

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



SEEDLING BIOMASS, SEED GERMINATION RESPONSES AND
CYTOLOGY OF *HAGENIA ABYSSINICA* (BRUCE) J.F. GMEL.

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**SEEDLING BIOMASS, SEED GERMINATION RESPONSES AND
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ABSTRACT

Seeds (botanical fruit) of *Hagenia abyssinica* (Bruce) J.F. Gmel. collected from several sites were used to study germination response of the same species to different concentrations of gibberellic acid, kinetin, glucose, and vitamin B₆ in the ranges of 10⁻³ M to 10⁻⁷ M. The controls were treated with distilled water. Most treatments were found to increase germination percentages of the species, in the presence of great variation among the different treatments. Of the chemicals used kinetin applied at 10⁻⁴ M were found to increase the germination percentages upto 80%. Vitamin B₆ applied at 10⁻⁶ M and gibberellic acid applied at 10⁻⁴ M also increased seed germination next to kinetin applied at 10⁻⁴ M. The results on germination percentages are discussed. In addition seedling biomass of *H. abyssinica* in time intervals (2, 4, and 6 months) were determined, and the results show that the shoots of the species grow at a faster rate but the root does not.

Somatic chromosomes from the radicles were studied using aceto-orcein, toluidine blue, and feulgen staining techniques. Pollen fertility was examined using the cotton blue lactophenol method. Although the chromosomes were rather small and difficult to study their morphology, it was possible to count them. The chromosome number for the species is 2n=40. The number of nucleoli was determined using silver nitrate as a stain. The maximum number of nucleoli observed was six, but the majority of the cells had only a single nucleolus. Meiosis in pollen mother cells showed 20 bivalence, and meiosis was found to be highly regular. Examination on a total of 4,900 pollen grains revealed that 95-97 percent of the pollen were fertile.

1 INTRODUCTION

In broad terms the environmental factors determining the variations in vegetation are moisture, topography, altitude, distribution of rainfall, and human activity. These vegetation types in Ethiopia have been described and classified by many authors. Among these are Pichi-Sermoli (1957), Mesfin Wolde-Mariam (1972), FAO (1984), Tewolde-Berhan Gebre-Egziabher (1988), Friis (1992) and Ensermu Kelbessa *et al.* (1992). Tewolde-Berhan Gebre-Egziabher (1988) classifies the Ethiopian vegetation into: (1) Moist evergreen montane forests; (2) Dry evergreen montane forests and associated grasslands; (3) Evergreen scrub; (4) Afro-alpine and subafro-alpine zone; (5) Broad leaved deciduous woodlands; (6) Small leaved deciduous woodlands; and (7) Semi-desert and desert vegetation.

But FAO (1984) use a different term for classification of Ethiopian vegetation. In both cases the forest regions of Ethiopia cover an altitudinal range from 450 to 3500 m, with annual rainfall ranges from 200 to 2000 mm. The occurrence of isolated mature trees in farm lands and around religious areas indicate that most forest resources have disappeared in the recent past.

In the early 1950's the Ethiopia forest coverage was about 16 per cent. In the early 1980's the forest coverage was reported to be 3.6 per cent and in 1989 it was estimated only to be 2.7 per cent (EFAP, 1994). The main reasons for the reductions of the forest area are uncontrolled exploitation, shifting cultivation, forest fires, the expansion of permanently cultivated areas, human settlement and population growth (Demel Teketay, 1992).

At present the remaining forests are mainly *Hagenia* forest, *Coniferous* forest, *Aningeria* forest, *Arundinaria* forest, *Olea* forest, and *Baphia* forest (Davidson, 1988). In the humid part of the *Juniperus* forest segment, *Hagenia* forest is best regarded as a fire subclimax of the *Juniperus* forest. Although in other cases the forest types are mixed on many of the east as well as central African mountains, a *Hagenia* zone covers the slopes above the *Arundinaria* forest. In Ethiopia most of the remaining *Hagenia* forests are found in drier habitats but considering the far-reaching degradation of the environment it seems reasonable to suggest that there may have been a distribution in more humid areas. The *Hagenia* forest is characterised by a 20 m high top canopy consisting of *Hagenia abyssinica*, *Rapanea simensis* and *Schefflera volkensii*. In the shrub layer *Hypericum revolutum* plays an important role (Breitenbach, 1963; Davidson, 1988).

Hagenia abyssinica (Bruce) J. F. Gmel. is a monospecific genus belonging to the family *Rosaceae*. The genus is most closely related to the monospecific genera *Leucosidea* Eckl. and Zeyh., *Bencomia* Webb and Bert., *Marcetella* Svent, and *Dendriopoterium* Svent. (Friis, 1992). Morphologically, it resembles *Boswellia*. *H. abyssinica* is easily recognised from distance by its typical parasol crown, its papery scaly bark with spongy-fibrous slash or imparipinnate leaves.

As *H. abyssinica* is found in undifferentiated Afromontane forest and dry single dominant Afromontane forest, it is considered as a typical example of Afromontane endemic (White, 1962). Its altitudinal range is between 2,450 to 3,250 m with annual rainfall of 1000 to 1500 mm (Hedberg, 1989); but according to Friis (1992), the altitudinal range can extend from 1,850 to 3,700 m. In Ethiopia, the species has a general distribution on the highlands

of Tigrai, Wello, Gonder, Gojam, Wellega, Shoa, Arsi, Bale, Sidamo, Kefa, and Harare; outside Ethiopia, it is distributed in Burundi, Rwanda, Sudan, Kenya, Uganda, Tanzania, Zaire, Malawi, and Zambia (Hedberg, 1989; Friis, 1992; Uhlig and Uhlig, 1991; Amsalu Biru, 1994)

The tree reaches up to 25 m high. The bark is red-brown, yellowish-brown or greyish to whitish (Thirakul, undated). Leaves are imparipinnate, clustered at the ends of very stout twigs, covered with golden villose hairs, and very prominent annual rings of stipple scars. The leaves are compound with 5 to 8 pairs of leaflets which are opposite and sessile plus an odd terminal one. The petiole is pinkish when young, thick and flattened with a length of 10 to 15 centimetre (cm). Young branches are pubescent with stiff hairs which are 2 to 5 mm long.

H. abyssinica is dioecious with distinct female and male trees. Flowers are large drooping panicles which measure upto 60 cm long. The receptacle is 'urn shaped'. Male flowers are orange to white, and female flowers are reddish in colour; as a result, the female inflorescence is attractive.

Because, at optimal environmental conditions leaf production, senescence and abscission rates are high, the tree is able to produce a large amount of litter per unit of time, which makes the species an effective nutrient pump. This means that it is useful in the process of fertile soil formation. As the tree is highly branched with a large number of leaves it is also useful for soil and rain-water conservation (Legesse Negash, 1995).

Medicinally, in Ethiopia, the dried female flowers or the whole panicles have long been used as anthelmintic drug under the name 'Koussou' or 'Kosso' (Dale and Greenway, 1961; Lounasmaa *et al.*, 1973; Berhanu Abegaz and Ermias Dagne, 1978; Paulos Ghebre Yohannes and Ermias Dagne, 1983). According to Lounasmaa *et al.*(1973) the flowers of *H. abyssinica* were at one time included in most European modern drug as an effective worm drug. In Ethiopia, this traditional medicine is used to treat tapeworm, a very common infestation among Ethiopians due to the age-long practice of eating raw beef by a large sector of the population. According to Pankhrust (1965) the drug is taken before meals and the amount taken varies depending on the age of the individuals and the region where the individual is present. In rural areas of Ethiopia, the drug is said to be highly effective and the demand is high (Kloos, 1976; Kloos *et al.*, 1978). However, there are serious side effects if an over dose of the drug is taken. The side effects range from feeling weak, nauseated and visual-deficits to death in extreme cases (Chernishov *et al.*, 1978; Edemariam Tsega *et al.*, 1978; Low *et al.*, 1985; Geraldine, 1993). Mesfin Tadesse (1986) also pointed out that flowers of this species can be used to cure skin disease.

In addition to its use as a traditional medicine, farmers also use the branches of the tree for maintaining their beehives. The leaves are also used as mulch and for making mattress in the rural area.

The wood of *H. abyssinica* is used for many purposes, such as for making display cabinets, cupboards, veneers, floors as well as for fuel. It is also used for truck body construction (Russ, 1944 cited in Legesse Negash, 1995). As Legesse Negash (1995) wrote, ".....in the Merkato of Addis Ababa, reportedly one of the biggest markets in Africa, it is not

uncommon to see low-grade timber that is on sell under the trade name of *Kosso* timber. Consequently, to satisfy these high demands (and also since the tree is rather branchy, and so has a short bole), many trees have to be felled at any one time to get the required volume of timber...”. This indicates that the tree is very much needed in the market.

At present, knowledges about the cytology of the species and the physiological requirements for a successful establishment of seedling of *H. abyssinica* is lacking, whereas the species is being selectively logged for timber, furniture making and other uses. Therefore, in Ethiopia this valuable tree species is highly endangered.

The main objective of this investigation is to provide some information about seedling biomass, seed germination responses, and cytology of the species.

2 LITERATURE REVIEW

2.1 METHODS OF PLANT PROPAGATION

Sexual and asexual developmental life cycles are means of plant propagation. The asexual developmental life cycle utilises various vegetative propagation methods like cutting, grafting, layering, budding, and micropropagation. Vegetative propagation methods enable to preserve the unique characteristics of any individual parent plant in the offspring plants, by preserving the genotypes of the parent plant intact in the offspring plant.

The sexual cycle utilises propagation by seeds. This results in the offspring plants receiving the genetic contribution of the two parents (Hartmann *et al.*, 1990). A seed contains an embryonic plant in an inactive form, and germination is its resumption of growth. The first external evidence of germination is the emergence of the growing radicle and its rapid development into the primary root. Thus, the criterion of germination is the emergence of the radicle. Germination can be defined as the stage in the life cycle of a plant when embryonic growth is continued or once more resumed, so as to lead to the emergence of the growing parts (Thomas *et al.*, 1960). In germinating seeds, three distinct stages are evident, namely: (a) imbibition of water (b) cell elongation and (c) increase in cell number. Germination starts only when both endogenous as well as exogenous factors become favourable (Colorado *et al.*, 1994).

2.2. FACTORS THAT AFFECT GERMINATION OF SEEDS

2.2.1 EXTERNAL FACTORS

2.2.1.1 TEMPERATURE

Though the ranges of temperature required for seeds to germinate varies depending upon the species, the germination of all seeds is prevented at very low or very high temperatures. There is an optimal temperature, below and above which germination is delayed but not prevented (Mayer and Poljakoff-Mayber, 1975). The optimal temperature may be taken to be that at which the highest percentage of germination is attained in the shortest time.

The effects of temperature on germination is not independent of other factors. Instances of interdependence between temperature and light are known for celery and other seeds (Mac Donald and Hart, 1981). High temperature affects the plant pigment, phytochrome, that mediates promotion of germination by light (Mac Donald and Hart, 1981; Karssen and Hilhorst, 1992). Phytochrome occurs either in a stable inactive form (Pr) with an absorption maximum at 660 nm or in an unstable physiologically active form (Pfr) with an absorption maximum at 730 nm. Increased temperature is known to accelerate inactivation of Pfr which may occur either by reversion to Pr or by destruction of the Pfr totally.

Temperature also helps to release seed dormancy of some species. For instance, hard seed-coat of *Rhus javanica* becomes permeable and the seed becomes germinable after exposure to high temperature of about 50 to 75° C for an appropriate length of time (Washitani,

1988). In dry seeds, temperature-dependent dormancy breaking mechanisms: (1) results in dormancy release during artificial dry storage or under natural conditions during the dry season; and, (2) controls the rate of hard seed coat breakdown in seeds which possess non-dormant embryos but are enclosed in hard seed coat.

In addition to the above effects, temperature also affects germination by affecting seed development (Pannangpetch and Bean, 1984). For example, increasing temperature during seed development (15-25° C) significantly increased seed germination in *Dactylis glomerata* (Probert, 1992).

2.2.1.2 WATER

The uptake of water by seeds, which occurs through imbibition, is the first process that takes place during seed germination. The composition of the seed, the availability of water in liquid or gaseous forms in the environment, and the permeability of the seed coat or fruit to water determine the extent to which imbibition occurs. Though protein is the chief water imbibing component of the seed, cellulose and pectin also contribute to the swelling of seeds, whereas it was found that starch does not add to the swelling (Mayer and Poljakoff-Mayber, 1975).

Germination is reduced by water stress. For example, germination of lettuce and wheat in saline soil is reduced by a decrease in water potential (Kaufmann and Ross, 1970; Berrie, 1984). On the other hand, germination is reduced under water-logged conditions. This is because, under water-logged conditions, there occurs oxygen stress. Besides the moisture

content of the growing medium, the moisture content of the dry seed itself also affects germination.

In some species the presence of hard seed-coat prevents imbibition of water by seeds. For example, the seeds of *Iris lorteti* and *Sesbania punicea* possess hard seed-coats that hinder germination by preventing water uptake (Bevilacqua *et al.*, 1987).

3.2.1.3 LIGHT

Although seeds of most cultivated plants usually germinate equally well in the dark and in the light much variability in the behaviour toward light is observed in other plants. Seeds may be divided into those which germinate: (a) only in the dark; (b) only in continuous light; (c) after being given a brief illumination; and, (d) those which are indifferent to the presence or absence of light during germination (Mayer and Poljakoff-Mayber, 1975). The light responses of seeds of many species have been shown to be markedly influenced by other factors in the environment, like some circumstances under which latent effects of light are manifested and associated with conditions of physiological stress. Water stress is a particular condition which can either induce light requirement or enhance the light inhibition for germination. In most cases, inhibitory effects of light on seed germination require long period of exposure and are believed to act through phytochrome.

It has been shown that very short exposure time to light during soil disturbances may induce germination (Karssen and Hilhorst, 1992). In the absence of light, the promotive effect of light for germination of seeds may be compensated for by high temperature and endogenous

nitrate (Takaki *et al.*, 1981; Takaki and Zaia, 1984). Some seeds naturally contain Pfr at the time of shedding or after a brief exposure to light during field disturbances. At high level of temperature treatment, a low level of Pfr is sufficient to promote germination.

Beside its effect through the phytochrome control, light can enhance germination indirectly by stimulating the synthesis of growth stimulating factors like gibberellic acids (Derkx and Karssen, 1993).

2.2.1.4 SALINITY

In soils of cultivated and natural habitats, where there is salinity stress, there occurs a reduction in germination percentage and delay in the onset of seed germination (Scorer *et al.*, 1985). On the other hand, although some seeds can germinate at high salt concentration, the higher salt concentration leads to reduction in germination percentage (Khan and Ungar, 1996).

The interaction of environmental parameters such as temperature and light modifies the effects of salinity on seed germination (Khan, 1966). The decrease in the percentage of germination may be due to: (1) a decrease in the rate of water uptake due to osmotic effect; (2) toxic effects associated with excess salinity; and (3) its effect on the activity of lipase (Reynolds and Thompson, 1973; Younis *et al.*, 1987).

2.2.2 INTERNAL FACTORS

2.2.2.1 OXYGEN AND CARBON DIOXIDE

As germination requires expenditure of energy which is produced by oxidation process, the presence or absence of oxygen affects germination. Most seeds are incapable of proper germination if the oxygen concentration is lower than in the atmosphere (Prasad *et al.*, 1983). As water logging results in oxygen deficiency, it leads to lowering of respiratory activity as well as the activity of several hydrolases necessary for immobilisation of storage food in seeds. Oxygen deficit results in the increment of the endogenous levels of the growth inhibitors, and in the decrement of the growth promoters like gibberellins and cytokinins (Mayer and Poljakoff-Mayber, 1975).

Unlike oxygen, most seeds fail to germinate if the partial pressure of carbon dioxide is greatly increased. However, in some cases increased carbon dioxide concentration was found to increase germination (Mayer and Poljakoff-Mayber, 1975). Further more, Karssen and Hilhorst (1992) reported that carbon dioxide breaks seed dormancy in *Trifolium subterraneum* optimally at 2.5 %, but germination is inhibited at 10 to 15 % of carbon dioxide concentration. Carbon dioxide interacts with ethylene such that it blocks the ability of ethylene to promote lettuce seed germination if seeds were imbibed in sodium chloride solution, thus being the first competitive inhibitor of ethylene action (Abeles, 1986).

2.2.2.2 GIBBERELLINS

Gibberellins are a large family of diterpene acids. The gibberellins are now known to be of wide spread occurrence in higher plants where they are generally accepted to function as hormones. Gibberellic acid, being a member of gibberellins, breaks dormancy and induces germination of several types of seeds (Chen and Park, 1973; Mayer and Poljakoff-Mayber, 1975; Fountain and Bewley, 1976; Dommes and Northcote, 1985a; Abeles, 1986; Groot *et al.*, 1988; Hsiao *et al.*, 1988). The mechanism by which gibberellic acid stimulates germination varies from species to species. The effect of gibberellic acid in promoting germination seems similar to that of light, but gibberellic acid is more effective than red light in reversing the high temperature inhibition of germination. For example, the inhibitory effect of high temperature treatment on celery seeds was completely removed by allowing the seeds to imbibe a mixture of GA₄ and GA₇ (Biddington and Thomas, 1978).

Gibberellic acid (GA₃) can also reverse the inhibition of germination that is caused by high osmotic pressure. For example, lettuce seeds which had 82 % germination in the dark (in the presence of water) gave only 22 % germination in 0.15 M mannitol (Mayer and Poljakoff-Mayber, 1975). But addition of 35 ppm GA₃ to the mannitol increased the germination percentage to 61. This shows that GA₃ reverses osmotic inhibition.

2.2.2.3 CYTOKININS

Cytokinin is a generic name used for chemical substances which promote cell division and exert other growth regulatory functions in the same manner as kinetin or zeatin. Cytokinin

consists of natural and synthetic compounds. Cytokinins are involved in breaking seed dormancy, facilitating growth rate, flower initiation, sex determination, fruit set, fruit growth, fruit ripening, and rooting (Skoog and Armstrong, 1970; Hall, 1973; Vlitos and Most, 1973; Leopold and Kriedemann, 1975; Biddington and Thomas, 1978).

Kinetin as a member of cytokinin promotes germination of seed of many species (Hsiao *et al.*, 1988). The mechanism by which kinetin enhances seed germination are:

1. At lower water potential it enhance water uptake by increasing either membrane permeability or the internal concentration of osmotically active solutes (Kaufmann and Ross, 1970);
2. It may reduce the moisture requirement of the tissue so that germination and growth can still take place under inadequate moisture supply or promote uptake of water under conditions of salt stress (Bozcuk, 1981);
3. It may reduce the strength of seed-coat and interacts with other inhibitors such as Absciscic acid and light to relieve their inhibitory effects (Kaufmann and Ross, 1970; Reynolds and Thompson, 1973).

2.2.2.4 ABSCISIC ACID

Absciscic acid prevents or delays the germination of many kinds of seeds. Because it is found in seeds of various plants, it is an important endogenous inhibitor of germination (Meyer *et al.*, 1973; Colorado *et al.*, 1994). Moreover, exogenous application of Absciscic acid inhibits germination of seeds (Meyer *et al.*, 1973; Dommès and Northcote, 1985b; Simmons, 1987).

As the mode of action of some plant growth regulators involves changes in RNA and protein synthesis, Absciscic acid regulates the synthesis of certain enzymes, related to germination, and the transcription of specific mRNA (Dommes and Northcote, 1985a, b; Rodriguez *et al.*, 1985; Simmons, 1987; Colorado *et al.*, 1994).

On the other hand, the primary action of Absciscic acid in inhibiting seed germination (in some seeds like mustard and rape) is through control of water uptake by the embryo tissue rather than control of RNA and protein synthesis (Schopfer *et al.*, 1979; Schopfer and Plachy, 1984). The inhibitory effect of Absciscic acid on seed germination is enhanced by increasing temperature and decreasing ion and water uptake (Liptay and Schopfer, 1983).

2.2.2.5 ETHYLENE

Because ethylene is produced by soil micro-organisms, plant roots and imbibed seeds, it is a common constituent of the soil environment (Etherington, 1983). Ethylene has been shown to promote seed germination though seeds of many plants do not respond to ethylene and some of its promotive effects are very small. Moreover, inhibitory effect of ethylene in germination has been reported (Abeles, 1986).

Generally, ethylene as a germination stimulator in many seeds is produced during pregermination period. It is known that factors which promote or prevent germination are associated with the control of embryonic growth. Thus the germination promoting activity of ethylene may be associated with its ability to increase the growth potential of the embryo (Hasegawa *et al.* 1994). That is, ethylene affects only the initial stages of germination by

promoting the expansion of radicle cells in the embryonic hypocotyle. Gallards *et al.*, (1994) found that ethylene does not seem to have a significant effect on the mitotic activity of the radicular meristems, rather it has a physiological function in the differentiation and elongation zones.

2.2.2.6 VITAMINS

Vitamins are organic compounds which, in low concentration, have catalytic and regulatory functions in cell metabolism. Normally, green plants do not suffer from vitamin deficiencies since they synthesise their own. Those organs of the plant that do not synthesise a sufficient amount of vitamin for their needs have it translocated to them from another organ. For this reason, plant organs such as roots isolated in tissue culture some times need the addition of certain vitamins (Devlin, 1968)

Members of the vitamin B complex do act as coenzymes in the enzymatic reactions by which carbohydrates, fats and proteins are metabolised. Vitamin B₆ (pyridoxine) is a necessary growth factor for most root tissue cultures (Robinson, 1973). Almestrand (1950, 1951) has observed a decrease in meristematic activity of isolated roots due to Vitamin B₆ deficiency. Addition of pyridoxine reverses the depressed growth effect of desoxypyridine in tomato root culture (Boll, 1954).

2.2.3 STORAGE CONDITIONS OF SEED

Storage conditions determine the length of time for which seeds can remain viable. Storage conditions that are required to maintain viability are different for different seeds. In some seeds drying causes very rapid loss in viability while in other cases only on drying will the seeds remain viable (Roberts, 1972; Villiers, 1974; Mayer and Poljakoff-Mayber, 1975).

Viability of seed is retained for long periods of time in seeds having a hard seed coat, but even under favourable storage conditions many seeds are relatively short lived. The main factors which influence the longevity of seeds in storage are temperature, moisture content and oxygen pressure (Roberts, 1961). Investigation on storage conditions has shown that the lower the temperature and the lower the moisture content the longer the period of viability. Most of the time, seeds loss viability if (1) the temperature is too low, (2) seeds are subjected to extreme desiccation, and (3) the moisture content is too high (above 30%).

2.3 CYTOLOGY

2.3.1 HETEROMORPHIC SEX-CHROMOSOMES

Heteromorphisms concerned with sexuality is not common in flowering plants as most of them are hermaphrodites. However, dioecy in plants is not negligible (Parker, 1990). There are four major ways by which heterogametic sex may be identified (Correns, 1928 cited in Westergaard, 1958):

1. Cytologically with heteromorphic sex-chromosomes;

2. Through sex-linked inheritance;
3. By competition (certation) experiments;
4. From crossings between dioecious and bisexual species.

Heteromorphic sex-chromosomes in higher plants were first described in *Melandrium rubrum* and *Rumex acetosa* (Blackburn, 1923, and Kihara and Ono, 1923 cited in Parker, 1990). Then after, different workers at different times claimed the presence of heteromorphic sex-chromosomes in flowering plants. For example, Allen (1940) suggested the presence of heteromorphic sex-chromosomes in about 90 species, whereas according to Parker (1990) "differentiated sex-chromosomes can be confidently claimed in only 5 families of flowering plants, in seven species and two species-groups...". Most of the time, one sex, which is mostly the male is heterozygous (heterogametic), the other (i.e. the female) being homozygous (homogametic).

In heteromorphism, the difference can be either in chromosome number, DNA amount or DNA quality or a combination of these. For instance, *Viscum fischeri* possesses male structural heterozygosity as well as numerical differences in which females have $2n=24$ while males have $2n=23$ (Barlow and Wiens, 1976). Mostly, unlike animals, sex-chromosomes in plants are the largest in the complement and most Ys or summed Y-multiples are larger than the X-chromosomes (Parker, 1990).

2.3.2 NUCLEOLI

The nucleolus is the structure visible in interphase nucleus and is formed by the action of the genes present in the nucleolar organiser region (NOR). Thus the nucleolar organiser region is part of a chromosome, and the nucleolus is a structure containing this chromosomal part and in addition the material which accumulates around the nucleolar organiser region. In other words, the NORs are the sites where ribosomal Deoxyribonucleic acid (rDNA) genes are localised. The nucleolus, far from being a stable organelle, may exhibit variations in size, shape and ultrastructural organisation during the cell cycle or as a result of experimental or pathological conditions. A careful study of the nucleolar organiser chromosomes at the appropriate stages of the cell cycle indicated that the nucleolus is organised at a specific site. In diploid species with one pair of nucleolus organising chromosomes, the resting nucleus has either two nucleoli or one nucleolus with a larger volume (Garber, 1979). The latter results from fusion of the two smaller nucleoli.

It has been established that the number of NORs as well as their locations on chromosomes are species specific characteristics. However, a diploid organism normally has a minimum of one homologous chromosomes, each bearing a single NOR. Nucleoli disappears during late prophase and reform in the following telophase. The maximum number of nucleoli can be detected during telophase. In the telophase-interphase progression, fusion of nucleoli takes place and thus most interphase nuclei contain less than the maximum number of nucleoli per nucleus. Thus, the frequency of interphase nuclei exhibiting the maximum number of nucleoli, that is, nucleoli where fusion of nucleoli has not taken place, is very small.

Nucleoli organiser regions on metaphase chromosomes and nucleoli stain darkly with silver nitrate. Thus, their number can be determined by silver nitrate staining method (Goodpasture and Bloom, 1975 cited in Hernandez-Verdun, 1983). However, direct localisation of NORs to chromosomes is difficult, if not impossible, in species having small chromosomes. As there is a correspondence between the NORs and the maximum number of nucleoli, however, one can infer the number of NORs from the maximum number of nucleoli.

The fibrillar centres, the dense fibrillar component, and the granular component together with the condensed chromatin and nucleolar spaces form the fine structure of nucleoli (Jordan, 1991). As Fakan and Hernandez-Verdun (1986) pointed out there are different organisational features of chromatin in the nucleolar organiser regions containing nuclear areas.

Ring shaped nucleoli, compact nucleoli, and nucleoli with nucleolenema are the different classes of nucleoli (Schwarzacher and Wachtler, 1983). But the different types of nucleoli are never found at the same time in one particular cell. A particular type of nucleolus is restricted to cells with a certain activity which shows that the different types of nucleoli reflect different levels of functional activity.

A description of nucleoli comes following the work of Ruzicka (1899) (cited in Schwarzacher and Wachtler, 1983) using toluidine blue and silver stain in studying the cell nucleus. Then after silver (particularly silver nitrate) is used to stain the nucleolus and

NORs (Tandler, 1959 ;Das, 1962). Now a days silver stain show that this stain specifically stain protein components of a cell.

3 MATERIALS AND METHODS

3.1 INFLORESCENCE COLLECTIONS

Mature female inflorescences were collected from Denkoro-Washa located at about 200 km west of Dessie (South Wello Administrative Region) in Borena District, and from Kolobo Farmers Association, about 25 km west of Addis Ababa on the main road to Wellega. The inflorescences were kept at room temperature for about three weeks in Plant Physiology laboratory of the Department of Biology. The seeds (botanically, fruits) were separated from the panicles by gently squashing portions of inflorescence between the hands.

The male inflorescence used to study pollen fertility and meiosis were collected from Dessie Tena Tabia, Denkoro-Washa Forest, Menagesha Forest (about 35 km west of Addis Ababa), Science Faculty Campus of Addis Ababa University, and Goro-Algee Forest (about 30 km northwest of Addis Ababa).

3.2 BIOMASS STUDY

3.2.1 TRANSPLANTATION OF SEEDLINGS

H. abyssinica seedlings that were used for biomass study were kindly provided by the Rapid Propagation of Trees Project, Addis Ababa University, Faculty of Science. The seedlings had been maintained in small polythene bags (7 cm diameter) for about 10 months. On August 26th 1996, ninety of these seedlings were picked at random from a bed of seedlings

and were transplanted into a larger (17 cm diameter) polythene bags containing a mixture of sand, soil and animal dung in a ratio of 3:3:2, respectively. The bags were pierced at several places for aeration and drainage purposes. The seedlings were maintained in a glasshouse (22.5 m², 3.10 m high) and were watered once a day.

3.2.2 BIOMASS MEASUREMENTS

For biomass measurements, the 90 seedlings were randomly divided into 3 groups of 30 seedlings each. On August 26, 1996 (date of transplantation) data on the shoot height and the number of leaflets were taken on all the three groups of seedlings. Again on the 26th of October 1996, similar data were taken on all the three groups. On the latter date, biomass measurements of shoot and root, and root height measurements were done for the first group of seedlings. Similarly, biomass measurements of shoots and roots, measurements of root height and shoot height, as well as counting of the number of leaflets were made for the second and third groups of seedlings on December 24th, 1996 and February 22nd, 1997, respectively. Further, for the third group dry biomass measurements were done at the end of the experiment (March, 1997). In this case the roots as well as the shoots were placed on the table in Physiology laboratory for about three weeks and then the shoots and roots were wrapped in paper envelope and kept in an oven at about 70° C until constant weight was obtained. Biomass measurements were made using triple beam balance. In all the cases, the shoots were cut off at soil surface level, and the soil substrate with the roots were taken from the harvested area for separating the roots from the soil. The roots after removing the polyethylene bags, were washed with tap water by placing them on 0.5 mm mesh sieve to

prevent loss of delicate root hairs and at the last the fibrous roots with their pieces were collected from the sieve for biomass measurement with the remaining roots.

During the course of the experiment, portions of the seedlings suffered from fungal and insect attack. Hundred grams of crude sulphur powder was dissolved in two litres of distilled water at 35° C and sprinkled on the parts of leaves attacked with fungi (Powdery mildew) in four days interval for about 40 days. In addition, to prevent insect (Aphid) attack, 100 ml of malathion diluted into 1000 ml distilled water, was sprinkled on portions of leaves where insects can be seen with naked eyes in five days interval for 15 days.

3.3 SEED GERMINATION

3.3.1 PREPARATION OF CHEMICALS

Gibberellic acid, kinetin, glucose and vitamin B₆ were used to investigate their effects on seed germination of seed of *H. abyssinica*. All the chemicals were "Plant Tissue Culture Tested " and were purchased in powder form from Sigma Chemical Company (ST. Louis, MO, USA). Stock solutions of 10⁻³ M gibberellic acid and kinetin were prepared by dissolving the required amount of the powder in 50-55 drops of 1M NaOH. These solutions were diluted with distilled water and then neutralised with 50-55 drops of 1M HCl. The pH of the solutions were adjusted to 7.0. The volume was adjusted to 500 ml by adding distilled water. Glucose and vitamin B₆ were dissolved in distilled water. Concentrations ranging from 10⁻⁴ M to 10⁻⁷ M were then prepared by serial dilution from the respective stock solutions. The controls were treated with distilled water.

3.3.2 SEED TREATMENTS

Two hundred and ten Petri dishes overlaid with tissue paper were prepared. On each Petri dish, thirty seeds were spread onto the moistened tissue paper. For each concentration (10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, and 10^{-7} M) of gibberellic acid, glucose, kinetin and vitamin B₆ there were 5 replicates which gave a total of 21 treatments (including the control) for seeds obtained from each administrative region (Shoa and Wello). The Petri dishes were labelled and placed randomly in the tissue culture room. A plastic sheet was then spread over the Petri dishes at a height of half a meter to reduce evaporation. The plastic sheet was pierced with pins at several places for aeration. Five millilitres of the corresponding test solutions was poured onto each Petri dish containing seeds. The temperature of the tissue culture laboratory during conducting the experiment was in the range of 25 to 27° C and was lit with a fluorescent lamps at a quantum flux density of ca $40 \mu \text{ mol m}^{-2} \text{ s}^{-1}$. Periodical supplements of 3 ml of the respective solutions were made as soon as these chemicals were depleted. The number of seeds germinated were recorded daily until no more seeds germinated. Radicle length was measured just before ending the germination experiment.

3.4 CYTOLOGICAL STUDY

3.4.1 PRE-TREATMENT AND MACERATION

Cytological investigations were made in Genetics laboratory. Seeds were germinated between moist tissue paper in Petri dishes for 10 to 15 days at room temperature. The radicles were cold treated by keeping the Petri dishes, containing germinating seeds

wrapped with wet tissue paper, in a beaker of ice for 15 hours. Then the radicles were fixed in methanol- acetic acid (3:1) for 24 hr at 4° C. The radicles were rinsed in distilled water for 30 minutes with 10 changes macerated in 6% aqueous solution of cellulase-pectinase at 35-37° C for 2 hr. The enzyme solution was decanted and the tips of the radicles were rinsed in distilled water before used for slide preparation.

Alternatively, root tips that were used for toluidine blue and Feulgen staining techniques were macerated with 1N HCl in a water bath at 60° C for 10-15 minutes, instead of enzyme treatment.

3.4.2 SLIDE PREPARATION

After pipetting the tips of enzyme treated radicles onto glass slide, the water was blotted off with filter paper, and the tips of radicles were mashed with tips of forceps in a few drops of fresh fixative. The cells were spread by a strong air blow on the slide. The slides were allowed to air dry at room temperature for 48 hours, before used for staining.

3.4.3 STAINS AND STAINING

Aceto-orcein stain solution was prepared by dissolving 2.0 g orcein in 100 ml of 45 % glacial acetic acid by gentle boiling. Then the solution was cooled, and filtered using Whatman filter paper number one. Also 1.5% of toluidine blue was prepared by dissolving 1.5 g of toluidine blue powder directly in 100 ml of distilled water. Feulgen stain solution from BDH suppliers was used.

After adding a drop of 2 % aceto-orcein on air dry slide preparations, the slides were covered with cover slip and were left for about 30 minutes to stain. After removing the excess stain by pressing on the slide with a thumb between tissue paper, the edges of the cover slip were sealed with paraffin wax, and the preparation was observed with the light microscope. For toluidine blue staining, the HCl treated radicles were stained with toluidine blue solution in a watch glass for about 10 minutes. The radicles were placed on a glass slide, the tips removed and squashed in a drop of the stain. The edge of the cover slip was sealed with paraffin wax. For Feulgen staining, the HCl treated radicles were rinsed in water and allowed to stain in Feulgen solution in the dark for a minimum of 3 hr. The stained tips were removed from the radicles on a glass slide and squashed in a drop of 45 % acetic acid. The edges of the cover slip is then sealed. Photomicrographs of the chromosomes were taken for chromosome analysis.

3.4.4 MEIOTIC CHROMOSOMES

To study parental meiosis, florets (collected from Science Faculty of Addis Ababa University) at the right stages of development were fixed in ethanol: chloroform: acetic acid (6:3:1) for about 24 hours at 4°C, and stored in 70% ethanol at 4°C temperature until used. Florets were stained in aceto-carmin for about four weeks at room temperature. The young pollen mother cells were released in a drop of 45% acetic acid on a glass slide and squashed under a cover slip. The preparations were made Semi-permanent by sealing with paraffin wax around the edges of the cover slip. Alternatively, the fixed florets were treated with 1N HCl at 60° C for about 10-15 minutes. These were either stained and squashed in toluidine blue or stained in Feulgen and squashed in 45 % acetic acid.

3.4.5 SILVER-STAINING

Radicles used to prepare air dry slides for nucleoli counting were not cold pre-treated, that is, the air dry slides were prepared from tips of radicles that were directly fixed in 3:1 methanol: acetic acid. Several drops of 50 % aqueous silver nitrate solutions were placed on air-dried slides and then covered with a piece of nylon cloth. The slides were then placed in a Petri dish lined with moist filter paper and incubated at 60° C for 5 hr, until the nylon cloth turned dark brown. Then the slides were taken out of the Petri dish, the nylon cloth was removed, and the slides were rinsed with tap water, air dried for 24 hr and mounted with Dpx mountant. Light microscope was used to count the nucleoli.

3.4.6 POLLEN FERTILITY

To determine the percentage of pollen fertility, freshly released pollen grains of *H. abyssinica* were placed in a drop of cotton-blue-lactophenol on a glass slide and covered with a cover slip. This preparation was done in the field at the site of the plant. Scoring was done later in the laboratory using a light microscope. In scoring, unstained and partially stained pollen grains were classified as sterile, and fully stained pollen grains as fertile. A total of 4, 929 pollen grains were counted.

3.5 STATISTICAL ANALYSES

For biomass measurements at seedling stage and germination physiology analysis of Variance (ANOVA) followed by post-hoc comparison using Least Significant Difference (LSD), and correlation analysis were done to compare the data. Mean relative growth rate (RGR) were estimated by the non-pairing method (Causton and Venus, 1981):

$$\text{Mean relative growth rate} = \frac{\text{Mean}(\ln W_2) - \text{Mean}(\ln W_1)}{t_2 - t_1}$$

where W and t are total weight and time (in this case month), respectively

4 RESULTS

4.1 BIOMASS AT SEEDLING STAGES

The increase in biomass production of the seedlings at different growth intervals (i.e., 2, 4 and 6 months of growth) is shown in Fig. 1. The biomass produced at these intervals were significantly different from each other at $p \leq 0.05$. The minimum and maximum values for shoot biomass were 14 g/seedling and 105 g/seedling, respectively. Also, the minimum and maximum values of root biomass were 3 g/seedling and 57 g/seedling, respectively.

By the end of the experiment, dry biomass of shoots and roots were measured, and mean relative growth rate of seedlings was calculated (Fig. 2). The ratio of root to shoot biomass for the three groups differs significantly, with that of the third group being the least. In addition, measurements of shoot height and root length showed a rapid increase after 6 months of starting the experiment in which the heights of the seedlings harvested in February, 1997 were much greater than the height of seedlings harvested in December 1996 and October 1996 (Fig. 3).

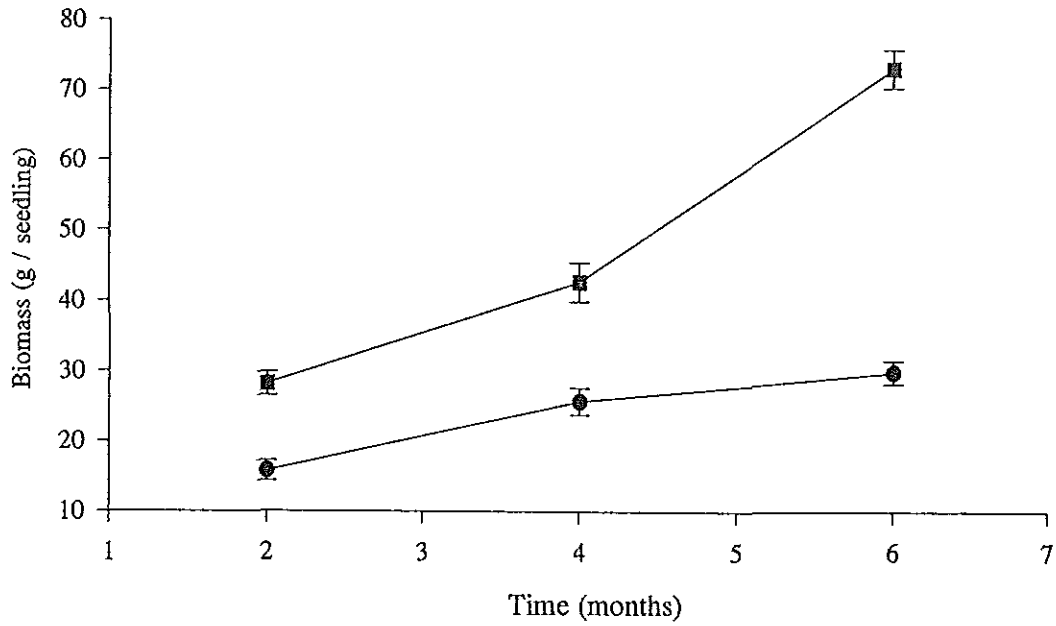


Figure 1. Biomass of shoots and roots of *H. abyssinica* at seedling stages at different time intervals. (Filled square is for shoots biomass; filled circle is for roots biomass).

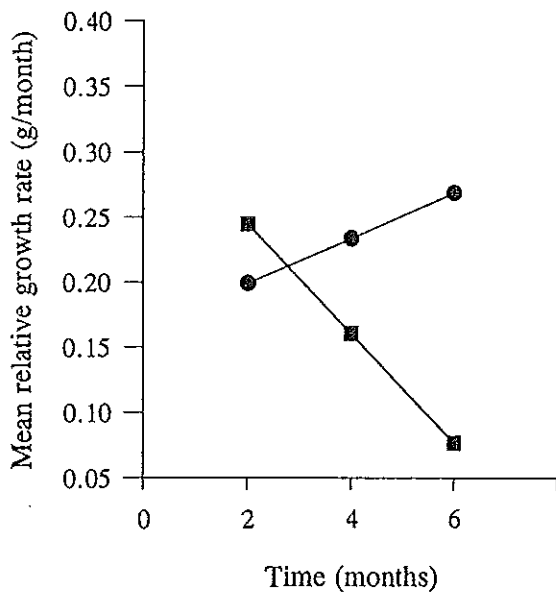


Figure 2. Mean relative growth rates of shoots and roots of *H. abyssinica*. Filled circles are values for shoots, and filled squares are for roots.

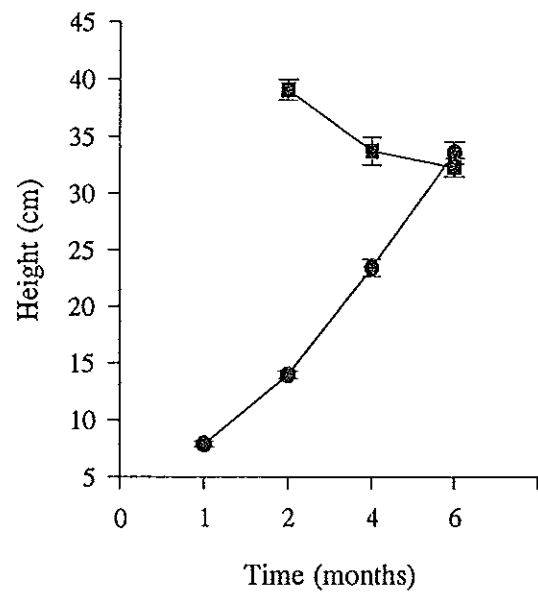


Figure 3. Time course of roots and shoots height of seedlings of *H. abyssinica*. Filled circles and squares are values for shoots and roots respectively.

4.2 GERMINATION RESPONSES OF SEEDS

The effect of the various concentrations of treatments on seed germination percentage of samples obtained from Shoa and Wello are shown in Fig. 4 and Fig. 5 respectively. Although germination percentage obtained after treatment with 10^{-7} M GA₃ was lower than the control (but not significantly), it was significantly lower than the rest concentrations of GA₃, Kinetin and Vitamin B₆ treatments (Appendix 1).

As to the Least Significance Difference (LSD) the results in Appendix 1 show, that there were significant difference in germination percentages of various treatments with control except between control and GA₃ treated at 10^{-7} M. Results in Appendix 2 also shows the same pattern of significance differences. Kinetin, and vitamin B₆ treated seeds gave significantly higher germination percentage (75.2% and 70%) at 10^{-4} M and 10^{-6} M respectively. The corresponding results on seeds collected from Wello in Appendix 2 indicate that kinetin treated seeds gave significantly higher germination percentage (80%) at 10^{-4} M (Fig. 5.). Also, significantly higher germination percentages were obtained when similar seeds were treated with 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M of GA₃ as well as 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, and 10^{-7} M, of vitamin B₆ and 10^{-3} M, 10^{-5} M, 10^{-6} M, and 10^{-7} M of kinetin.

The highest mean radicle length of seeds germinated was obtained from seeds collected from Wello treated with 10^{-4} M of glucose. Table 1(A and B) show values for the mean radicle length of seeds germinated with various treatments at different concentrations of

gibberellic acid, glucose, kinetin, and vitamin B₆ of seeds collected from Shoa and Wello.

In most cases there exist significant variation.

Table 1. Mean length of radicles of *H. abyssinica* seeds germinated in response to the various treatments. (SE = Standard error).

A) Seeds collected from Shoa.

Chemicals (M)	Mean radicle length of seeds germinated \pm SE			
	Gibberellic acid	Glucose	Kinetin	Vitamin B ₆
10 ⁻³	1.3 \pm 0.002	1.3 \pm 0.003	0.8 \pm 0.023	1.2 \pm 0.005
10 ⁻⁴	1.4 \pm 0.004	1.85 \pm 0.012	0.9 \pm 0.003	1.18 \pm 0.005
10 ⁻⁵	1.4 \pm 0.005	1.5 \pm 0.010	1.2 \pm 0.03	1.4 \pm 0.006
10 ⁻⁶	1.2 \pm 0.005	1.40 \pm 0.007	1.2 \pm 0.005	1.75 \pm 0.009
10 ⁻⁷	1.5 \pm 0.004	1.50 \pm 0.007	1.23 \pm 0.004	1.7 \pm 0.009
Control	1.2 \pm 0.003	1.2 \pm 0.003	1.2 \pm 0.003	1.2 \pm 0.003

B) Seeds collected from Wello

Chemicals (M)	Mean radicle length of germinated seeds \pm SE			
	Gibberellic acid	Glucose	Kinetin	Vitamin B ₆
10 ⁻³	1.16 \pm 0.012	1.18 \pm 0.003	0.8 \pm 0.023	1.4 \pm 0.003
10 ⁻⁴	1.5 \pm 0.003	1.95 \pm 0.012	0.9 \pm 0.0032	1.5 \pm 0.006
10 ⁻⁵	1.34 \pm 0.001	1.65 \pm 0.004	1.1 \pm 0.004	1.5 \pm 0.008
10 ⁻⁶	1.18 \pm 0.008	1.60 \pm 0.005	1.4 \pm 0.005	1.6 \pm 0.002
10 ⁻⁷	1.2 \pm 0.006	1.5.00 \pm 0.008	1.3 \pm 0.004	1.4 \pm 0.007
Control	1.35 \pm 0.004	1.35 \pm 0.004	1.35 \pm 0.004	1.35 \pm 0.004

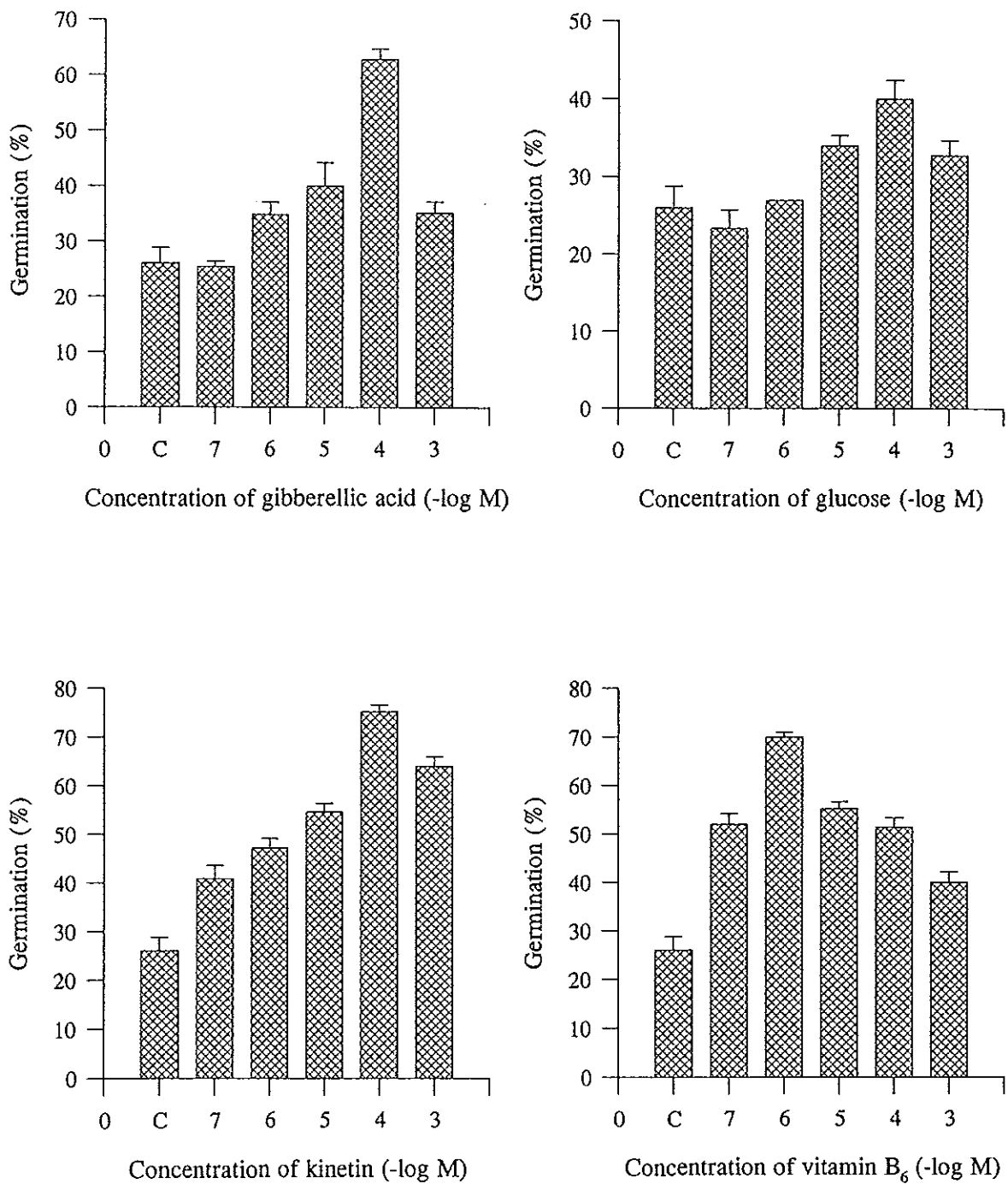


Figure 4. Mean (+SE) final germination percentages of *H. abyssinica* seeds in response to the various treatments of seeds obtained from Shoa. In the above figures C stands to control.

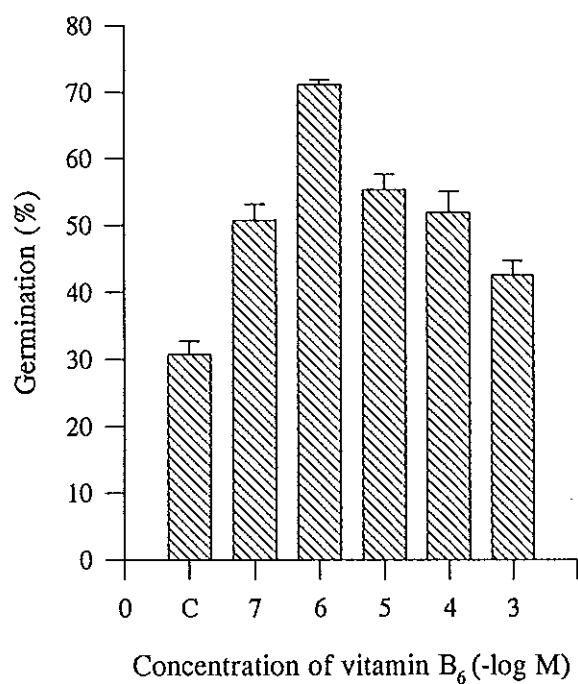
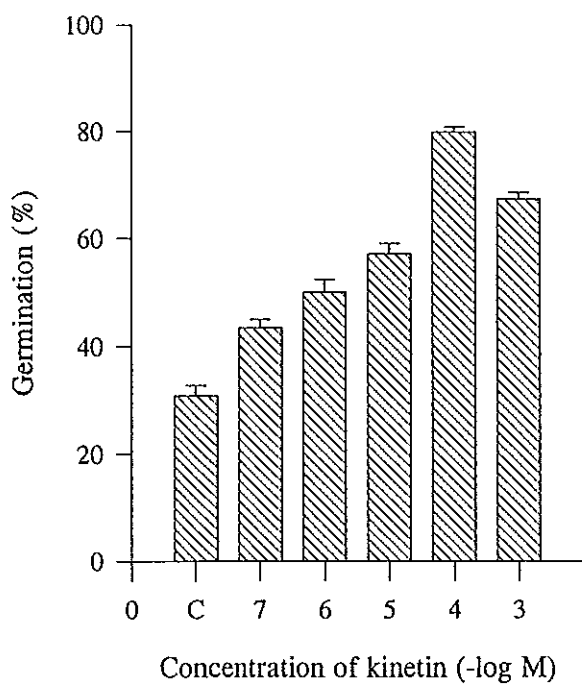
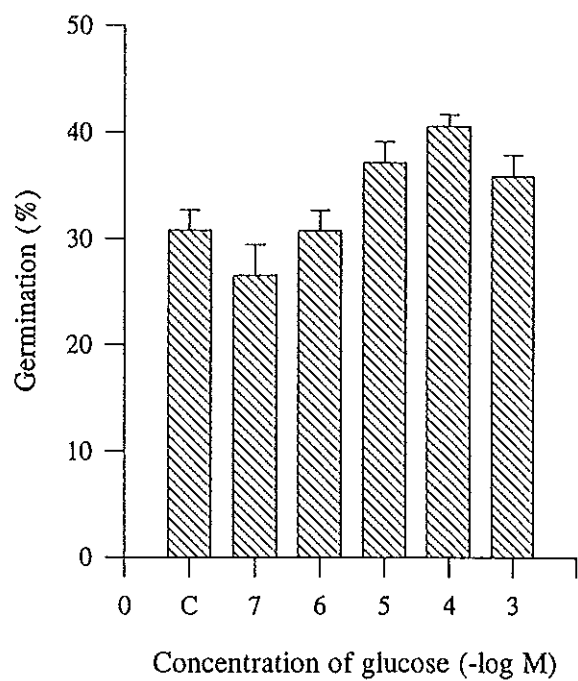
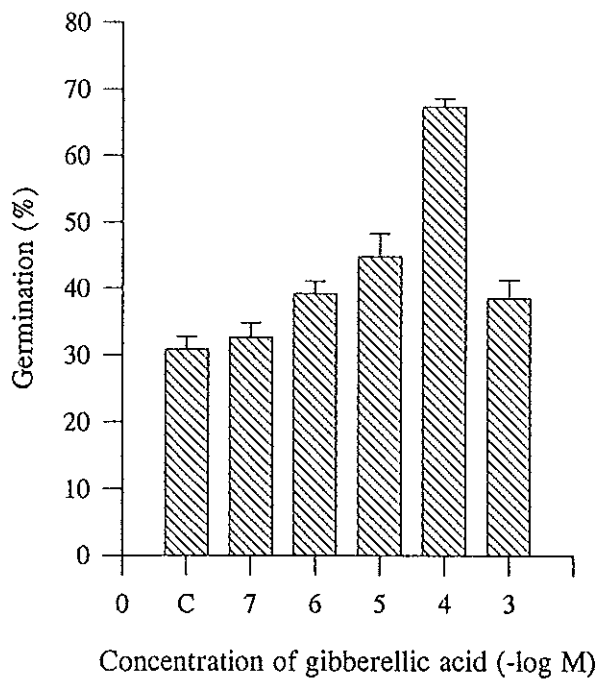


Figure 5. Mean (\pm SE) final germination percentages of *H. abyssinica* seeds in response to the various treatments of seeds collected from Wello. In the above figures C stands to control.

Figure 4 and Figure 5 also show the percentage of seeds germinated of those collected from Shoa and Wello and treated with glucose alone respectively. Significantly higher germination percentages were obtained from samples collected from Wello (40.6 % applied at 10^{-4} M) and Shoa (40 % applied at 10^{-4} M). The next higher germination percentages were obtained for those seeds treated with glucose at 10^{-5} M (37.2 % and 34 % from Wello and Shoa respectively). In case of samples obtained from Shoa seeds treated with glucose at 10^{-3} M also showed significant variation to the control. There were no significant variation among seeds treated with glucose at 10^{-6} M, 10^{-7} M and controls.

On the other hand, daily records on germination value showed no germination was observed within the first week for any of the treatments including the control. Seeds germinated maximally on the eighth day after treatment with gibberellic acid, kinetin, and vitamin B₆ at various concentrations and it stopped 20 days after incubation of the seeds (Fig. 6 and Fig.7).

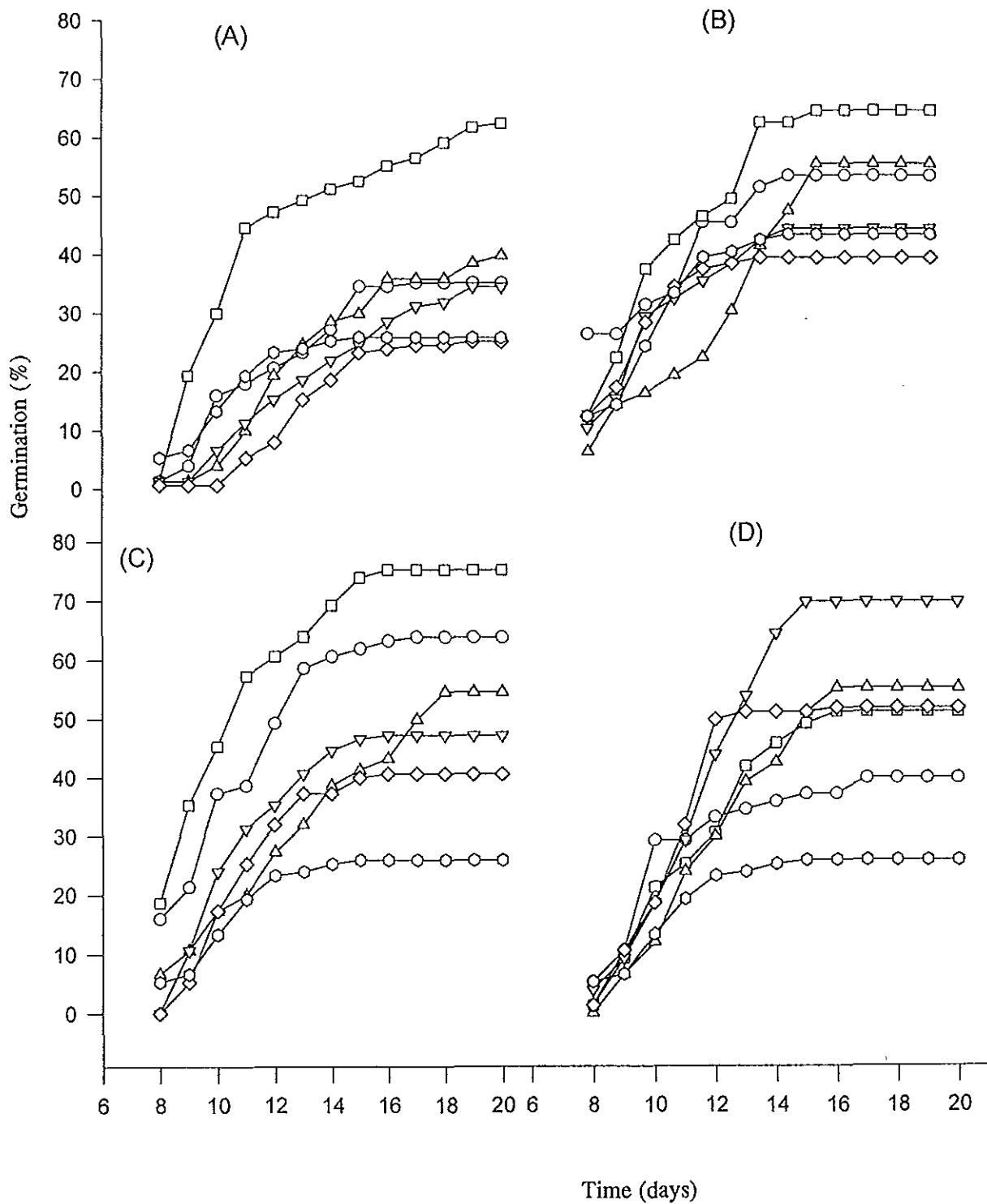


Figure 6. Mean daily germination percentages as a function of time *H. abyssinica* seeds in 10^{-3} M(\square), 10^{-4} M(\circ), 10^{-5} M(\triangle), 10^{-6} M(∇) and 10^{-7} M(\diamond) of GA₃ (A), glucose (B), kinetin (C), vitamin B₆ (D) and Control(\square) of seeds obtained from Shoa.

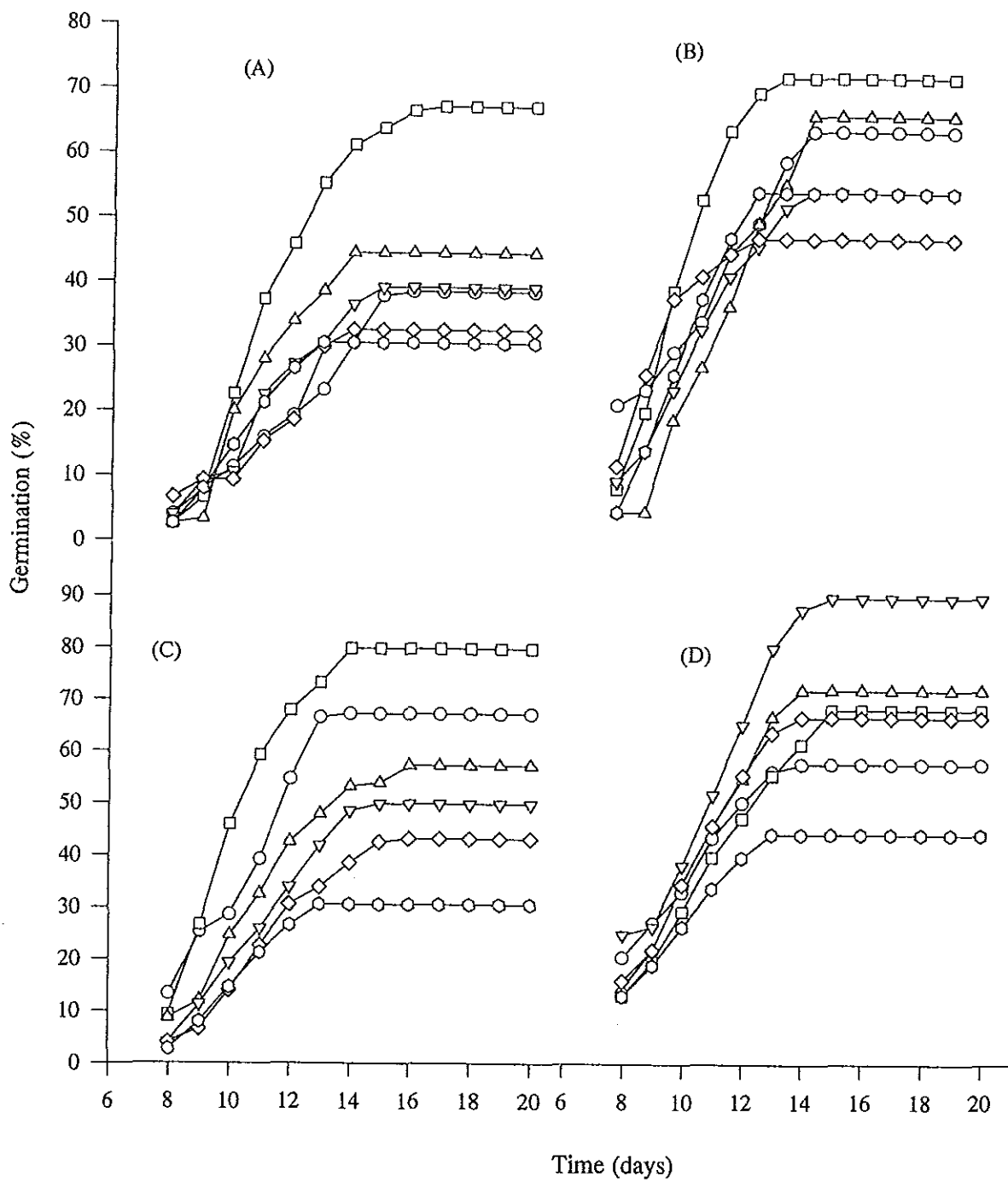


Figure 7. Mean germination percentages as a function of time of *H. abyssinica* seeds in $10^{-3}M$ (○), $10^{-4}M$ (□), $10^{-5}M$ (△), $10^{-6}M$ (▽), and $10^{-7}M$ (◇) of GA3 (A), glucose (B), kinetin (C), vitamin B₆ (D) and control(○). Seeds were collected from Wello.

4. 3 CYTOLOGY

4. 3. 1. SOMATIC CHROMOSOMES

To determine the number of chromosomes of the species radicles of about 1800 germinants and root tips from 120 seedlings were analysed. Most cells investigated (3380 or 92.91 % out of 3638 cells) had a diploid number of 40 chromosomes (Fig. 8a and 8b). In some cells the number of chromosomes were less than 40, which might have resulted due to loss of some chromosomes during slide preparations. In addition, out of 18 twin germinants encountered, in two cases one member of the twin had chromosome number of 20, which is a haploid condition.

In maximally compacted metaphase chromosomes morphological features such as centromeres and chromosome arms are not discernible (the size of these chromosomes was about $1\mu\text{m}$), such chromosomes appeared as compact dots (Fig. 8a). Differential condensation of parts of chromosomes was observed during the prophase and prometaphase stages, in which parts of chromosomes around the centromere condensed ahead of the distal parts of chromosome arms. As a result of this differential condensation, the region of the chromosome around the centromere stained darker whereas the proximal regions stained lightly and thus appeared diffused (the size of these chromosomes was about $2.2\mu\text{m}$) (Fig. 8b). In some cases the chromosomes exhibited more than one condensed regions alternating with less condensed regions. When stained, such chromosomes had banded appearance with alternating dark and light regions (the size of these chromosomes was about $3\mu\text{m}$) (Fig. 8c).

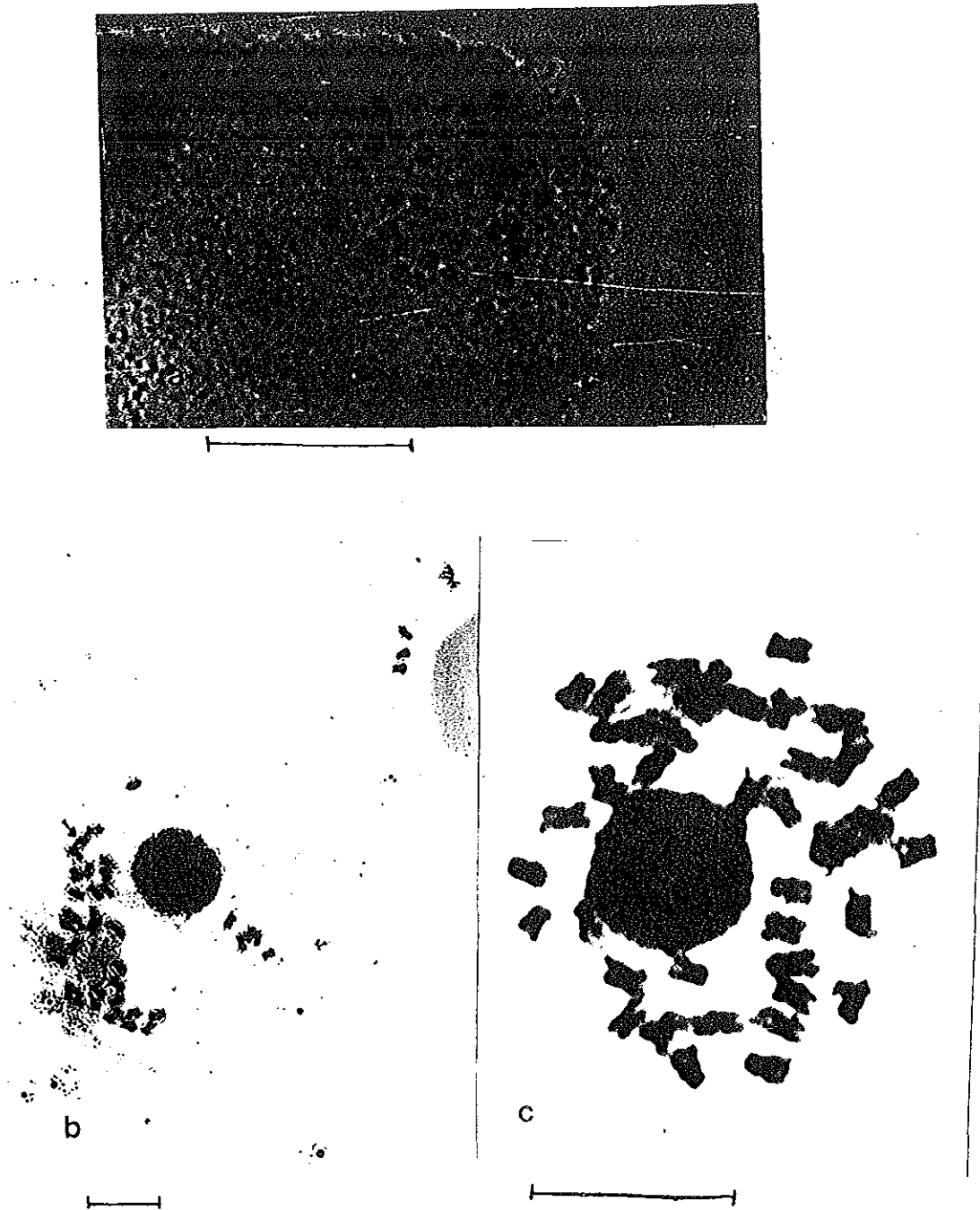


Figure 8. Mitotic chromosomes of *H. abyssinica*. Compacted mitotic metaphase(a). Prophase/Prometaphase (b and c). Arrow in (b) indicate an artefact which is not a chromosome. The bar represents 10 μm .

It is Feulgen and toluidine blue stains that allowed to count and take photograph of meiotic chromosomes of *H. abyssinica*, but use of aceto-carmin as stain did not gave good results. Aceto-carmin is not able to stain may be due to the hard covering of pollen mother cells of the species.

4. 3. 2. MEIOTIC CHROMOSOMES

During meiosis usually 20 bivalents are formed (Fig. 9a). Rarely, the members of a single bivalent exhibit delayed separation (lagging). It is only in two cases out of 800 cells (0.25 %) that laggards were observed. Chromosome behaviour was highly regular both in the first and second meiotic divisions with approximately more than 99 % of the pollen mother cells showing regular meiosis (Fig. 9a-e). Furthermore, figure 9b and figure 9c shows that meiosis within an anther is highly synchronised, that is almost all the micro-spore mother cells within an anther are at similar meiotic stage at any stages of microsporogenesis. The tetrads also appear quite normal (Fig. 9e).

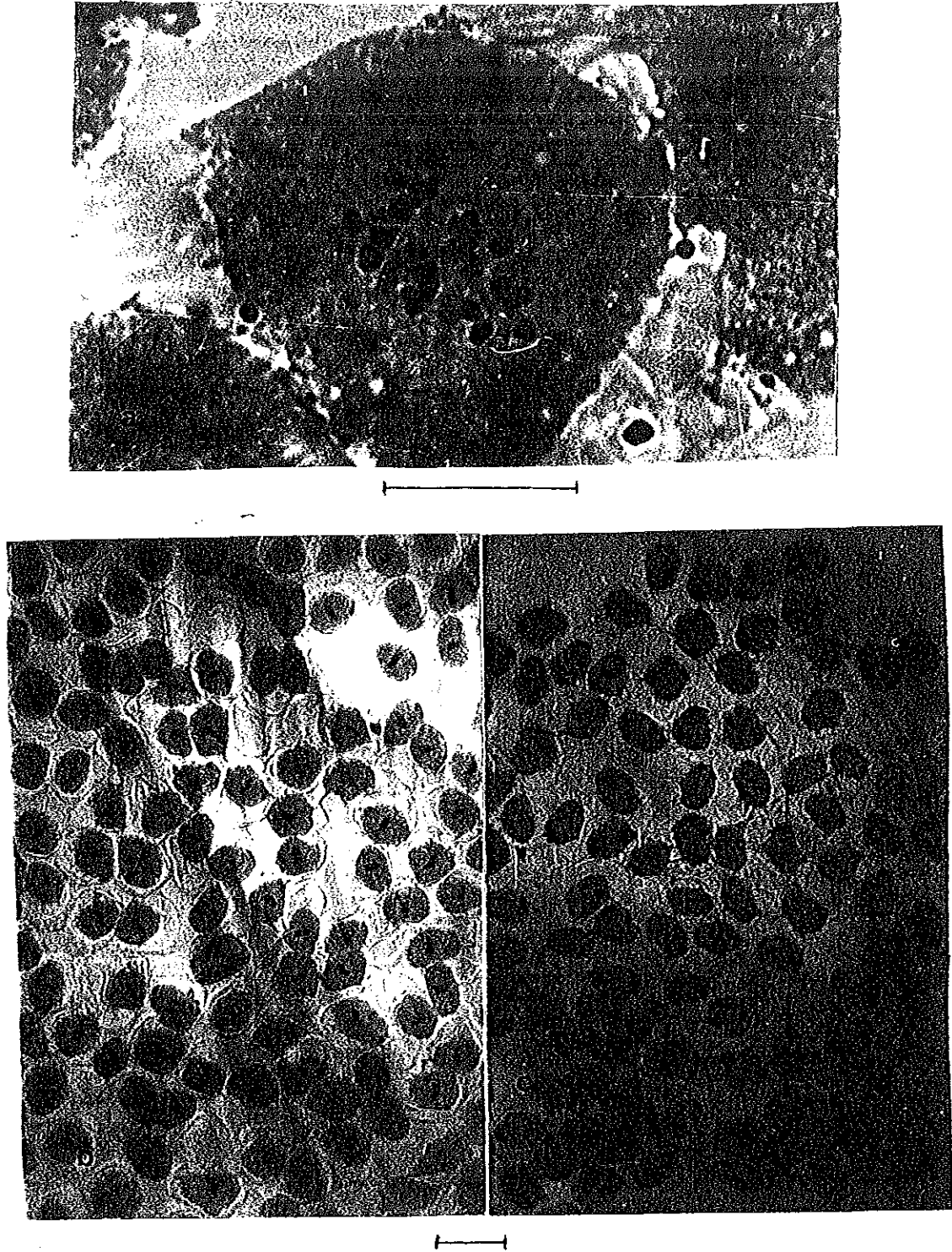


Figure 9(A). Various phases of pollen mother cell meiosis of *H. abyssinica*. (a) bivalents at metaphase I; (b) groups of pollen mother cells at metaphase I (lateral view); (c) group of pollen mother cells at telophase I. The bar represents 10 μ m.

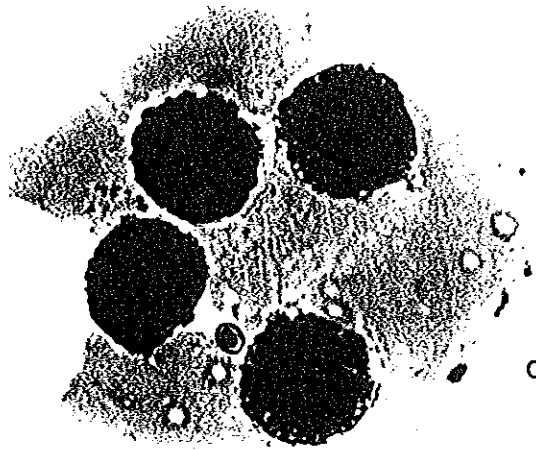
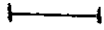
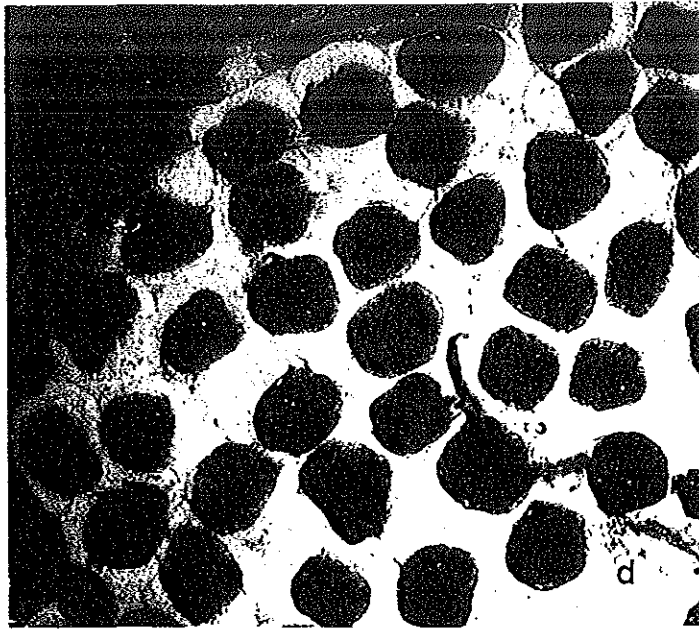


Figure 9(B). Various phases of pollen mother cell meiosis of *H. abyssinica*. (d) group of pollen mother cells at telophase II; (e) a tetrad of microspores. The bar represents 10 μ m.

4. 3. 3. NUMBER OF NUCLEOLI

Silver-staining revealed that the number of nucleoli per nucleus is variable. The maximum number observed was six in mitotic telophase nuclei (Fig. 10a and Fig. 10b). A count of nucleoli made on 1677 randomly selected cells of the radicles from 200 germinants showed that 54 % of the cells had only one nucleolus each, and about 96 % of the total number of cells examined had 1 up to 3 nucleoli per nucleus (Table 2).

Three main parts of the cell showed different degrees of staining with silver: (1) central nucleoli that are wholly darkly silver stained; (2) nucleus which is dark brown silver stained; and (3) cytoplasm some what yellowish silver stained (Fig. 10c).

Table 2. Number of nucleoli observed in cells with respect to their proportion.

Number of nucleoli	Frequencies	Percent
1	910	54.26
2	553	32.98
3	149	8.88
4	50	2.98
5	5	0.30
6	10	0.60

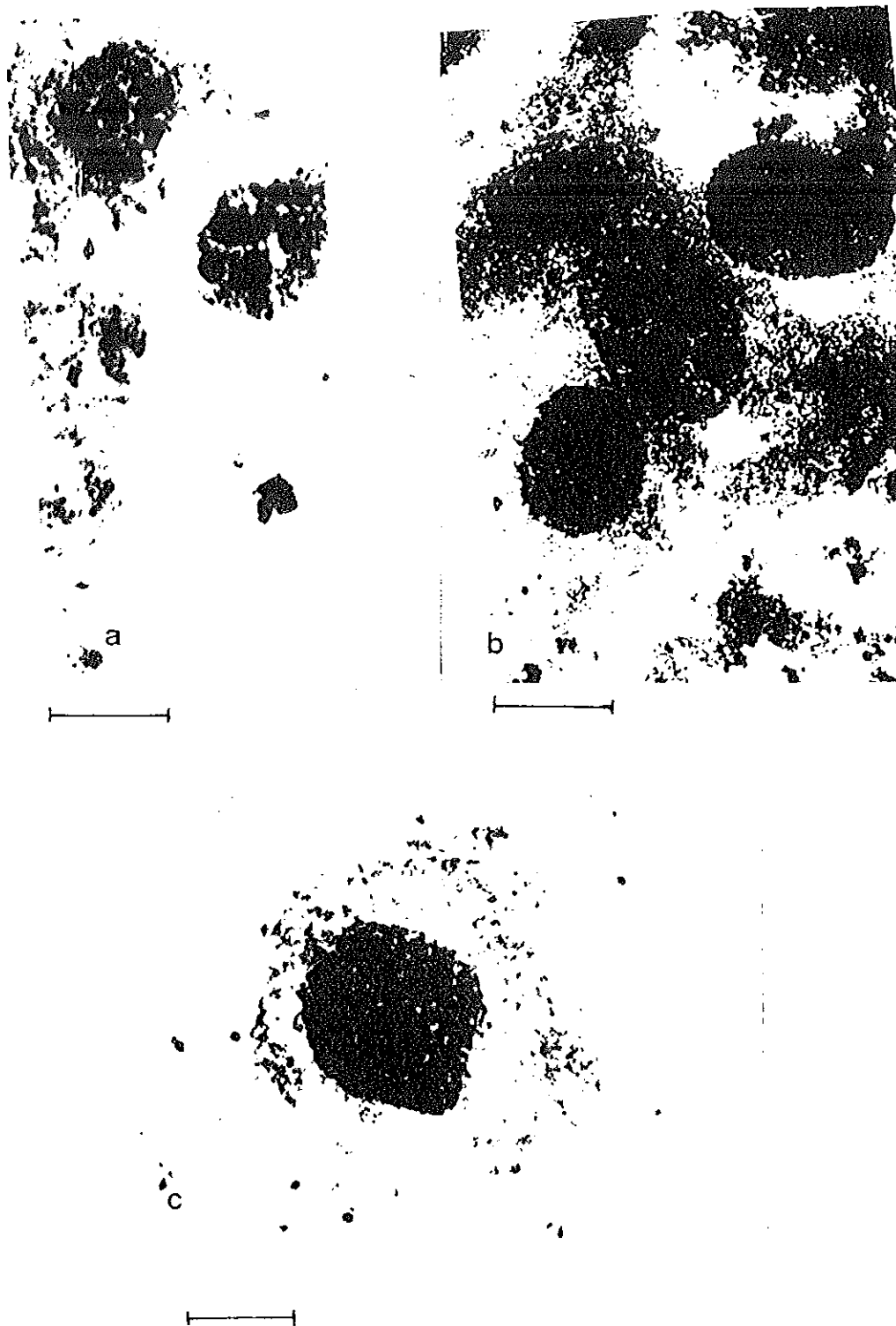


Figure 10. Number of Silver-stained nucleoli of *H. abyssinica*. (a) telophase cell with six nucleoli in each nucleus; (b) two interphase nuclei with one and two nucleoli; (c) interphase cell showing different staining of the cytoplasm, nucleus and nucleolus. The bar represents 10µm.

4. 3. 4 POLLEN FERTILITY

To test the fertility of pollen, 929-1000 pollen grains from each sample sites totally from nine plants were scored. The results showed that the fertility of pollen range from 95 to 97.2 % (Table 3). The stain also allowed to observe that the pollen is triporate.

Table 3. Fertility of pollen from different sites.

Number	Sample site	Stained pollen number	Unstained pollen number	% of pollen fertility	Number of plants
1	AAU, Science Faculty	972	28	97.20	1
2	Goro-Algee Forest	950	50	95.00	2
3	Dessie Tena-Tabia	970	30	97.00	2
4	Denkoro-Washa Forest	901	28	96.986	2
5	Menagesha Forest	963	27	96.30	2

5 DISCUSSION

5.1 BIOMASS AT SEEDLING STAGES

The different parameters used to evaluate the above and below ground biomass production showed that seedlings of the tree of *H. abyssinica* grow faster under appropriate environmental conditions. The shoot exhibited larger relative growth rate (Fig. 2). For example, the ratio of root to shoot of this species show a continuous decrease with increase in age of the species. Shetron and Spindler (1983) who work in other species report that in comparing root to shoot ratios, values less than one indicate above ground parts have more development than roots. The work of Hunt (1976) also indicate the same results. Shoot to root ratios for a particular species vary with chronological age, rooting environment, stage of development and whether they are mono or dicots. But with woody species the most common situation is a continuous increase in root to shoot ratio.

Correlation analyses of dry biomass and fresh biomass of shoot (correlation coefficient, $r = 0.96$) indicate that fresh biomass of seedling is highly correlated with dry biomass of the species. This result show that determination of fresh biomass at seedling stage is useful to estimate the growth potential of this species. On the other hand the difference in root weight at different intervals as well as in individual seedlings may be due to the difference in nutrient content of the soil. The results of seedling biomass of this species is in agreement with the work of Balagopalan and Alexander (1981), who work in other species. But it is very difficult to distinguish between ontogenetic and seasonal changes in this work.

5.2 EFFECTS OF GIBBERELLIC ACID, KINETIN, VITAMIN B₆ AND GLUCOSE

Seeds treated with different concentrations of kinetin showed better germination response. Except in one case (i.e. GA₃ applied at 10⁻⁷ M) all the other treatments increased the final germination percentage of the seeds. Seeds treated with kinetin applied at 10⁻³ M and 10⁻⁴ M, GA₃ applied at 10⁻⁴ M and vitamin B₆ applied at 10⁻⁶ M gave significantly higher final germination percentages as compared with the controls.

Seeds treated with kinetin at 10⁻³ M and 10⁻⁴ M yielded significantly different percentage germination from all treatments of seeds obtained from Shoa and Wello respectively (Appendices 2 and 3). But in all cases it is kinetin applied at 10⁻⁴ M that resulted to higher final germination percentage. On the other hand, when we consider the daily germination percentage, seeds start to germinate on the eighth day after incubation, and terminate mostly on the seventh day. However, seeds treated with GA₃ at 10⁻⁴ M and 10⁻⁵ M germinate upto the 20th day.

As to the mean radicle length, seeds treated with kinetin at 10⁻³ M and 10⁻⁴ M had less radicle length than the control as well as with other treatments of the chemicals. The least radicle length being 0.80 ± 0.0023 cm and 0.90 ± 0.0032 cm for seeds treated with kinetin at 10⁻³ M and 10⁻⁴ M respectively. The highest value for mean radicle length of seeds treated with gibberellic acid, kinetin, vitamin B₆ and controls was 1.75 ± 0.09 cm when seeds were treated with vitamin B₆ at 10⁻⁶ M and 10⁻⁷ M.

A considerable number of studies have been carried out on the effects of exogenously supplied GA₃ and kinetin on seed germination (Koller *et al.*, 1962; Khan, 1966; Khan, 1971; Anderson, 1970; Wareing and Saunders, 1971; Mayer and Shain, 1974; Legesse Negash, 1992, 1993). According to Koller *et al.*(1962) the effects of Kinetin and GA₃ on seed germination differ in that: (1) kinetin is effective on in combination with light, while GA₃ may affect germination in both light and darkness. Khan (1966) also reported that kinetin breaks dormancy in *Xanthium* seeds in the presence of light. (2) the presence of kinetin reverse the effects of red light but not that of GA₃; and, (3) kinetin increases germination only in combination with light of longer wave-lengths, while GA₃ may do so over the long range.

Gibberellic acids are mainly involved in the initiation of germination and germination processes (Khan, 1971). For instance, use of excess GA₃ initiates production of α -amylase in aleurone layer of barley (Chrispeels and Varner, 1967), as well as germination in the intact grains of the same variety of barley. Although these processes may be inhibited by inhibitors (like addition of abscisic acid, coumarin and other inhibitors) when a cytokinin, such as kinetin or benzyladenine, is added to the inhibited system the enzyme production as well as germination are almost fully recovered (Khan, 1971). Khan (1971) formulated a model for the hormonal mechanisms of seed dormancy and germination using gibberellin, cytokinin and inhibitors. The model shows that if an inhibitor is present, it inhibits the effects of gibberellin so germination does not take place, but addition of cytokinin blocks the effects of inhibitor and permits germination to take place.

On the other hand, when we consider the effect of vitamin B₆ it stimulates seed germination of *H. abyssinica* at lower concentration (that is 10⁻⁶ M). This idea is in agreement with the report of Bonner and Bonner (1948) cited in Devlin (1968) who explained that vitamin B₆ is physiologically useful in the participation of pyridoxal phosphate as a coenzyme in amino acid metabolism. Meister (1954) also explained that pyridoxine initiate growth at a lower concentration. This might be one of the reason that vitamin B₆ stimulate seed germination of *H. abyssinica*.

According to Khan (1971), at any time it is more appropriate to say that individual hormones in seeds are at a physiologically effective or a physiologically ineffective concentration. The effective or ineffective concentrations of hormones in a seed in turn can depend on many metabolic and environmental factors.

Glucose was found to have significant effect in increasing final germination percentage of seeds collected from Shoa (applied at 10⁻³ M, 10⁻⁴ M and 10⁻⁵ M and Wello (applied at 10⁻⁴ M and 10⁻⁵ M) as compared to controls. But, seeds treated with glucose at concentrations of 10⁻⁶ M and 10⁻⁷ M (for seeds obtained from Shoa) and 10⁻³ M, 10⁻⁶ M and 10⁻⁷ M (for seeds collected from Wello) showed no significance effects in increasing final germination percentage. In both cases seeds treated with glucose at 10⁻⁴ M gave high germination percentages (i.e. around 40%).

Glucose at a concentration of 10⁻³ M yielded the highest daily germination percentage eight days after incubation. The results were significantly different from the other concentrations of glucose, as well as from the controls. Mayer and Shain (1974) pointed out that glucose

may be required for glucose metabolism. Glucose metabolism is required from the rapid respiratory rise in barley seed in the early stages of germination which is due to glucose respiration (Abdul-Baki, 1969). However, the rate of glucose metabolism in different plant is different (Abdul-Baki and Anderson, 1970; Anderson, 1970; Anderson and Abdul-Baki, 1971). In the early stages of germination, glucose seems to leach out readily from germinating seeds. Even in *Phaseolus*, there is no apparent break down of cotyledon storage materials for the first few hours (days) of germination (Collins and Wilson, 1972). As a respiratory substrate is vital for the early event of germination, the addition of exogenous glucose brings about early germination.

In addition, measurements of mean radicle length showed that seeds treated with glucose at 10^{-4} M gave significantly higher value (1.95 ± 0.012 cm) as compared to the control. This can be addressed for the fact that as the size of the seeds are very small exogenous application of glucose might be used in carbohydrate metabolism until the first true leaf appears and starts to photosynthesize.

5.3 CYTOLOGY

Because of the limitations rendered by the small chromosomes size and the lack of significant morphological differentiation between the chromosomes it has not been possible to give detailed cytogenetical characterisation of the species including karyotype and number of satellite chromosomes. For the same reasons, it has not been possible to determine whether the sex determination mechanism in *H. abyssinica* has heteromorphic chromosome basis. However, this is the first report on chromosome number and number of

nucleoli of *H. abyssinica*. As the size of chromosomes of the species is very small it is very difficult to identify homologous chromosomes on the basis of their size or centromeric position. On the other hand, Prophase and prometaphase chromosomes are larger.

Twin seedlings were observed in *H. abyssinica* seeds germinated for chromosomal study. Two of the twin seedlings had haploid chromosome number of 20. It is known in other plant that twin seedlings result from the presence of more than one embryo in the seed, a condition known as polyembryony. The origins of polyembryony are the cleavage of the proembryo followed by each cleavage developing into separate embryos or the development of more than one embryo sac within an ovule. Another origin of polyembryony is the development of one of the cells in the embryo sac, rather than the egg, into an embryo, with or without being fertilised. This could be the antipodal cells or the synergids, with the latter being the most common source of additional embryo. If such an embryo arises from unfertilised cells of the embryo sac, they give rise to haploid embryos and ultimately haploid seedlings (Bhojwani and Bhatnagar, 1992).

Before the development of anther (pollen) culture techniques, twin seedlings were the main source of haploid plants. Haploids are useful for genetic study since haploid condition allows the expression of recessive genes. Also the presence or absence of chromosome pairing in haploids during meiosis is indicative of whether the source species is polyploid or diploid. Another importance of haploids is that it is used for the production of completely homozygous individuals in a short period of time by doubling the chromosome number. The haploid seedling of *H. abyssinica* are worth investigation for its possible use for genetic study or improvement of the plant through breeding in the future.

As Risely (1981) pointed out in other species, the chromosomes exist in a relatively extended configuration consisting of interspersed regions of diffuse (euchromatin) and condensed (heterochromatin) chromatin. At metaphase the chromosomal filaments attain their highest degree of compaction and become resolvable as individual units. Without using any special staining techniques that produce specific banding patterns on mitotic chromosomes, in rare cases we observe alternating staining pattern on mitotic chromosomes. The chromosomes exhibit regions of darkly stained chromatin alternating with regions of lightly stained chromatin, which might be as a result of the presence of heterochromatin and euchromatin. The bands deeply stained chromatin (chromomeres) is specific for individual chromosomes and it is important for combined genetic and morphological analysis of a particular chromosomal regions. Similar phenomenon have been observed in some other species, for example Cheng has observed in *Brassica* species (Cheng, 1996). It is not known whether the observed differential condensation of chromosomes of *H. abyssinica* is induced by cold treatment used to arrest mitosis at metaphase or whether it is a normal condition in untreated cells as well.

The stages of meiosis have been followed in a large number of organisms both in plants and animals. As in most other plants, meiosis in *H. abyssinica* is highly synchronised. This high level of synchronisation of cell population provides a hint for biochemical analyses. The high pollen fertility observed is in agreement with the high meiotic regularities in pollen mother cells. The work on other plant species, like in *Hordeum* (Bothmer *et al.*, 1988) and *Guizotia Cass* (Kifle Dagne, 1994) also indicate that meiotic regularities are related with fertility of most of the parental landraces.

The number of nucleoli arising in telophase is always constant and is characteristics of the species (Heitz, 1931 cited in Goessens, 1984). However fusion of nucleoli could occur early in telophase. Thus the number of nucleoli in the interphase cell can be much lower than the number of nucleolar organiser regions (NORs) presence because of a marked tendency for nucleoli to fuse. But generally the maximum number of nucleoli per cell should correspond to the number of NORs (Sato and Asano, 1951 cited in Sato *et al.*, 1980; Kifle Dagne and Heneen, 1992). Accordingly, since the maximum number of nucleoli observed in *H. abyssinica* as revealed using silver nitrate stain was six, there are six major nucleolar organiser regions in the diploid complement. Assuming that no chromosome is carrying more than one NORs in a duplicated form, from the maximum number of nucleoli observed it can be inferred that *H. abyssinica* has at least six chromosomes with NORs.

De Mol (1926)(cited in Goessens, 1984) correlate nucleolus and chromosomes with polyploids. He showed that an increase in nucleolar number is correlated with degree of ploidy. Although the maximum number of nucleoli was six 87 % of the cells were with nucleolar number of 1-2, the species is diploid.

6 CONCLUSIONS AND RECOMMENDATIONS

On the basis of this work the following conclusions can be made:

- 1 Once seeds are collected at their appropriate developmental stages, gibberellic acid, kinetin, and vitamin B₆ were found to have significant effect on increasing seed germination percentages.
- 2 When seeds were treated with glucose, the germination percentages were increased significantly ($p \leq 0.05$) than the control but less significantly as compared to gibberellic acid, kinetin, and vitamin B₆.
- 3 Cytological investigation showed that *H. abyssinica* is diploid with $2n = 40$.
- 4 The maximum number of nucleoli observed were six which also show that the number of major nucleolar organiser regions for the species are six, which indicates the presence of three pairs of NOR chromosomes.
- 5 Feulgen stain was found to be the best for staining chromosomes of the species followed by toluidine blue stain. It is very difficult to use aceto-carmin as a stain for the pollen mother cells of *H. abyssinica*.
- 6 Meiosis on the male side is highly regular which results in the high fertility of the pollen

On the other hand, the following recommendations can be made:

- 1 Selection of the right plant growth regulators may also be important to get the highest germination percentage results.
- 2 The mechanism of sex determination of the species must be further investigated.
- 3 The nature of the twin seedlings need to be further investigated.

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Appendix 1. Results of Post-Hoc comparison on the effects of various treatments on seed germination of *Hagenia abyssinica* of seeds collected from Shoa. (Probabilities for least significance difference (LSD) Test are present in a matrix in which differences are significant at $p \leq 0.05$. Underlined and highlighted values indicate insignificance differences; T = treatment in molar concentration; MG = mean germination percentage GA₃ = Gibberellic acid; Glu = Glucose; Kin = Kinetin; Vit = Vitamin B₆; Con = Control).

																					MG	T.	
1																					35.2	10 ³	GA ₃
2	.000																				62.0	10 ⁴	GA ₃
3	<u>.111</u>	.000																			40.0	10 ⁵	GA ₃
4	<u>.894</u>	.000	<u>.085</u>																		34.8	10 ⁶	GA ₃
5	.001	.000	.000	.002																	25.4	10 ⁷	GA ₃
6	<u>.423</u>	.000	.018	<u>.504</u>	.015																32.8	10 ⁸	Glu.
7	<u>.111</u>	.000	<u>1.00</u>	<u>.085</u>	.000	.018															40.0	10 ⁹	Glu.
8	<u>.689</u>	.000	.048	<u>.789</u>	.005	<u>.689</u>	.048														34.0	10 ¹⁰	Glu.
9	.007	.000	.000	.011	<u>.593</u>	<u>.055</u>	.000	.021													27.0	10 ¹¹	Glu.
10	.000	.000	.000	.000	<u>.504</u>	.002	.000	.001	<u>.231</u>												23.4	10 ¹²	Glu.
11	.000	<u>.689</u>	.000	.000	.000	.000	.000	.000	.000	.000											64.0	10 ¹³	Kin.
12	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000										75.2	10 ¹⁴	Kin.
13	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000	.002	.000									54.6	10 ¹⁵	Kin.
14	.000	.000	.018	.000	.000	.000	.018	.000	.000	.000	.000	.000	.015								47.2	10 ¹⁶	Kin.
15	<u>.064</u>	.000	<u>.789</u>	.048	.000	.009	<u>.789</u>	.025	.000	.000	.000	.000	.000	.035							40.8	10 ¹⁷	Kin.
16	<u>.111</u>	.000	<u>1.00</u>	<u>.085</u>	.000	.018	<u>1.00</u>	.048	.000	.000	.000	.000	.000	.018	<u>.789</u>						40.0	10 ¹⁸	Vit.
17	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	<u>.287</u>	<u>.163</u>	.001	.000						51.4	10 ¹⁹	Vit.
18	.000	.015	.000	.000	.000	.000	.000	.000	.000	.000	.004	.000	<u>.841</u>	.009	.000	.000	<u>.206</u>				55.2	10 ²⁰	Vit.
19	.000	.018	.000	.000	.000	.000	.000	.000	.000	.000	.048	<u>.085</u>	.000	.000	.000	.000	.000	.000			70.0	10 ²¹	Vit.
20	.000	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	<u>.386</u>	<u>.111</u>	.000	.000	<u>.841</u>	<u>.287</u>	.000		52.0	10 ²²	Vit.
21	.003	.000	.000	.004	<u>.841</u>	.025	.000	.009	<u>.738</u>	<u>.386</u>	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	26.0	0	Con
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		

Appendix 2. Results of Post-Hoc comparison on the effects of various treatment on seed germination of *H. abyssinica* of seeds collected from Welio. (Probabilities for LSD Test are presented in a matrix form (Differences are significant at $P \leq 0.05$). Underlined and highlighted values indicate insignificance differences; T = treatment in molar concentration; MG = mean germination percentage; GA₃ = Gibberellic acid; Glu = Glucose; Kin = Kinetin; Vit = Vitamin B₆; Con = Control).

																					MG	T.	
1																					38.6	10 ³	GA ₃
2	.000																				67.4	10 ⁴	GA ₃
3	.042	.000																			44.8	10 ³	GA ₃
4	<u>.842</u>	.000	<u>.066</u>																		39.2	10 ⁴	GA ₃
5	.049	.000	.000	.000																	32.6	10 ⁷	GA ₃
6	<u>.390</u>	.000	.004	<u>.291</u>	<u>.262</u>																36.0	10 ³	Glu
7	<u>.508</u>	.000	<u>.166</u>	<u>.643</u>	.009	<u>.130</u>															40.6	10 ⁴	Glu
8	<u>.643</u>	.000	.013	<u>.508</u>	<u>.130</u>	<u>.691</u>	<u>.262</u>														37.2	10 ³	Glu
9	.011	.000	.000	.006	<u>.551</u>	<u>.088</u>	.002	.036													30.8	10 ⁴	Glu
10	.000	.000	.000	.000	.049	.002	.000	.001	<u>.166</u>												26.6	10 ⁷	Glu
11	.000	<u>1.00</u>	.000	.000	.000	.000	.000	.000	.000	.000											67.4	10 ³	Kin
12	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000										80.0	10 ⁴	Kin
13	.000	.001	.000	.000	.000	.000	.000	.000	.000	.000	.001	.000									57.2	10 ³	Kin
14	.000	.000	<u>.088</u>	.001	.000	.000	.002	.000	.000	.000	.000	.000	.019								50.0	10 ⁴	Kin
15	<u>.114</u>	.000	<u>.643</u>	<u>.166</u>	.001	.016	.355	.042	.000	.000	.000	.000	.000	.031							43.4	10 ⁷	Kin
16	<u>.187</u>	.000	<u>.467</u>	<u>.262</u>	.001	.031	.508	.076	.000	.000	.000	.000	.000	.016	<u>.791</u>						42.6	10 ³	Vit
17	.000	.000	.019	.000	.000	.000	.000	.000	.000	.000	.000	.000	<u>.088</u>	<u>.508</u>	.005	.002					52.0	10 ⁴	Vit
18	.000	.000	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	<u>.551</u>	<u>.076</u>	.000	.000	<u>.262</u>				55.4	10 ³	Vit
19	.000	<u>.210</u>	.000	.000	.000	.000	.000	.000	.000	.000	.000	<u>.210</u>	.004	.000	.000	.000	.000	.000			71.2	10 ⁴	Vit
20	.000	.000	.000	.000	.000	.000	.001	.000	.000	.000	.000	.000	.036	<u>.791</u>	.016	.008	<u>.691</u>	<u>.130</u>	.000		50.8	10 ⁷	Vit
21	.011	.000	.000	.006	<u>.551</u>	<u>.088</u>	.002	.036	<u>1.00</u>	<u>.166</u>	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	30.8	0	Con
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		