt strengths (full MS, half MS, and one-third MS) on shoot multiplication, combination of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l)and TDZ (0.25 and 0.5 mg/l) was used. BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and NAA (0.1 mg/l)combination was also used to compare rate of shoot multiplication in solid and liquid MS medium. For the third experiment regenerated plantlets were cultured on shoot multiplication medium containing different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) in combination with 0.25 mg/l KN and different concentration of KN(0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) combined with 0.25 mg/l BAP.

Six shoots per culture vessel and five replications for each treatment were used. The culture vessels were properly sealed, labeled and randomly placed in the growth room chambers with the same culture conditions (temperature, photoperiod and light intensity) as that of the initiation experiment. PGR free medium was used as a control. Sub-culturing was carried out at monthly interval by transferring the newly multiplied micro-shoots to fresh medium.The number of shoots per explant, mean shoot height and number of leaves per shoot were recorded every four weeks.

4.1.7. Callus induction

Young rhizomes (0.5-1.0 cm long) from in vitro multiplied shoots were cut into small pieces and punched using forceps, wounded and cultured on MS medium containing 30 g/l sucrose and different concentration of 2, 4-D alone (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0

mg/l), 2,4-D in combination with 0.25 mg/l BAP and 0.5 mg/l BAP, 2,4-D combined with 0.5 KN and 0.5 NAA for callus induction. Ten pieces of rhizomes per Petri dish with three replications were used. The culture was maintained in full darkness for eight weeks at room temperature. The first subculturing was done after 45 days and the rest subculturing was carried out every two weeks on the same fresh callus induction medium.The number of rhizomes that induced callus (percentage of response) and morphology of callus were observed and recorded.

4. 1. 8. Shoot regeneration

After three months, the calli produced were transferred to shoot regeneration medium containing various concentrations of BAP (0.5, 1.0, 2.0, 3.0 mg/l) in combination with 0.25 mg/l and 0.5 mg/l IAA and different concentrations of KN (0.5, 1.0, 2.0, 3.0 mg/l) combined with 0.25 mg/l and 0.5 mg/l IAA. Then, the cultures were transferred to a 16 hour photoperiod and covered with loose transparent soft papers for fifteen days. The cultures were then uncovered and maintained under reduced light conditions at 25 ± 2°C for another 15 days after which they were transferred to full light intensity with white fluorescent lamp and 16 hour photoperiod. After four months, the number of calli that regenerated shoot, shoot length and number of regenerated shoots per explant were recorded.

4. 1.9. Rooting

Rooting of the already multiplied shoots of *A. corrorima* was conducted on half strength MS basal medium supplemented with different concentrations of IBA, IAA and NAA alone (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l).Rooting of shoots in both solid and liquid media was conducted on halfstrength MS basal medium containing different concentrations of IAA (1.0, 2.0, 3.0, 4.0mg/l) alone. For the third experiment rooting was conducted using well developed shoots on half strength MS media supplemented with various concentrations of NAA (1.0, 2.0, 3.0 mg/l) in combination with IBA (0.25 mg/l and 0.5 mg/l).PGR free half strength MS basal medium was used as control in all cases. Six replications with five explants per Magenta culture vessel and one explantper test tube in 30 replications were used for solid and liquid medium respectively. Finally, the cultures were placed randomly on growth room and the number and length of roots were recorded after four weeks of culture.

4.1.10. Acclimatization

Plantlets with well developed shoots and roots were washed gently under running tap water to remove the agar and transferred to pots containing compost, field soil and sand with a ratio of 1:2:1. Each plastic pot was labeled and covered with polyethylene bags to ensurehigh humidity and kept in the greenhouse. The polyethylene bags were removed after two weeks. One hundredtwentyplantlets were acclimatized in the first experiment. Fifty rooted plantlets were acclimatizedfrom each of solid and liquid mediafor the second experiment.One hundred plantlets were planted in the greenhouse for the third experimentand after about one month, percent of plantlets successfully hardened were calculated

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4.1.11. Experimental design and statistical data analysis

The design used for all the experiments was a complete randomized design (CRD) and the data was analyzed using the statistical data analysis software SPSS 20.0. Data were analyzed using one way ANOVA, followed by the Duncan‟s multiple range tests for mean separation at 5% probability.

4.2. Chromosome count of *A. corrorima*

4.2.1.Plant material and root collection

Actively growing root tips (1-2 cm) of*A. corrorima* were collected from pot plants acclimatized in the greenhouse and used for chromosome count.

4.2.2. Pretreatment of Roots for Chromosome Preparation

The root tips collected in the early morning or evening were treated with 8-hydroxyquanoline for 3-4 hours or kept in ice-cold water for 24 hours to inhibit spindle formation. The chemical (8-hydroxyquanoline) was decanted and the root tips were rinsed in distilled water and transferred to fixative (ethanol: acetic acid, 3:1) and kept at 4°C overnight. The root tips were removed from the fixative and rinsed several times with distilled water again before maceration with enzyme (4% cellulase + 4% pectinase) solution at 37°C. After maceration, the tips of the roots were detached from the rest part of the root. Then the enzyme solution was decanted and the root tips were rinsed in distilled water and kept in distilled water for about 15 minutes.

The detached root tips were then taken with forceps and placed on microscope slides.The water was removed by absorbent tissue paper and the tips were mashed in a drop offixative with flat-end needle and spread with strong air blow. The slides were then airdried at room temperature. The air dried slides were then stained in Giemsa stain (pH = 6.8) for 45 minutes to one hour. Then the slides rinsed and air dried. The samples on each slide was mounted in distyrene, a plasticizer and xylene (DPX) mounting medium and covered with 24mm×50mm cover-slip. The slides were then examined with light microscope for metaphase chromosomes and photographs of wellspread metaphase plates were taken. Chromosome counts were performed fromwell spread chromosomes.

4.3. Genetic diversity of *Aframomum corrorima* using ISSR markers

4.3.1. Plant materials

Korarima leaf samples for DNA extraction were collected from South Ari, North Ari and Basketo especial weredas of South Nations Nationalities and Peoples‟ region and Mecha, JabiTehnan and Guangua wereda‟s of Amhara region from Ethiopia(Figure 2;Table 1).

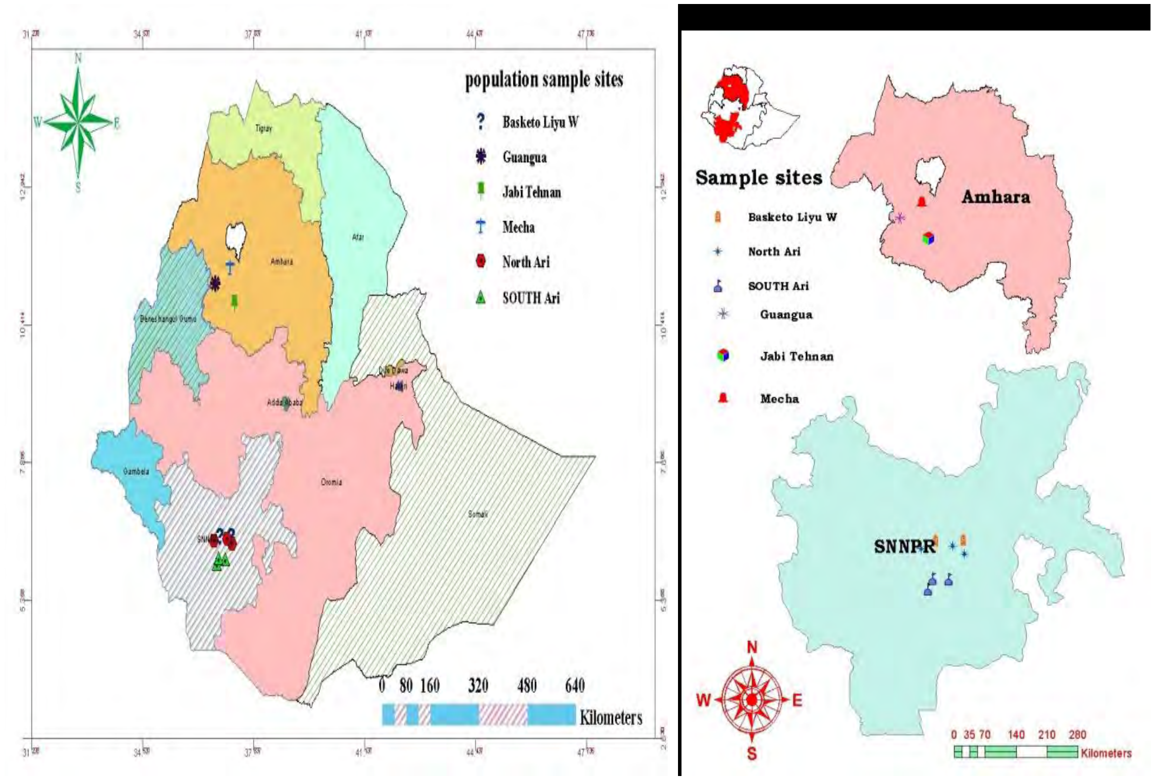


Figure 2.Map of Ethiopia showing sample collection areas in SNNP and Amhara regions.

Table 1. Sampling area details of *A. corrorima* populations in the present study

Population	Wereda	Region	Altitude (M)	Latitude (N)	Longitude (E)	Sample Size
Bikolo Abay	Mecha	Amhara	1900	11^{O} 22.361'	037^{O} 02.428'	10
Finote Selam	Jabiehnan	Amhara	1818	10^{O} 40.508'	037^{O} 15.103'	10
Chagne	Guangua	Amhara	1659	10^{O} 56.880'	036^{O} 31.683'	8
Gelila I	North Ari	SNNP	2205	06^{O} 19.272'	036^{O}62.629'	8
Gelila II	North Ari	SNNP	2043	06^{O} 21.465'	036^{O}60.232'	10
Gelila III	North Ari	SNNP	2257	06^{O} 12.888'	036^{O}38.181'	8
Wubamer I	South Ari	SNNP	1996	06^{O}04.181'	036^{O}57.243'	9
Wubamer II	South Ari	SNNP	1752	05^{O} 58.176'	036^{O}38.478'	10
Wubamer III	South Ari	SNNP	1656	05^{O} 58.588'	036^{O}34.926'	9
Basketo I	Basketo	SNNP	1803	06^{O}16.955	036^{O}36.920'	10
Basketo II	Basketo	SNNP	1910	06^{O} 24.517'	036^{O} 61.630'	10

4.3.2. Sampling technique

Young leaves of 102 individuals (8 to 10 individuals from each population) of*Aframomum corrorima* representing 11 populations were collected separately in zip locked plastic bag in April 2014 and dried with silica gel. The study populations were subjected to genetic diversity analysis to evaluate the level of polymorphism within populations and divergence among populations.

4.3.3. DNA extraction from leaf samples

Genomic DNA was extracted from silica gel-dried leaf-tissue by using Cetyltrimethyl Ammonium Bromide (2% Cetyltrimethyl ammonium bromide, 1% Polyvinylpyrrolidone, 1.0 MTris HCl, 0.5M EDTA, 5.0 M NaCl, 0.2% β-Mercapto-ethanol) extraction protocolbased on Borsch *et al*.(2003) with minor modifications.Dried leaf sample (50 mg)was weighed and pulverized thoroughly using a clean mortar and pestle with the addition of liquid nitrogen. Then, the powder was transferred into an eppendorf cap and 700 µl of warm CTAB solution was added to the powdered sample and the sample was incubated for 30 minutes at 65°C. Then the sample was centrifuged for 7 minutes at 13000 rpm.

The supernatant was transferred to a new eppendorf cap and 700 µl CTAB solution was added to the tissue pellet for the second extraction and stirred slightly with a new 1000 µl pipette tip and incubated for 30 min at 65°C.Subsequently 600 µl chloroform was added to the cap immediately with supernatant and shaken carefully a few times upside down for approximately 5 minutes and centrifuged for 7 min at 13000 rpm.After that the clear supernatant was transferred to a new eppendorf-cap and the chloroform extraction was repeated to remove all impurities. Then, cooled isopropanol (4°C), approximately **2/3** of the solution volume was added and shaken carefully and allowed to freeze for more than 2 h at -20°C. This was followed by centgrifugation at 13000 rpm for 13 min andthe liquid was aspirated using yellow tips without touching the pellet.

To the pellet 200 µl of 70 % ethanol was added and centrifuged for 13 min at 13000 rpm. The ethanol was aspirated by tips and the DNA pellet was dried at room temperature for 15 minutes and dissolved in 100 µl TE (1x) and stored at 4°C over night. Then, 50 µl cooled 7.5 M ammonium acetate solution was added and mixed carefully. Coldabsoluteethanol was added and mixed carefully and frozen for more than 2 h at -20°C, centrifugedfor 35 min at 13000 rpm and fluid was carefully aspirated. Then, 200 µl of 70% ethanol was added and the inner cap surface was rinsed by turning the cap and centrifuge for 13 min at 13000 rpm and the supernatant discarded and the pellet was dried at room temperature. Then, the pellet was dissolved in 100 µl TE and these four steps were repeated with 3 M sodium acetate solution instead of 7.5 M ammonium acetate solution (4°C, half the volume), then centrifuged at 13000 rpm for 13 min and the supernatantdiscardedand the pellet was dried at room temperature and dissolved in 100 µl TE andstored at 4°C until used forPCR.

4.3.4. Measurment of DNA concentration and quality

An agarose gel was prepared for test gel electrophoresis.Genomic DNA (2 µl)samples with 2µl loading dye was loaded on to the gel and electrophoresed at constant voltage of 80V for 45 minutes. Electrophoresis was conducted in 1x TBE buffer using gel tank. The gel was stained for 30 min with 50 µl ethidiumbromide (10mg/ml) after well mixed with 450 ml distilled water. Then test gel was de-stained for 30 minutes in 450ml of distilled water. The second extraction of all samples was selected for further PCR amplification based on the result of the test gel. Selection of the extracts was based on DNA quantity (band intensity) and quality (absence or presence of minimum smear).

Absorbance ratio at 260/280 and 260/230 was analyzed for all the genomic DNA extracted to measure the quality and concentration of DNA for further analysis by using NanoDrop spectrophotometer. Almost all the genomic DNA extracted for this study showed absorbance ratio between 1.8 and 2.2 and above 90% of the samples showed concentration of more than 100ng/µl DNA.

4.3.5. Primer selection and optimization

A total of 13 ISSR primers which were available at plant Biotechnology Laboratory, Addis Ababa University were used for the initial testing of variability and reproducibility. For optimization andscreening of primers, one individual was selected from each population with 25 ng/µl concentration. All pre-selected 13 primers were checked for reproducibility and polymorphism. Finally, four di-nucleotide primers (primer 809, 818, 836 and 848), one tri- nucleotide primer (primer 866) and one penta-nucleotide primer (primer 880) (Table 2) were selected based on polymorphism and reproducibility.

Table 2. List of primers, annealing temperature, primer sequence, amplification pattern and repeat motif used for optimization.

Primers	Annealing temperature in °C	Sequence	Amplification Pattern	Repeat Motif
807	45	AGAGAGAGAGAGAGAGT	Not reproducible	Dinucleotide
809	48	AGAGAGAGAGAGAGAGG	Good	Dinucleotide
810	45	GAGAGAGAGAGAGAGAT	Not polymorphic	Dinucleotide
812	45	GAGAGAGAGAGAGAGAA	Not polymorphic	Dinucleotide
818	48	CACACACACACACACG	Good	Dinucleotide
834	45	AGAGAGAGAGAGAGAGTY	Not Reproducible	Dinucleotide
835	48	AGAGAGAGAGAGAGAGYC	Not polymorphic	Dinucleotide
836	45	AGAGAGAGAGAGAGAGYA	Good	Dinucleotide
844	48	CTCTCTCTCTCTCTCTRC	Not polymorphic	Dinucleotide
848	48	CACACACACACACACARG	Good	Dinucleotide
854	48	TCTCTCTCTCTCTCTCRG	Not polymorphic	Dinucleotide
866	55	CTCCTCCTCCTCCTCCTC	Good	Trinucleotide
880	45	GGAGAGGAGAGGAGA	Good	Pentanucleotide

Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T)

4.3.6. PCR Amplification

PCR amplification was carried out in a 25µl reaction mixture containing 2µl template DNA (25 ng), 17.0µl ddH20, 0.3µl of dNTP mix (20 mM), 2.5µl Taq buffer (10xThermopol reaction buffer), 2.5 µl $MgCl_2$ (2 mM), 0.4µl primer (20pmol/µl) and 0.3µl Taq Polymerase (5u/µl). The amplification program was set as 4 minutes preheating and initial denaturation at 94^{o}C, followed by 40 cycles of 15 seconds at 94^{o}C, 1 minute primer annealing at 45^{o}C/48^{o}C/55^{o}C based on primers used, 1 minute and 30 seconds extension at 72 ^{o}C and the final extension for 7 minutes at 72^{o}C. The PCR products were stored at 4 ^{o}C until loading on gel for electrophoresis.

4.3.7. Gel Visualization

The PCR amplification products were electrophoresed on 1.67 % (w/v) agarose gel which consists of 2.0µl ethidium bromide. TBE buffer (108 g Tris base; 55 gm Boric acid; 40ml EDTA, pH 8.57 components per liter of ddH_2O) was used as running buffer and for gel preparation. PCR product (10µl)of each sample with 2µl loading dye (6x concentrated) (0.041% bromophenol blue, 0.041% xylene cynol and 6% glycerol) was loaded on a gel. DNA marker (100 bp molecular ladder) was used to estimate size of the fragments. The electrophoresis was done at 100 volts for about 3 hours. The DNA was stained for 30 minutes with 10mg/ml ethidium bromide (EtBr) which was mixed and distained with 450 ml distilled water. The gel DNA bands were scored through UV light illuminator and photographed by BiodocAnalyse 2.0 with digital canon camera.

4.3.8. Molecular data analysis

ISSR bands were scored manually for each individual accession from the gelphotograph.The bands were recorded as distinct characters, presence „1" or absence „0" and „?" for missing and ambiguous data. Based on recorded bands different softwares were used for analysis. GenAlEx6.502 (genetic analysis in excel) was used to calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, gene diversity and Shannon–Weaver diversity index (H). Analysis of molecular variance (AMOVA) was used tocalculate variation among and within population using GenAlEx6.502 (Peakall and Smouse, 2012).NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al.,* 1999) softwares were used to calculate Jaccard"s similarity coefficient which is calculated with the formula:-

$$S_{ij} = \frac{a}{a+b+c}$$ Where,

'a ' is the total number of bands shared between individuals i and j,

'b' is the total number of bands present in individual i but not in individual j and

'c' is the total number of bands present in individual j but not in individual i.

The un-weighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare the individual genotypes and generate phenogram using NTSYS- pc version 2.02 (Rohlf, 2000). The neighbor joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 software (Pavlicek *et al.,* 1999). To further examine the patterns of variation among individual samples, a principal coordinated analysis (PCoA) was performed based on Jaccard‟s coefficient (Jaccard, 1908). The calculation of Jaccard‟s coefficient was made with NTSYS- pc version 2.02.The first three axes were later used to plotwith STATISTICA version 12.0 software (Stat soft.Inc.,2015).

5. Results

5.1. Effect of plant growth regulators on in vitro propagation of *A. corrorima*using shoot tip explant

5.1.1. In vitro seed germination

During the in vitro establishment, seeds and seedlings of *A. corrorima* were monitored regarding the fungal and bacterial contamination as well as for germination of the seeds. Seeds were washed in detergent, disinfected with 70% ethanol for 60 seconds and further sterilized using different concentrations of Clorox for 30 minutes (10%, 25%, 50%, and 100%)and sowed in plant growth regulator free MS medium (Figure 3). Seeds were rinsed 3-5 times in distilled water after the alcohol and Clorox treatments for removal of surface sterilizing agent.Seeds started to germinate after one and half months and 25% Clorox showed mean germination andcontamination rate of 70 % and 30% respectively. Increased strength of Clorox reduced the germination rate of seeds.Similarly,when the concentration of Clorox was increased, the contamination rate of the seeds was reduced. Ten percent Clorox showed the highest contamination (80%) and the least germination (30%) of seeds followed by the control treatment (0%).Seeds treated in 100% Clorox for 30 minutesdid not show contamination and germination (Table 3).

Table 3. Percentage of contaminated and clean culture for seed germination at various concentrations of Clorox at 30 min exposure time.

Clorox concentration (%)	No. of magenta with contaminated seed	percentage of clean seeds germinate
0	10	0%
10	8	20%
25	3	70%
50	1	10
100	0	0

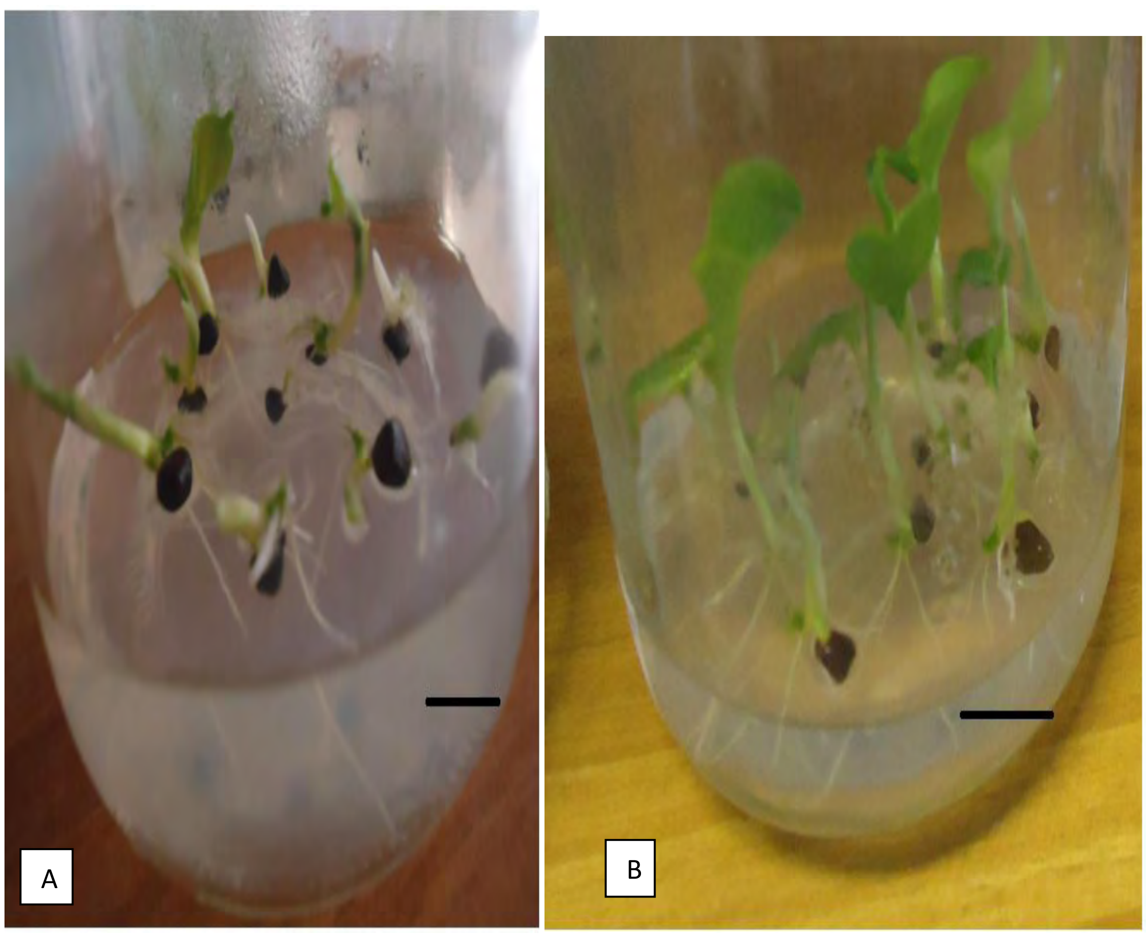


Figure 3. Seed Germination on plant growth regulator free MS medium(A) = 2 months of germination (B) = 3 months of Germination. Bars = 1 cm

5.1.2. Culture initiation

Shoot tips started to develop leaves after a week of culturing (Figure 4). After 30 days of growth on the initiation medium,90 % of explants survived and responded better on MS medium containing 1.0 mg/l BAP in combination with 0.1 mg/l NAA. Explants used as control also showed high number of survived shoots (56%). Generally, the addition of1.0, 2.5 and 3.0 mg/l BAP in combination with 0.1mg/l NAA resulted in higher percentage of plantlet survival(Table 4). Based on this result, 1.0 mg/l BAP in combination with 0.1 mg/l NAA was used as an optimum PGRs for shoot initiation.The highest mean shoot number (1.40 ± 0.09) and shoot length(2.27±0.14 cm)was obtained on MS medium supplemented with 1.0 mg/l BAP in combination with 0.1 mg/l NAA, however, 1.0, 2.0, 2.5 and 3.0 mg/l BAP with 0.1 mg/l NAA showed no significant difference in mean shoot number. Medium containing 0.5 mg/l BAP in combination with 0.1 mg/l NAA showed the least shoot number (1.00±0.00). There is also no significant difference in shoot number among treatments 0.5, 1.5, 2.5 mg/l BAP with 0.1 mg/l NAA and the control.

Table 4.Effect of BAP and NAA on shoot initiation of *A. corrorima*

PGR (mg/l) BAP	NAA	Mean no. of shoots per explants	Mean shoot length (cm)	Mean leaf number per explants	Survival of plantlets in %
0.0	0.0	1.13 ± 0.06^{bc}	2.13 ± 0.21^{ab}	2.03 ± 0.17^{ab}	56
0.5	0.1	1.00 ± 0.00^{c}	1.90 ± 0.13^{ab}	1.93 ± 0.18^{b}	50
1.0	0.1	1.40 ± 0.09^{a}	2.27 ± 0.14^{a}	2.17 ± 0.17^{ab}	90
1.5	0.1	1.23 ± 0.08^{bc}	1.73 ± 0.13^{b}	2.37 ± 0.16^{ab}	66.67
2.0	0.1	1.27 ± 0.08^{ab}	1.85 ± 0.13^{ab}	2.70 ± 0.16^{ab}	73
2.5	0.1	1.26 ± 0.09^{abc}	2.15 ± 0.14^{ab}	3.03 ± 0.72^{a}	86.67
3.0	0.1	1.27 ± 0.10^{ab}	2.20 ± 0.15^{ab}	2.17 ± 0.19^{ab}	83.33

Means within columns having different letters in superscript are significantly different at $p<0.05$.

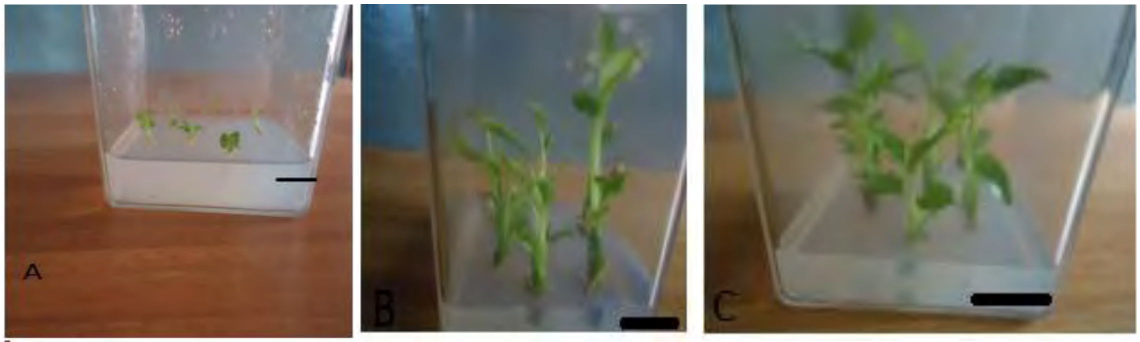


Figure 4. Response of shoot tip explants of Korarima on initiation mediumcontaining different concentrations of BAP in combination with 0.1 NAA. (A) = 1.0 mg/l BAP and 0.1mg/l NAA after 7 days of growth, (B)= Controlafter 45 days of growth and (C)= 3.0mg/l BAP and 0.1 mg/l of NAA after 40 days of growth. Bars = 1 cm.

5.1.3. Shoot multiplication

5.1.3.1. Effect of BAP and KN on shoot multiplication

The shoots on medium containing different concentrations of BAP and KN alone gave different response on mean shoot number, shoot length and leaf number (Table 5 & Figure 5). The highest mean shoot number obtained was 3.03±0.27 and 3.00±0.23 on medium containing 2.5 mg/l KN and 1.5 mg/l BAP respectively. Medium containing 0.5, 1.0, 1.5, 2.0 and 3.0 mg/l KN showed no significant difference in shoot number per explant. The highest mean shoot length of 6.62±0.17 cm was recorded on medium containing 1.0 mg/l KN and the next highest shoot length was 5.60±0.18 on MS medium supplemented with 0.5 mg/l KN. The least mean shoot length (2.07 ± 0.13 and 2.68 ± 0.23 cm) wasexhibited by medium containing 0.5 and 3.0 mg/l BAP respectively. The least shoot length obtained in medium supplemented with KN (3.70± 0.19) washigher than the highest shoot length on medium supplemented with BAP (3.66±19 cm).Medium containing 2.5 mg/l BAP and KN showed the highest leaf number of 7.73± 0.55 and

7.67± 0.62respectively. The least mean leaf number (2.30±0.28) was observed on MS medium containing0.5 mg/l BAP.

Table 5.Effect of BAP and KN alone on shoot multiplication of *A. corrorima*

PGR (mg/l) BAP	Mean no. of shoots per explants	Mean shoot length (cm)	Mean number of leaf per explant
00	2.05±0.21^{bc}	3.84±0.25^{a}	4.52±0.33^{b}
0.5	2.83±0.24^{b}	2.07±0.13^{b}	2.30±0.28^{c}
1.0	2.53±0.29^{b}	3.42±0.15^{a}	6.93±0.77^{a}
1.5	3.00±0.23^{a}	3.52±0.15^{a}	7.03±0.67^{a}
2.0	2.07±0.20^{bc}	3.55±0.14^{a}	5.4±0.47^{b}
2.5	2.77±0.23^{b}	3.66±0.19^{a}	7.73±0.55^{a}
3.0	1.79±0.15^{c}	2.68±0.23^{b}	2.50±0.25^{c}
KN			
0.5	2.33±0.34^{b}	5.60±0.18^{b}	5.07±0.55^{cd}
1.0	1.77±0.16^{b}	6.62±0.17^{a}	4.47±0.34^{d}
1.5	1.93±0.12^{b}	3.93±0.18^{de}	5.47±0.46^{bcd}
2.0	2.21±0.28^{b}	4.50±0.22^{c}	7.08±0.83^{ab}
2.5	3.03±0.27^{a}	4.40±0.19^{cd}	7.67±0.62^{a}
3.0	1.87±0.17^{b}	3.70±0.19^{e}	6.27±0.57^{abc}

Means within columns and treatments having different letters in superscript are significantly different at $p<0.05$.

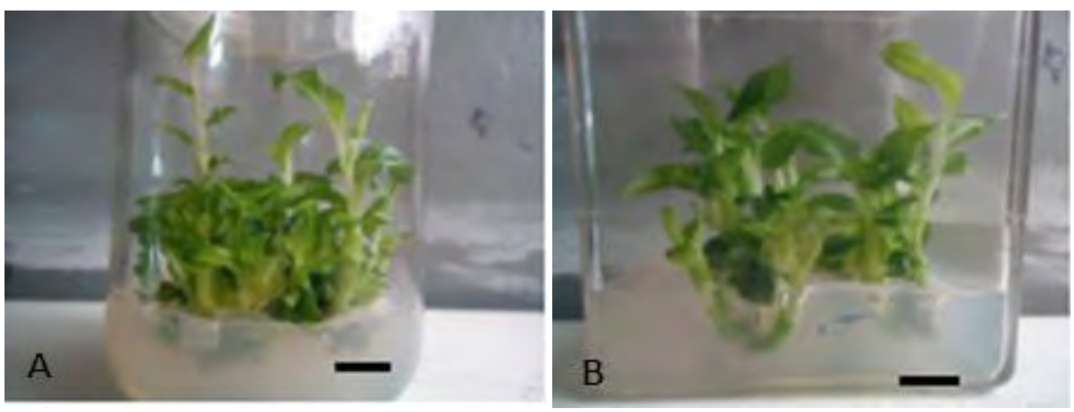


Figure 5. Shoot multiplication on MS medium supplemented with BAP and KN alone (A) 1.5mg/l BAP and (B) 2.5 mg/l KN. Bars = 1 cm.

5.1.3.2. Synergistic effect of BAP and KN on shoot multiplication

The shoots on multiplication media supplemented with different concentrations of BAP in combination with KN resulted in different responsesin shoot number, shoot length and leaf number (Table 6 & Figure 6). The best shoot multiplication medium from the combined treatments and the better stage of multiplication that provided highest number of shoots was obtained. The highest mean number of shoots was 5.13 ± 0.64 on the MS multiplication medium containing 1.5 mg/l BAP and 2.0 mg/l KN. The second highest mean shoot number per explants (4.79±0.36)was obtained on medium containing 1.0 mg/l BAP and 1.5 mg/l KN. The multiplication rate was relatively less and produced 1.83 ± 0.27 mean number of shoots on the multiplication medium containing 0.5 mg/l BAP and 1.0 mg/l KN. The maximum and minimum shoot length (5.63±0.26 and 2.42±0.16) were observed on media containing 3.0 mg/l BAP and 1.5 mg/l KN, and 2.5 mg/l BAP and 1.0 mg/l KN respectively. Highest and lowest leaf numbers (9.73±0.69 and 4.07±0.34) were recorded on media containing 1.5mg/l BAP and 2.5mg/l KN, and 2.0 mg/l BAP and 1.5 mg/l KN.Increasing BAP from 0.5 to 1.0 with 1.5 KN resulted in significant increase in shoot number per explants from 2.53±0.29 to 4.79±0.36. Further increase in BAP concentration from 1.5 to 3.0 mg/l with 1.5 KN did not result significant difference in mean shoot number per explants. Similarly, significant increase in shoot number was observed by increasing BAP from 1.0 to 1.5 with 0.5 KN, 1.0 to 1.5 BAP with 2.0 KN, 2.0 to 2.5 BAP with 2.0 KN and 0.5 to 1.0 BAP with 2.5 KN.

Table 6.Synergistic effect of BAP and KN on shoot multiplication of shoot tip explants of *A. corrorima*

PGR (mg/l)		Mean no. of shoots per explants	Mean shoot length (cm)	Mean no. of leaves per explants
BAP	KN			
0.0	0.0	3.05 ± 0.30^{efghijkl}	3.84 ± 0.25^{defg}	4.51 ± 0.33^{kl}
0.5	0.5	2.77 ± 0.23^{fghijklm}	3.10 ± 0.24^{hijkl}	7.06 ± 0.67^{defghi}
1.0	0.5	3.27 ± 0.23^{efghij}	2.52 ± 0.13^{lm}	$7.10\pm0.44d^{efghi}$
1.5	0.5	4.57 ± 0.37^{abcd}	3.83 ± 0.15^{defg}	9.57 ± 0.63^{ab}
2.0	0.5	3.27 ± 0.22^{efghij}	4.20 ± 0.21^{cde}	5.23 ± 0.46^{ijkl}
2.5	0.5	3.17 ± 0.25^{efghij}	3.60 ± 0.17^{efgh}	4.68 ± 0.46^{kl}
3.0	0.5	3.83 ± 0.31^{bcdef}	2.83 ± 0.25^{jklm}	5.63 ± 0.63^{hijkl}
0.5	1.0	1.83 ± 0.27^{m}	2.71 ± 0.18^{klm}	4.79 ± 0.49^{jkl}
1.0	1.0	2.75 ± 0.22^{fghijklm}	3.28 ± 0.12^{ghigk}	7.38 ± 0.52^{cdefgh}
1.5	1.0	4.50 ± 0.43^{abcd}	3.68 ± 0.20^{efgh}	9.70 ± 0.70^{a}
2.0	1.0	2.30 ± 0.22^{ijklm}	3.43 ± 0.15^{fghij}	6.77 ± 0.69^{efghij}
2.5	1.0	3.33 ± 0.40^{efghi}	2.42 ± 0.16^{m}	6.73 ± 0.77^{efghij}
3.0	1.0	2.96 ± 0.21^{efghijklm}	4.87 ± 0.20^{b}	6.38 ± 0.52^{fghijk}
0.5	1.5	2.53 ± 0.29^{ghijklm}	4.17 ± 0.21^{cde}	4.77 ± 0.37^{jkl}
1.0	1.5	4.79 ± 0.36^{ab}	3.87 ± 0.18^{defg}	9.42 ± 0.64^{ab}
1.5	1.5	3.03 ± 0.28^{efghijkl}	4.18 ± 0.17^{cde}	9.20 ± 0.71^{abc}
2.0	1.5	2.00 ± 0.29^{klm}	3.28 ± 0.14^{ghijk}	4.07 ± 0.34^{l}
2.5	1.5	2.47 ± 0.20^{hijklm}	$3.93\pm0.16d^{efg}$	6.40 ± 0.47^{fghijk}
3.0	1.5	2.57 ± 0.29^{ghijklm}	5.63 ± 0.26^{a}	4.53 ± 0.43^{kl}
0.5	2.0	3.21 ± 0.23^{efghij}	2.93 ± 0.17^{ijklm}	5.88 ± 0.50^{ghijkl}
1.0	2.0	3.57 ± 0.43^{ghijklm}	3.57 ± 0.43^{efghi}	5.70 ± 0.63^{ghijkl}
1.5	2.0	5.13 ± 0.64^{a}	3.05 ± 0.11^{hijklm}	8.80 ± 0.87^{abcd}
2.0	2.0	2.23 ± 0.22^{ijklm}	4.10 ± 0.15^{cdef}	5.60 ± 0.50^{hijkl}
2.5	2.0	3.80 ± 0.38^{bcdef}	3.45 ± 0.15^{fghij}	7.73 ± 0.71^{bcdefg}
3.0	2.0	3.90 ± 0.32^{bcde}	3.88 ± 0.23^{defg}	7.00 ± 0.53^{defghi}
0.5	2.5	$2.67\pm0.25f^{ghijklm}$	4.69 ± 0.22^{bc}	5.70 ± 0.46^{ghijkl}
1.0	2.5	4.67 ± 0.64^{abc}	3.63 ± 0.17^{efgh}	8.25 ± 0.99^{abcdef}
1.5	2.5	3.67 ± 0.31^{cdefg}	3.43 ± 0.18^{fghij}	9.73 ± 0.69^{a}
2.0	2.5	2.27 ± 0.26^{ijklm}	3.83 ± 0.23^{defg}	4.87 ± 0.47^{jkl}
2.5	2.5	2.43 ± 0.27^{hijklm}	3.25 ± 0.18^{ghijk}	5.83 ± 0.55^{ghijkl}
3.0	2.5	3.13 ± 0.28^{efghijk}	$4.60\pm0.22bc$	6.80 ± 0.51^{efghij}
0.5	3.0	2.47 ± 0.18^{hijklm}	2.63 ± 0.14^{klm}	6.93 ± 0.48^{defghi}
1.0	3.0	3.20 ± 0.34^{efghij}	3.57 ± 0.16^{efghi}	8.60 ± 0.82^{abcde}
1.5	3.0	2.37 ± 0.31^{ijklm}	4.48 ± 0.17^{bcd}	5.60 ± 0.65^{hijkl}
2.0	3.0	1.93 ± 0.23^{lm}	3.92 ± 0.14^{defg}	4.10 ± 0.34^{l}
2.5	3.0	2.90 ± 0.37^{efghijklm}	4.23 ± 0.15^{cde}	7.00 ± 0.74^{defghi}
3.0	3.0	3.17 ± 0.31^{efghij}	3.90 ± 0.21^{defg}	6.13 ± 0.62^{ghijk}

Means within columns having different letters in superscript are significantly different at $P < 0.05$

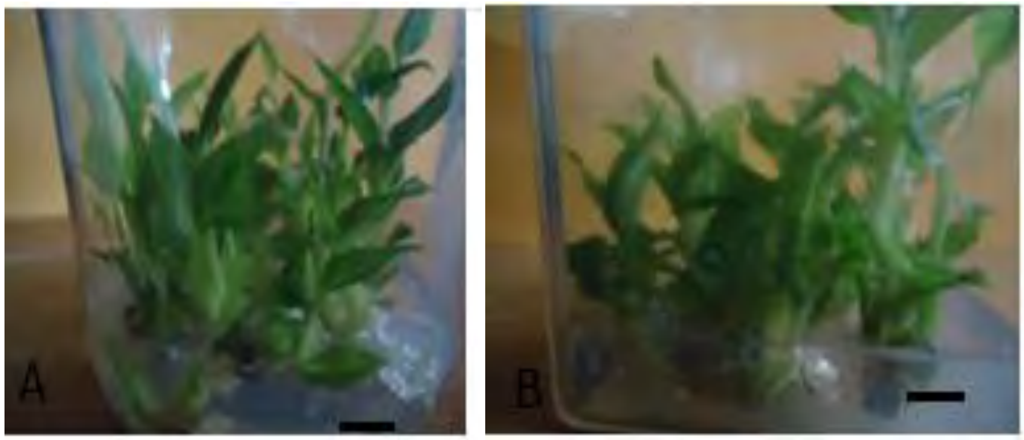


Figure 6.Shoot multiplication on MS medium supplemented with BAP and KN

(A) 1.5mg/l BAP + 2.0 mg/l KN (B) 1.0mg/l BAP + 1.5mg/l KN. Bars = 1cm

5.1.3.3. Synergistic effect of BAP and NAA on shoot multiplication

Different concentrations of BAP in combination with NAA caused varied effects on shoot multiplication (Table 7 & Figure 7). The highest number of microshoots (3.87 ± 0.32) was recorded on MS medium supplemented with 2.5 mg/l BAP and 0.25 mg/l NAA. There is no significant difference in mean shoot number per explants among treatments 0.5 mg/l BAP with 0.25 mg/l NAA, 1.0 mg/l BAP with 0.25 mg/l NAA, 2.5 mg/l BAP with 0.25 mg/l NAA, 0.5 mg/l BAP with 0.5 mg/l NAA and 2.0 mg/l BAP with 0.5 mg/l NAA. Growth regulators free MS medium showed the longest shoots (5.24 ± 0.26). BAP concentration of 1.0 mg/l in combination with 0.25 mg/l NAA resulted in the highest leaf number (9.83 ± 1.75).In contrast, the least mean number of shoots (1.67±0.15) was obtained at concentration of 3.0 mg/l BAP in combination with 0.5 mg/lNAA. The concentration of 3.0 mg/l BAP and 0.25 mg/l NAA showed the least shoot length and leaf number of 2.03±20 and 1.90±0.23 respectively. Increasing BAP from 2.0 to 2.5 mg/l with 0.25 NAA showed significant increase in shoot number per explants. Further increase in BAP from 2.5 to 3.0 mg/l with 0.25 NAA resulted significant decrease in shoot number per explants.

Table 7.Effect of BAP and NAAcombinationson shoot multiplication of *A. corrorima* using shoot tip explants

PGR (mg/l) BAP	NAA	Mean no. of shoots per explants	Mean shoot length (cm)	Mean no. of leaves per explants
0.0	0.0	2.52 ± 0.31^{bcd}	5.24 ± 0.26^{a}	5.93 ± 0.50^{bc}
0.5	0.25	3.33 ± 0.35^{ab}	3.48 ± 0.15^{bcd}	6.20 ± 0.67^{bc}
1.0	0.25	3.07 ± 0.28^{abc}	3.80 ± 0.20^{bc}	9.83 ± 1.75^{a}
1.5	0.25	3.13 ± 0.28^{abc}	3.95 ± 0.13^{b}	5.80 ± 0.42^{bc}
2.0	0.25	2.66 ± 0.21^{bcd}	3.36 ± 0.15^{cde}	7.69 ± 0.61^{b}
2.5	0.25	3.87 ± 0.32^{a}	3.18 ± 0.11^{de}	7.90 ± 0.65^{ab}
3.0	0.25	1.93 ± 0.16^{de}	2.03 ± 0.20^{f}	1.90 ± 0.23^{e}
0.5	0.5	3.07 ± 0.21^{abc}	3.52 ± 0.15^{bcd}	6.00 ± 0.48^{bc}
1.0	0.5	2.30 ± 0.19^{cde}	3.43 ± 0.16^{bcde}	2.90 ± 0.17^{de}
1.5	0.5	2.80 ± 0.26^{bc}	3.47 ± 0.14^{bcd}	6.23 ± 0.56^{bc}
2.0	0.5	3.25 ± 0.33^{ab}	2.90 ± 0.17^{e}	7.50 ± 0.92^{b}
2.5	0.5	2.93 ± 0.25^{bc}	3.08 ± 0.19^{de}	6.13 ± 0.58^{bc}
3.0	0.5	1.67 ± 0.15^{e}	3.37 ± 0.22^{cde}	4.40 ± 0.39^{cd}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

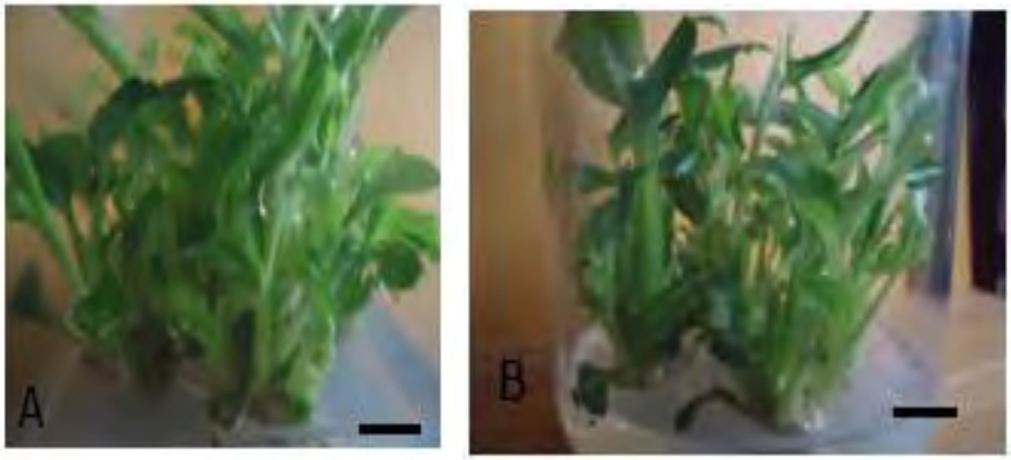


Figure 7. Shoot multiplication on MS medium supplemented with BAP and NAA(A) 2.5mg/l BAP + 0.25 mg/l NAA (B) 2.0mg/l BAP + 0.5mg/l NAA. Bars = 1cm

5.1.3.4. The combined effect of BAP and IBA on shoot multiplication

MS medium containing 3.0mg/l BAP and 0.5mg/l IBA was found to show maximum mean shoot number of 3.84±0.54 and the minimum shoot number of 1.47±0.13 was observed on MS medium supplemented with 1.5 mg/l BAP and 0.25 mg/l IBA (Table 8 & Figure 8). The longest and shortest shoots 5.04±0.26 cm and 2.00±0.13 cm were recorded on medium containing 1.5 mg/l BAP and 0.5 mg/l IBA, and 3.0mg/l BAP and 0.25mg/l IBA respectively. MS medium containing 2.0 mg/l BAP and 0.25mg/l IBA showed the highest leaf number of 8.90±0.70 and BAP concentration of 1.5 mg/l in combination with 0.25 mg/l IBA showed the least number of leaves (2.73±0.33). Increasing BAP from 1.0 to 1.5 mg/l with 0.25 mg/l IBA showed significant decrease in shoot number per explants from 2.47 ± 0.25, however further increase BAP from 2.0 to 3.0 mg/l with 0.25 IBA resulted similar mean shoot number per explants. Minimizing BAP from 3.0 to 2.5 mg/l with 0.5 mg/l IBA showed decrease in mean shoot number per explants from 3.84 ± 0.54 to 2.50 ± 0.22. BAP from 0.5 to 2.5 mg/l resulted similar mean shoot number per explants.

Table 8.Effect of different concentrations and combinations of BAP and IBA on shoot multiplication of *A. corrorima*

PGR (mg/l) BAP	IBA	Mean no. of shoots per explants	Mean shoot length (cm)	Mean no. of leaves per explant
0.0	0.0	2.87 ± 0.26^{b}	2.64 ± 0.33^{g}	3.06 ± 0.39^{fg}
0.5	0.25	2.23 ± 0.21^{b}	3.42 ± 0.16^{def}	4.73 ± 0.47^{def}
1.0	0.25	2.47 ± 0.25^{b}	3.75 ± 0.14^{cde}	7.33 ± 0.75^{ab}
1.5	0.25	1.47 ± 0.13^{c}	3.18 ± 0.16^{efg}	2.73 ± 0.33^{g}
2.0	0.25	2.93 ± 0.29^{b}	3.85 ± 0.16^{cd}	8.90 ± 0.70^{a}
2.5	0.25	2.30 ± 0.19^{b}	4.30 ± 0.20^{bc}	5.77 ± 0.43^{bcde}
3.0	0.25	2.75 ± 0.34^{b}	2.00 ± 0.13^{h}	4.33 ± 0.77^{efg}
0.5	0.5	2.71 ± 0.27^{b}	3.23 ± 0.19^{efg}	4.50 ± 0.36^{ef}
1.0	0.5	2.73 ± 0.30^{b}	3.17 ± 0.16^{efg}	5.15 ± 0.61^{cde}
1.5	0.5	2.46 ± 0.22^{b}	5.04±0.26a	6.63 ± 0.59^{bc}
2.0	0.5	2.60 ± 0.29^{b}	4.83 ± 0.19^{ab}	7.50 ± 0.66^{ab}
2.5	0.5	2.50 ± 0.22^{b}	2.86 ± 0.20^{fg}	6.29 ± 0.59^{bcd}
3.0	0.5	3.84 ± 0.54^{a}	4.85 ± 0.20^{ab}	6.50 ± 0.46^{bc}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

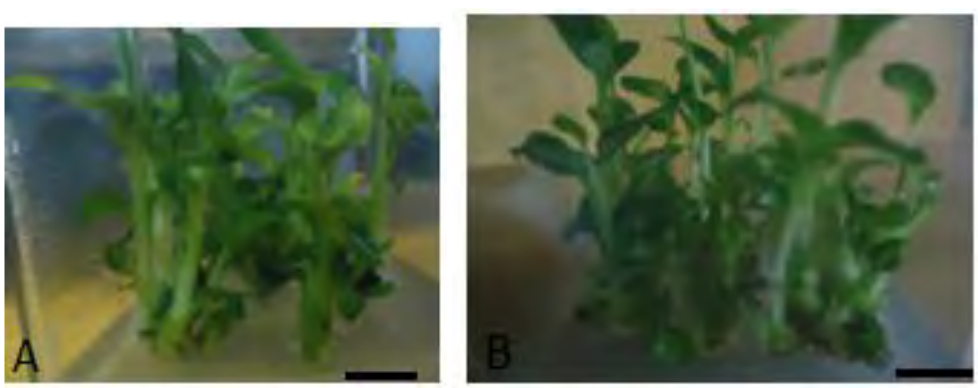


Figure 8. Shoot multiplication on MS medium supplemented with BAP and IBA(A) 2.5mg/l BAP + 0.5 mg/l IBA (B) 3.0mg/l BAP + 0.5mg/l IBA. Bars = 1cm

5.1.3.5. The combined effect of KN and NAA on shoot multiplication

Higher concentrations of KN in combination with NAA (3.0 mg/l KN with 0.25 and 0.5mg/l NAA) showed least number of shoots per explant (1.87±0.20/0.23) (Table 9). The highest shoot number of 3.23±0.38 was observed in MS medium containing 0.5mg/l KN and 0.25mg/l NAA. The second highest mean number of shoots per explant (2.93 ± 0.38) was observed on medium containing 0.5mg/l KN and 0.5mg/l NAA(Figure 9). Increasing KN from 0.5 to 2.5 mg/l with 0.25 mg/l NAA showed no significant difference in mean shoot number per explants. Further increase in KN from 2.5 to 3.0 mg/l with 0.25 mg/l NAA resulted significant decrease in mean shoot number per explants from 2.87 ± 0.34 to 1.87 ± 0.23. The longest shoot was recorded in growth regulator free MS medium (5.30±0.26). The second longest and shortest shoots (4.98±0.25 cm and 3.75±0.17 cm) were recorded in media supplemented with 2.0mg/l KN and 0.5 mg/l NAA and 0.5mg/l KN and 0.5 mg/l NAA respectively. MS medium containing 2.0 mg/l KN and 0.25 mg/l NAA showed the highest mean leaf number (7.60±0.65), whereas 1.5mg/l KN and 0.5mg/l NAA showed the least mean leaf number of 3.75±0.47.

Table 9. Synergistic effect of KN and NAA on shoot multiplication of *A. corrorima* using shoot tip explants

PGR (mg/l) KN	NAA	Mean no. of shoots per explants	Mean shoot length (cm)	Mean no. of leaves per explants
0.0	0.0	2.47±0.30^{abc}	5.30±0.26^{a}	5.87±0.49^{abcd}
0.5	0.25	3.23±0.38^{a}	3.87±0.16^{d}	6.00±0.63^{abc}
1.0	0.25	2.43±0.24^{abc}	4.22±0.20^{cd}	7.20±0.72^{ab}
1.5	0.25	2.87±0.28^{ab}	4.23±0.19^{cd}	5.27±0.36^{bcde}
2.0	0.25	2.83±0.24^{ab}	4.32±0.18^{cd}	7.60±0.65^{a}
2.5	0.25	2.87±0.34^{ab}	4.20±0.21^{cd}	5.43±0.62^{bcde}
3.0	0.25	1.87±0.23^{c}	4.15±0.18^{cd}	5.00±1.04^{cde}
0.5	0.5	2.93±0.38^{ab}	3.75±0.17^{d}	4.67±0.51^{cde}
1.0	0.5	2.30±0.26^{bc}	4.57±0.20^{bc}	4.57±0.51^{cde}
1.5	0.5	1.92±0.20^{c}	3.96±0.17^{cd}	3.75±0.47^{e}
2.0	0.5	2.37±0.25^{abc}	4.98±0.25^{ab}	6.13±0.69^{abc}
2.5	0.5	2.30±0.25^{bc}	3.83±0.17^{d}	4.63±0.56^{cde}
3.0	0.5	1.87±0.20^{c}	3.89±0.15^{d}	3.90±0.31^{de}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

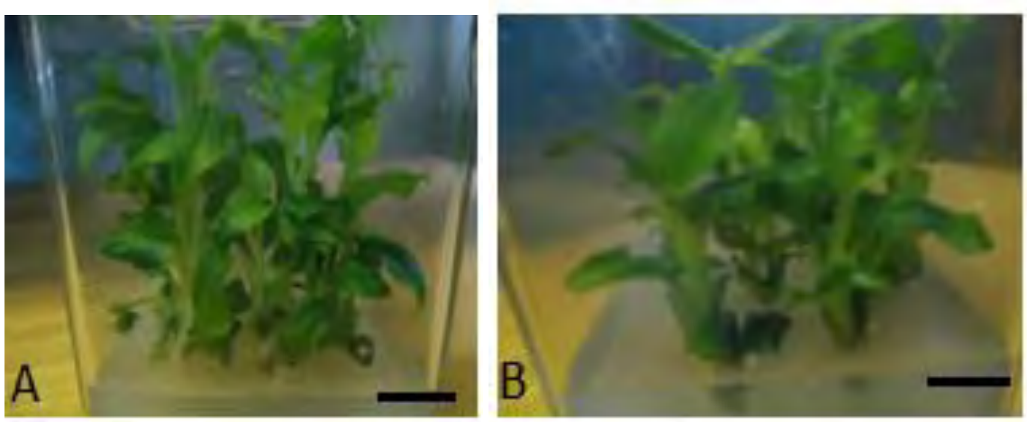


Figure 9. Shoot multiplication on MS medium supplemented with KN and NAA(A) 0.5mg/l KN + 0.25 mg/l NAA (B) 1.5mg/l KN + 0.25mg/l NAA. Bar = 1cm.

5.1.3.6. The combined effect of KN and IBA on shoot multiplication

The highest number of shoots (2.58 ± 0.22),shortest shoot length (3.25±0.20 cm) and minimum leaf number (2.96 ± 0.34) were obtained on a medium containing 1.5 mg/l KN combined with 0.25 mg/l IBA(Figure 10). The least mean shoot number (1.50±0.15) was observed on MS medium containing 1.5 mg/l KN in combination with 0.5 mg/l IBA (Table 10). MS medium containing 0.5mg/l KN and 0.25 mg/l IBA showed the longest shoots with mean shoot length of 5.75±0.17 cm. The maximum (6.53±0.61) mean leaf number was recorded on MS media supplemented with 2.0mg/l KN and 0.5 mg/l IBA.

Table 10. Combined effect of KN and IBA on shoot multiplication of *A. corrorima*

PGR (mg/l)		Mean no. of shoots	Mean shoot length	Mean no. of leaves
KN	IBA	per explants	(cm)	per explant
0.0	0.0	$2.47±0.30^{ab}$	$5.29±0.26^{abcd}$	$5.87±0.49^{ab}$
0.5	0.25	$1.80±0.18^{bcd}$	$5.75±0.17^{a}$	$5.50±0.48^{abcd}$
1.0	0.25	$2.23±0.26^{abcd}$	$4.23±0.18^{ef}$	$6.37±0.72^{a}$
1.5	0.25	$2.58±0.22^{a}$	$3.25±0.20^{g}$	$2.96±0.34^{f}$
2.0	0.25	$1.58±0.17^{cd}$	$4.23±0.26^{ef}$	$3.58±0.32^{ef}$
2.5	0.25	$1.57±0.16^{cd}$	$4.65±0.18d^{e}$	$3.83±0.29^{ef}$
3.0	0.25	$1.90±0.16^{abcd}$	$4.17±0.27^{ef}$	$4.33±0.40^{cdef}$
0.5	0.5	$2.08±0.28^{abcd}$	$5.52±0.20^{ab}$	$6.25±0.49^{ab}$
1.0	0.5	$2.10±0.31^{abcd}$	$4.97±0.16^{bcd}$	$4.80±0.44^{bcde}$
1.5	0.5	$1.50±0.15^{d}$	$4.93±0.23^{bcd}$	$4.07±0.30^{def}$
2.0	0.5	$2.27±0.21^{abc}$	$3.78±0.17^{fg}$	$6.53±0.61^{a}$
2.5	0.5	$1.97±0.22^{abcd}$	$4.82±0.22^{cde}$	$5.70±0.50^{abc}$
3.0	0.5	$1.80±0.19^{bcd}$	$5.33±0.21^{abc}$	$4.83±0.40^{bcde}$

Means within columns having different letters in superscript are significantly different at $p<0.05$.

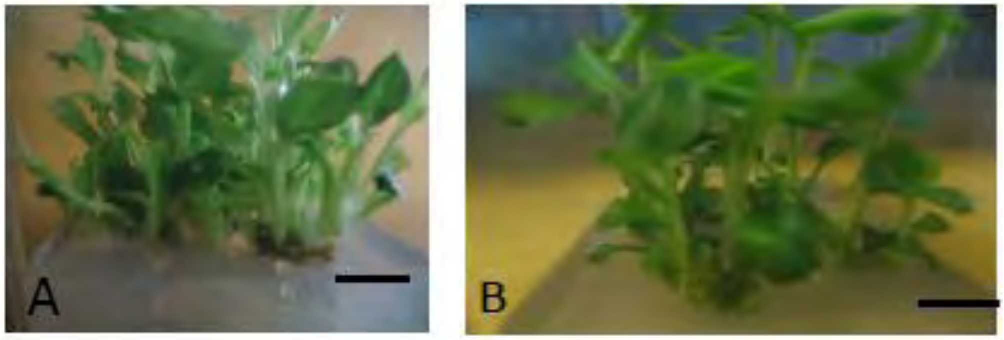


Figure 10. Shoot multiplication on MS medium supplemented with KN and IBA(A) 1.5 mg/l KN + 0.25 mg/l IBA (B) 2.0 mg/l KN + 0.5 mg/l IBA. Bars = 1cm.

5.1.4. Rooting

5.1.4.1. The effect of NAA, IBA and IAA

Those shoots with 1.0 – 2.0cm in height were taken and transferred onto rooting medium thatcontains half strength MS supplemented with different concentrations of NAA, IBA and IAA (Table11& Figure 11). The maximum rooting percentage per treatment (92.36±3.32, 90.56±3.63and 90.00±3.26) were recorded in media supplemented with 1.5 mg/l IBA, 2.0mg/l and 2.5 mg/l NAA respectively. The concentrations of 2.5 mg/l IBA, 1.0 mg/l IAA and 2.5 mg/l NAA produced the minimum rooting percentage per treatment (19.44±5.77, 32.78±7.21and 32.64±5.98)respectively.Roots of maximum and minimum mean length were produced on MS medium fortified with 0.1 mg/l NAA and 2.5 mg/l IBA having average length of 2.45±0.20 cm and 0.42±0.14 cm respectively.

Table 11.Effect of NAA, IBA and IAA alone on rooting

PGR(mg/l)	Mean no. of roots per explants	Mean root length (cm)	Mean rooting response of each treatment in percent
NAA			
0.0	2.25 ± 0.39^{c}	1.35 ± 0.20^{cd}	35.42 ± 5.15^{c}
0.25	5.29 ± 0.66^{b}	1.96 ± 0.23^{ab}	70.83 ± 6.67^{b}
0.5	2.13 ± 0.46^{c}	0.79 ± 0.16^{d}	32.64 ± 5.98^{c}
1.0	5.03 ± 0.43^{b}	2.45 ± 0.20^{a}	75.00 ± 4.36^{b}
1.5	5.50 ± 0.66^{b}	1.63 ± 0.17^{bc}	74.31 ± 6.02^{b}
2.0	8.13 ± 0.69^{a}	2.00 ± 0.20^{ab}	90.56 ± 3.63^{a}
2.5	8.27 ± 0.72^{a}	2.00 ± 0.21^{ab}	90.00 ± 3.26^{a}
3.0	7.57 ± 0.66^{a}	2.25 ± 0.22^{ab}	89.44 ± 3.95^{a}
IBA			
0.0	2.25 ± 0.39^{de}	1.35 ± 0.20^{bc}	35.41 ± 5.15^{d}
0.25	5.63 ± 0.48^{bcd}	1.81 ± 0.18^{b}	81.25 ± 5.15^{ab}
0.5	7.23 ± 0.67^{ab}	2.30 ± 0.20^{a}	85.00 ± 4.47^{ab}
1.0	5.03 ± 0.56^{cd}	0.87 ± 0.09^{de}	71.67 ± 6.04^{bc}
1.5	8.71 ± 1.00^{a}	1.50 ± 0.19^{bc}	92.36 ± 3.32^{a}
2.0	4.63 ± 0.85^{d}	0.50 ± 0.05^{e}	61.81 ± 7.06^{c}
2.5	1.20 ± 0.36^{e}	0.42 ± 0.14^{e}	19.44 ± 5.77^{e}
3.0	7.04 ± 1.05^{abc}	1.06 ± 0.16^{cd}	78.47 ± 6.69^{ab}
IAA			
0.0	2.25 ± 0.39^{b}	1.35 ± 0.20^{ab}	35.41 ± 5.15^{b}
0.25	5.73 ± 0.60^{a}	1.77 ± 0.21^{a}	76.11 ± 5.69^{a}
0.5	4.13 ± 0.53^{a}	1.25 ± 0.16^{ab}	60.00 ± 6.27^{a}
1.0	2.13 ± 0.50^{b}	0.50 ± 0.12^{c}	32.78 ± 7.21^{b}
1.5	4.90 ± 0.63^{a}	1.28 ± 0.25^{ab}	65.45 ± 5.44^{a}
2.0	5.40 ± 0.52^{a}	1.55 ± 0.19^{a}	76.00 ± 5.41^{a}
2.5	5.10 ± 0.57^{a}	0.85 ± 0.12^{bc}	70.59 ± 5.63^{a}
3.0	5.83 ± 0.90^{a}	1.63 ± 0.18^{a}	74.44 ± 5.16^{a}

Means within columns and treatments having different letters in superscript are significantly different at $p<0.05$.

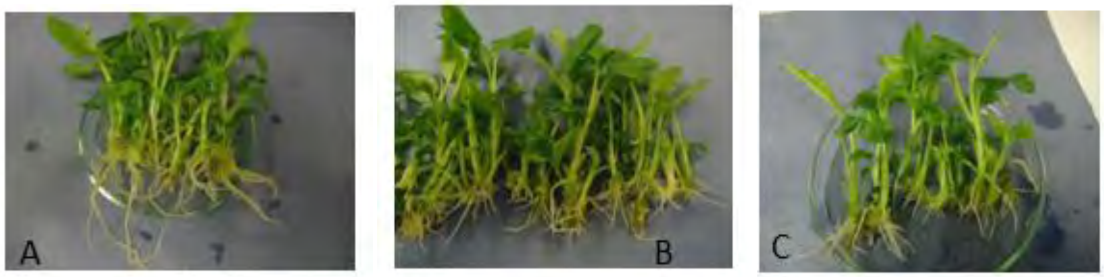


Figure 11. Root induction on half strength MS mediumenriched with NAA, IBA or IAA. (A) 3.0 mg/l NAA (B) 0.5 mg/l IBA(C) 3.0 mg/l IAA

5.1.5. Acclimatization

From one hundred twenty plantlets 109 (90.83%) were survived after one month and no aberrant plants were observed.

Figure 12. Acclimatization of in vitro rooted microshoots. (A) Acclimatized plantlets after 3 days(B) Acclimatized plantlets covered with polyethylene bag (C) Established plants after 30 days. Bars = 1cm.

5.2. The effects of sugar concentration, salt strength and gelling agent on in vitro propagation of *A. corrorima* using shoot tip explants

5.2.1. In vitro response of *A. corrorima* to media with different sucrose concentration

Variation in shoot response was observed in different sugars and concentrations (Table 12a and Table 12b). The highest mean number of shoots (6.67±0.55) was recorded in the medium containing 3% sucrose concentration (Figure 13A). The smallest mean number of shoots (3.40±0.27) recorded from MS medium containing 3% sucrose was higher than the highest for 2% sucrose (3.38±0.28) and 1% sucrose (3.07±0.24). In MS medium containing 3% common table sugar mean shoot number of 4.10±0.44 was recorded (Figure 13B).The highest shoot length (2.95±0.13 cm) was obtained on MS medium containing 3% table sugar. The second highest shoot length (2.81±0.09 cm) was recordedon medium containing 3% sucrose. The shortest shoot length (2.02±0.13 cm) was observed on MS medium containing 2% sucrose.It can be asserted that sucrose at 30 g/l (recommended concentration for plant tissue culture) found to be optimum for *A. corrorima* micropropagation.

Table 12a. Effect of different concentrations of sucrose on shoot multiplication of *A.corrorima* with media containing different combinations of BAP and TDZ

PGR (mg/l)		2% sucrose		1% sucrose	
BAP	TDZ	Mean no. of shoot/explant	Mean shoot length (cm)	Mean no. of shoot/explant	Mean shoot length (cm)
0.5	0.1	3.21 ± 0.30^{def}	2.02 ± 0.13^{fg}	1.67 ± 0.56^{g}	2.06 ± 0.10^{efg}
1.0	0.1	2.79 ± 0.26^{efg}	2.48 ± 0.17^{bcde}	2.63 ± 0.35^{fg}	2.28 ± 0.13^{cdef}
1.5	0.1	3.29 ± 0.30^{def}	2.60 ± 0.15^{abcd}	2.27 ± 0.19^{fg}	1.77 ± 0.09^{g}
2.0	0.1	3.38 ± 0.28^{def}	2.21 ± 0.13^{def}	3.07 ± 0.24^{def}	2.27 ± 0.12^{cdef}
2.5	0.1	2.92 ± 0.24^{def}	2.48 ± 0.14^{bcde}	2.73 ± 0.17^{fg}	1.95 ± 0.12^{fg}
3.0	0.1	3.00 ± 0.25^{def}	2.28 ± 0.15^{cdef}	2.97 ± 0.18^{def}	1.95 ± 0.09^{fg}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

Table 12b. Comparison of the effect of sucrose and table sugar on shoot multiplication of *A. corrorima*

PGR (mg/l)		3% sucrose3% common table sugar			
		Mean no. of shoot/explant	Mean shoot length (cm)	Mean no. of shoot/explant	Mean shoot length (cm)
BAP	TDZ				
0.5	0.1	4.79 ± 0.39^{bc}	2.81 ± 0.09^{ab}	4.10 ± 0.44^{cd}	2.62 ± 0.17^{abcd}
1.0	0.1	3.40 ± 0.27^{def}	2.50 ± 0.09^{bcd}	4.03 ± 0.53^{cde}	2.80 ± 0.18^{ab}
1.5	0.1	6.67 ± 0.55^{a}	2.68 ± 0.11^{abc}	3.37 ± 0.31^{def}	2.48 ± 0.11^{bcde}
2.0	0.1	4.96 ± 0.58^{bc}	2.81 ± 0.13^{ab}	3.30 ± 0.32^{def}	2.95 ± 0.13^{a}
2.5	0.1	4.80 ± 0.54^{bc}	2.65 ± 0.15^{abc}	3.43 ± 0.35^{def}	2.65 ± 0.16^{abc}
3.0	0.1	5.57 ± 0.50^{b}	2.52 ± 0.09^{abcd}	4.03 ± 0.46^{cde}	2.82 ± 0.13^{ab}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

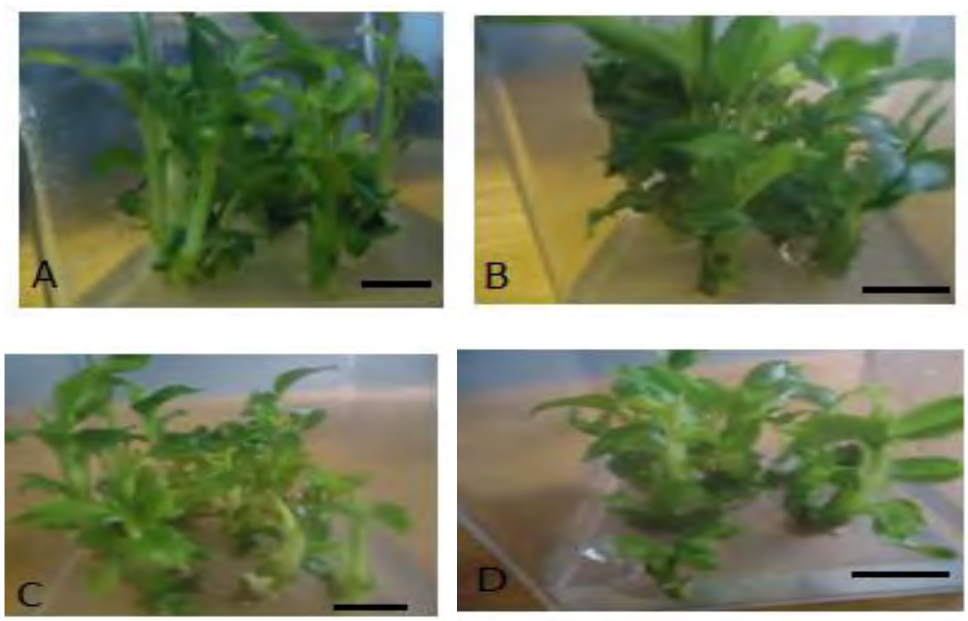


Figure 13. Shoot multiplicationon MS medium containing different concentrations of carbon sources. (A) 3% sucrose (B) 3% table sugar (C) 2% sucrose (D) 1% sucrose. Bars = 1cm

5.2.2. In vitro response of *A. corrorima* to different salt strength

The mean number of shootlets produced per explant was the highest in the case of full salt strength (5.27±0.53) as compared with half salt strength (3.37±0.43) and one-third salt strength (4.13±0.42) (Table 13 and Figure 14). The mean longest shoot was found when using full strength MS medium (3.08±0.20 cm) followed by half strength (2.68±0.18 cm) and one-third strength (2.20±0.22 cm). Thus, it can be concluded that the best treatment for increasing the shoot number and length of *A. corrorima* was full MS salt strength.

Table 13. Effect of different salt strength of MS medium on shoot multiplication of *A. corrorima* in media containing different concentrations of BAP and TDZ combinations

PGR (mg/l)		Full MS		Half (1/2) MS		One-third (1/3) MS	
BAP	TDZ	Mean no. of shoot/explant	Mean shoot length (cm)	Mean no. of shoot/explant	Mean shoot length (cm)	Mean no. of shoot/explant	Mean shoot length (cm)
0.5	0.25	2.63±0.25hij	2.81±0.18ab	3.10±0.18defghij	2.02±0.17efghij	3.93±0.34cdef	2.15±0.10defg
1.0	0.25	2.47±0.19hij	2.20±0.14defg	2.83±0.28fghij	2.70±0.16abc	3.17±0.22defgihj	2.20±0.10defg
1.5	0.25	5.07±0.75ab	2.70±0.18abc	2.30±0.22j	2.68±0.18abc	3.17±0.33defghij	2.00±0.14efghij
2.0	0.25	2.67±0.22ghij	3.06±0.21a	2.70±0.23ghij	1.95±0.16efghijk	3.50±0.30defghi	1.92±0.08efghijkl
2.5	0.25	2.70±0.24ghij	2.60±0.14bcd	2.80±0.21fghij	1.78±0.12ghijklm	4.03±0.40cde	2.08±0.11efgh
3.0	0.25	2.33±0.18ij	3.08±0.20a	2.97±0.29efghij	1.62±0.12hijklmn	2.70±0.22ghij	2.17±0.13defg
0.5	0.5	4.87±0.39abc	2.30±0.11cdef	2.70±0.26ghij	1.98±0.12efghij	3.29±0.33defghij	1.83±0.11fghijkl
1.0	0.5	5.13±0.52ab	2.30±0.15cdef	2.97±0.27efghij	1.33±0.09mn	3.27±0.30defghij	1.98±0.12efghij
1.5	0.5	5.27±0.53a	2.38±0.15bcde	2.67±0.23ghij	1.27±0.06n	3.04±0.29defghij	2.08±0.11efgh
2.0	0.5	3.83±0.38cdefg	2.23±0.22defg	2.97±0.23efghij	1.50±0.09klmn	3.42±0.18defghij	1.60±0.05ijklmn
2.5	0.5	3.58±0.40defgh	2.17±0.22defg	3.37±0.43defghij	1.55±0.09jklmn	4.13±0.42bcd	2.05±0.11efghi
3.0	0.5	3.93±0.32cdef	1.95±0.15efghijk	2.70±0.23ghij	1.47±0.08lmn	3.04±0.28defghij	1.77±0.13ghijkm

Means within columns having different letters in superscript are significantly different at $p<0.05$.

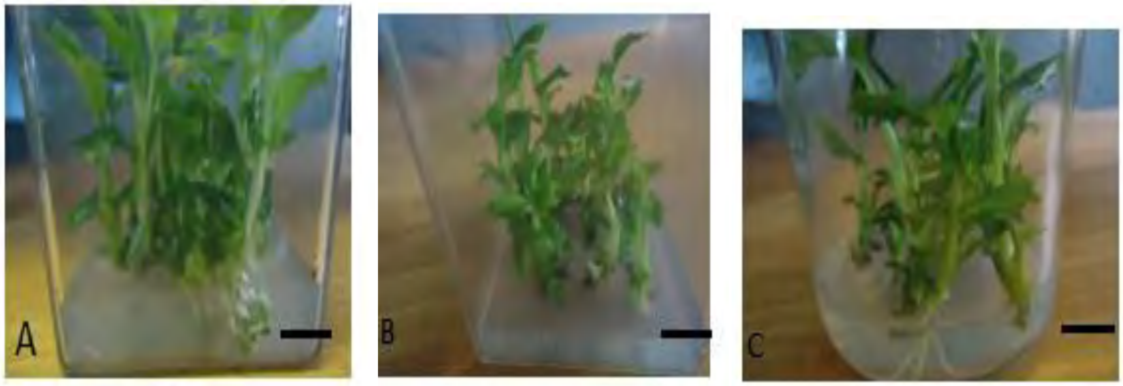


Figure 14. Shoot multiplication on MS medium containing different salt strength. (A) Full MS medium (B) Half MS medium (C) One-third MS medium. Bars = 1cm.

5.2.3. In vitro response of *A. corrorima* to liquid and solid media

Data indicated that higher mean number of shoots (4.41±0.65) and mean shoot length (5.86±0.27 cm) were obtained from liquid culture in comparison with solid medium (Table 14 & Figure 15). The mean shoot number and mean shoot length obtained from solid medium was 3.00±0.25 and 3.60±0.23 cm respectively. The highest shoot number was obtained in both liquid and solid media using 2.5 mg/l BAP in combination with 0.1 mg/l NAA.

Table 14. Mean number of shoots and shoot length per explant on solid and liquid media containingdifferent combinations of BAP and NAA

PGR (mg/l)		Solid medium		Liquid medium	
BAP	NAA	Mean no. of shoot/explant	Mean shoot length (cm)	Mean no. of shoot/explant	Mean shoot length (cm)
0.0	0.0	2.05 ± 0.16^{c}	4.44 ± 0.16^{b}	2.90 ± 0.12^{bc}	4.65 ± 0.14^{b}
0.5	0.1	2.38 ± 0.24^{c}	3.60 ± 0.23^{c}	2.08 ± 0.31^{c}	4.48 ± 0.21^{b}
1.0	0.1	2.83 ± 0.24^{bc}	3.45 ± 0.14^{c}	3.64 ± 0.46^{ab}	5.86 ± 0.27^{a}
1.5	0.1	2.53±0.35c	3.35 ± 0.20^{c}	4.31 ± 0.52^{a}	3.67 ± 0.24^{c}
2.0	0.1	2.07 ± 0.19^{c}	3.05 ± 0.17^{c}	3.62 ± 0.50^{ab}	4.60 ± 0.38^{b}
2.5	0.1	3.00 ± 0.25^{bc}	3.18 ± 0.12^{c}	4.41 ± 0.65^{a}	5.02 ± 0.21^{b}
3.0	0.1	2.70 ± 0.23^{bc}	3.40 ± 0.19^{c}	3.67 ± 0.21^{ab}	3.07 ± 0.14^{c}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

Figure 15. Shoot multiplication of *A. corrorima* on solid and liquid MS medium (A) solid MS medium (B & C) liquid MS medium. Bars = 1cm.

5.2.4. Rooting

The shoots cultured on half strength MS basal solid and liquid media supplemented with different concentrations of IAA showed different rooting responses (Figure 16). Liquid medium showed higher mean number of roots than solid medium.The highest mean number of roots per shoot was 7.60±0.56and 18.50±1.15 inmedium containing 4.0 mg/l

IAA in solid and liquid media respectively (Table 15). Thehighest mean root length was observed on 4.0 mg/l IAA (4.03±0.15cm and 1.50±0.11 cm)in liquid and solid media respectively. However, a relatively small number of roots were observed on bothgrowth regulators free solid and liquid media. Moreover, MS medium containing 3.0 mg/l IAA produced lowest number of roots in liquid medium (8.20 ± 0.41) and solid medium (5.27 ± 0.68).

Table 15.Number and length of roots on half strength MS medium containing different concentrations of IAA in solid and liquid media

PGR (mg/l) IAA	Liquid medium		Solid medium	
	Mean no. of root/explants	Mean root length (cm)	Mean no. of root/explant	Mean root length (cm)
00	2.43 ± 0.41^{f}	0.97 ± 0.18^{e}	3.54 ± 0.43^{ef}	1.75 ± 0.17^{c}
1.0	17.13 ± 1.22^{a}	3.67 ± 0.13^{ab}	5.57 ± 0.54^{de}	1.35 ± 0.07^{cde}
2.0	10.87 ± 0.78^{b}	3.52 ± 0.30^{b}	5.37 ± 0.78^{e}	1.03 ± 0.11^{de}
3.0	8.20 ± 0.41^{c}	3.50 ± 0.17^{b}	5.27 ± 0.68^{cd}	1.20 ± 0.10^{de}
4.0	18.50 ± 1.15^{a}	4.03 ± 0.15^{a}	7.60 ± 0.56^{a}	1.50 ± 0.11^{cd}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

Figure16. Root induction of *A. corrorima* on half MS medium solid and liquidmedia. (A) Solid MS medium (B&C) Liquid MS medium

5.2.5. Plantlet acclimatization

Fifty plantlets from each of solid and liquid medium were planted in pots and the survival percentage was found to be 86% and 78% for solid media and liquid media respectively after one month.

Figure 17. Acclimatization of in vitro rooted microshoots from solid and liquid media (A) Plantlets from liquid medium after three days of plantingB) Plantlets from liquid medium covered with polyethylene bag C) Established plants after 35 days from liquid media (D)Plantlets transferred to potsfrom solid media (E) Plantlets covered with polyethylene bag from solid media (F) Established plants after 25 days from solid media.

5.3. Callus induction and in vitro shoot regeneration of *A. corrorima* using rhizome explants

5.3.1. Culture of explants on callus induction media

5.3.1.1. Effect of 2,4-D

Callus formation was observed after 6-8 weeks of culture. Callus formation on the explants occurred at wounding sites and then continued to grow until it covered the entire rhizome tissue. The best response of callus induction (80.00 ± 5.77%) was observed on MS medium containing 0.5 mg/l 2,4-D and the least response of callus induction (43.33 ± 8.82 %) was recorded on MS medium containing 5.0 mg/l 2,4-D (Table16). Rhizome pieces on each medium containing 2,4-D in the range of 0.5 - 5.0 mg/l responded differently. No callus was obtained on growth regulators free MS medium.The calli were friable with white or white-yellow color (Figure 18). In this study embryogenic nodular callus was observed and somatic embryos were formed only from this nodular callus type. The results indicated that an increase in concentration of 2,4-D showed decline in the percentage of callus formation.

Table 6. Effect of 2,4-D on callus induction from rhizome explants of *A. corrorima*

2,4-D (mg/l)	Callus induction (%)	Color	Texture
0.0	0±0.00	-	-
0.5	80.00±5.77^{a}	White-yellow	Friable
1.0	70.00±5.77^{ab}	White	Friable
2.0	66.67±8.82^{ab}	White-yellow	Friable
3.0	56.67±8.82^{ab}	White-yellow	Friable
4.0	50.00±11.55^{b}	White	Friable
5.0	43.33±8.82^{b}	White	Friable

Means within columns having different letters in superscript are significantly different at $p<0.05$.

5.3.1.2. Synergistic effect of 2,4-D and BAP

After six weeks, the cellular clumps consisting of small whitish friable callus grew on the rhizome explants (Figure 18). The callus was then maintained under the same conditions with subcultures at 2weeks interval in fresh medium after 45 days of callus culture. It was observed that the treatments with5.0 mg/l 2,4-D + 0.25 mg/l BAP presented the highest induction (80.00±5.77 %) and the best visual characteristics followed by 5.0mg/l 2,4-D + 0.5 mg/l BAP of 76.67±6.67 %. The minimum callus induction (30.00±5.77 %) was recorded with treatments 0.5 mg/l 2,4-D + 0.25 mg/l BAP. No callus production was observed during the culture period in the control.The result showed that percentage of callus formation tended to increase as an increase inconcentrations of 2,4-D in combination with BAP (Table 17).

Table 17. Synergistic effect of 2,4-D and BAP on callus induction from rhizome explants of *A.corrorima*

2,4-D (mg/l)	BAP (mg/l)	Callus induction (%)	Color	Texture
2,4-D	BAP			
0.0	0.0	0±0.00	-	-
0.5	0.25	30.00±5.77^{e}	White	Friable
1.0	0.25	40±5.77^{de}	White	Friable
2.0	0.25	40±5.77^{de}	White-yellow	Friable
3.0	0.25	50.00±5.77^{cde}	White	Friable
4.0	0.25	70.00±5.77^{abc}	White-yellow	Friable
5.0	0.25	80.00±5.77^{a}	White	Friable
0.5	0.5	43.33±6.67^{de}	White	Friable
1.0	0.5	53.33±6.67^{bcd}	White	Friable
2.0	0.5	60.00±10.00^{abcd}	White-yellow	Friable
3.0	0.5	60.00±10.00^{abcd}	White-yellow	Friable
4.0	0.5	73.33±8.82^{abc}	White-yellow	Friable
5.0	0.5	76.67±6.67^{ab}	White	Friable

Means within columns having different letters in superscript are significantly different at $p<0.05$.

5.3.1.3. Synergistic effect of 2,4-D with KN and/or NAA

Callus initiation occurred at the cut surfaces of the rhizomes during 6-8 weeks of culture in most of the explants. The induction percentage, texture and color of callus were different on different media (Tables 18). The maximum callus induction after 60 days (76.67%) was observed on MS medium with treatments 2,4-D (5.0 mg/l) + KN (0.5 mg/l) and2,4-D (0.5 mg/l) + NAA (0.5 mg/l).Lowest percentage of callus induction (46.67±8.82%) was recorded on the medium containing 5.0 mg/l 2,4-D + 0.5 mg/l NAA.

The primary callus was whitish or whitish-yellow but after 3 months grew into grey-white and after 4 months appeared green or brown. On the control medium the explants dried and callus induction was not noticed.Result shows that high concentration of 2,4-D in combination with KN and low concentration of 2,4-D in combination with NAA increased the percentage of callus induction.

Table 18.Synergistic effect of 2,4-D withKN and NAA on callus induction from rhizome explants of *A.corrorima*

PGR (mg/l)		Callus induction (%)	Color	Texture
2,4-D	KN			
0.0	0.0	0±0.00	-	-
0.5	0.5	63.33±8.82[a]	White	Friable
1.0	0.5	56.67±8.82[a]	White-yellow	Friable
2.0	0.5	53.33±8.82[a]	White-yellow	Friable
3.0	0.5	66.67±12.02[a]	White-yellow	Friable
4.0	0.5	70.00±5.77[a]	White-yellow	Friable
5.0	0.5	76.67±6.67[a]	White	Friable
2,4-D	NAA			
0.5	0.5	76.67±8.82[a]	White	Friable
1.0	0.5	70.00±11.55[ab]	White-yellow	Friable
2.0	0.5	70.00±5.77[ab]	White	Friable
3.0	0.5	63.33±6.67[ab]	White-yellow	Friable
4.0	0.5	53.33±8.82[ab]	White	Friable
5.0	0.5	46.67±8.82[b]	White-yellow	Friable

Means within columns and treatments having different letters in superscript are significantly different at $p<0.05$.

Figure 18. Callus induction from rhizome explants using auxin and cytokinins (A) 5.0 mg/l 2,4-D after 2 weeks, (B) 2.0 mg/l 2,4-D + 0.5 mg/l KN after 2 months, (C) 3.0 mg/l2.4-D + 0.25 mg/l BAP after 3 months. Bars = 1cm

5.3.2. Plantlet regeneration

The embryogenic callus obtained from the previous step was cultured on MS medium supplemented with BAP and KN in combination with IAA. Somatic embryos were obtained after 10 weeks of callus sub-culture. The fully developed somatic embryos were continuously sub-cultured in magenta GA-7 culture vessel containing MS medium with the same combination of PGR (BAP and KN with IAA). Embryos were differentiated into 1-4 plantlets per explant. The maximum shoot regeneration percentage (25.33±1.99%) was achieved on a medium containing 0.5mg/l BAP in combination wtih 0.25 mg/l IAA (Table 19). The lowest shoot regeneration percentage (14.67 ± 1.46%) was recorded on a medium containing 3.0 mg/l BAP and 0.25 mg/l IAA.In some of the treatments only callus formation was observed and did not show any regeneration of plantlets from callus (Table 19).Fully developed plantlets were obtained after 60 daysof culture of somatic embryos on regeneration media (Figure 19).

Table 19. Effect of BAP and kinetin in combination with IAA on shoot regeneration from callus

Growth regulators (mg/l)		Regeneration percent	Mean no. of shoot per callus	Mean length of shoot (cm)
BAP	IAA			
0.0	0.0	0 ± 0.00	0.00	0.00
0.5	0.25	25.33 ± 1.99^{a}	2.93 ± 0.23^{a}	0.99 ± 0.06^{a}
1.0	0.25	19.00 ± 1.62^{bc}	1.97 ± 0.20^{bc}	0.84 ± 0.05^{abc}
2.0	0.25	-	-	-
3.0	0.25	14.67 ± 1.46^{c}	1.47 ± 0.12^{c}	0.62 ± 0.03^{d}
0.5	0.5	-	-	-
1.0	0.5	21.67 ± 1.86^{ab}	2.10 ± 0.20^{b}	0.93 ± 0.06^{ab}
2.0	0.5	-	-	-
3.0	0.5	17.17 ± 1.91^{bc}	1.63 ± 0.15^{bc}	0.75 ± 0.05^{bcd}
KN	IAA			
0.5	0.25	21.83 ± 2.12^{a}	2.46 ± 0.26^{a}	0.87 ± 0.07^{a}
1.0	0.25	-	-	-
2.0	0.25	19.17 ± 1.63^{ab}	2.40 ± 0.16^{a}	0.82 ± 0.05^{ab}
3.0	0.25	-	-	-
0.5	0.5	22.67 ± 1.85^{a}	2.37 ± 0.19^{ab}	0.86 ± 0.05^{a}
1.0	0.5	17.33 ± 1.17^{ab}	1.57 ± 0.10^{c}	0.66 ± 0.03^{ab}
2.0	0.5	15.83 ± 1.05^{b}	1.93 ± 0.14^{bc}	0.72 ± 0.0^{4}
3.0	0.5	-	-	-

Means within columns and treatments having different letters in superscript are significantly different at $p<0.05$.

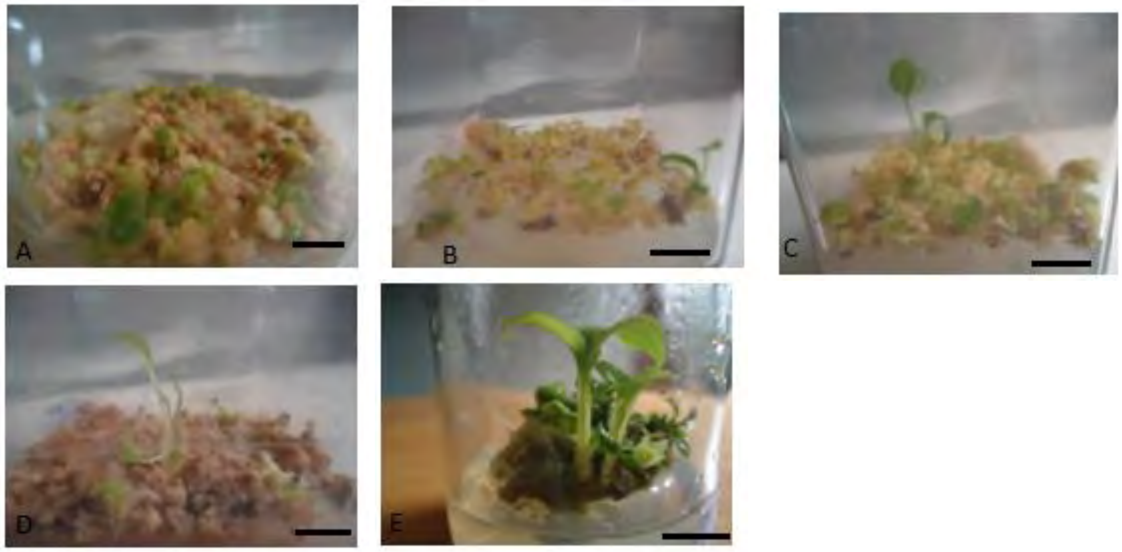


Figure 19. Shoot regeneration from callus using BAP and IAA (A) 0.5 mg/l BAP + 0.25 mg/l IAA (B) 1.0 mg/l BAP + 0.5 mg/l IAA (C) 0.5 mg/l KN +0.25 mg/l IAA (D) 0.5 mg/l KN + 0.5 mg/l IAA (E) 1.0 mg/l BAP + 0.25 mg/l IAA

5.3.3. Shoot multiplication

When the regenerated plantletsare cultured on MS medium containing BAP in combination with KN enhanced shoot proliferationwas observed (Figure 20).Higher concentration of BAP (3.0 mg/l) in combination with lower concentration of KN (0.25 mg/l) showed better response to shoot multiplication of *A. corrorima (*mean shoot number of 4.90±0.53) followed by 2.0 mg/l BAP in combination with 0.25 mg/lKN(4.86±0.50 mean shoot number).The lowest meanshoot number(1.97±0.16)and highest mean shoot length (4.30±0.37 cm) were recorded on growth regulators free MS medium which was used as control (Table 20).

Table 20. Mean shoot number, length and leaf number of *A. corrorima* on medium supplemented with BAP and KN

PGR (mg/l) BAP	KN	Mean shoot no. per explants	Mean shoot length (cm)	Mean leaf no. per explants
0.0	0.0	1.97 ± 0.16^{c}	4.30 ± 0.37^{a}	5.87 ± 0.49^{c}
0.50.25		3.87 ± 0.35^{ab}	3.62 ± 0.19^{bcd}	9.87 ± 0.76^{bc}
1.0	0.25	3.43 ± 0.28^{b}	3.90 ± 0.19^{ab}	9.43 ± 0.68^{bc}
1.5	0.25	3.67 ± 0.40^{b}	3.70 ± 0.17^{bc}	10.00 ± 0.95^{bc}
2.0	0.25	4.86 ± 0.50^{a}	3.97 ± 0.14^{ab}	12.70 ± 0.97^{ab}
2.5	0.25	3.50 ± 0.33^{b}	3.63 ± 0.18^{bcd}	8.93 ± 0.69^{bc}
3.0	0.25	4.90 ± 0.53^{a}	3.47 ± 0.13^{bcd}	13.27 ± 0.98^{ab}
0.25	0.5	4.07 ± 0.37^{ab}	3.13 ± 0.15^{cd}	14.80±4.35a
0.25	1.0	3.30 ± 0.29^{b}	3.57 ± 0.17^{bcd}	9.20 ± 0.69^{bc}
0.25	1.5	3.60 ± 0.32^{b}	3.47 ± 0.17^{bcd}	10.37 ± 0.79^{bc}
0.25	2.0	3.57 ± 0.31^{b}	3.03 ± 0.16^{d}	9.33 ± 0.79^{bc}
0.25	2.5	3.83 ± 0.26^{ab}	3.07 ± 0.14^{d}	10.37 ± 0.50^{bc}
0.25	3.0	3.30 ± 0.29^{b}	3.03 ± 0.16^{d}	9.23 ± 0.58^{bc}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

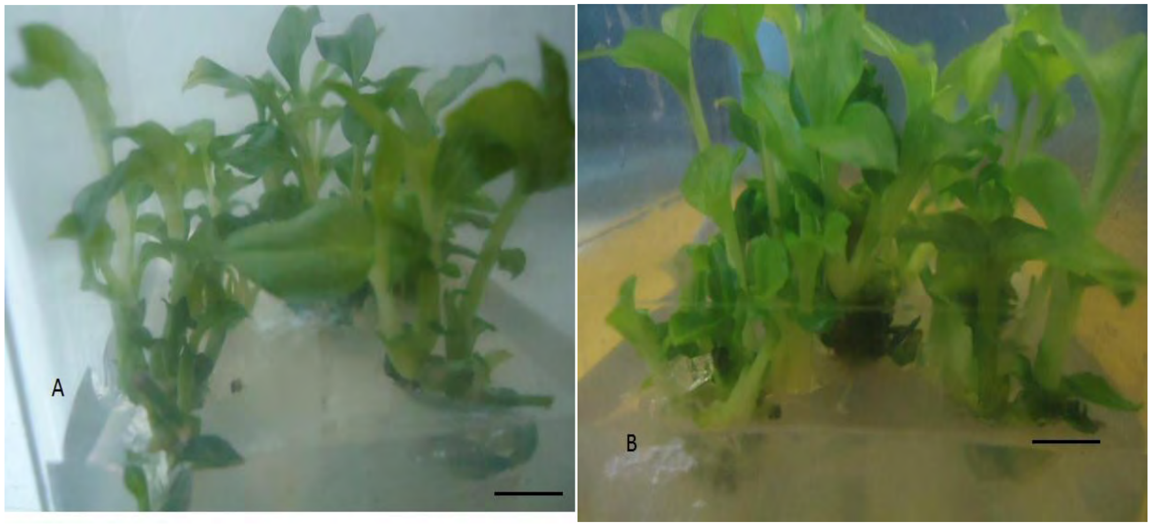


Figure 20. In Vitro shoot multiplication on MS mediumcontaining BAP in combination withKN (A) 0.5 mg/l BAP and 0.25 mg/l KN (B) 0.25 mg/l BAP and 0.5 KN. Bars = 1cm

5.3.4. **Rooting**

Highest mean root number per shootof9.77±0.78 was observed on MS medium containing 0.5 mg/l NAA and 0.5 mg/l IBA (Table 21; Figure 21). The least rooting response, however, was observed on growth regulators free MS medium with mean root number and length of 3.54±0.43 and 1.75±0.17 respectively. The highest mean root length (4.17±0.66 cm) and rooting response (96.67±1.86%) was recorded on medium supplemented with 2.0 mg/l NAA in combination with 0.5 mg/l IBA.

Table 21.Synergisticeffect of NAA and IBA on root induction of *A. corrorima*

PGR (mg/l)		Mean root no. per explants	Mean root length (cm)	Rooting response in %
NAA	IBA			
0.0	0.0	3.54 ± 0.43^{c}	1.75 ± 0.17^{c}	55.56 ± 5.73^{c}
0.5	0.5	9.77 ± 0.78^{a}	2.78 ± 0.09^{b}	93.33 ± 3.05^{ab}
1.0	0.5	8.83 ± 0.92^{ab}	2.40 ± 0.15^{bc}	85.00 ± 4.55^{ab}
1.5	0.5	8.23 ± 1.02^{ab}	2.38 ± 0.14^{bc}	82.22 ± 4.86^{b}
2.0	0.5	9.37 ± 0.61^{ab}	4.17 ± 0.66^{a}	96.67 ± 1.86^{a}
2.5	0.5	8.93 ± 0.69^{ab}	2.50 ± 0.10^{bc}	91.11 ± 3.27^{ab}
3.0	0.5	7.20 ± 0.57^{b}	2.43 ± 0.11^{bc}	90.00 ± 2.73^{ab}

Means within columns having different letters in superscript are significantly different at $p<0.05$.

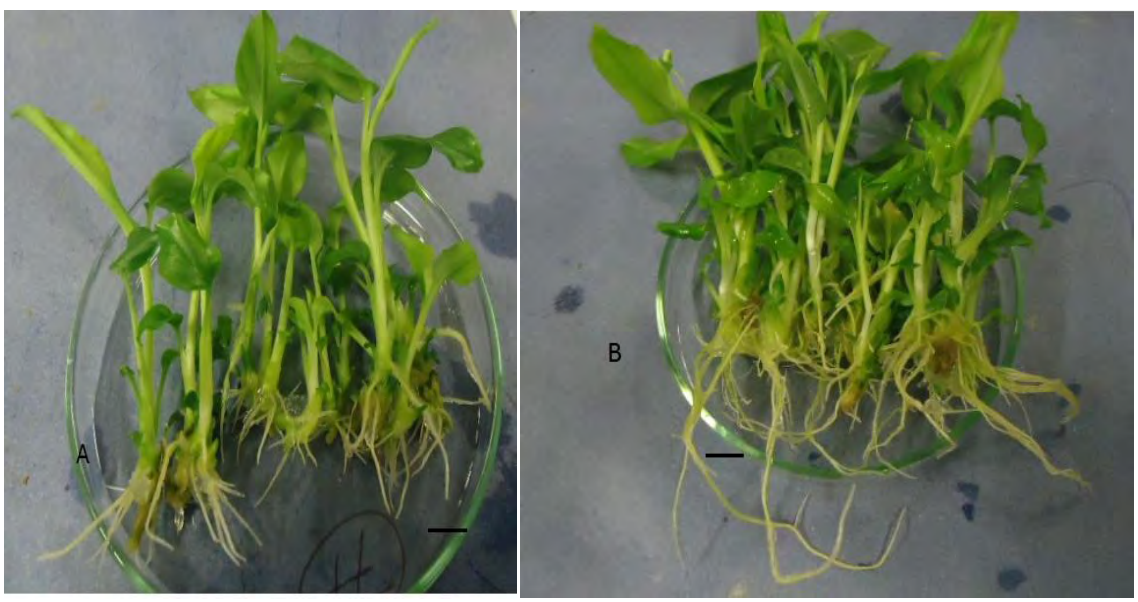


Figure 21. In Vitro rooting of *A. corrorima*in half strength MS medium fortified with NAA and IBA combination (A) 1.0 mg/l NAA and 0.5 mg/l IBA (B) 2.0 mg/l NAA comgined with 0.5 mg/l IBA

5.3.5. Acclimatization

From one hundred plantletsafter 30 days of acclimatization in greenhouse,89% survival rate of the plantlets was recorded.

Figure 22. Acclimatization of *A. corrorima* (A) after 3 days (B) after 30 days (C) after 45 days. Bars = 1 cm.

5.5. Genetic diversity of *A. corrorima* using ISSR markers

5.5.1. Chromosome count of A. corrorima

In the present study analysis of somatic chromosome numberwas confirmed in mitotic division at metaphase stage from in vitro grown plantlets acclimatized in the greenhouse.The chromosome study showed that *A. corrorima* has a diploid chromosome number of 2n =48 (Figure 23).

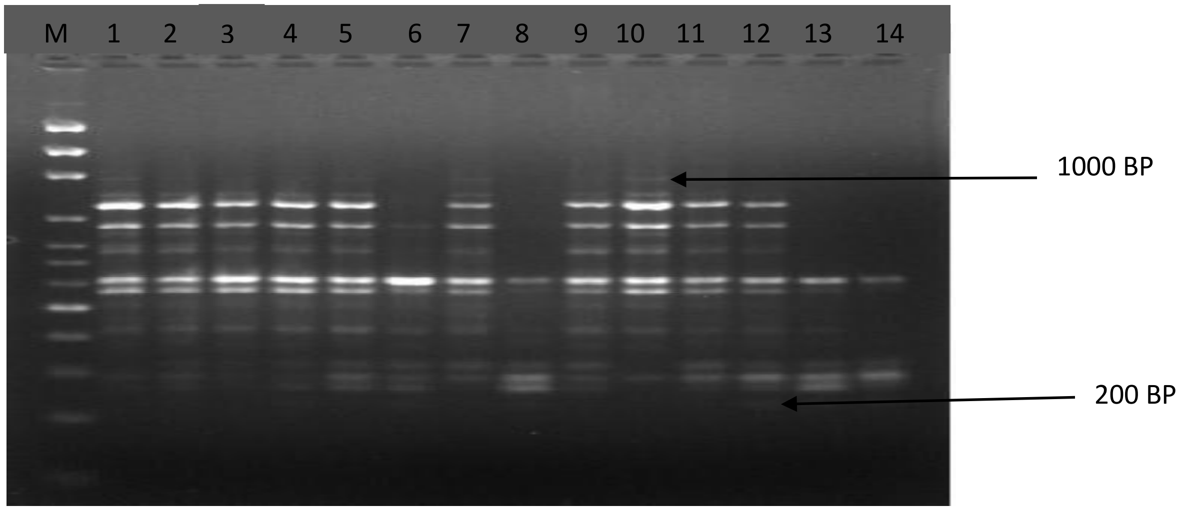
Figure 23. Mitotic somatic chromosome of *A. corrorima*

5.5.2. Banding patterns of the ISSR primers

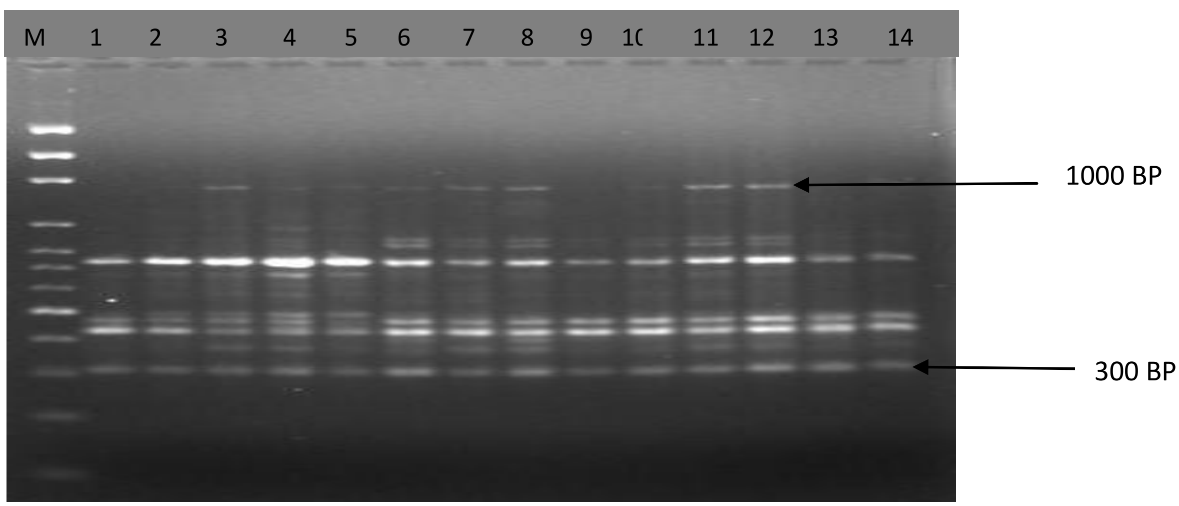
Out of 13 ISSR primers tested, six primers produced clear amplified products and discernible banding pattern (Table 22).The size of the bands amplified using the six primers were in the range of 200 bp to 1000base pairs (Figure 24). A total of 83 bands were scored from six primers and the number of polymorphic bands per primer ranged from 11 (primer 848 and 866) to 17 (primer 836) with an average of 13.83.

Table 22. The six primers used, their repeat motifs, amplification quality and number of bandsscored

Primers	Repeat motif	Amplification Pattern	No. of scored bands
809	(AG)8G	Good	14
818	(CA)8G	Good	15
836	(AG)8YA	Good	17
848	(CA)8RG	Good	11
866	(CTC)6	Good	11
880	(GGAGA)3	Good	15
Total			**83**



A



B

Figure 24. PCR products from twenty fourkorarima genotypes viewed using agarose gel electrophoresis (A) Lanes 1 - 14 from Basketo and West Gojam zones using ISSR 818 primer and (B) Lanes1-14 from South Omo zone using ISSR 880 primer. M: 100bp DNA ladder.

5.5.3. Polymorphism based on ISSR analysis

The percentage of polymorphic loci (PPL) for all primers was 100%. In this study thelowestheterozygosity value was 0.3623 from primer 866and the highest (0.4492) was observed with primer 848. Similarly, the maximum and minimum Shanon‟s information index was 0.6391 (primer 848) and 0.5383 (primer 866) respectively (Table 23).

Table 23. Number of scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H), Shanon information Index (I) of 102 *A. corrorima* accessions based on six ISSR primers used

Primers	NSB	NPL	PP	H ± SD	I± SD
809	14	14	100	0.4164 ± 0.0806	0.6044 ± 0.0875
818	15	15	100	0.4035 ± 0.0859	0.5901 ± 0.0948
836	17	17	100	0.4235 ± 0.1263	0.6047 ± 0.1606
848	11	11	100	0.4492 ± 0.0747	0.6391 ± 0.0836
866	11	11	100	0.3623 ± 0.1329	0.5383 ± 0.1615
880	15	15	100	0.4309 ± 0.1207	0.6143 ± 0.1479

5.5.4. Genetic diversity

Comparisons between populations showed that Basketo II has the highest percent polymorphism (80.72%) followed by Basketo I (75.90%) while populations from Gelila II were observed to have the least percent polymorphism with 63.86%. The second least percent polymorphism (67.47%) was shown by populations from Finote Selam and Wubamer I (Table 25).Genetic diversity expressed as gene diversity value showed higher variability for Basketo IIpopulation with 0.34, followed by Basketo I population with 0.32 values. Gelila IIpopulation was observed to be the least diverse with gene diversity value of 0.25. The total diversity index values for the total populations were found to be 0.30 (Table 24). The same diversity patterns were also observed for Shannon diversity index, whereby Basketo II and Basketo I populations showed the highest value with 0.49 and 0.46, respectively. The least variability was shown by Gelila II population (0.37).

Table 24.Number of polymorphic loci (NPL), percentage of polymorphic loci (PP), Nei‟s gene diversity (H) and Shannon‟s information index (I) of korarima using data generated from all populations

Populations	NPL	PP%	H ± SD	I ± SD
Basketo II	67	80.72	0.3418 ± 0.1903	0.4918 ± 0.2637
Finote Selam	56	67.47	0.2822 ± 0.2123	0.4076 ± 0.2994
Chagne	60	72.29	0.2930 ± 0.2095	0.4245 ± 0.2910
Bikolo Abay	60	72.29	0.2818 ± 0.2018	0.4128 ± 0.2826
Gelila I	59	71.08	0.2890 ± 0.2033	0.4204 ± 0.2869
Gelila II	53	63.86	0.2528 ± 0.2122	0.3691 ± 0.2993
Gelila III	61	73.49	0.2568 ± 0.1948	0.3836 ± 0.2728
Wubamer I	56	67.47	0.2791 ± 0.2122	0.4040 ± 0.2986
Wubamer II	61	73.49	0.3138 ± 0.2052	0.4511 ± 0.2880
Wubamer III	59	71.08	0.2742 ± 0.2036	0.4022 ± 0.2854
BasketoI	63	75.90	0.3161 ± 0.2011	0.4562 ± 0.2805
Over all	83	100.00	0.3041	1.1441

5.5.5. Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) was carried out on the overall ISSR data score of korarimapopulations without grouping (Table 25). AMOVA without grouping population revealed that higher percentage of variation (76%) is attributed to the within population variation while the remaining variation is due to the among population variation (24%). The variations were found to be highly significant at P<0.001.

Table 25. Analysis of molecular variance (AMOVA) of *Aframomum corrorima*populations from Southern and North western Ethiopia without grouping

Source of Variation	Dergee of fredom	Sum of squares	Variance Components	Percentage of Variation	Fixation Indices	P
Among populations	10	510.102	4.127	24%	0.244	0.001
Within populations	91	1162.819	12.778	76%		
Total	101	1672.922	16.905	100%		

5.5.6. Genetic similarity

Inter-population genetic distance (D*)* ranged from 0.1198 to 0.3548,while inter-population genetic similarity sorted between 0.7013 and 0.8871 for all the 11 populations (Table 26). Among the pair-wise population comparisons made within studied population samples from Bikolo Abay and Wubamer II showed the highest genetic distance (0.3548) and least genetic similarity (0.7013) and samples from Finote Selam and Gelila II showed the least genetic distance (0.1198) and highest genetic similarity (0.8871).

Table 26.Nei‟s unbiased measure of genetic identity (similarity) and distance (dissimilarity) based on Nei (1978)

pop ID	Bas I	Bas II	FSChBK			G- I	G-II	G-III	W-I	W-II	W-III
Bas I	****	0.8146	0.8257	0.8190	0.7862	0.8478	0.8506	0.7826	0.8332	0.8461	0.8067
BasII	0.2051	****	0.8614	0.8853	0.8133	0.7364	0.8108	0.7517	0.8212	0.7638	0.7589
FS	0.1915	0.1492	****	0.8485	0.8227	0.8180	0.8871	0.7658	0.8078	0.7661	0.7826
Ch	0.1997	0.1219	0.1643	****	0.8131	0.7709	0.8139	0.7323	0.8482	0.8063	0.7925
BK	0.2405	0.2066	0.1952	0.2069	****	0.7754	0.7927	0.7411	0.7750	0.7013	0.7797
G- I	0.1651	0.3060	0.2009	0.2602	0.2544	****	0.8401	0.7771	0.7733	0.7683	0.8105
G-II	0.1618	0.2098	0.1198	0.2060	0.2323	0.1743	****	0.8164	0.7783	0.7982	0.8552
G-III	0.2451	0.2855	0.2668	0.3116	0.2997	0.2522	0.2029	****	0.8114	0.7755	0.8607
W-I	0.1825	0.1970	0.2135	0.1647	0.2549	0.2571	0.2506	0.2090	****	0.8849	0.8238
W-II	0.1671	0.2695	0.2664	0.2153	0.3548	0.2636	0.2254	0.2542	0.1223	****	0.8417
W-III	0.2147	0.2759	0.2452	0.2325	0.2488	0.2101	0.1564	0.1500	0.1938	0.1723	****

Where: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

5.5.7. Clustering analysis

UPGMA and neighbor joining analysis were used to construct dendrogram for the eleven populations and 102 individuals based on 83 PCR bands amplified by six ISSR primers(Figure 26; Figure 27).UPGMA analysis based on *A. corrorima* populations (Figure25) revealed three major groups and one outlier (Gelila III population). The first major cluster again forked into three sub groups (Basketo I, Gelila I and Gelila II populations) while the second contained Basketo II, Bikolo Abay, Finote Selam and Chagne. The third major cluster comprises of Wubamer I, Wubamer II and Wubamer III populations which embodies the South Omo zone of South Ari wereda.

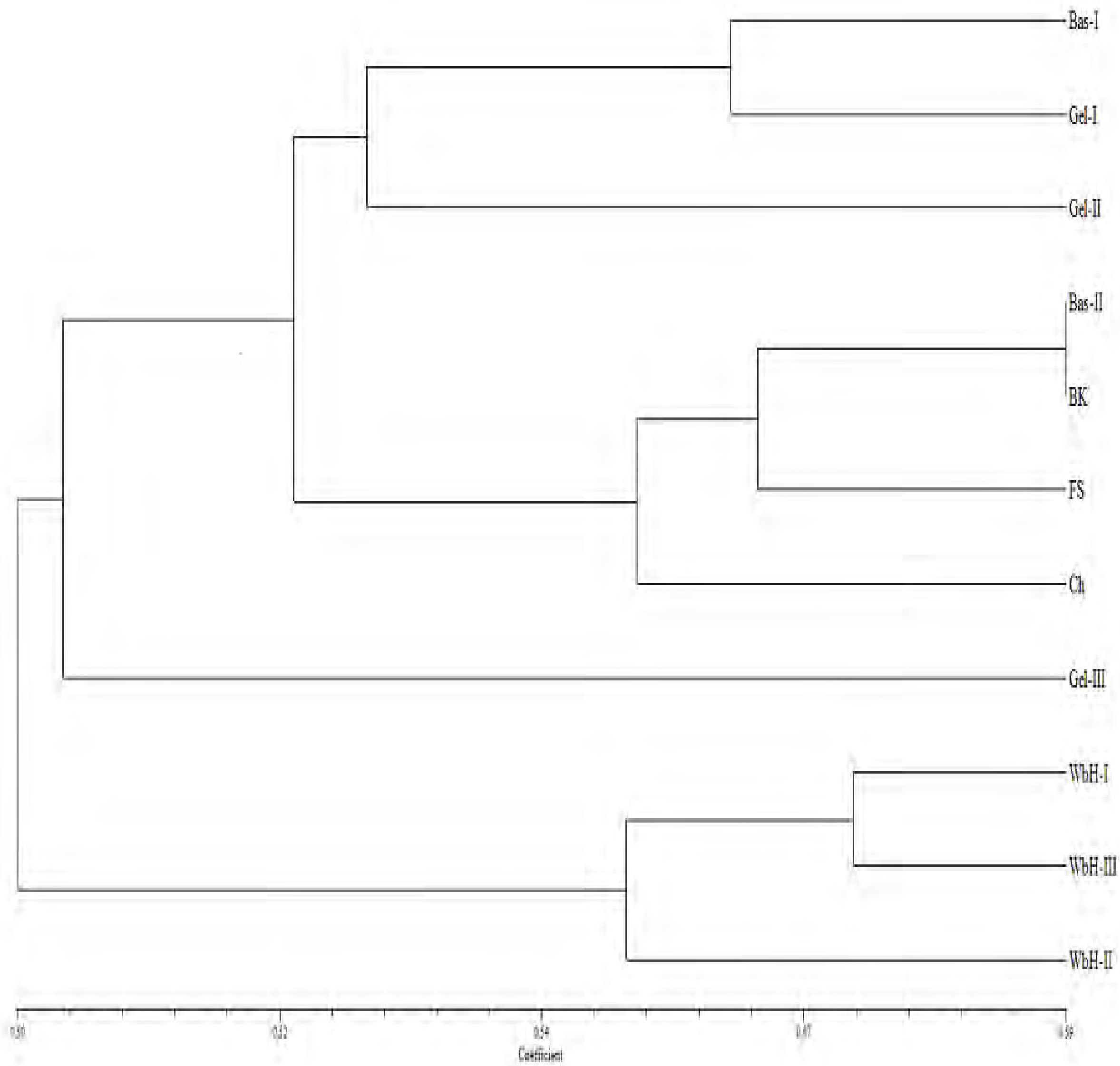


Figure 25.UPGMA based dendrogram obtained from 11 populations using 6 ISSR primers.Key: Bas1-Basketo 1, Bas II-Basketo 2, FS- Finote Selam, Ch- Chagne, Bk-Bikolo Abay, Gel I- Gelila 1, Gel II- Gelila 2, Gel III- Gelila 3, WbH I-Wubamer 1, WbH II-Wubamer 2, WbH III-Wubamer 3.The UPGMA algorithm is based on Jaccard"s coefficients obtained after pair wise comparison of the presence-absence fingerprint

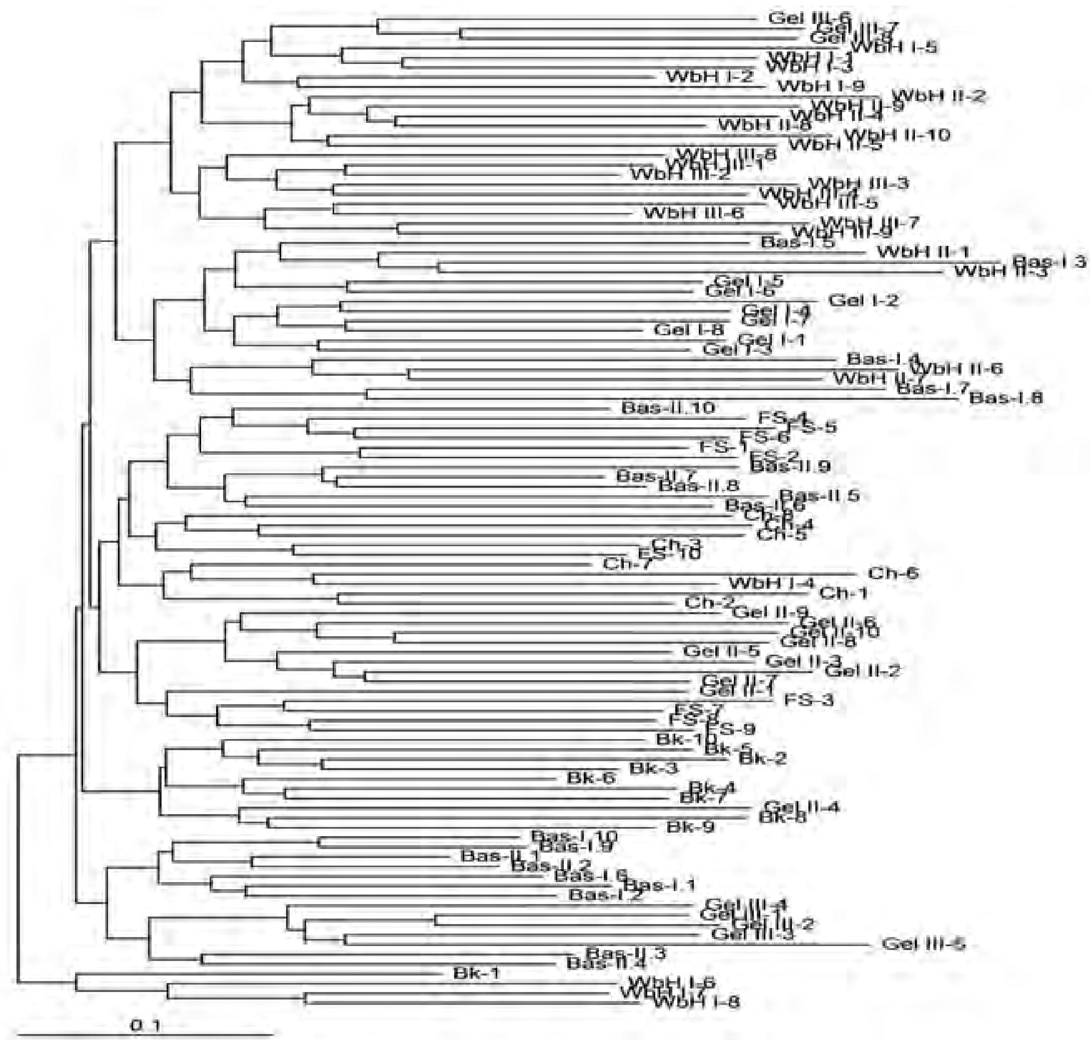


Figure 26**.** Neighbor-joining analysis of 102 individuals based on 83 PCR bands amplified by six ISSRprimers.Key: Bas1-Basketo 1, Bas II-Basketo 2, FS- Finote Selam, Ch- Chagne, Bikolo Abay, Gel I- Gelila 1, Gel II- Gelila 2, Gel III- Gelila 3, Wb I-Wubamer 1, Wb II-Wubamer 2, Wb III-Wubamer 3.The neighbor joining algorithm is based on Jaccard‟s cœfficients obtained after pair wise comparison of the presence-absence fingerprint

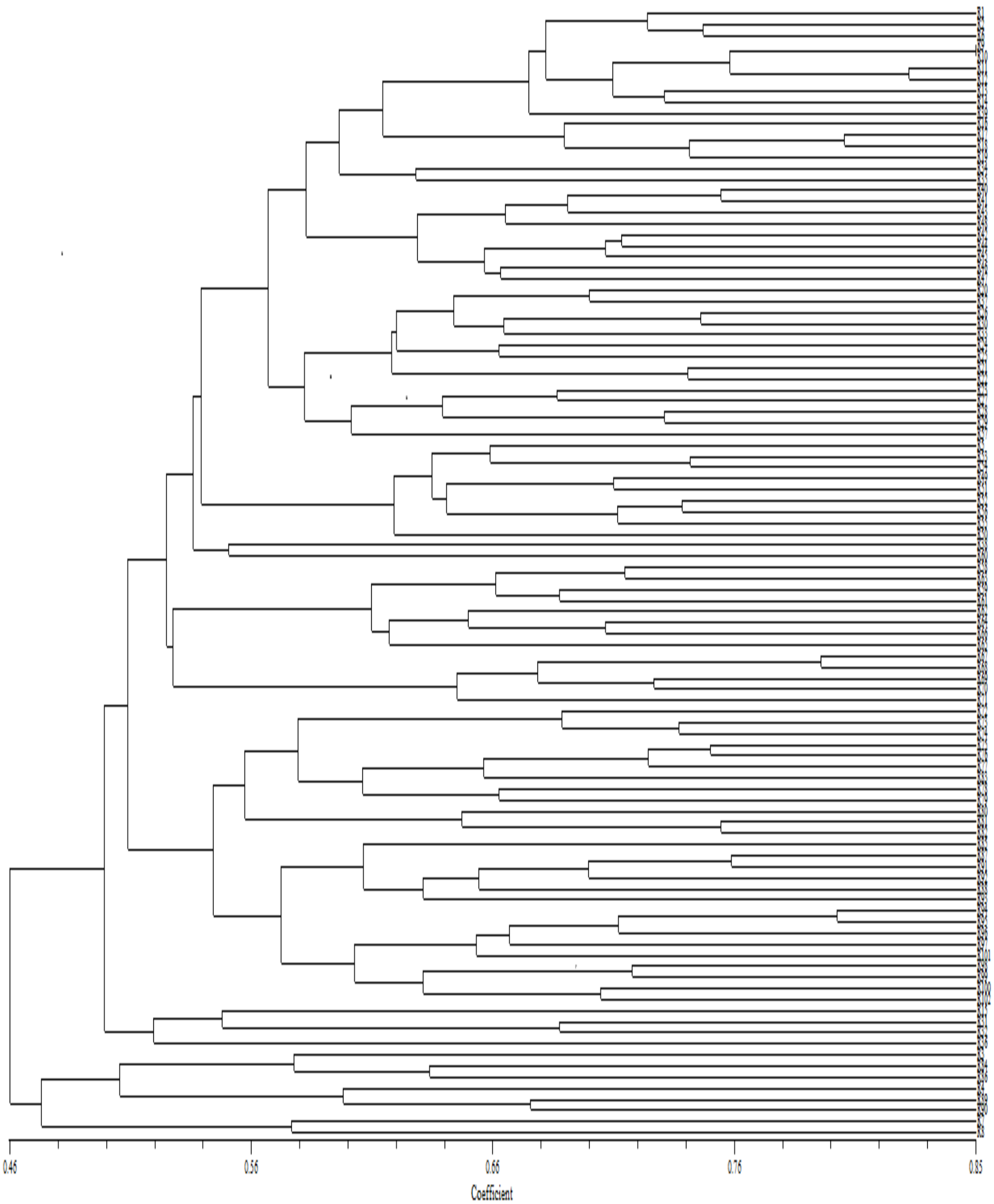


Figure 27**.** Dendrogram showing genetic relationships among 102 korarima plants constructed by UPGMA clustering analysis using6 ISSR markers

Key: Bas1-Basketo 1, Bas II-Basketo, FS- Finote Selam, Ch- Chagne, Bikolo Abay, Gel I- Gelila I, Gel II- Gelila II, Gel III- Gelila III, Wb I-Wubamer I, Wb II-Wubamer II, Wb III-Wubamer III.

5.5.8. Principal coordinate (PCoA) analysis

All the data obtained using the six ISSR primers were used in PCoA plotting. The analysis was carried out using GenAlEx6.502 and Statistica version 12.0 softwares. The first three coordinates of the PCO having eigenvalues of **1**4.87, 9.83 and 9.23 with variance of 8.93%, 5.90% and 5.55% respectivelyused to show the grouping of individuals using three coordinates (Figure 29). All the 11 populations were observed to form separate clusters. However, some individuals from Gelila I observed to be intermixed with Chagne and Bikolo Abay populations. Using two coordinates (Figure 28), almost similar result was observed like that of three coordinates.

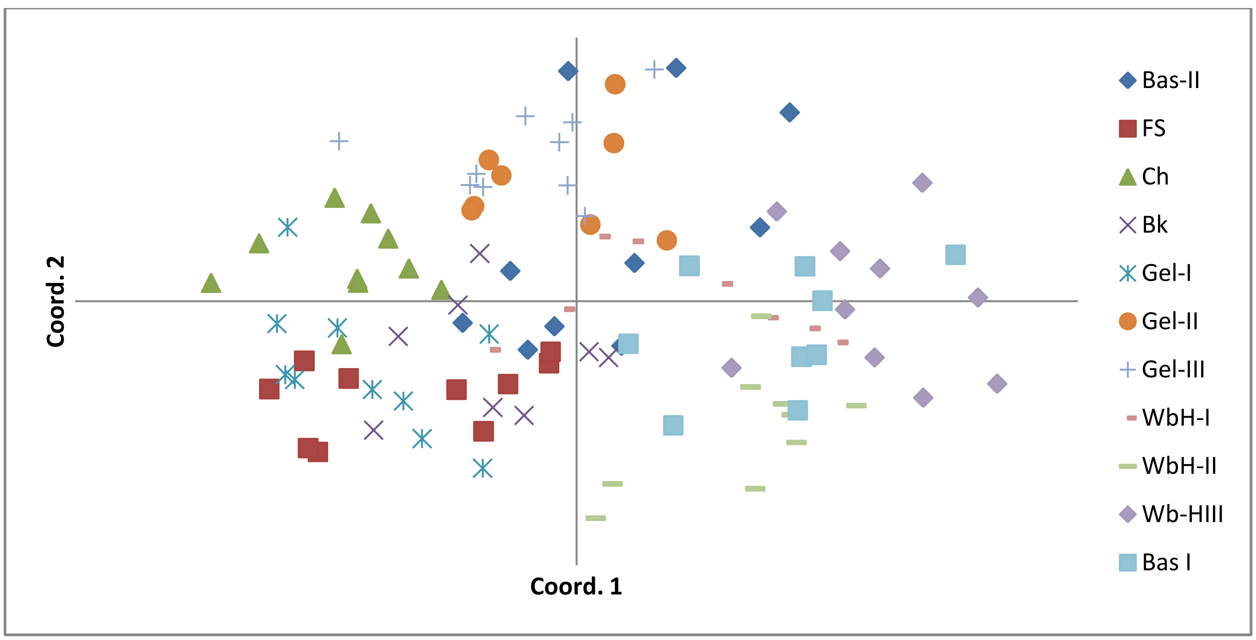


Figure 28**.**Two dimensional representation of principal coordinate analysis of genetic relationships among 102 individuals
Key: Bas I-Basketo 1, Bas II-Basketo 2, FS- Finote Selam, Ch- Chagne, Bikolo Abay, Gel I- Gelila 1, Gel II- Gelila 2, Gel III- Gelila 3, Wb I-Wubamer 1, Wb II-Wubamer 2,Wb III-Wubamer 3.

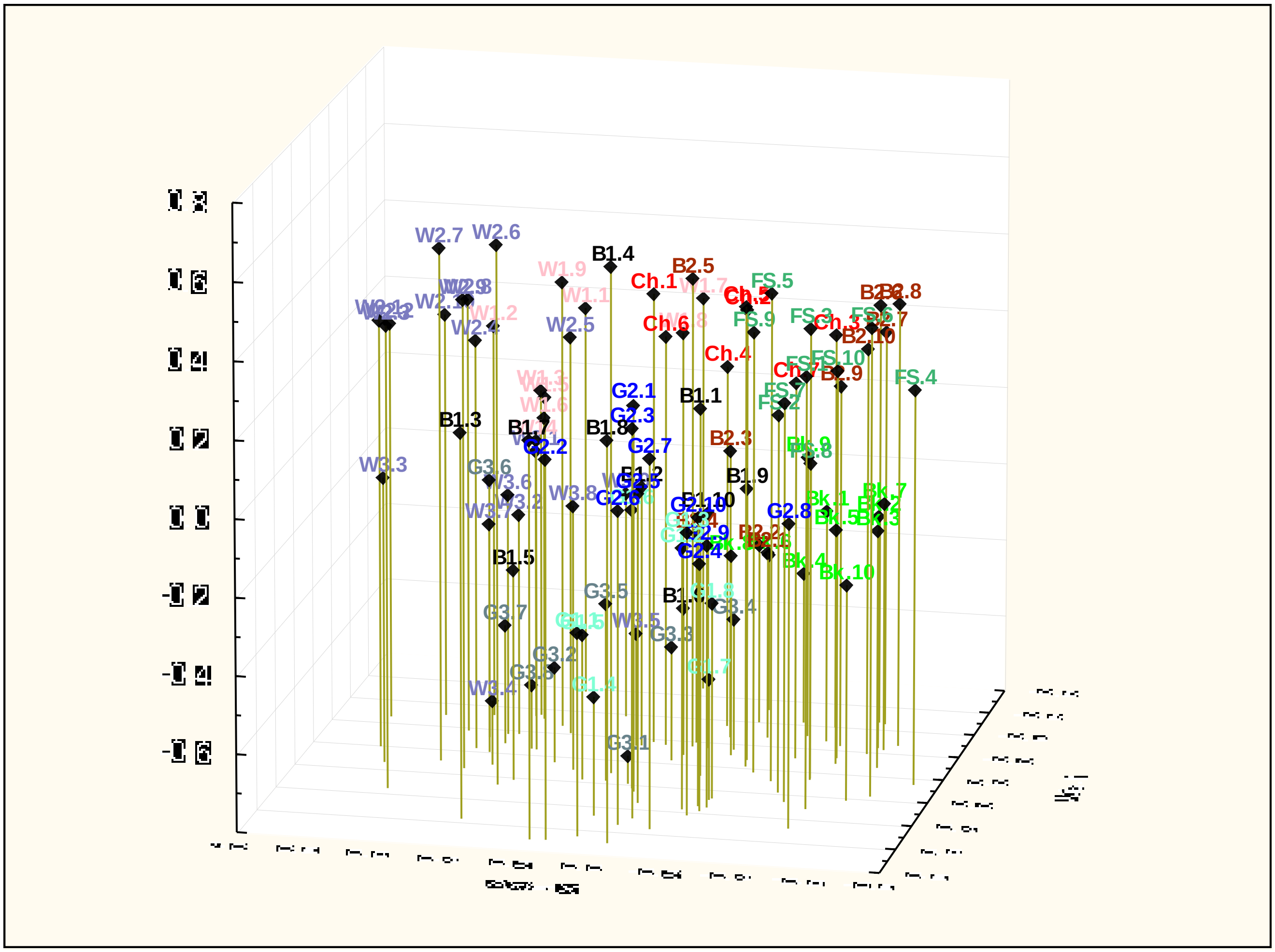


Figure 29**.**Three dimensional representation of principal coordinate analysis of genetic relationships among 102 individuals

Key: B1-Basketo 1, B2-Basketo 2, Fs-Finote Selam, Ch-Chagne, Bk- Bikolo Abay, G1-Gelila 1, G2- Gelila 2, G3- Gelila 3, W1- Wubamer 1, W2- Wubamer 2, W3- Wubamer 3

6. Discussion

6.1. Micropropagation of *A. corrorima*

6.1.1. Seed germination

Successful surface sterilization is one of the most critical steps of in vitro culture.The present result showed that the seeds of *A. corrorima* have revealed different asepsis response to different concentrations of Clorox for 30 minutesexposure (Table 3). Clorox (25%) for 30 minutes proved to be the best sterilizing treatment in comparison to 10%, 50% and 100% Clorox. It secured maximum number of aseptic cultures and the maximum rate of germination (70%). Treatment of korarima seeds using 10% Clorox for 30 minutes resulted in 20% aseptic cultures. Though, an increased dose of Clorox reduced the rate of contamination from 50 -100 % but it caused death of seeds and resulted in decline of seed germination. Clorox 50% treatment showed only 10 % contamination and 10% germination of seeds and in fact 80% of them fail to germinate. Therefore, it seems that the optimum concentration of Clorox for korarima which showed the maximum germination rate and the minimum contamination is 25%. Similar findings were reported on surface sterilization using Clorox in *Echinops kebericho* (Begashaw Manahlie and Tileye Feyissa, 2014) and*Cordeauxia edulis* (Chanie Derso and Tileye Feyissa, 2015). Our results are similar to those reported by Sharmin *et al*. (2013)in *Curcuma aromatic* by using a 0.1% mercuric chloride solution, more than 70% of explants were free of contamination after 4 weeks of culture.

6.1.2. Shoot initiation

Various literatures supported that cytokinin: auxin ratio is the deciding factor in the establishment of efficient reproducible protocol. Higher cytokinin and low auxin concentration helps to multiple shoot proliferation. Cytokinins as a plant growth regulator causes shoot induction by stimulating cell division and decreasing apical dominance (Jain and Ochatt, 2010).Multiple shoot bud formation from the young shoot tip explant of *Clitoria ternatea* on 0.5 mg/l BAP with lower concentrations of NAA or IAA has also been reported by Kalamani and Gomez (2002).Culture initiation experiment on *A. corrorima* was conducted on MS medium supplemented with 0.1 mg/l NAA in combination with six levels of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l). Best result was obtained in case of 1.0 mg/l BAP with 0.1mg/l NAA for shoot number and shoot length (cm) (Table 4). Combination of BAP and NAA facilitated multiple axillary shoot initiation and elongation was reported in*Prunella vulgaris* (Rasool *et al*., 2009) and *Cucurbita pepo* (Ananthakrishnan *et al*., 2003).In this experiment 1.0 mg/l BAP + 0.1 mg/l NAA combination was the best treatment which resulted the maximum shoot number (1.40 ± 0.09)and survival of plantlets (90%) as well as the highest shoot length (2.27 ± 0.14 cm).Yohannes Seyoum and Firew Mekbib (2014) reported that the cotyledonary node explants of Yeheb (*Cordeauxia edulis*) cultured on MS medium supplemented with 2.0 mg/l BAP resulted in the highest rate of shoot initiation (89 %) and the highest number of shoots per culture after nine weeks. Similar result was obtained by Adane Gebeyehu (2015) by using combination of 5 mg/l BAP and 0.5 mg/l NAA which resulted in the highest proliferation of 1.75 shoots per clump in 30 days of culture in banana (*Musa spp.)* cv. Giant Cavendish.The efficient shoot formation of korarima achieved from in vitrogerminated seedling shoot tips in this study will be useful for mass propagation.

6.1.3. Shoot multiplication

Micropropagation is an advanced technique for producing a large number of genetically uniform and pathogen free plants in limited time and space (Zobayed and Saxena, 2003). In vitroclonal propagation of species through tissue culture has been frequently based on the successful adjustment of the type and combinations of plant growth hormones (Uranbey*et al*., 2005). In vitro shoot multiplication implies multiplication from one culture to many cultures and then many cultures to further many cultures which is essentially a major criterion for successful commercial micropropagation. The use of different types and concentrations of plant growth regulators highly affected the in vitro growth and development of korarima. Once the cultures were established,in vitroshoots were further multiplied on cytokinin alone or in combination with auxin supplemented media. Highest number of shoots per explant (5.13 ± 0.64) was recorded in MS media containing 1.5 mg/l BAP in combination with 2.0 KN followed by 2.0 mg/l BAP + 0.25 mg/l NAA (3.87 ± 0.32) and 3.0 mg/l BAP + 0.5 mg/l IBA (3.83 ± 0.54). Primarily, cytokinins have a major role on plant development, such as the regulation of shoot formation and multiplication and the promotion of cell division and expansion (Mok and Mok, 2001), which is in conformity with the results of this study. The highest mean shoot length of the in vitroshoots was observed on MS basal medium containing 1.0 mg/l KN with an average length of 6.62 ± 0.17 cm although the number of shoots showed in this treatment was lower. Solomon Eyob (2009) reported 3.0 mg/l BAP + 1.0 mg/l KN combination was the best for shoot multiplication of *A. corrorima* (7.67 shoots per explant). Waman *et al*. (2016) noticed highest multiplication (4.6 shoots per explant) in medium supplemented with higher concentration of BAP and shoot multiplication increased with increase in concentration of BAP and KN in silk banana. The minimum shoot number of 1.47 ± 0.13 was obtained on MS medium containing 1.5 mg/l BAP and 0.25 mg/l IBA.Lower number of shoots with this lower concentration of BAP and IBA combination suggests that BAP and IBA combination has the capacity to induce more shoots with increased concentrations.

The maximum and minimum average shoot numbers of 3.00 ± 0.22 and 1.79 ± 0.15 were obtained on medium containing 1.5 and 3.0 mg/l BAP respectively.The effect of BAP on shoot formation had been reported by Purohit *et al*. (1995) for medicinal and aromatic plant species C*hlorophytum borivilianum.*The average shoot number on media containing KN alone ranged from 1.77 ± 0.16 to 3.03 ± 0.77. Ashraf *et al*. (2014) reported maximum number of shoots (8.48) in *Chlorophytum borivilianum* on MS media containing KN alone.MS medium supplemented with 3.0 mg/l KN has already been reported to induce the highest multiple shoot formation in *Hypericum perforatum* (Sood *et al*., 2015). About 1.83 ± 0.27 to 5.13 ± 0.64shoots with 2.42 ± 0.16 to 5.63 ± 0.26 cm were produced on media containing BAP combined with KN.Rahiel Hagos and Hailay Gebremdhin (2015) showed 6.0 mg/l of BA alone and the combination of 1.5 mg/l of KN with 3.0 mg/l of BA was the best medium for shoot multiplication in *A. corrorima* which had a good response to regenerate the largest number of shoots with an average number of 10.33 and 9.67 shoots per explant, respectively. BAP combined with KN showed a synergistic effect producing high rate of shoot multiplication and elongation in *Bambusa glaucescens*

(Shirin and Rana, 2007).In this study, it can be concluded that BAP enhanced shoot number whereas KN promoted shoot elongation, but when in combination they worked synergistically to produce optimal shoot multiplication and elongation of *A. corrorima.*

BAP and KN were supplemented with NAA and IBA to study the effect of cytokinin/auxin interaction on axillary bud induction. MS media containing combination of BAP with NAA showed mean shoot number of 1.67 ± 0.15 to 3.87 ± 0.32 and length of 2.00 ± 0.13 cm to 5.04 ± 0.26 cm. Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation. Some species may require a low concentration of auxin in combination with high levels of cytokinins to increase shoot proliferation (Kaviani, 2014; Baker *et al.* 2014).The mean shoot number and length obtained from media fortified with BAP and IBA combinationranged from1.47 ± 0.13 to 3.84 ± 0.54 and 2.03 ± 0.20 to 5.24 ± 0.26 respectively. Average shoot number of 1.87 ± 0.23/22 to 3.23 ± 0.38 and length of 2.03 ± 0.20 cm to 5.24 ± 0.26 cm were obtained on media containing combination of KN and NAA. Medium containing KN and IBAachieved1.5 ± 0.15 to 2.58 ± 0.22 mean number ofshoots with 3.25 ± 0.20 to 5.75 ± 0.17 cmlengths.Sahooand Rout (2014) also reported that a combination of BAP and NAA enhanced multiple shoot proliferation from shoot tip explants of *A. barbadensis*. The MS medium containing BAP and NAA was the best medium for aloe micropropagation (Wenping *et al*., 2004).

6.1.4. Rooting and acclimatization

All auxins induced rooting, the number of roots and the length of roots however varied with the auxins tested and their concentrations (Table 11). Seven levels of each of auxins (IAA, IBA, and NAA) were used in half strength MS medium for root induction of *A. corrorima*. Among the three different auxins evaluated, maximum response (92.36%) with highest root number (8.71) and length (1.50 cm) were obtained on half strength MS medium augmented with 1.5 mg/l IBA. Similar result was obtained by Yesmin *et al*. (2015) by using 0.2 mg/l IBA. As compared to IAA, IBA and NAA induced higher rooting. In korarima, Solomon Eyob (2009)achieved 75.56% rootingby transferring an individual shoot to MS medium containing 1.0 mg/l IBA which was the bestto induce rooting from in vitroshoots of korarima. Rooting response using IBA was also reported in several plants like *Celastrus paniculatus* (Rao and Purohit, 2006) and *Anogeissus sericea* (Yusuf, 2005). The mineral concentration in the culture medium affects rooting characteristic and some researchers have proposed that reduction of salt strength to half strength improved rooting (Theriou and Economou, 1993). The reason behind increasing rooting rate on half strength culture medium might be due to a disorder in carbohydrate to nitrogen in nutrient medium, which lead to decreasing nitrogen level in shoot and then improving rooting rate, initiation roots, increasing root number and lengths (Fotopoulos and Sotiropoulos, 2005).After sufficient development of roots, plantlets were successfully transplanted into small plastic pots containing soil, sand and compost (Figure 12). After one month acclimatization of plants in the greenhouse 90.83% were survived. Wondyifraw Tefera and Wannakrairoj (2004) reported similar acclimatization result

(93%survival). The results showed that the better rooting response and the soil mixture preparedmight have contributed to the high survival rates of the plantlets.

6.2. Effect of different factors on the micropropagation of korarima

6.2.1. Effect of different concentrations of carbon source

Sucrose is the most widely used carbon source in most plant species, as it is the main sugar translocated in the phloem (Konat´e *et al*., 2013).Carbohydrates serve as an energy source in the tissue culture medium and act to supplement osmotic pressure in the culture of plant cells (Huh *et al*., 2016). Sucrose is the main source of carbon energy for in vitrocultures. Plant cells and tissues in a culture medium lack autotrophic ability and therefore, need external carbon for energy (Razdan, 1993). The addition of an external carbon source to the medium enhances the proliferation of cells and regeneration of green shoots (Karim*et al*., 2007). In this study the effect of different concentrations of sucrose, 1%, 2% and 3% (w/v) and 3% table sugar on in vitro *A. corrorima* growth was investigated and presented in Table 12a and 12b. The maximum average shoot number (6.67±0.55) was obtained in media containing 3% sucrose and 3% common table sugar induces the second maximum shoot number (4.10±0.44). Increasing the sugar concentration from 1% to 3% has a visible effect in increasing shoot number per explant.

The positive effects of sucrose on growth of explants under in vitrocondition is linked with its high solubility in water, its electrical neutrality andits lack of inhibitory effect on the majority of biochemical processes (Gauchan, 2012).Koné*etal*. (2015) reported that above the concentration of 3% sucrose, no significant difference was observed. From these results, a 3% sucrose concentration in the basal medium seems to be sufficient for normal plant growth. Kim *et al*. (2016) reported that highest shoot multiplication and elongation were obtained in media supplemented with 4% sucrose.However, high sucrose concentrations may limit growth, due to an increase in the osmotic potential in the medium caused by sucrose (Rejšková *et al*., 2007).

With the different concentrations of carbon sources tested, a non significant difference was recorded for the shoot length of korarima. Among the different carbon sources used in this experiment, the highest plant height (2.95±0.13 cm) was observed on medium containing 3% table sugar followed by 2.81±0.09 on media containing 3% sucrose. The most common carbohydrate used in tissue culture work is sucrose, at a concentration of 3% as recommended by Murashige and Skoog (1962).Generally, sucrose is the carbohydrate used most of the time in the culture medium of tissues cultivated in vitro(Sul and Korban, 2004), because of the facilitated absorption of sucrose through the cellular membrane (Borkowska and Szezebra, 1991). Ilczuk *et al*. (2013) reported that shoots cultured on medium without carbon source did not produce roots, indicating the importance of sugar in root formation.

6.2.2. Effect of different salt strength

The types of basal media affected all shoot, leaf and root numbers, as well as shoot and root lengths of korarima. The results showed that full strength MS medium resulted in the highest number of shoots per explant, mean length of shoots and the values were (5.27 ± 0.53 shoot/explant and 3.08 ± 0.20 cm) respectively. Wondyifraw Tefera and

Wannakrairoj (2004) showed in korarima that the highest average number of shoots (6.2 shoots per explant), leaf number (13.5) and shoot length (5.37 cm) in full strength MS medium. They also reported that the maximum root number (7.3) in half strength MS and root length (4.19 cm) in half nitrogen MS medium. Martins *et al*. (2015) reported that the explants cultivated in liquid and solid medium withoutMS-mineraldid not display shoot formation.

The medium having one-third MS mineral produced the second highest for number of shoots per explant (4.13±0.42). The medium containing half strength MS salt showed the lowest number of shoots per explant and mean length of shoots and the values were (2.30 ± 0.22 shoot/explant and 1.27 ± 0.06 cm).These results confirmed that some plant species have enough levels of endogenous hormones and do not require a high level of exogenous growth regulators for growth (Hussey, 1982). Mineral components in the growth medium are vitally important for the in vitroregeneration process in plants (Williams, 1993). Some mineral compounds are related to endogenous cytokinin biosynthesis. An increase in nitrate resources may induce the expression of genes responsible for the biosynthesis of cytokinins, resulting in accumulation of these hormones in plants (Takei *et al*., 2004; Wang *et al*., 2004). Cytokinins are primarily responsible for breaking apical dominance and consequent lateral shoot induction.Cytokinins promote cell division and lateral bud development, while auxins inhibit it. The breaking apical dominance is fundamental in the first cell division (Pasternak *et al*., 2000).

6.2.3. Effect of agar

In this study we examined whether the agar in the growth medium plays fundamental roles in shoot multiplication and rooting of *A. corrorima*. The highest amount of multiplication and root initiation were obtained in the liquid medium. Explants cultivated on liquid medium had higher shoot number per explant (4.41± 0.65) and longer average shoot length (5.86 ± 0.27). The solid mediums produced mean shoot number per explant and shoot length of 3.00 ± 0.25 and 3.60 ± 0.23 cm respectively. Liquid MS medium showed best rooting response with average root number and root length of 18.50 ± 1.15 and 4.03 ± 0.15 cm respectively. In solid medium, the highest mean root number and length were 7.60 ± 0.56 and 1.75 ± 0.17 cm respectively.Using liquid media in micropropagation processes is considered to be the ideal solution for reducing plantlet production costs and for considering automation (Debergh, 1983).Higher shoot number and length obtained in the liquid medium due to the fact that they could uptake and accumulate nutrients and plant growth regulators (PGRs) from liquid media easier than from the solid media. By using liquid medium, it may be possible to reduce costs to a level lower than solid medium and liquid medium is better than solid medium in growth.

The use of agar may interfere with shooting and rooting response in some plant species. It may reduce the formation of lateral shoots (Ivanova and Staden, 2011). The employment of a gelling agent in the medium may decrease water availability, mineral salts and plant growth regulators (Debergh, 1983) and may also decrease endogenous cytokinin (Ivanova *et al*., 2006). Mengarda *et al*. (2009) worked with different species of plants and obtained higher multiplication rates with stationary liquid medium relative to medium solidified with agar.The employment of a gelling agent is important for some plant species, and it may assist with plant formation without physiological disturbances such as hyperhydricity (Ivanova and Staden, 2011). Hyperhydricity during plant formation on liquid medium or on medium with low agar concentrations has already been noted in some *in vitro* plant species (Casanova *et al*., 2008).

6.2.4. Rooting and acclimatization

Rooting is an important process to the success of micropropagation. Without effective rootsystem, plant acclimatization will be difficult and the rate of plant propagation may be severely affected (Goncalves *et al*., 1998). Concentrations of auxins, culture conditions and medium strength are various factors supposed to influence in vitrorooting. Our results showed that ½ MS medium supplemented with IAA (4.0 mg/l) induced the highest number of roots (18.50±1.15 and 7.60 ± 0.56) on liquid and solid medium respectively (Table 15). Plants cultured on the low cost liquid medium developed a significantly high number of roots compared to those cultured on the solid medium. Liquid medium seems to be more effective for shoot regeneration and root induction, which is due to better aeration (Hung *et al*., 2006).Wated *et al*. (1997) compared performance of agar-solidified medium and interfacial membrane rafts floating on liquid medium for shoot multiplication and root induction. The results showed shoot multiplication was highest on membrane rafts floating on the liquid medium, and also plants rooted much better.In vitrorooted plantlets were carefully removed from culture vessels and washed under running tap water to remove agar and media remaining without damaging the roots. These rooted plantlets were transferred to soil, compost and sand mixture in pots and covered with polyethylene bags and placed in the greenhouse for acclimatization to outer environmental condition and 86% and 78% survival was obtained after 4 weeks from the liquid and solid media respectively (Figure 17).The total plant height and leaf size in plantlets derived from liquid media is higher than those derived from solid medium; this is the possible reason why plantlets derived from liquid media showed a relatively lower survival rate due to high rate of transpiration.

6.3. In vitro regeneration of korarima

6.3.1. Callus induction

The callogenic response from rhizome explants was observed at different plant growth regulators concentration either singly or in combination (Tables 16; 17 and 18). Callogenic response started from injuries of the plant tissue. Plant growth regulator (PGR) free basal MS medium did not show induction of callus and explants died after few days. Callus induction, somatic embryogenesis and plant regeneration were initiated in *A. corrorima* using rhizomes cultured on MS media. The highest frequencies of callus induction (80%) was obtained using the MS medium supplemented with 0.5 mg/l 2,4-D alone and 5.0 mg/l 2,4-D in combination with 0.25 BAP. BAP (0.25 mg/l) in combination with 2,4-D (0.5 mg/l)showed the lowest callus induction frequency ($30 \pm 5.77\%$). Significant difference for callogenesis under the same nutritional condition indicated that the callus induction quality is genotype dependent. The strong influence of genotypes on callus induction and plant regeneration was also observed in different plants (Duangsee and Bunnag, 2014; Mahajan and Sharma, 2015)**.**Nin *et al*. (1996) reported no callogenic response from leaf explant on PGR-free medium of *Artimesia annua*. 2, 4-D alone and in combination with other PGRs produced white, white yellow, soft and friable callus. But in most combinations of PGRs embryogenic callus was observed. Nin *et al*. (1996) reported best callogenic response with BAP and NAA in the medium for *A. absinthium.*

In this study, the maximum amount of white and white yellow friable callus was obtained from rhizome explants cultured on MS media containing different concentrations of 2, 4-D alone and in combination with BAP, NAA and KN. According to previous reports, 2, 4-D in combination with BAP proved to be effective plant growth regulators for callus induction in many medicinal plant species (Anjali *et al*., 2000; Chen *et al*., 2000; Manickam *et al*., 2000). Successful callus induction resulted from the combined interaction of plant genotype, plant physiological condition, explant source as well as the nutritional and regulatory conditions provided by the specific medium (Vinod *et al*., 2004).The combination of auxins and kinetin ratio plays a key role in differentiation and organogenesis in plants (Kumar *et al*., 2011). It seems that the ratio is critical in inducing mitotic activity in the explants leading to callus formation. Trivedi *et al*. (2015) reported that young leaves of *Withania somnifera*formed friable callus which was soft, oval and white on MS media with NAA (2mg/l) and kinetin (0.5mg/l).

In this study an increase in concentration of 2,4-D showed decline in the percentage of callus formation. Reports indicate that callus induction was found to increase with increase in the concentration of 2,4-D (Zang *et al*., 2016; Tahir *et al*., 2011).In *Barringtonia racemosa*leaf explantsincreasing the concentration of 2,4-D till 2.0 mg L^{-1} had reduced percentage of callus inductionand gave compact and brownish callus (Dalila*et al*., 2013).An explanation for the considerably lower growth at higher auxin concentrations with increasing culture time could be growth inhibition caused by the enrichment of 2,4-D in the tissues, as was shown in *Arabidopsis* (Meijer *et al*., 1999).

6.3.2. Regeneration

The cytokinin and auxin type and concentration greatly influenced axillary shoot regeneration from nodal explants. Medium without growth regulator (control) gave no regeneration response; the explants swelled and became necrotic two weeks after culture. MS media with 0.5 mg/l BAP plus 0.25 IAA treatments yielded maximum shoot regeneration percentage (25.33±1.99 %) and maximum number of multiple shoots per callus (2.93±0.23). At four weeks, shoots developed in this medium also were longest (0.99±0.06cm). Verma *et al.* (2011) obtained 95% of shoot regeneration in MS media supplemented with a combination with BAP and GA3 in cotyledonary node explants of rice (*Oryza sativa* L.). No shoot regeneration was observed in 37.5% of the treatments used in this study. Rajakumar (2013) reported that plant regeneration from seed derived callus of rice is not influenced by physiological factors but also seems to be genetically controlled.

6.3.3. Shoot multiplication

Shoot cultures were multiplied by repeatedly subculturing the original shoot tip explants on shoot multiplication medium (MS supplemented with BAP and KN combination) after every 4 weeks of culture. In vitroshoots responded differently on various concentration and combination of plant growth regulators in medium (Table 20). Addition of 3.0 mg/l BAP with 0.25 mg/l KNon MS media showed the the highest shoot number (4.90 ± 0.53). Medium supplemented with 2.0 mg/l BAP + 0.25mg/l KN generated the second highest shoot number of 4.86 ± 0.50. In this experiment the lowestmean shoot number (1.97 ± 0.16) and the highest mean shoot length (4.30 ± 0.37) were recorded on growth regulators free medium.Solomon Eyob (2009) reported that the maximum mean number of shoots per explant (11) was obtained when grown on medium containing 0.5 mg/l TDZ.Rahiel Hagos and Hailay Gebremdhin (2015) indicated PGR-free medium had the longest mean shoot length (3.11 cm) followed by 0.5 mg/l of KN alone (3.10 cm). Shoot length varied amongst the treatments studied and treatments which stimulated multiple shoot formation hindered shoot elongation. As stated by Waman *et al*. (2016) shoot length in silk banana was found to be highest in medium supplemented with low dose of KN (8.69 cm).Solomon Eyob(2009) in korarima showed that supplementation of 0.5 mg/l TDZ with 3 mg/l BA also increased shoots number (10) perexplant but the shoots were short. The longest shoots were obtained in the control (3.73 cm), and followed by 3 mg/l BA (3.07 cm).Wondyifraw Tefera and Wannakrairoj (2004) obtained best result with respect toshoot proliferation (16.6 shoots/ explant) from the combined use of 2 mg/l IMA (imazalil) and 0.5 mg/l TDZ in korarima.They obtained highest multiplication of shoots (7.32 shoots/explant) from the use of 4.0 mg /l IMA in combinationwith 0.1 mg/l BA. As stated by Sharmin *et al*. (2013), in *Curcuma aromatic*the maximum number of microshoots (an average of 13 shoots per explant) was obtained in MS medium supplemented with 1.0 mg/L BAP. The differential response of plantsin PGR response and shoot induction efficiency is probably due to genotype, as the plant material was obtained from diverse geographical locations (Sharmin *et al*., 2013).

6.3.4. Rooting and acclimatization

Rooting is an important prerequisite especially from shoots of in vitroorigin and there by a successful protocol needs to be developed for high root frequency. Auxin concentration and the type of auxins also played a significant role for in vitroroot induction and development of root length. The in vitro regenerated shoots during shoot proliferation were transferred to half MS rooting media supplemented with different levels of IBA and NAA combination.Half-strength MS medium with 0.5 mg/l IBA + 0.5 mg/lNAA produced the highest percentage of rooting (96.67±1.86 %)(Table 21). Plant growth regulator free MS media showed the least percentage of root induction (55.56±5.73%).The effectiveness of IBA with NAA in rooting of invitroregenerated shoots has been well documented in *Gymnema sylvestra* (Komalavalli and Rao, 2000).In vitrogenerated plantlets have weak stomatal movement. They are dependent on complete media provided to them; hence they have weak photosynthetic regulations. They can get infected by microorganisms (Aitken, 1995). As such, they were grown up in controlled environment with suitable temperature range, humidity and light exposure and ultimately they need to be hardened to achieve high rate of survival. In primary hardening, well rooted in vitrogrown plantlets were transferred to greenhouse with suitable potting mixture containing field soil, compost and sand in 2:1:1 ratio respectively and survival rate was 89% when plantlets were ready for field trial.This successful acclimatization results depend on the reduction of light intensity, temperature and gradual reduction humidity.

6.5. Genetic diversity of korarima

6.5.1.Chromosome number of A. corrorima

In this experiment mitotic study was carried out in root tip cells of korarima. The root tips of *A. corrorima* showed the somatic chromosome number of 2n= 48. Our results are similar to those reported for this species (2n=2x=48) by Wannakrairoj and Wondyifraw Tefera (2013). The diploid chromosome number of *Elettaria cardamomum* which is the very close relative of *A. corrorima* was found to be 2n = 48 (Anjali *et al*., 2016). The lack of information on chromosome morphology of the species could be attributed to the small chromosome size. Although Zingiberaceae includes a large number of economically and medicinally important plants, karyomorphological studies on these plants is limited. This may be due to the small size of chromosomes and their large number, which hamper their detailed karyotype study (Islam, 2004). Existing reports on chromosome numbers indicate both intra–and interspecific chromosomal variation, that might have contributed to speciation in these predominantly vegetatively propagating plants. Coupled with this, the presence of polyploids and aneuploids and instances of naturalization of hybrids in the wild, have resulted in a huge variation in the basic chromosome number in most of the genera (Stace, 2000).

6.5.2. Diversity within and among korarima populations

Assessment of genetic diversity is crucial for any crop improvement program and for the conservation of plant genetic resources in their natural habitats (Choudhary *et al*., 2013). In the present study, the extent of genetic variability and relatedness among the accessions of *A. corrorima* collected from the Southern and Northwestern parts of Ethiopia were determined by using ISSR markers. ISSR markers have been successfully utilized for assessing the genetic diversity and revealed a remarkable molecular discrimination between the 11 korarima populations under study. Our analysis of the genetic polymorphism obtained with ISSR markers demonstrated that the highest percentage of polymorphic loci (80.72%), largest number of polymorphic loci (67) and the highest gene diversity (0.3418) and Shannon‟s information index (0.4918) were found in population from Basketo II. The second highest values were observed for populations from Basketo I. Therefore, the sampled individuals from Basketo wereda could be considered to possess a higher genetic variation as compared to the other populations indicating that the populations were subjected to genetic isolation. Similar results were also reported in many other medicinal plant species such as *Torreya jackii* (Li and Jin, 2007) and *Rheum palmatuma* (Wang *et al*., 2012). The higher variability of Shannon index for korarima accessions may be influenced by different cultivation locations where the samples were collected and due to the dynamic agrogeographical conditions.

High number of alleles and high polymorphism are very important for correct estimation of genetic diversity of a germplasm. The degree of polymorphism showed the extent of diversity and effectiveness of the markers (Pfeifer *et al.,* 2011) and consequently, polymorphic information is related to expected heterozyosity and is usually determined from allele frequency. In the present study, high genetic variation was observed among different accessions of korarima using ISSR markers (for example, Shannon‟s information index (Table 24) ranged from 0.37 to 0.49. Shanon‟s information index (0.23

to 0.54) of high genetic variation was reported in Indian cardamom using ISSR analysis (Anjali *et al*., 2016). The higher genetic differentiation of population within a species is driven by various factors such as genetic isolation or genetic drift, natural selection, pollination and breeding system and geographic distribution range (Hogbin and Peakall, 1999; Schall *et al*., 1998). The geographic distribution and topographical barriers can lead to difficulties in seed dispersal resulting in limited gene flow among populations (Hamrick and Godt, 1996). A large amount of variability was observed in these sampled populations, exhibiting high intraspecific genetic diversity. Our results are in agreement with the findings of Wang and Zhang, (2013), through the study conducted on the genetic diversity of castor bean (*Ricinus communis* L.) based on ISSR markers.

The analysis of molecular variance (AMOVA) indicated that the majority of genetic variation occurred at the population level. The genetic differentiation occurred within and among populations was 76% and 24% respectively (Table 26). Based on AMOVA analysis, genetic variation within *A. corrorima* population was higher than among populations. Although korarima is mainly self-pollinated, cross-pollination by insects is possible due to the presence of large nectaries at the top of the ovaries (Jansen, 2002). Some degree of outcrossing could explain the high genetic diversity observed in the studied korarima accessions. Moreover, the presence of long distance marketing of korarima within and among different regions by farmers and local traders have their own effect on the observed variation of cultivated korarima.The high value of within-population genetic variation in *A.corrorima* is in accordance with other studies in different plant species (Pereira *et al.*, 2015; Jena *et al*., 2015).

Genetic diversity within a species is shaped over long periods of time through evolutionary genetic processes acting in combination on a species (Rajora and Mosseler, 2001). Korarima is widely distributed in the different eco-climatic zones of Ethiopia, which is suggestive of a broad genetic base (Pant *et al*., 2012; Haji *et al*., 2014). Reduction and fragmentation in forest plants due to over-exploitation in the forest cover could be one of the main causes that led to an increase in genetic differentiation and reduced gene flow between populations.

Although the present study indicated the presence of a relatively high genetic diversity among landraces of korarima from various geographical locations of Ethiopia, some genotypes situated geographically far apart were grouped together in the same cluster. These results are in agreement with earlier studies which showed that geographical separation did not generally result in greater genetic distance (Wei *et al*., 2008). This could be a consequence of exchange of genetic materials among the neighboring farmers as well as traders in the region. The human factor has been previously shown to be responsible for the lack of correlation between genetic and geographical distance (Stankiewicz *et al*., 2001). The increasing demand for korarima by exporters in Ethiopia, the movement of traders and exchange of germplasm across different regions could be a possible explanation for the spreading of korarima seeds.

The higher proportion of polymorphism in the present study could suggest the extent of geneticdiversity among the various korarima germplasm of Ethiopia. The diversity could mainly be attributed to diverse agro-climatic conditions in the country. Accessions from different regionswere sometimes closely related and accessions from the same region had different genetic background. Even so, the intraregional diversity could be as a valuable source as interregionaldiversity for korarima improvement (Benson *et al*., 2013). Since areas of high genetic diversity contribute more accessions than those with a low diversity for further and future collection, breeding and conservation activities high priority should be given to areas with high genetic diversity.

6.5.3. Clustering analysis and relationships of korarima populations

Genetic similarity between collected populations is found to be between 0.7013 and 0.8871 (Table 26). This indicates that there is high genetic variability among populations, which can be advantageous in programs of genetic improvement. The highest genetic distance (0.3548) was observed between Bikolo Abay and Wubamer II populations which might be attributed to having long geographical distance. However, this could also be due to the exchange of plant materials across the regions during the korarima cultivation and long distance seed transport for marketing. Therefore, their difference in cluster could imply their being originated from different sources, while grouping in the same cluster would mean genetic affinity among individuals in the same group. The genetic distances between populations is a valuable parameter to conserve and use a given germplasm in breeding activities. It was proved that crosses between unrelated parents show stronger hybrid than crosses between closely related genotypes (Solomon *et al*., 2007). The majority of the landraces from similar geographical regions were grouped together in the same clusters with the exception of Gel III, which was found to be an out-group from any of the population and stand alone (Figure 25).

On the basis of UPGMA and NJ, most individuals of the respective population were observed to form moderately clustered groups with few intermixing from the other population (Figure 26). Korarima individuals assembled from different localities and regions form strict grouping based on their geographic origin. The two-dimensional plot generated from PCoA also supported the clustering pattern of the UPGMA dendrogram (Figure 27) with some minor disagreements. This reflected a higher genetic diversity in the studied collection, which was confirmed by a principle component analysis. Results of this analysis showed a wider genetic distribution of genotypes in the studied collection. In the three-dimensional PCoA plot, generally, similar groupings with the UPGMA dendrogram and additional information were also revealed. The first three principal axis accounted for the total variation indicating the complex multidimensional nature of ISSR variation (Figure 28).

In the present study, PCO results based on two and three coordinates showed similar results except some population dispersed overall the 2D and 3D. Few groups from the PCO results have shown recovered similar groupings with that of the UPGMA and NJ results. The fact that they are grouped in the same cluster implies their close relationship in genetic characters. Therefore, their differences in cluster could imply they originated from different sources. A dendrogram based on the Jaccard"s similarity index showed that a distribution pattern of variability between species was related to geographical origin. However, Basketo II populations are intermixed with the northwest populations which suggested that Basketo II population has the highest genetic similarity with the

Northwestern populations. The northwestern areas are not major producers of korarima, seeds from Basketo II population area might be transported by people to Gojam area and this may have its own impact on the clustering of Basketo II population with those populations from Gojam area.

The results of the PCoA corresponded largely to the results obtained through cluster analysis. Both PCoA and UPGMA cluster analysis confirmed the clustering of all 11 populations into three groups, corresponding to the geographic distribution patterns of the populations. The genetic variation data would be very useful for improvement of the korarima through conventional breeding programs as well as molecular breeding approaches such as marker assisted selection. Our results indicate that ISSR markers are very informative and cost-effective in determining genetic diversity among diverse accessions of korarima germplasm growing in Ethiopia. This evidence is congruent with previous reports on the usefulness of ISSR markers in differentiating rice genotypes (Joshi *et al*., 2000; Sarla *et al*., 2005).

7. Conclusion

The present result showed that the sodium hypochlorite concentrations used for the surface sterilization of *A. corrorima* seeds for 30 minutes were 10%, 25%, 50% and 100% and the percentage which yielded the most germinated seeds was that of 25% followed by 10% and 50% had the minimal germination.Combination of different levels of BAP (0.5 – 3.0 mg/l) and 0.1 mg/l NAA were used for culture initiation and the highest percentage of shoot survival (90%) was obtained by 1.0 mg/l BAP + 0.1 mg/l

NAA. Optimum culture conditions for shoot proliferationon MS medium containing different concentrations BAP and KN alone, combination of BAP and KN with each other and in combination with NAA and IBA were used. Plant growth regulators significantly ($P < 0.05$) affected the mean shoot number, shoot length and leaf number per explant. The highest mean shoot number, shoot length and leaf number per explant were 5.13 ± 0.64, 6.62 ± 0.17 and 9.73 ± 0.69 and obtained from the the medium containing 1.5 mg/l BAP + 2.0 KN, 1.5 mg/l BAP + 2.5 mg/l KN and 1.0 mg/l KN alone. Based on our findings, combination of BAP and KN showed superior performance for shoot multiplicaiton of *A. corrorima*.

Observations indicated that the multiplication of shoots of *A.corrorima* plantlets in vitroare affected by the type of exogenous carbon source added to the medium. The laboratory grade sucrose showed higher effect to support the growth of korarima plantlets under in vitro condition at a level of 30g/l. Common table sugar was able to support the second highest growth of korarima plantletsat the same concentration with laboratory sucrose. Sucrose is the prime importance for cell growth but significant cost incurred by analytical sucrose makes the technique expensive for commercial micropropagation. However, the costs of media can be brought down by using locally available and cheap common table sugar.

Our investigation also showed that liquid medium was better than solid medium for in vitro proliferation of *A. corrorima.*The mean shoot and root number and length were significantly higher in liquid medium due to the fact that they could uptake and accumulate nutrients and plant growth regulators (PGRs) from liquid media easier than from the solid media. Decreasing agar concentration increased mineral availability and growth. Growth is related to soluble mineral uptake. Our results revealed that MS medium, which is always used for conventional in vitro solid media, is effective. Also, when it is used as a liquid medium without agar for the formation of shoots in vitro, (hydroponic system) where an inorganic nutrient solution is usually used. The use of liquid MS medium instead of agar solidified medium reduces the cost of culturing.

Korarima shoot tip explants were cultured on MS salt at concentration of full strength, half strength and one-third strength. The highest shoot number per explant (5.27 ± 0.53) was observed on the full strength MS medium containing 1.5 mg/l BAP in combination with 0.5 mg/l TDZ. BAP (2.5 mg/l) in combination with TDZ (0.5 mg/l) produced the maximum shoot number in half-MS (3.37±0.43) and one-third MS media (4.13 ± 0.42).

The results of this study also showed callus induction and shoot regeneration from the rhizome segments of *A. corrorima* at various degrees. MS medium supplemented with 0.5 mg/l 2,4-D and 5.0 mg/l 2,4-D in combination with 0.25 BAP were found to be the best for callus induction frequency (80.00±5.77 %), whereas 0.5 mg/l 2,4-D in combination with 0.25 BAP showed the minimum callus induction frequency (30.00±5.77 %). The plantlet regeneration frequency in this experiment ranged from 0% to 25%.Generally, development of in vitro propagation protocol for *A. corrorima* provides the possibility to conserve germplasm and used for mass propagation and genetic improvement of this economically important spices and indigenous medicinal plant.

The present study showed that this plant has a somatic chromosome number of 2n = 48. This chromosome study will be helpful to confirm the chromosome number of the species and also beneficial for further research in cytogenetics. Since there are persistent problems in cytological analysis owing to the small size and large number of chromosomes, chromosome number reports without karyomorphological analysis might lead to uncertainty regarding the true evolutionary nature of the chromosomes of korarima.

The ISSR markers used were polymorphic and able to reveal the diversity among accessions of korarima from southern and Northwestern Ethiopia, which offer valuableinformation for conservation and management of genetic resources, utilizing them in breeding programs and analyzing the evolutionary and historical development of cultivars. Also, grouping of the korarima landraces was in concordance with their geographical distribution areas. The genotypes originating from the same region, often located in the same group. Finally, our study established that the ISSR marker could be used as an efficient tool for studying genetic diversity among korarima accessions collected from diverse geographical locations, but different molecular marker systems need to be combined for an accurate and comprehensive assessment of genetic diversity.

8. Recommendations

Based on the results of the present study, the following recommendations are made:

- Micropropagation technique using 1.5 mg/l BAP in combination with 0.1 TDZat 3% sucrose concentration should be applied to solve the problem associated with the propagation of the crop.
- Further investigations are required to test the efficiency of survival rate of plantlets in the field condition
- Agronomic performance of korarima derived from tissue culture regenerated plantlets should be tested.
- The developed in vitro regeneration protocol can be used for genetic transformation of this plant
- Study of karyotype, meiosis, chromosome banding and molecular cytogenetic of korarima growing in different regions are required.
- For better understanding of the genetic diversity and phylogeny of *A. corrorima* future studies should focus on a larger number of populations and accessions collected from more geographical regions.
- Further molecular analysis with other molecular marker system like AFLP needs to be conducted to better understand and estimate the gene flow, and determine the size of a population and levels of inbreeding.
- To reduce genetic erosion and diversity loss in this species, germplasm storage and conservation using seed bank is required.

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Appendices

Appendix 1. The interaction effect of different sugar levels with BAP and/or TDZ on multiplication of korarima

Descriptives[a]

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Shootnumber	1.00	30	1.6667	.56466	.11526	1.4282	1.9051	1.00	3.00
	2.00	30	2.6333	1.92055	.35064	1.9162	3.3505	1.00	10.00
	3.00	30	2.2667	1.01483	.18528	1.8877	2.6456	1.00	5.00
	4.00	30	3.0667	1.31131	.23941	2.5770	3.5563	1.00	6.00
	5.00	30	2.7333	.90719	.16563	2.3946	3.0721	1.00	4.00
	6.00	30	2.9667	.99943	.18247	2.5935	3.3399	1.00	5.00
	7.00	30	3.2083	1.47381	.30084	2.5860	3.8307	1.00	7.00
	8.00	30	2.7917	1.25036	.25523	2.2637	3.3196	1.00	5.00
	9.00	30	3.2917	1.48848	.30384	2.6631	3.9202	2.00	6.00
	10.00	30	3.3750	1.37722	.28112	2.7935	3.9565	1.00	6.00
	11.00	30	2.9167	1.17646	.24014	2.4199	3.4134	1.00	5.00
	12.00	30	3.0000	1.38962	.25371	2.4811	3.5189	1.00	7.00
	13.00	30	4.7917	1.93321	.39462	3.9753	5.6080	1.00	9.00
	14.00	30	3.4000	1.47625	.26952	2.8488	3.9512	1.00	7.00
	15.00	30	6.6667	2.98656	.54527	5.5515	7.7819	2.00	16.00
	16.00	30	4.9583	2.82041	.57571	3.7674	6.1493	1.00	12.00
	17.00	30	4.8000	2.97576	.54330	3.6888	5.9112	1.00	12.00
	18.00	30	5.5667	2.72515	.49754	4.5491	6.5843	1.00	12.00
	19.00	30	4.1000	2.42615	.44295	3.1941	5.0059	1.00	10.00
	20.00	30	4.0333	2.89451	.52846	2.9525	5.1142	1.00	13.00
	21.00	30	3.3667	1.69143	.30881	2.7351	3.9983	1.00	8.00
	22.00	30	3.3000	1.74494	.31858	2.6484	3.9516	1.00	8.00
	23.00	30	3.4333	1.92414	.35130	2.7148	4.1518	1.00	10.00
	24.00	30	4.0333	2.51181	.45859	3.0954	4.9713	1.00	12.00
	Total	720	3.6146	2.20323	.08499	3.4477	3.7815	1.00	16.00
Shootlength	1.00	30	2.0625	.49591	.10123	1.8531	2.2719	1.00	3.00
	2.00	30	2.2833	.71539	.13061	2.0162	2.5505	1.50	4.00

	3.00	30	1.7667	.46855	.08555	1.5917	1.9416	1.00	3.00
	4.00	30	2.2667	.67891	.12395	2.0132	2.5202	1.50	4.00
	5.00	30	1.9500	.66111	.12070	1.7031	2.1969	1.00	3.00
	6.00	30	1.9500	.49741	.09081	1.7643	2.1357	1.00	3.00
	7.00	30	2.0208	.63381	.12938	1.7532	2.2885	1.00	3.50
	8.00	30	2.4792	.82724	.16886	2.1299	2.8285	1.00	4.00
	9.00	30	2.6042	.75151	.15340	2.2868	2.9215	1.50	5.00
	10.00	30	2.2083	.64127	.13090	1.9375	2.4791	1.00	3.50
	11.00	30	2.4792	.69905	.14269	2.1840	2.7744	1.50	4.00
	12.00	30	2.2833	.80605	.14716	1.9824	2.5843	1.00	4.00
	13.00	30	2.8125	.43769	.08934	2.6277	2.9973	2.00	3.50
	14.00	30	2.5000	.49130	.08970	2.3165	2.6835	2.00	4.00
	15.00	30	2.6833	.62261	.11367	2.4508	2.9158	2.00	4.00
	16.00	30	2.8125	.63951	.13054	2.5425	3.0825	2.00	4.00
	17.00	30	2.6500	.80032	.14612	2.3512	2.9488	2.00	6.00
	18.00	30	2.5167	.49971	.09123	2.3301	2.7033	1.50	3.00
	19.00	30	2.6167	.90671	.16554	2.2781	2.9552	1.00	5.00
	20.00	30	2.8000	.97025	.17714	2.4377	3.1623	1.00	6.00
	21.00	30	2.4833	.57959	.10582	2.2669	2.6998	2.00	3.50
	22.00	30	2.9500	.69914	.12764	2.6889	3.2111	1.50	4.50
	23.00	30	2.6500	.89201	.16286	2.3169	2.9831	1.50	5.00
	24.00	30	2.8167	.72497	.13236	2.5460	3.0874	1.50	4.00
	Total	720	2.4442	.74972	.02892	2.3874	2.5010	1.00	6.00
	1.00	30	3.4167	1.38051	.28179	2.8337	3.9996	1.00	7.00
	2.00	30	4.9000	2.52368	.46076	3.9576	5.8424	1.00	12.00
	3.00	30	6.1667	2.69205	.49150	5.1614	7.1719	2.00	13.00
	4.00	30	5.7667	1.85106	.33796	5.0755	6.4579	3.00	10.00
	5.00	30	5.0000	2.36352	.43152	4.1174	5.8826	1.00	11.00
	6.00	30	4.9000	1.91815	.35021	4.1837	5.6163	2.00	10.00
	7.00	30	5.5000	3.53861	.72232	4.0058	6.9942	1.00	14.00
Leafnumber	8.00	30	5.8750	2.70768	.55270	4.7316	7.0184	2.00	12.00
	9.00	30	5.5833	2.65259	.54146	4.4632	6.7034	1.00	12.00
	10.00	30	6.2083	2.90396	.59277	4.9821	7.4346	2.00	12.00
	11.00	30	5.2083	2.63718	.53831	4.0948	6.3219	2.00	12.00
	12.00	30	5.8000	3.57578	.65285	4.4648	7.1352	2.00	15.00
	13.00	30	13.1250	4.26627	.87085	11.3235	14.9265	4.00	22.00
	14.00	30	8.6667	3.85364	.70358	7.2277	10.1056	3.00	21.00
	15.00	30	15.4667	4.66634	.85195	13.7242	17.2091	6.00	24.00

	16.00	30	13.5833	6.86463	1.40124	10.6847	16.4820	3.00	30.00
	17.00	30	11.3667	5.86329	1.07049	9.1773	13.5561	2.00	28.00
	18.00	30	13.3000	5.96050	1.08823	11.0743	15.5257	3.00	25.00
	19.00	30	7.3667	4.23844	.77383	5.7840	8.9493	2.00	18.00
	20.00	30	8.5333	3.98907	.72830	7.0438	10.0229	2.00	19.00
	21.00	30	8.4667	4.53898	.82870	6.7718	10.1615	2.00	20.00
	22.00	30	7.8333	2.99521	.54685	6.7149	8.9518	4.00	16.00
	23.00	30	7.0000	3.62938	.66263	5.6448	8.3552	2.00	17.00
	24.00	30	8.5333	4.44688	.81188	6.8728	10.1938	4.00	21.00
	Total	720	7.8512	4.92040	.18981	7.4785	8.2239	1.00	30.00

a. Some or all bootstrap sample results are missing, so no bootstrap estimation has been performed for this table.

Shoot number

	Treatment	N	Subset for alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a,b]	1.00	30	1.6667						
	3.00	30	2.2667	2.2667					
	2.00	30	2.6333	2.6333					
	5.00	30	2.7333	2.7333					
	8.00	30	2.7917	2.7917	2.7917				
	11.00	30		2.9167	2.9167	2.9167			
	6.00	30		2.9667	2.9667	2.9667			
	12.00	30		3.0000	3.0000	3.0000			
	4.00	30		3.0667	3.0667	3.0667			
	7.00	30		3.2083	3.2083	3.2083			
	9.00	30		3.2917	3.2917	3.2917			
	22.00	30		3.3000	3.3000	3.3000			
	21.00	30		3.3667	3.3667	3.3667			
	10.00	30		3.3750	3.3750	3.3750			
	14.00	30		3.4000	3.4000	3.4000			
	23.00	30		3.4333	3.4333	3.4333			
	20.00	30			4.0333	4.0333	4.0333		
	24.00	30			4.0333	4.0333	4.0333		
	19.00	30				4.1000	4.1000		
	13.00	30					4.7917	4.7917	
	17.00	30					4.8000	4.8000	
	16.00	30					4.9583	4.9583	
	18.00	30						5.5667	
	15.00	30							6.6667
	Sig.		.054	.071	.052	.065	.125	.180	1.000
Means for grou									
a. Uses Harmor									
b. The group si:									
Duncan[a,b]									

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 27.692.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Shootlength

	Treatment	N	Subset for alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a,b]	3.00	30	1.7667						
	5.00	30	1.9500	1.9500					
	6.00	30	1.9500	1.9500					
	7.00	24	2.0208	2.0208					
	1.00	24	2.0625	2.0625	2.0625				
	10.00	24		2.2083	2.2083	2.2083			
	4.00	30		2.2667	2.2667	2.2667	2.2667		
	2.00	30		2.2833	2.2833	2.2833	2.2833		
	12.00	30		2.2833	2.2833	2.2833	2.2833		
	8.00	24			2.4792	2.4792	2.4792	2.4792	
	11.00	24			2.4792	2.4792	2.4792	2.4792	
	21.00	30			2.4833	2.4833	2.4833	2.4833	
	14.00	30				2.5000	2.5000	2.5000	
	18.00	30				2.5167	2.5167	2.5167	2.5167
	9.00	24				2.6042	2.6042	2.6042	2.6042
	19.00	30				2.6167	2.6167	2.6167	2.6167
	17.00	30					2.6500	2.6500	2.6500
	23.00	30					2.6500	2.6500	2.6500
	15.00	30					2.6833	2.6833	2.6833
	20.00	30						2.8000	2.8000
	13.00	24						2.8125	2.8125
	16.00	24						2.8125	2.8125
	24.00	30						2.8167	2.8167
	22.00	30							2.9500
	Sig.		.160	.130	.052	.068	.066	.144	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 27.692.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Leaf number

	Treatment	N	Subset for alpha = 0.05								
			1	2	3	4	5	6	7	8	9
Duncan[a,b]	1.00	30	3.4167								
	2.00	30	4.9000	4.9000							
	6.00	30	4.9000	4.9000							
	5.00	30	5.0000	5.0000	5.0000						
	11.00	30	5.2083	5.2083	5.2083						
	7.00	30	5.5000	5.5000	5.5000	5.5000					
	9.00	30	5.5833	5.5833	5.5833	5.5833					
	4.00	30	5.7667	5.7667	5.7667	5.7667					
	12.00	30		5.8000	5.8000	5.8000					
	8.00	30		5.8750	5.8750	5.8750					
	3.00	30		6.1667	6.1667	6.1667					
	10.00	30		6.2083	6.2083	6.2083	6.2083				
	23.00	30		7.0000	7.0000	7.0000	7.0000	7.0000			
	19.00	30			7.3667	7.3667	7.3667	7.3667			
	22.00	30				7.8333	7.8333	7.8333			
	21.00	30					8.4667	8.4667			
	20.00	30					8.5333	8.5333			
	24.00	30					8.5333	8.5333			
	14.00	30						8.6667			
	17.00	30							11.3667		
	13.00	30							13.1250	13.1250	
	18.00	30							13.3000	13.3000	
	16.00	30								13.5833	13.5833
	15.00	30									15.4667
	Sig.		.051	.095	.056	.058	.051	.169	.076	.679	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 27.692.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 2. The interaction effect of different salt strength with BAP and/or TDZ on multiplication of korarima

Descriptives[a]

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Shoot number	1.00	30	2.6250	1.24455	.25404	2.0995	3.1505	1.00	6.00
	2.00	30	2.4667	1.04166	.19018	2.0777	2.8556	1.00	5.00
	3.00	30	5.0667	4.10158	.74884	3.5351	6.5982	1.00	22.00
	4.00	30	2.6667	1.18419	.21620	2.2245	3.1088	1.00	5.00
	5.00	30	2.7000	1.31700	.24045	2.2082	3.1918	1.00	5.00
	6.00	30	2.3333	.86811	.17720	1.9668	2.6999	1.00	4.00
	7.00	30	4.8667	2.16131	.39460	4.0596	5.6737	2.00	9.00
	8.00	30	5.1333	2.83735	.51803	4.0738	6.1928	1.00	12.00
	9.00	30	5.2667	2.88795	.52726	4.1883	6.3450	1.00	13.00
	10.00	30	3.8333	2.06920	.37778	3.0607	4.6060	1.00	9.00
	11.00	30	3.5833	1.97631	.40341	2.7488	4.4179	1.00	9.00
	12.00	30	3.9333	1.76036	.32140	3.2760	4.5907	1.00	8.00
	13.00	30	3.1000	.99481	.18163	2.7285	3.4715	2.00	5.00
	14.00	30	2.8333	1.51050	.27578	2.2693	3.3974	1.00	6.00
	15.00	30	2.3000	1.20773	.22050	1.8490	2.7510	1.00	7.00
	16.00	30	2.7000	1.23596	.22565	2.2385	3.1615	1.00	7.00
	17.00	30	2.8000	1.15669	.21118	2.3681	3.2319	1.00	6.00
	18.00	30	2.9667	1.58622	.28960	2.3744	3.5590	1.00	7.00
	19.00	30	2.7000	1.41787	.25887	2.1706	3.2294	1.00	7.00
	20.00	30	2.9667	1.47352	.26903	2.4164	3.5169	1.00	7.00
	21.00	30	2.6667	1.24106	.22659	2.2032	3.1301	1.00	7.00
	22.00	30	2.9667	1.24522	.22735	2.5017	3.4316	1.00	6.00
	23.00	30	3.3667	2.35597	.43014	2.4869	4.2464	1.00	11.00
	24.00	30	2.7000	1.26355	.23069	2.2282	3.1718	1.00	6.00
	25.00	30	3.9333	1.85571	.33881	3.2404	4.6263	1.00	7.00
	26.00	30	3.1667	1.20583	.22015	2.7164	3.6169	1.00	6.00
	27.00	30	3.1667	1.60615	.32785	2.4885	3.8449	1.00	7.00
	28.00	30	3.5000	1.69685	.30980	2.8664	4.1336	1.00	9.00
	29.00	30	4.0333	2.17324	.39678	3.2218	4.8448	1.00	10.00
	30.00	30	2.7000	1.14921	.20982	2.2709	3.1291	1.00	5.00

	31.00	30	3.2917	1.60106	.32682	2.6156	3.9677	1.00	7.00
	32.00	30	3.2667	1.65952	.30299	2.6470	3.8863	1.00	8.00
	33.00	30	3.0417	1.42887	.29167	2.4383	3.6450	1.00	7.00
	34.00	30	3.4167	.88055	.17974	3.0448	3.7885	2.00	5.00
	35.00	30	4.1333	2.28539	.41725	3.2800	4.9867	1.00	9.00
	36.00	30	3.0417	1.36666	.27897	2.4646	3.6188	1.00	6.00
	Total	1080	3.3236	1.91879	.05973	3.2064	3.4408	1.00	22.00
	1.00	30	2.8125	.88234	.18011	2.4399	3.1851	2.00	5.00
	2.00	30	2.2000	.77237	.14101	1.9116	2.4884	1.00	4.00
	3.00	30	2.7000	.96132	.17551	2.3410	3.0590	1.50	5.00
	4.00	30	3.0633	1.17429	.21439	2.6248	3.5018	1.50	7.00
	5.00	30	2.6000	.77013	.14061	2.3124	2.8876	1.00	4.00
	6.00	30	3.0833	.97431	.19888	2.6719	3.4947	1.50	5.00
	7.00	30	2.3000	.61026	.11142	2.0721	2.5279	1.50	3.50
	8.00	30	2.3000	.84690	.15462	1.9838	2.6162	1.00	4.00
	9.00	30	2.3833	.83752	.15291	2.0706	2.6961	1.50	4.50
	10.00	30	2.2333	1.20153	.21937	1.7847	2.6820	1.00	6.00
	11.00	30	2.1667	1.07001	.21842	1.7148	2.6185	1.00	4.50
	12.00	30	1.9500	.82368	.15038	1.6424	2.2576	1.00	4.00
	13.00	30	2.0167	.95125	.17367	1.6615	2.3719	1.00	4.00
	14.00	30	2.7000	.85702	.15647	2.3800	3.0200	1.00	5.00
	15.00	30	2.6833	.98684	.18017	2.3148	3.0518	1.00	4.50
Shoot length	16.00	30	1.9500	.88425	.16144	1.6198	2.2802	1.00	4.00
	17.00	30	1.7833	.63901	.11667	1.5447	2.0219	1.00	3.00
	18.00	30	1.6167	.63901	.11667	1.3781	1.8553	1.00	3.00
	19.00	30	1.9833	.64971	.11862	1.7407	2.2259	1.00	4.00
	20.00	30	1.3333	.51417	.09387	1.1413	1.5253	1.00	3.00
	21.00	30	1.2667	.31441	.05740	1.1493	1.3841	1.00	2.00
	22.00	30	1.5000	.49130	.08970	1.3165	1.6835	1.00	3.00
	23.00	30	1.5500	.47976	.08759	1.3709	1.7291	1.00	3.00
	24.00	30	1.4667	.41384	.07556	1.3121	1.6212	1.00	2.00
	25.00	30	2.1500	.55940	.10213	1.9411	2.3589	1.50	4.00
	26.00	30	2.2000	.55086	.10057	1.9943	2.4057	1.00	3.00
	27.00	30	2.0000	.69156	.14116	1.7080	2.2920	1.50	4.00
	28.00	30	1.9167	.45644	.08333	1.7462	2.0871	1.50	3.00
	29.00	30	2.0833	.61705	.11266	1.8529	2.3137	1.50	4.00
	30.00	30	2.1500	.69667	.12719	1.8899	2.4101	1.50	4.00
	31.00	30	1.8333	.54507	.11126	1.6032	2.0635	1.00	3.50
	32.00	30	1.9833	.67573	.12337	1.7310	2.2357	1.00	4.00

	33.00	30	2.0833	.52475	.10711	1.8618	2.3049	1.00	3.00
	34.00	30	1.6042	.25449	.05195	1.4967	1.7116	1.00	2.00
	35.00	30	2.0500	.57760	.10545	1.8343	2.2657	1.50	4.00
	36.00	30	1.7708	.62518	.12761	1.5068	2.0348	1.00	3.00
	Total	1080	2.0929	.85518	.02662	2.0407	2.1452	1.00	7.00
Leaf number	1.00	30	4.4583	2.81269	.57414	3.2706	5.6460	1.00	11.00
	2.00	30	2.7333	1.33735	.24417	2.2340	3.2327	.00	6.00
	3.00	30	4.5667	3.82986	.69923	3.1366	5.9968	1.00	21.00
	4.00	30	4.0667	1.72073	.31416	3.4241	4.7092	1.00	8.00
	5.00	30	3.0000	1.66091	.30324	2.3798	3.6202	1.00	7.00
	6.00	30	3.2917	1.08264	.22099	2.8345	3.7488	2.00	5.00
	7.00	30	6.0667	3.00498	.54863	4.9446	7.1887	.00	14.00
	8.00	30	5.2000	2.86958	.52391	4.1285	6.2715	2.00	14.00
	9.00	30	5.1667	2.82944	.51658	4.1101	6.2232	.00	13.00
	10.00	30	4.4000	1.73404	.31659	3.7525	5.0475	1.00	8.00
	11.00	30	4.5417	2.50181	.51068	3.4852	5.5981	1.00	11.00
	12.00	30	4.3667	2.56614	.46851	3.4085	5.3249	.00	12.00
	13.00	30	5.0333	2.99981	.54769	3.9132	6.1535	.00	15.00
	14.00	30	3.8667	2.14530	.39168	3.0656	4.6677	.00	9.00
	15.00	30	4.0000	2.61297	.47706	3.0243	4.9757	1.00	12.00
	16.00	30	3.1333	1.79527	.32777	2.4630	3.8037	.00	8.00
	17.00	30	3.4000	2.41547	.44100	2.4980	4.3020	.00	11.00
	18.00	30	3.7000	2.21515	.40443	2.8728	4.5272	.00	11.00
	19.00	30	4.0667	2.47656	.45216	3.1419	4.9914	.00	11.00
	20.00	30	3.6000	2.06113	.37631	2.8304	4.3696	.00	9.00
	21.00	30	2.5333	1.33218	.24322	2.0359	3.0308	.00	5.00
	22.00	30	4.1333	2.06336	.37672	3.3629	4.9038	1.00	10.00
	23.00	30	3.3333	2.12267	.38755	2.5407	4.1260	1.00	10.00
	24.00	30	2.9667	1.84733	.33728	2.2769	3.6565	.00	8.00
	25.00	30	9.3333	4.15504	.75860	7.7818	10.8849	3.00	19.00
	26.00	30	7.2000	2.85754	.52171	6.1330	8.2670	2.00	14.00
	27.00	30	7.4167	3.00603	.61360	6.1473	8.6860	2.00	13.00
	28.00	30	7.2667	6.06251	1.10686	5.0029	9.5304	1.00	23.00
	29.00	30	6.7000	5.25980	.96030	4.7360	8.6640	2.00	22.00
	30.00	30	4.8000	2.55154	.46584	3.8472	5.7528	1.00	11.00
	31.00	30	7.2500	4.06737	.83025	5.5325	8.9675	3.00	19.00

	32.00	30	6.1333	4.24860	.77568	4.5469	7.7198	2.00	20.00
	33.00	30	6.2917	3.09950	.63268	4.9829	7.6005	2.00	16.00
	34.00	30	6.2083	2.06375	.42126	5.3369	7.0798	2.00	11.00
	35.00	30	7.6333	4.87416	.88990	5.8133	9.4534	1.00	18.00
	36.00	30	4.5208	3.82349	.78047	2.9063	6.1354	1.00	14.00
	Total	1080	4.8716	3.37270	.10499	4.6656	5.0776	.00	23.00

a. Some or all bootstrap sample results are missing, so no bootstrap estimation has been performed for this table.

Shoot number

	Treatment	N	Subset for alpha = 0.05									
			1	2	3	4	5	6	7	8	9	10
	15.00	30	2.3000									
	6.00	30	2.3333	2.3333								
	2.00	30	2.4667	2.4667	2.4667							
	1.00	30	2.6250	2.6250	2.6250							
	4.00	30	2.6667	2.6667	2.6667	2.6667						
	21.00	30	2.6667	2.6667	2.6667	2.6667						
	5.00	30	2.7000	2.7000	2.7000	2.7000						
	16.00	30	2.7000	2.7000	2.7000	2.7000						
	19.00	30	2.7000	2.7000	2.7000	2.7000						
	24.00	30	2.7000	2.7000	2.7000	2.7000						
	30.00	30	2.7000	2.7000	2.7000	2.7000						
Duncan[a,b]	17.00	30	2.8000	2.8000	2.8000	2.8000	2.8000					
	14.00	30	2.8333	2.8333	2.8333	2.8333	2.8333					
	18.00	30	2.9667	2.9667	2.9667	2.9667	2.9667	2.9667				
	20.00	30	2.9667	2.9667	2.9667	2.9667	2.9667	2.9667				
	22.00	30	2.9667	2.9667	2.9667	2.9667	2.9667	2.9667				
	33.00	30	3.0417	3.0417	3.0417	3.0417	3.0417	3.0417	3.0417			
	36.00	30	3.0417	3.0417	3.0417	3.0417	3.0417	3.0417	3.0417			
	13.00	30	3.1000	3.1000	3.1000	3.1000	3.1000	3.1000	3.1000			
	26.00	30	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667			
	27.00	30	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667			
	32.00	30	3.2667	3.2667	3.2667	3.2667	3.2667	3.2667	3.2667			
	31.00	30	3.2917	3.2917	3.2917	3.2917	3.2917	3.2917	3.2917			

23.00	30	3.3667	3.3667	3.3667	3.3667	3.3667	3.3667	3.3667			
34.00	30	3.4167	3.4167	3.4167	3.4167	3.4167	3.4167	3.4167			
28.00	30		3.5000	3.5000	3.5000	3.5000	3.5000	3.5000			
11.00	30			3.5833	3.5833	3.5833	3.5833	3.5833			
10.00	30				3.8333	3.8333	3.8333	3.8333	3.8333		
12.00	30					3.9333	3.9333	3.9333	3.9333		
25.00	30					3.9333	3.9333	3.9333	3.9333		
29.00	30						4.0333	4.0333	4.0333		
35.00	30							4.1333	4.1333	4.1333	
7.00	30								4.8667	4.8667	4.8667
3.00	30									5.0667	5.0667
8.00	30									5.1333	5.1333
9.00	30										5.2667
Sig.		.063	.051	.063	.050	.054	.070	.061	.054	.052	.447

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 28.421.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Shoot length

	Treatment	N	Subset for alpha = 0.05													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
	21.00	30	1.2667													
	20.00	30	1.3333	1.3333												
	24.00	30	1.4667	1.4667	1.4667											
	22.00	30	1.5000	1.5000	1.5000	1.5000										
	23.00	30	1.5500	1.5500	1.5500	1.5500	1.5500									
	34.00	30	1.6042	1.6042	1.6042	1.6042	1.6042	1.6042								
	18.00	30	1.6167	1.6167	1.6167	1.6167	1.6167	1.6167	1.6167							
	36.00	30		1.7708	1.7708	1.7708	1.7708	1.7708	1.7708	1.7708						
	17.00	30		1.7833	1.7833	1.7833	1.7833	1.7833	1.7833	1.7833						
	31.00	30			1.8333	1.8333	1.8333	1.8333	1.8333	1.8333	1.8333					
	28.00	30			1.9167	1.9167	1.9167	1.9167	1.9167	1.9167	1.9167	1.9167				
	12.00	30				1.9500	1.9500	1.9500	1.9500	1.9500	1.9500	1.9500				
	16.00	30				1.9500	1.9500	1.9500	1.9500	1.9500	1.9500	1.9500				
	19.00	30					1.9833	1.9833	1.9833	1.9833	1.9833	1.9833				
	32.00	30					1.9833	1.9833	1.9833	1.9833	1.9833	1.9833				
	27.00	30					2.0000	2.0000	2.0000	2.0000	2.0000	2.0000				
Duncan[a,b]	13.00	30					2.0167	2.0167	2.0167	2.0167	2.0167	2.0167				
	35.00	30						2.0500	2.0500	2.0500	2.0500	2.0500				
	29.00	30							2.0833	2.0833	2.0833	2.0833				
	33.00	30							2.0833	2.0833	2.0833	2.0833				
	25.00	30								2.1500	2.1500	2.1500	2.1500			
	30.00	30								2.1500	2.1500	2.1500	2.1500			
	11.00	30								2.1667	2.1667	2.1667	2.1667			
	2.00	30								2.2000	2.2000	2.2000	2.2000			
	26.00	30								2.2000	2.2000	2.2000	2.2000			
	10.00	30								2.2333	2.2333	2.2333	2.2333			
	7.00	30									2.3000	2.3000	2.3000	2.3000		
	8.00	30									2.3000	2.3000	2.3000	2.3000		
	9.00	30										2.3833	2.3833	2.3833	2.3833	
	5.00	30											2.6000	2.6000	2.6000	
	15.00	30												2.6833	2.6833	2.6833
	3.00	30												2.7000	2.7000	2.7000
	14.00	30												2.7000	2.7000	2.7000
	1.00	30													2.8125	2.8125
	4.00	30														3.0633
	6.00	30														3.0833
	Sig.		.130	.051	.053	.056	.051	.064	.053	.061	.058	.058	.056	.081	.056	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 28.421.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Leafnumber

	Treatment	N	Subset for alpha = 0.05									
			1	2	3	4	5	6	7	8	9	10
	21.00	30	2.5333									
	2.00	30	2.7333	2.7333								
	24.00	30	2.9667	2.9667	2.9667							
	5.00	30	3.0000	3.0000	3.0000							
	16.00	30	3.1333	3.1333	3.1333	3.1333						
	6.00	30	3.2917	3.2917	3.2917	3.2917	3.2917					
	23.00	30	3.3333	3.3333	3.3333	3.3333	3.3333					
	17.00	30	3.4000	3.4000	3.4000	3.4000	3.4000					
	20.00	30	3.6000	3.6000	3.6000	3.6000	3.6000					
	18.00	30	3.7000	3.7000	3.7000	3.7000	3.7000					
	14.00	30	3.8667	3.8667	3.8667	3.8667	3.8667					
	15.00	30	4.0000	4.0000	4.0000	4.0000	4.0000					
	4.00	30	4.0667	4.0667	4.0667	4.0667	4.0667					
	19.00	30	4.0667	4.0667	4.0667	4.0667	4.0667					
	22.00	30	4.1333	4.1333	4.1333	4.1333	4.1333					
	12.00	30	4.3667	4.3667	4.3667	4.3667	4.3667	4.3667				
Duncan[a,b]	10.00	30	4.4000	4.4000	4.4000	4.4000	4.4000	4.4000	4.4000			
	1.00	30	4.4583	4.4583	4.4583	4.4583	4.4583	4.4583	4.4583			
	36.00	30		4.5208	4.5208	4.5208	4.5208	4.5208	4.5208			
	11.00	30		4.5417	4.5417	4.5417	4.5417	4.5417	4.5417			
	3.00	30		4.5667	4.5667	4.5667	4.5667	4.5667	4.5667			
	30.00	30			4.8000	4.8000	4.8000	4.8000	4.8000			
	13.00	30				5.0333	5.0333	5.0333	5.0333	5.0333		
	9.00	30					5.1667	5.1667	5.1667	5.1667		
	8.00	30					5.2000	5.2000	5.2000	5.2000		
	7.00	30						6.0667	6.0667	6.0667	6.0667	
	32.00	30						6.1333	6.1333	6.1333	6.1333	
	34.00	30						6.2083	6.2083	6.2083	6.2083	
	33.00	30							6.2917	6.2917	6.2917	
	29.00	30								6.7000	6.7000	
	26.00	30									7.2000	
	31.00	30									7.2500	
	28.00	30									7.2667	

	27.00	30									7.4167	
	35.00	30									7.6333	
	25.00	30										9.3333
	Sig.		.052	.067	.067	.056	.056	.057	.050	.075	.102	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 28.421.

Appendix 3. Comparison of solid and liquid MS media on multiplication of korarima

Descriptives

				Statistic	Bootstrap[a]			
					Bias	Std. Error	95% Confidence Interval	
							Lower	Upper
Shoot number	1.00	N		30	0	5	21	40
		Mean		2.3750	.0010	.2409	1.8892	2.8095
		Std. Deviation		1.17260	-.02827	.09888	.93980	1.31893
		Std. Error		.23936				
		95% Confidence Interval for Mean	Lower Bound	1.8799				
			Upper Bound	2.8701				
		Minimum		1.00				
		Maximum		4.00				
	2.00	N		30	0	5	20	40
		Mean		2.8333	-.0003	.2349	2.3571	3.2901
		Std. Deviation		1.28877	-.02732	.11578	1.02606	1.49686
		Std. Error		.23530				
		95% Confidence Interval for Mean	Lower Bound	2.3521				
			Upper Bound	3.3146				
		Minimum		1.00				
		Maximum		5.00				
	3.00	N		30	0	5	20	41
		Mean		2.5333	-.0006	.3485	1.9130	3.2631
		Std. Deviation		1.90703	-.08051	.36908	1.04444	2.51279
		Std. Error		.34818				
		95% Confidence Interval for Mean	Lower Bound	1.8212				
			Upper Bound	3.2454				
		Minimum		1.00				
		Maximum		8.00				
	4.00	N		30	0	5	20	41
		Mean		2.0667	-.0089	.1828	1.7143	2.4515
		Std. Deviation		1.01483	-.02538	.10220	.78956	1.18626
		Std. Error		.18528				
		95% Confidence Interval for	Lower Bound	1.6877				

	Mean	Upper Bound	2.4456				
	Minimum		1.00				
	Maximum		4.00				
5.00	N		30	0	5	20	41
	Mean		3.0000	-.0037	.2469	2.5200	3.5000
	Std. Deviation		1.38962	-.03818	.21614	.95452	1.77675
	Std. Error		.25371				
	95% Confidence Interval for Mean	Lower Bound	2.4811				
		Upper Bound	3.5189				
	Minimum		1.00				
	Maximum		7.00				
6.00	N		30	0	5	20	41
	Mean		2.7000	.0051	.2278	2.2668	3.1600
	Std. Deviation		1.23596	-.02606	.15164	.88418	1.49107
	Std. Error		.22565				
	95% Confidence Interval for Mean	Lower Bound	2.2385				
		Upper Bound	3.1615				
	Minimum		1.00				
	Maximum		5.00				
7.00	N		30	0	5	21	39
	Mean		2.0500	-.0038	.1637	1.7347	2.4122
	Std. Deviation		1.24090	-.02189	.17094	.89757	1.57850
	Std. Error		.16020				
	95% Confidence Interval for Mean	Lower Bound	1.7294				
		Upper Bound	2.3706				
	Minimum		1.00				
	Maximum		7.00				
8.00	N		30	0	5	21	39
	Mean		2.0769	.0078	.3138	1.5385	2.7890
	Std. Deviation		1.59808	-.08554	.44172	.76969	2.36306
	Std. Error		.31341				
	95% Confidence Interval for Mean	Lower Bound	1.4314				
		Upper Bound	2.7224				
	Minimum		1.00				
	Maximum		8.00				
9.00	N		30	0	5	21	41

	Mean		3.6429	-.0238	.4651	2.7744	4.6206
	Std. Deviation		2.45273	-.09048	.43695	1.47672	3.16804
	Std. Error		.46352				
	95% Confidence Interval for Mean	Lower Bound	2.6918				
		Upper Bound	4.5939				
	Minimum		1.00				
	Maximum		11.00				
10.00	N		30	0	5	20	41
	Mean		4.3103	.0040	.5383	3.3441	5.4284
	Std. Deviation		2.79161	-.08658	.49205	1.75009	3.67554
	Std. Error		.51839				
	95% Confidence Interval for Mean	Lower Bound	3.2485				
		Upper Bound	5.3722				
	Minimum		1.00				
	Maximum		13.00				
11.00	N		30	0	5	21	41
	Mean		3.6207	-.0162	.4970	2.6670	4.6188
	Std. Deviation		2.70468	-.12123	.53378	1.42416	3.54820
	Std. Error		.50225				
	95% Confidence Interval for Mean	Lower Bound	2.5919				
		Upper Bound	4.6495				
	Minimum		1.00				
	Maximum		12.00				
12.00	N		30	0	5	20	41
	Mean		4.4138	.0057	.6581	3.2801	5.7998
	Std. Deviation		3.51036	-.20541	.92598	1.43397	4.95613
	Std. Error		.65186				
	95% Confidence Interval for Mean	Lower Bound	3.0785				
		Upper Bound	5.7491				
	Minimum		1.00				
	Maximum		17.00				
13.00	N		30	0	5	20	41
	Mean		3.6667	.0027	.2168	3.2310	4.0882
	Std. Deviation		1.15470	-.03825	.18687	.75109	1.49652
	Std. Error		.21082				
	95% Confidence Interval for	Lower Bound	3.2355				

		Mean	Upper Bound	4.0978				
		Minimum		1.00				
		Maximum		7.00				
	14.00	N		30	0	5	21	39
		Mean		2.9000	-.0004	.1211	2.6795	3.1343
		Std. Deviation		.95136	-.02253	.14934	.67997	1.23171
		Std. Error		.12282				
		95% Confidence Interval for Mean	Lower Bound	2.6542				
			Upper Bound	3.1458				
		Minimum		1.00				
		Maximum		7.00				
	Total	N		420	0	0	420	420
		Mean		2.9505	-.0017	.0921	2.7742	3.1355
		Std. Deviation		1.94641	-.01101	.16322	1.63617	2.27999
		Std. Error		.09026				
		95% Confidence Interval for Mean	Lower Bound	2.7732				
			Upper Bound	3.1279				
		Minimum		1.00				
		Maximum		17.00				
Shoot length	1.00	N		30	0	5	21	40
		Mean		3.6042	.0058	.2305	3.1591	4.0570
		Std. Deviation		1.13232	-.03044	.12736	.83358	1.33813
		Std. Error		.23113				
		95% Confidence Interval for Mean	Lower Bound	3.1260				
			Upper Bound	4.0823				
		Minimum		1.50				
		Maximum		5.50				
	2.00	N		30	0	5	20	40
		Mean		3.4500	.0078	.1477	3.1732	3.7664
		Std. Deviation		.78069	-.01633	.08427	.59623	.92399
		Std. Error		.14253				
		95% Confidence Interval for Mean	Lower Bound	3.1585				
			Upper Bound	3.7415				
		Minimum		2.00				
		Maximum		5.00				
	3.00	N		30	0	5	20	41
		Mean		3.3500	-.0064	.1989	2.9288	3.7407
		Std. Deviation		1.07599	-.02909	.15086	.75608	1.36033

	Std. Error		.19645				
	95% Confidence Interval for Mean	Lower Bound	2.9482				
		Upper Bound	3.7518				
	Minimum		1.00				
	Maximum		6.00				
4.00	N		30	0	5	20	41
	Mean		3.0500	.0057	.1698	2.7500	3.4285
	Std. Deviation		.91303	-.02329	.10975	.65360	1.08494
	Std. Error		.16670				
	95% Confidence Interval for Mean	Lower Bound	2.7091				
		Upper Bound	3.3909				
	Minimum		2.00				
	Maximum		5.00				
5.00	N		30	0	5	20	41
	Mean		3.1833	.0014	.1128	2.9532	3.4117
	Std. Deviation		.63631	-.01408	.07275	.47408	.76126
	Std. Error		.11617				
	95% Confidence Interval for Mean	Lower Bound	2.9457				
		Upper Bound	3.4209				
	Minimum		2.00				
	Maximum		4.50				
6.00	N		30	0	5	20	41
	Mean		3.4000	-.0087	.1950	3.0000	3.7833
	Std. Deviation		1.05373	-.02553	.11939	.78826	1.25287
	Std. Error		.19238				
	95% Confidence Interval for Mean	Lower Bound	3.0065				
		Upper Bound	3.7935				
	Minimum		1.50				
	Maximum		5.50				
7.00	N		30	0	5	21	39
	Mean		4.4417	-.0029	.1504	4.1518	4.7500
	Std. Deviation		1.20764	-.01524	.10200	.98767	1.39163
	Std. Error		.15591				
	95% Confidence Interval for Mean	Lower Bound	4.1297				
		Upper Bound	4.7536				
	Minimum		1.50				
	Maximum		7.00				
8.00	N		30	0	5	20	39
	Mean		4.4808	.0023	.2147	4.0770	4.8946
	Std. Deviation		1.06283	-.02251	.11074	.81003	1.24323

	Std. Error		.20844				
	95% Confidence Interval for Mean	Lower Bound	4.0515				
		Upper Bound	4.9101				
	Minimum		2.50				
	Maximum		6.00				
9.00	N		30	0	5	21	41
	Mean		5.8571	.0010	.2673	5.3335	6.3888
	Std. Deviation		1.41328	-.03468	.14376	1.09310	1.66641
	Std. Error		.26708				
	95% Confidence Interval for Mean	Lower Bound	5.3091				
		Upper Bound	6.4052				
	Minimum		3.00				
	Maximum		8.00				
10.00	N		30	0	5	20	41
	Mean		3.6724	-.0037	.2376	3.1806	4.1557
	Std. Deviation		1.30436	-.02398	.15937	1.00137	1.61896
	Std. Error		.24221				
	95% Confidence Interval for Mean	Lower Bound	3.1763				
		Upper Bound	4.1686				
	Minimum		1.50				
	Maximum		7.00				
11.00	N		30	0	5	20	40
	Mean		4.6034	-.0176	.3909	3.9168	5.4838
	Std. Deviation		2.07183	-.13630	.59324	1.05159	3.11309
	Std. Error		.38473				
	95% Confidence Interval for Mean	Lower Bound	3.8154				
		Upper Bound	5.3915				
	Minimum		1.50				
	Maximum		13.00				
12.00	N		30	0	5	20	41
	Mean		5.0172	-.0066	.2065	4.6035	5.4397
	Std. Deviation		1.12981	-.04444	.15752	.74785	1.37898
	Std. Error		.20980				
	95% Confidence Interval for Mean	Lower Bound	4.5875				
		Upper Bound	5.4470				
	Minimum		3.00				
	Maximum		8.00				
13.00	N		30	0	5	20	41
	Mean		3.0667	-.0045	.1393	2.8001	3.3599
	Std. Deviation		.73968	-.02316	.10436	.52286	.92175

		Std. Error		.13505				
		95% Confidence Interval for Mean	Lower Bound	2.7905				
			Upper Bound	3.3429				
		Minimum		2.00				
		Maximum		5.00				
	14.00	N		30	0	5	21	39
		Mean		4.6500	-.0056	.1439	4.3594	4.9230
		Std. Deviation		1.11728	-.01016	.08125	.93376	1.25523
		Std. Error		.14424				
		95% Confidence Interval for Mean	Lower Bound	4.3614				
			Upper Bound	4.9386				
		Minimum		3.00				
		Maximum		7.00				
	Total	N		420	0	0	420	420
		Mean		4.0495	-.0004	.0653	3.9248	4.1774
		Std. Deviation		1.39416	-.00372	.07194	1.26540	1.54337
		Std. Error		.06465				
		95% Confidence Interval for Mean	Lower Bound	3.9224				
			Upper Bound	4.1765				
		Minimum		1.00				
		Maximum		13.00				
Leaf number	1.00	N		24	0	5	21	40
		Mean		6.0417	-.0057	.6582	4.8263	7.4539
		Std. Deviation		3.19618	-.13831	.74254	1.55665	4.36486
		Std. Error		.65242				
		95% Confidence Interval for Mean	Lower Bound	4.6920				
			Upper Bound	7.3913				
		Minimum		3.00				
		Maximum		16.00				
	2.00	N		30	0	5	20	40
		Mean		5.8333	-.0153	.5110	4.8333	6.9128
		Std. Deviation		2.76784	-.10404	.40828	1.87788	3.50080
		Std. Error		.50534				
		95% Confidence Interval for Mean	Lower Bound	4.7998				
			Upper Bound	6.8669				
		Minimum		2.00				
		Maximum		14.00				
	3.00	N		30	0	5	20	41

	Mean		4.6667	-.0006	.5991	3.6004	5.9655
	Std. Deviation		3.29402	-.15729	.71025	1.63312	4.42585
	Std. Error		.60140				
	95% Confidence Interval for Mean	Lower Bound	3.4367				
		Upper Bound	5.8967				
	Minimum		.00				
	Maximum		15.00				
	N		30	0	5	20	41
4.00	Mean		4.7000	-.0085	.4076	3.9445	5.5711
	Std. Deviation		2.27657	-.09995	.39791	1.40577	2.97935
	Std. Error		.41564				
	95% Confidence Interval for Mean	Lower Bound	3.8499				
		Upper Bound	5.5501				
	Minimum		2.00				
	Maximum		12.00				
	N		30	0	5	20	41
5.00	Mean		5.6000	.0001	.4428	4.7149	6.4997
	Std. Deviation		2.41547	-.04952	.24972	1.89313	2.85106
	Std. Error		.44100				
	95% Confidence Interval for Mean	Lower Bound	4.6980				
		Upper Bound	6.5020				
	Minimum		2.00				
	Maximum		11.00				
	N		30	0	5	20	41
6.00	Mean		5.2333	-.0166	.5402	4.2611	6.3445
	Std. Deviation		3.02499	-.13302	.64827	1.54113	4.10677
	Std. Error		.55229				
	95% Confidence Interval for Mean	Lower Bound	4.1038				
		Upper Bound	6.3629				
	Minimum		1.00				
	Maximum		15.00				
	N		30	0	5	21	40
7.00	Mean		5.2667	.0034	.3938	4.5557	6.0666
	Std. Deviation		2.92196	-.07188	.46789	1.90822	3.71153
	Std. Error		.37722				
	95% Confidence Interval for	Lower Bound	4.5118				

	Mean	Upper Bound	6.0215				
	Minimum		2.00				
	Maximum		16.00				
	N		30	0	5	20	39
	Mean		4.4231	-.0069	.4427	3.6924	5.3998
	Std. Deviation		2.24808	-.15725	.68702	.82212	3.38675
	Std. Error		.44088				
8.00	95% Confidence Interval for Mean	Lower Bound	3.5151				
		Upper Bound	5.3311				
	Minimum		1.00				
	Maximum		13.00				
	N		30	0	5	20	40
	Mean		5.8571	-.0287	.6277	4.6454	7.1851
	Std. Deviation		3.40712	-.12043	.54456	2.12035	4.27284
	Std. Error		.64389				
9.00	95% Confidence Interval for Mean	Lower Bound	4.5360				
		Upper Bound	7.1783				
	Minimum		2.00				
	Maximum		15.00				
	N		30	0	5	20	41
	Mean		6.0690	.0169	.6946	4.7412	7.4837
	Std. Deviation		3.53484	-.08306	.44242	2.61069	4.33221
	Std. Error		.65640				
10.00	95% Confidence Interval for Mean	Lower Bound	4.7244				
		Upper Bound	7.4135				
	Minimum		.00				
	Maximum		15.00				
	N		30	0	5	20	40
	Mean		5.0000	-.0242	.6751	3.7273	6.3780
	Std. Deviation		3.64496	-.20392	.83876	1.94951	5.13024
	Std. Error		.67685				
11.00	95% Confidence Interval for Mean	Lower Bound	3.6135				
		Upper Bound	6.3865				
	Minimum		.00				
	Maximum		18.00				
12.00	N		30	0	5	20	41

	Mean		8.7241	-.0173	1.0332	6.7858	10.8749
	Std. Deviation		5.50906	-.22439	1.00308	3.22138	7.09890
	Std. Error		1.02301				
	95% Confidence Interval for Mean	Lower Bound	6.6286				
		Upper Bound	10.8197				
	Minimum		2.00				
	Maximum		25.00				
13.00	N		30	0	5	20	41
	Mean		7.1667	-.0010	.5750	6.0008	8.3666
	Std. Deviation		3.18491	-.09492	.45371	2.25306	4.02162
	Std. Error		.58148				
	95% Confidence Interval for Mean	Lower Bound	5.9774				
		Upper Bound	8.3559				
	Minimum		1.00				
	Maximum		16.00				
14.00	N		30	0	5	21	40
	Mean		5.2667	.0015	.3715	4.6072	6.0476
	Std. Deviation		2.92196	-.05171	.44524	1.99028	3.70957
	Std. Error		.37722				
	95% Confidence Interval for Mean	Lower Bound	4.5118				
		Upper Bound	6.0215				
	Minimum		2.00				
	Maximum		16.00				
Total	N		420	0	0	420	420
	Mean		5.6473	-.0056	.1613	5.3484	5.9784
	Std. Deviation		3.33288	-.01810	.19618	2.94596	3.70364
	Std. Error		.15456				
	95% Confidence Interval for Mean	Lower Bound	5.3436				
		Upper Bound	5.9510				
	Minimum		.00				
	Maximum		25.00				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Homogeneous Subsets

Shoot number

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a,b]	7.00	30	2.0500		
	4.00	30	2.0667		
	8.00	26	2.0769		
	1.00	30	2.3750		
	3.00	30	2.5333		
	6.00	30	2.7000	2.7000	
	2.00	30	2.8333	2.8333	
	14.00	30	2.9000	2.9000	
	5.00	30	3.0000	3.0000	
	11.00	30		3.6207	3.6207
	9.00	30		3.6429	3.6429
	13.00	30		3.6667	3.6667
	10.00	30			4.3103
	12.00	30			4.4138
	Sig.		.085	.072	.130

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.931.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Shoot length

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a,b]	4.00	30	3.0500		
	13.00	30	3.0667		
	5.00	30	3.1833		
	3.00	30	3.3500		
	6.00	30	3.4000		
	2.00	30	3.4500		
	1.00	30	3.6042		
	10.00	30	3.6724		
	7.00	30		4.4417	
	8.00	30		4.4808	
	11.00	30		4.6034	
	14.00	30		4.6500	
	12.00	30		5.0172	
	9.00	30			5.8571
	Sig.		.073	.084	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.931.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Leaf number

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a,b]	8.00	30	4.4231		
	3.00	30	4.6667		
	4.00	30	4.7000		
	11.00	30	5.0000		
	6.00	30	5.2333		
	7.00	30	5.2667		
	14.00	30	5.2667		
	5.00	30	5.6000	5.6000	
	2.00	30	5.8333	5.8333	
	9.00	30	5.8571	5.8571	
	1.00	30	6.0417	6.0417	
	10.00	30	6.0690	6.0690	
	13.00	30		7.1667	7.1667
	12.00	30			8.7241
	Sig.		.100	.095	.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.931.

Appendix 4: Comparison of solid and liquid MS media on rooting of korarima

Descriptive

				Statistic	Bootstrap[a]			
					Bias	Std. Error	95% Confidence Interval	
							Lower	Upper
Root number	1.00	N		30	0	5	20	41
		Mean		17.1333	.0231	1.2374	14.6300	19.5379
		Std. Deviation		6.68366	-.13151	.74976	5.07633	8.03845
		Std. Error		1.22026				
		95% Confidence Interval for Mean	Lower Bound	14.6376				
			Upper Bound	19.6291				
		Minimum		2.00				
		Maximum		29.00				
	2.00	N		30	0	5	20	41
		Mean		10.8667	-.0041	.7591	9.3337	12.2999
		Std. Deviation		4.26480	-.14713	.71025	2.69428	5.46649
		Std. Error		.77864				
		95% Confidence Interval for Mean	Lower Bound	9.2742				
			Upper Bound	12.4592				
		Minimum		.00				
		Maximum		18.00				
	3.00	N		30	0	5	20	40
		Mean		8.2000	.0205	.4186	7.4400	9.0798
		Std. Deviation		2.23453	-.05460	.23546	1.71833	2.64204
		Std. Error		.40797				
		95% Confidence Interval for Mean	Lower Bound	7.3656				
			Upper Bound	9.0344				
		Minimum		5.00				
		Maximum		13.00				
	4.00	N		30	0	5	20	40
		Mean		18.5000	.0260	1.1417	16.2778	20.8312
		Std. Deviation		6.29587	-.15083	.54916	5.03319	7.16768
		Std. Error		1.14946				
		95% Confidence Interval for Mean	Lower Bound	16.1491				
			Upper Bound	20.8509				
		Minimum		7.00				

	Maximum		28.00				
5.00	N		30	0	5	20	41
	Mean		2.4333	.0023	.4128	1.6255	3.2497
	Std. Deviation		2.25424	-.04989	.25382	1.72910	2.73860
	Std. Error		.41157				
	95% Confidence Interval for Mean	Lower Bound	1.5916				
		Upper Bound	3.2751				
	Minimum		.00				
	Maximum		8.00				
6.00	N		30	0	5	20	40
	Mean		5.5667	-.0145	.5452	4.5004	6.6774
	Std. Deviation		2.97905	-.07357	.34962	2.19064	3.52602
	Std. Error		.54390				
	95% Confidence Interval for Mean	Lower Bound	4.4543				
		Upper Bound	6.6791				
	Minimum		2.00				
	Maximum		13.00				
7.00	N		30	0	5	20	40
	Mean		5.3667	-.0125	.7761	3.7694	6.8745
	Std. Deviation		4.27086	-.12037	.63592	3.03804	5.48520
	Std. Error		.77975				
	95% Confidence Interval for Mean	Lower Bound	3.7719				
		Upper Bound	6.9614				
	Minimum		.00				
	Maximum		18.00				
8.00	N		30	0	5	20	40
	Mean		5.2667	.0321	.6530	4.0018	6.6188
	Std. Deviation		3.72256	-.05460	.47327	2.66203	4.55264
	Std. Error		.67964				
	95% Confidence Interval for Mean	Lower Bound	3.8766				
		Upper Bound	6.6567				
	Minimum		.00				
	Maximum		14.00				
9.00	N		30	0	5	20	40
	Mean		7.6000	.0140	.5510	6.5716	8.6996
	Std. Deviation		3.04676	-.08621	.49715	2.00220	3.92180

		Std. Error		.55626				
		95% Confidence Interval for Mean	Lower Bound	6.4623				
			Upper Bound	8.7377				
		Minimum		.00				
		Maximum		16.00				
	10.00	N		30	0	5	21	40
		Mean		3.5417	.0137	.4281	2.7368	4.4444
		Std. Deviation		2.10546	-.05124	.34831	1.34072	2.68544
		Std. Error		.42978				
		95% Confidence Interval for Mean	Lower Bound	2.6526				
			Upper Bound	4.4307				
		Minimum		.00				
		Maximum		9.00				
	Total	N		300	0	0	300	300
		Mean		8.5476	.0005	.3983	7.7517	9.3810
		Std. Deviation		6.61337	-.01693	.33699	5.92039	7.24349
		Std. Error		.38570				
		95% Confidence Interval for Mean	Lower Bound	7.7885				
			Upper Bound	9.3067				
		Minimum		.00				
		Maximum		29.00				
Root length	1.00	N		30	0	5	20	41
		Mean		3.6667	-.0033	.1273	3.4139	3.9230
		Std. Deviation		.69893	-.01839	.11075	.45727	.88709
		Std. Error		.12761				
		95% Confidence Interval for Mean	Lower Bound	3.4057				
			Upper Bound	3.9277				
		Minimum		2.00				
		Maximum		5.00				
	2.00	N		30	0	5	20	41
		Mean		3.5167	-.0004	.2974	2.8754	4.0440
		Std. Deviation		1.62143	-.04544	.21499	1.10368	1.93502
		Std. Error		.29603				
		95% Confidence Interval for Mean	Lower Bound	2.9112				
			Upper Bound	4.1221				
		Minimum		.00				

	Maximum		6.00				
3.00	N		30	0	5	20	40
	Mean		3.5000	.0015	.1636	3.1740	3.8181
	Std. Deviation		.93772	-.02120	.09723	.72286	1.09794
	Std. Error		.17120				
	95% Confidence Interval for Mean	Lower Bound	3.1499				
		Upper Bound	3.8501				
	Minimum		2.00				
	Maximum		5.00				
4.00	N		30	0	5	20	40
	Mean		4.0333	-.0005	.1492	3.7335	4.3103
	Std. Deviation		.80872	-.01734	.05961	.66906	.89443
	Std. Error		.14765				
	95% Confidence Interval for Mean	Lower Bound	3.7314				
		Upper Bound	4.3353				
	Minimum		3.00				
	Maximum		5.00				
5.00	N		30	0	5	20	41
	Mean		.9667	-.0053	.1735	.6344	1.3205
	Std. Deviation		.97108	-.02811	.10803	.70374	1.13106
	Std. Error		.17729				
	95% Confidence Interval for Mean	Lower Bound	.6041				
		Upper Bound	1.3293				
	Minimum		.00				
	Maximum		3.00				
6.00	N		30	0	5	20	40
	Mean		1.3500	-.0021	.0750	1.2027	1.5000
	Std. Deviation		.39719	-.00833	.03714	.30192	.45346
	Std. Error		.07252				
	95% Confidence Interval for Mean	Lower Bound	1.2017				
		Upper Bound	1.4983				
	Minimum		1.00				
	Maximum		2.00				
7.00	N		30	0	5	20	40
	Mean		1.0333	-.0050	.1118	.8077	1.2498
	Std. Deviation		.61495	-.01686	.07553	.43697	.74410

	Std. Error		.11227				
	95% Confidence Interval for Mean	Lower Bound	.8037				
		Upper Bound	1.2630				
	Minimum		.00				
	Maximum		2.00				
8.00	N		30	0	5	20	40
	Mean		1.2000	-.0020	.1003	1.0000	1.3965
	Std. Deviation		.55086	-.01356	.07614	.37072	.67344
	Std. Error		.10057				
	95% Confidence Interval for Mean	Lower Bound	.9943				
		Upper Bound	1.4057				
	Minimum		.00				
	Maximum		2.00				
9.00	N		30	0	5	20	40
	Mean		1.5000	.0035	.1056	1.3036	1.7083
	Std. Deviation		.60172	-.01414	.08460	.44263	.76267
	Std. Error		.10986				
	95% Confidence Interval for Mean	Lower Bound	1.2753				
		Upper Bound	1.7247				
	Minimum		.00				
	Maximum		3.00				
10.00	N		30	0	5	21	40
	Mean		1.7500	.0005	.1660	1.4287	2.0833
	Std. Deviation		.83406	-.01869	.09398	.63499	1.00318
	Std. Error		.17025				
	95% Confidence Interval for Mean	Lower Bound	1.3978				
		Upper Bound	2.1022				
	Minimum		.00				
	Maximum		3.00				
Total	N		300	0	0	300	300
	Mean		2.2619	-.0049	.0879	2.0915	2.4286
	Std. Deviation		1.47530	-.00421	.04317	1.38748	1.55655
	Std. Error		.08604				
	95% Confidence Interval for Mean	Lower Bound	2.0926				
		Upper Bound	2.4312				
	Minimum		.00				

Maximum	6.00					

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Homogeneous Subsets

Root number

	Treatment	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Duncan[a,b]	5.00	30	2.4333					
	10.00	30	3.5417	3.5417				
	8.00	30		5.2667				
	7.00	30		5.3667				
	6.00	30		5.5667	5.5667			
	9.00	30			7.6000	7.6000		
	3.00	30				8.2000		
	2.00	30					10.8667	
	1.00	30						17.1333
	4.00	30						18.5000
	Sig.		.304	.087	.060	.578	1.000	.206

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 29.268.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Root length

	Treatment	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a,b]	5.00	30	.9667				
	7.00	30	1.0333	1.0333			
	8.00	30	1.2000	1.2000			
	6.00	30	1.3500	1.3500	1.3500		
	9.00	30		1.5000	1.5000		
	10.00	30			1.7500		
	3.00	30				3.5000	
	2.00	30				3.5167	
	1.00	30				3.6667	3.6667
	4.00	30					4.0333
	Sig.		.125	.060	.096	.492	.106

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 29.268.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.