1-INTRODUCTION

Cultivated strawberry, (food of youth), (*Fragaria* x *ananassa* Duch.), belongs to the family Rosaceae and is a member of the genus *Fragaria.* It is a perennial stoloniferous, herbaceous species representing the most important soft fruit worldwide (Hancock, 1990). It has been commercially cultivated in many countries in the world (Biswas, *et al.*2008). The fruit is an achene attached to a juicy, enlarged receptacle. It is one of the most popular fruits in the world and per capita consumption is increasing annually. Strawberry have been growing wild for millennia in temperature regions throughout the world, whereas cultivated Strawberry originated from an accidental cross between *Fragaria chiloensis* L.P.Mill. and *Fragaria virgintana* Duch. The first species is a white fruited type brought to France from Chile in 1714 and the second species is a red-fruited species brought from Virginia colony to Europe in 1600s (Aharoni *et al*. 2004). Strawberry is one of the most important fruit plants for both fresh consumption and food processing in the temperate and subtropical regions with a global production of over 43.66 million tons(FAO,2010) and a production area of about 255000 ha (FAO, 2008).

The attractive appearance of, delicious flavor and taste and seasonal availability of the fruits, make strawberries an excellent fruit crop. It has become one of the popular and favorite fruits for its high nutritional value, and relatively reasonable price.

The fruits are consumed in different ways fresh or frozen and are also processed and used in very large number of food products such as juice, jam, jellies, yogurt, syrups, toppings, sweet, wine, baked goods and are highly valued as dessert fruits. The leaves are used in blended herbal tea. The fruit is a rich source of vitamins, minerals and numerous dietary components (Driscoll's, 2004). The leaves and roots are believed to have medicinal qualities. The fruits are rich in phytochemical

compounds, mainly rich in ellagic acid, have a wide range of biological activity (Mass, *et al.*1991; Hannum, 2004).

Sudan imported about 1005 tons of fruits and about 16600 plantlets (Personal communication, 2010, Fedral Ministry of Agriculture's, records 2010). Strawberry is gaining some economic importance as a new promising fruit crop in Sudan. Expansion is faced by shortage of planting material and stock plants. The potential demand for propagules of desired varieties for planting and expansion of plantations is enormous.

Strawberry is propagated sexually by seeds and asexually by rooted runners. Seed propagation is rarely used because of genetic variability among plants produced. The method of rooted runners is the method most used for the vegetative propagation of strawberry (Hartmann, *et al.* 2002). Healthy stock plants are not available in the country and importing mother plant from abroad is neither practical nor desirable. It is expensive and result in introduction and spread of plant and soil transmissible diseases and insect pests. The current method of vegetative propagation by rooted runners is unsuitable and inadequate to provide the volume of plant material required for establishment of new fields. Runners are limited in certain times of the year because plants produce runners during the vegetative phase and are vulnerable and susceptible to pathogenic infection. Continuous planting of runners from old mother plants for a long time would lead to accumulation of diseases and virus. Maintaining stock mother plants requires considerable labor, space, and time and is difficult during the hot months of the summer. Plant micro-propagation by tissue culture has increased in importance in recent years because it offers major production and marketing advantages over traditional propagation methods. It is often faster than conventional propagation methods, promotes volume production and results in healthier plants. Strawberry appears amenable to tissue culture propagation. Application of tissue culture to

strawberry would eliminate the required maintenance of large numbers of stock plants. It is possible to maintain healthy stock plants *in vitro* for extended periods, and to eliminate seasonal constraints and enable propagation of large numbers of disease-free clonal material from a single stock on a year-round basis. Large scale commercial propagation by tissue culture has been used widely in the strawberry industry. A million or more plants can be produced in a year from a single shoot tip (Boxus, 1974). Micro-propagation through axillary shoot proliferation (Elmana, *et al*. 2003; Sakila, *et al.* 2007; Hasan, *et al.* 2010), direct shoot formation from leaf disks (Liu and Sanford, 1988; Sovariet, *et al.* 1993; Nehra *et al*, 1989; Kordestani and Karami, 2008) or indirect shoot regeneration via an intermediary callus pathway (Jones, *et al.*1988; Nehra, *et al.* 1990; Rugini and Orlando, 1992; Biswas, *et al*. 2007a) has been achieved. Various explants types have been used for culture initiation in strawberry tissue culture. The most widely used tissue culture in any true-to-type clonal propagation is the use of shoot tips or stem segments. Shoot tips have been used by (Khanam, *et al.* 1998; Ritu, *et al.* 2001; Elmana, *et al.* 2003; Biswas, *et al*. 2010; Mozafari and Gerdakaneh, 2012) whereas (Sakila, *et al* 2007; Hasan *et al.* 2010; Mir, *et al.* 2010; Moradi, *et al.* 2011) used nodal segments for culture initiation in strawberry. Other less commonly used explants include leaf disks (Liu and Sanford, 1988; Nehra, *et al.*1990; Sovariet, *et al.*1993; Biswas *et al.* 2007a); flower buds (Foucault and Letouze, 1987; Gerdakaneh, *et al.* 2009); stipules (Rugini and Orlando, 1992); achenes (Lis, 1990); immature embryos (Wang, *et al.* 1984); cotyledon (Miller and Chandler,1990); anther (Hou,1992). Micro-propagated strawberry plants are comparatively better in different characters under field conditions. They have enhanced vigor, larger crown size, and greater number of runners, earlier flowering time and higher yield of berries than conventionally propagated runner plants (Swartz, *et al.* 1981; Cameron and Hancock, 1985; 1986; Karhu and Hakala, 2002; Singh, *et al.* 2004).

Beside propagation, tissue culture techniques have been used for production of disease-free plants (Mullin, *et al.* 1974; Kauahal, *et al.* 2004; Biswas, *et al.* 2007b) and in plant breeding and crop improvement programmes (Barcelo, *et al*. 1998; Adel and Sawy, 2007).

Strawberry research in Sudan is quite limited (Elmana, *et al.* 2003; Ahmed, 2009). Three decades ago the Agricultural Research Corporation (ARC) initiated program in Hudieba, Elfaki-hashim, and Jabel Marrah at the end of sixties of the last century with the objective of evaluating some strawberry cultivars under the conditions of Sudan. All cultivars introduced were European and some of them were "Bell Rubi", "Govela", "Sequeia", "Aliso", and "Gurdeiner". Recently a variety trial at Umdoom, (Personal communication, 2010, Arab Authority for Agricultural Investment and Development) reported that yields were reasonable but not as high as the 48 ton/ha which is common in many strawberry producing countries.

In the view of the potential commercial value of strawberries, it is highly desirable to develop efficient, rapid, reliable, cost-effective and large scale multiplication techniques through tissue culture. Plant species that regenerate organs easily *in vivo* are expected to respond similarly *in vitro,* and strawberry is not an exception. Since not all cultivars will respond in the same way to a generalized medium and/or culture condition, the objective of this research is to optimize efficient tissue culture procedures for *in vitro* multiplication of the strawberry cultivar "Festival"

2-LITERATURE REVIEW

2.1-History, Origin and distribution:

Fragaria ananassa (Duch.) is a natural hybrid of *Fragaria chiloensis* (L.) P. Mill. and *Fragaria virginiana* (Duch.). *Fragaria* is a member of the Rosaceae family. Recorded history of the *Fragas*, fruit of the strawberry, dates back to 23-79 A.D. in the writings of Pliny (Darrow, 1966). The woodland strawberry of Northern Europe, *Fragaria vesca* (L.), was cultivated as early as 1300 in France. It was appreciated as much for its flowers as for the fruit. Cultivation also took place in England where documentation of the history of the name 'strawberry' is found in literature from the tenth to fifteenth centuries. Anglo-Saxons named the strawberry as a descriptor denoting the way the runners strew or stray from the mother plant. In the 1500s other species and subspecies of *Fragaria* were discovered in Europe.

Early colonists in North America cultivated their native strawberry, *Fragaria virginiana*. The North American strawberry was a hardy plant with the ability to withstand cold temperatures and drought. In the early 1600s *Fragaria virginiana* was imported to Europe from North America. In the 1700s French explorers found a wild strawberry in Chile. The South American strawberry plants produce large fruits but were not well suited to a wide range of climates. The European importation of the Chilean strawberry, *Fragaria chiloensis*, in 1714 was the most important event in the history of the large-fruited strawberry grown today (Darrow, 1966). A French army officer named Amedee Francois Frezier nursed five F. *chiloensis* plants back to Europe from Concepcion, Chile. The plants were inter-planted alongside with F. *virginiana* (Hancock, 1996). A natural hybrid developed and its combination of hardy plant and large fruit, made plant breeders to take notice. The hybrid quickly spread through Europe. Frenchman, Antoine Nicolas Duchesne is credited with identification of the natural hybrid of the *Fragaria* x

ananassa. Duchesne was a contemporary of the famous Swedish taxonomist Carolus Linnaeus (1707-1778), also known as the Father of Taxonomy. Duchesne's father was the chief gardener at Versailles in France. His interest in horticulture began at an early age. He studied the strawberry and discovered that there were two main groups with several sub-groups. His study led him to the discovery that the large fruited and flavorful strawberry sweeping Europe at the time was a hybrid of the species, F. *chiloensis* and F. *virginiana*. Duchesne's discovery changed the name from F. *ananass*a to *Fragaria* x *ananassa*. The Chilean parent plant was suited to a mild climate whereas the North American parent was more adapted to heat, drought, and cold weather conditions. European plant breeding from the mid-1700s throughout the following one hundred years provided selections from the progeny of crosses between the Chilean and North American varieties resulting in the large-fruited cultivars we enjoy today. The Europeans called the large fruited strawberries *Ananas* because of their resemblance to pineapple in fragrance and shape. *Ananas* strawberries were introduced to the American colonies in the late 1700s and the hardy North American strawberries were crossed with them to produce large fruit and hardy plants. Plant breeders have developed strawberry cultivars with more disease resistance, better tasting, larger, and redder berries to meet consumer demands (Darrow, 1966).

2.2- Taxonomy:

Strawberries are diploid, tetraploid, hexaploid, octoploid and even decaploid. The woodland strawberry F. *vesca* and most of the native species around the world are diploids. They range from dioecious to hermaphroditic and self-fertile to self-infertile. Three known tetraploids are F. *moupinensis*, F. *orientalis*, and F. *corymbosa*. F. *moupinensis,* and F. *orientalis* are pistillate, staminate and hermaphroditic. Only male plants of F. *corymbosa* have been found. The hexaploid strawberry is the F. *moschata*. It is known for its musky flavor and has perfect flowers when cultivated. *Fragaria chiloensis* and *Fragaria virginiana* are both octoploid (2n=56). F. *chiloensis* is dioecious with some hermaphrodites. F. *virginiana* is dioecious and hermaphroditic (Hancock, 1996). Both have many sub species. The ploidy level of these ancestors is credited with the large number of highly adapted, natural varieties and cultivars of strawberries existing today (Darrow, 1966).

2.3. Botany of the strawberry:

Fragaria x *ananassa* is a member of the Rosaceae family which also includes raspberry and blackberry. The *Fragaria* x *ananassa* is a perennial which arises from a crown of meristematic tissue or compressed stem tissue. Leaves, stems, runners, axillary crowns, inflorescences, and roots all arise from the crown.

2.3.1 -Roots:

The strawberry plant has shallow fibrous root system that arises from short stems near soil surface. Horizontal roots extend about one foot on all sides of the base of the plant. Adventitious roots arise from the crown primarily in late summer. They extend several inches into the soil and form lateral roots which are primary means of absorbing water and nutrients. Lateral roots usually live one or two years, while primary roots live two to three years. The largest concentration of roots occurs in

the upper three inches of the soil. Each plant maintains twenty to thirty primary roots, the average length being four to (15cm). Strawberry plants are herbaceous perennials consisting of leaves and roots attached to a compressed stem or crown. A plant may have between 25 and 100 primary root, with thousands of small lateral roots arising from the primary root. Secondary roots are short lived, whereas primary roots can remain functional for a year. New primary roots are initiated above old roots of the crown (Pritts and Handley, 1998).

The root system is composed of two types of roots, large adventitious roots which differentiate slowly from crown, and fibrous branch root, which differentiate from the large roots, (White, 1927). Rapid development of fibrous branch roots is vital to transplant health plants as strawberry roots rarely have plant hairs. Instead of root hairs, they possess many-branched feeder rootlets with fascicles, which appear like root hairs. Feeder rootlets are composed of primary tissue, and have a live expectancy of a few days to a few weeks, and are concerned primarily with water and nutrient absorption.

2.3.2-Crown and branches:

The strawberry plant grows from a shortened, central stem called a crown whose terminal is the growing point. Buds formed on the crown produce leaves, flowers, runners, branch crowns and adventitious roots. Runners are branches from the main stem; Branches are mostly short and usually profuse, later spread to 30-50cm. Runners (stolons) arise from the crown. A runner is a true stem with tissue specialized to conduct water and nutrients. The runner plant can use its own root system shortly after formation of roots in branches (Rom and Dana, 1960). Runners and branch crown are essentially shoots, which develop from axillary buds that formed at the base of each leaf. Environmental conditions were found to strongly influence type of shoot to develop. Runner's development is stimulated by long day length and warm temperatures. Initial growth of runners results from

development of first internodes. Branch crown development on the other hand, is stimulated by short day lengths and cooler temperatures and that occurs later in the season than runner formation (Pritts and Handley, 1998).

2.3.3-Leaves:

Strawberry leaves are trifoliate, consisting of three leaflets each with its own short stem attached to the main leaf petiole. Individual leaf may live from 1 to 3 months (or longer) and vary widely in leaf thickness, size, stomata density and physiological characteristics. Leaves of Fragaria *chiloensis* and *Fragaia virginiana* are evergreen and are grown in winter, whereas the *Fragaria ananassa* hybrid exhibits intermediate characteristics (Galletta and Himelrick, 1990).

The leaves are arranged spirally, such that every long petiole arises above the crown. Leaflets are round to oblong, with serrated edges and a thick cuticle layer (Pritts and Handley, 1998).

2.3.4-Flower cluster:

The flower cluster of the strawberry is made up of two main branches at the crotch of which a single flower is produced, that is, the primary flower is the first of the cluster to bloom. Each of the two branches is similarly made up of two other branches with their single flowers at crotches. These flowers being the second of the cluster to bloom and are known as secondary flowers. Each of these four branches again divides at the crotches to produce the third group of flowers to bloom; known as tertiary flowers.

The flowers located in the cymose cluster, are either perigynous bisexual (hermaphrodite) or unisexual (monecious). The flower has five white petals. The sepal number of the primary flowers is commonly 7 or 8, that of secondary flowers are 6, and tertiary flowers are 5. There is a direct relation between the order of blooming, the number of flowers parts produced, the size of fruit, and the time of ripening. Also, fruit size is the result of the number of fruitlets borne on each berry

(Hoemaker, 1954). The androecium of strawberry fruitlets is made up of 20 to 35 stamens (multistamenate), arranged together with petals and sepals in spiral pattern in three whorls. The gynoecium part, on the other hand, is made up of 60 to 600 pistils (multipistillate). Within each pistil there is a carpel containing a single ovary. The pistils are arranged spirally on the receptacle. Fertilization occurs 24 to 48 hours after pollination, (Pritts and Handley, 1998).

Buds in the axils of leaves produce flower cluster when temperature is cool and days are relatively short. Different varieties tend to produce cluster of particular type. Thus some varieties produce clusters with many flowers while others produce cluster with few flowers. The clusters of some varieties branch close to the crown while others branch far out the stem (Luby, *et al.* 1991).

Each hermaphroditic flower contains a receptacle composed of hundreds of ovaries. The receptacle is surrounded by a ring of stamens, followed by a ring of five white petals. Plants of some strawberry species such as *Fragaria chiloensis* tend to be dioecious, but males occasionally produce perfect flowers and fruits. Strawberry plants are self-fertile and pollination is assisted by wind, gravity and insects (Pritts and Handley, 1998).

2.3.5-Fruits:

Strawberry fruit is an aggregate with a collection of many external achenes (seeded fruitlets). An aggregate fruit is formed by the simulation ripening of a number of separate ovaries, all belonging to a single flower and adhering as a unit on a common receptacle (Hoemaker, 1954). Actually, the strawberry fruit is an enlarged receptacle and is not reproductive materials. As a result, it is not really a fruit in the botanical sense. Strawberry is harvested at full ripeness when greenish white fruit turn to rich red color (Adriance and Brison, 1955).

Ripening of fruit takes about 30 days from pollination, depending on temperature, genotype and degree of pollination. Fruits change colour during development from

green to white to red. Strawberries are considered to be non-climacteric fruit because they do not exhibit a rise in respiration on ripening and do not respond to ethylene with accelerated ripening. Fruits of most wild species weigh less than 29g, whereas commercial cultivars can produce fruit weighing 30-69g (Pritts and Handley, 1998).

The edible portion of the strawberry is the swollen receptacle whereas the true fruit are the seed (achenes) on the surface. Therefore, strawberry is not a true berry in the botanical sense because the consumed tissue is not derived from the ovary.The growth of the receptacle is dependent on successful fertilization of the ovules with its size and shape dependent on the number of achenes formed. Strawberry plants are day length dependent with cultivars being long day, short day or day neutral (Darnell, 2003).

2.4. Environmental requirements:

2.4.1. Ecology:

The least winter-hardy strawberry plants grow better on the places where snow accumulated in winter time and are often destroyed by frost during snowless winters. Temperature lower than -10 to -15° C without snow cover lead to withering of leaves; under -12 to -17° C cause the destruction of weak-root plants and plants perish completely with temperature lower -20° C without snow. Flowering in May-June (often appearance of the first flowers in time with slight frosts leads to the loss of the most valuable part of harvest), bear fruit in July. The plants prefer dewy fertile soils with subsoil waters not nearer than 1 m from soil surface. It fruits in the second year after planting and gives high harvest during 4 years (http://agroatlas.ru/en/content/cultural/fragaria-ananassa/k/map).

2.4.2. Soil:

The strawberry can be grown on any type of soil from poor sand to heavy clay provided that proper moisture, organic matter and drainage are present. Strawberry ripens somewhat earlier on sandy soils than on clay soils. There is definite cultivar adaptation to soils. Some cultivars grow better on heavier soils and others on light soils. Water should not be stagnant in the field. Since most of its roots are found in the top 15 cm soil, this layer should be kept porous and rich in humus. Strawberry is not much sensitive to soil reaction. However, it prefers a slight acidic soil. At higher pH, there is less root growth. There should be no underlying lime layer up to 15-20 cm, otherwise burning of leaves occurs. In drier areas, alkali soils must be avoided, thus, sandy loam to loamy soil with pH 5.7-6.5 is ideal for strawberry cultivation.

Strawberries grow and produce satisfactorily in a wide range of soil types, but sandy loam and sandy clay-loam soils are ideal for building and shaping the 8-inch-deep raised beds that are critical to the success of the strawberry plasticulture system. Soils with high clay content or those that are rocky or very stony are more difficult for bedding, fumigation and plastic-mulch equipment. As a general rule, growers should consider using plug plants on soils with high clay content for semi-mechanical transplanting with a water wheel rather than bare-rooted, fresh dug plants which require hand transplanting. Sandy soils will require more careful irrigation and nutrient management. Research is ongoing to identify useful soil amendments, such as chicken compost, and testing of summer cover crops to improve soil structure.

2.4.3. Effect of photoperiod on growth and flowering of Strawberry:

Strawberries are often grouped according to their flowering habit. Traditionally, in temperate regions, this has consisted of a division between "June-bearing" strawberries, which bear their fruit in the early summer and "ever-bearing" strawberries, which often bear several crops of fruit throughout the season.

Research has shown recently that strawberries actually occur in three basic flowering habits: short-day, long-day, and day-neutral. These refer to the day-length sensitivity of the plant and the type of photoperiod that induces flower formation. Day-neutral cultivars produce flowers regardless of the photoperiod. (http://40bp.blogspot.com).(http://www.strawberry.dreab.com).

2.5. World production:

The United States is the largest strawberry producer in the world, providing approximately 27% of the world's strawberries Table (2.1). The future of strawberry production and sales is very positive. Increased demand in the future from Canada, Japan and Mexico should increase export demand on the United States growers. Strawberry production, grower price, trade (import and export), and consumption are expected to continue to increase over the next several years.

Table (2.1) World strawberry production in tones(Source FAO, 2010)

Country	2006	2007	2008	2009	2010
USA	1,090,440	1,109,220	1,148,530	1,270,620	1,292,780
Turkey	211,127	250,316	261,078	291,996	299,940
Spain	330,485	269,139	281,240	263,700	275,300
Egypt	128,349	174,414	200,254	242,776	238,432
Korea, South	205,307	203,227	192,296	203,772	231,803
Mexico	191,843	176,396	207,485	233,041	226,657
Japan	190,700	191,400	190,700	184,700	177,500
Poland	193,666	174,578	200,723	198,907	176,748
Germany	173,230	158,658	150,854	158,563	166,911
Russia	227,000	230,400	180,000	185,000	165,000
Italy	143,315	160,558	155,583	163,044	153,875
Morocco	112,000	120,000	130,000	355,020	140,600
Total world	3,973,243	4,001,721	4,136,802	4,596,614	4,366,889

2.6-Cultural practices:

2.6.1- Weed Control:

Hand-hoeing and hand-weeding are very important in strawberry plantings. There are several weed-control materials for the strawberry, but in general home garden plantings are best weeded without the use of chemicals. It is difficult to apply the chemical at the proper rate without the necessary equipment and there is the danger of doing damage to adjacent vegetable and flower plants (spray drift). Subsequent crops following strawberries in the garden may also be sensitive to these chemicals. The basic methods of controlling weeds are:

1. Machine cultivation plus hoeing and hand pulling;
2. Mulching with suitable material;
3. Chemical herbicides.

Herbicide should not be applied when plants are blooming, or when runner plants are taking root, and during late summer and early fall when fruit buds are being formed.

2.6.2. Fumigation:

Fumigants such as methyl bromide and chloropicrin have been used in combinations with plastic mulch row covers since the early 1980s for broad spectrum soil-borne pest and disease control in strawberry plasticulture. New land that has been subject to good crop rotations and best management practices (such as cover cropping and good drainage strategies) can, under optimum conditions, generate yields that are 85 to 95 percent of the yields in fumigated soil. Weed control, however, can be a serious problem. Strawberry plasticulture production on the same site year after year is not advisable without pre-plant fumigation because

of potential weed and disease problems. (http://strawberryplants.org/wp-content/uploads).

2.6.3. Planting Material:

Strawberry planting material is available in three forms depending on the strawberry producing region. Plants may be acquired as plugs, fresh dugs and cutoffs. Mountains and foothills plantations primarily use plugs. In fact, the mountains are restricted to using plugs since the fresh dugs are not available until after their last acceptable planting date. Growers in the coastal plains primarily use fresh dugs. These types of planting material are all "fresh" as opposed to dormant. Fresh dugs and cutoffs are bare-rooted plants, while plugs are runner tips that have been rooted in a peat-based media and feature an intact root-ball. The distinction between fresh dugs and cutoffs is that fresh dugs retain the vegetative foliage while the foliage of cutoffs has been removed prior to digging from the propagation bed. Plugs are the most expensive type of planting material, while cutoffs are the least expensive.

Strawberry plants are asexually propagated through tissue-culture. The plants are grown out for up to four generations in the nursery. Runners harvested after two years of growth in the field nursery are considered "registered," while those grown for three years in the field are referred to as "certified." The most vigorous plants are the first year nursery plants, called foundation or white tag plants; however these are cost prohibitive to producers and serve only as nursery stock. Each year thereafter, there is reduced vigor.

2.6.3.1. Plugs:

The prices of strawberry plugs are nearly twice the cost of fresh dug or cutoff strawberry plants, but they have the advantage of being suitable for mechanical transplanting with a water-wheel or disposable pot mulch planter. Part-time

growers, who do not have the time to oversee the continuous overhead watering of fresh dugs during the first week following field transplanting, should use plugs. Also, less experienced growers are encouraged to consider planting plugs because they are more "mistake-proof" than highly perishable, fresh dug plants. Transplanting dates for plugs can also be slightly later than for fresh dugs without as great a yield reduction. This is because plugs establish more quickly than fresh dugs after transplanting.

2.6.3.2. Fresh dugs:

Freshly dug plants, commonly referred to as fresh dugs, are most often transplanted by hand requiring an availability of affordable labor. To establish these highly perishable fresh dugs, growers must rely on intense overhead sprinkling for one to two weeks, depending on weather and the condition of the freshly dug plants. Fresh dugs must be irrigated within 30 minutes of planting. Fresh dugs exposed to cooler temperatures, chilling in the nursery or both, will require less time for establishment than fresh dugs produced in warmer climates.

2.6.3.3. Cutoffs:

These cutoffs have accumulated considerable chilling in the nursery and the leaves have to be removed prior to digging and harvesting (http:// .ncsu.edu/ enterprises/strawberries/plant material).

2.6.4. Cultavition:

Strawberry cultivars vary widely in size, color, flavor, shape, degree of

Fertility, season of ripening, liability to disease and constitution of plant. Some vary in foliage, and some vary materially in the relative development of their sexual organs. In most cases, the flowers appear hermaphroditic in structure, but function as either male or female. For purposes of commercial production, plants are propagated from runners (stolons) and, in general, distributed as either bare-rooted plants or plugs. Cultivation follows one of two general models, annual

plasticulture or a perennial system of matted rows or mounds. A small amount of strawberries are also produced in greenhouses during the off season.

The bulk of modern commercial production uses the plasticulture system. In this method, raised beds are formed each year, fumigated, and covered with plastic to prevent weed growth and erosion. Plants, usually obtained from northern nurseries, are planted through holes punched in this covering, and irrigation tubing is run underneath. Runners are removed from the plants as they appear, in order to encourage the plants to put most of their energy into fruit development. At the end of the harvest season, the plastic is removed and the plants are plowed into the ground. Because strawberry plants more than a year or two old begin to decline in productivity and fruit quality, this system of replacing the plants each year allows for improved yields and denser plantings. However, because it requires a longer growing season to allow for establishment of the plants each year, and because of the increased costs in terms of forming and covering the mounds and purchasing plants each year, it is not always practical in all areas.

The other major method, which uses the same plants from year to year growing in rows or on mounds, is most common in colder climates. It has lower investment costs, and lower overall maintenance requirements. Yields are typically lower than in plasticulture. (http://www.strawberry.dreab.com).

There are 4 training systems-matted row, space row, hill and plastic film mulch).

2.6.4.1. Matted row:

This is the simplest and least expensive method. The runners are usually planted at 90 cm x 45 cm spacing. After the initial growth of the first year, runners are allowed to cover the vacant space all around the mother plants ultimately covering the whole vacant space and giving the appearance of a mat. It is generally followed in heavy soils which are free from weeds. In this system, more number of plants can be accommodated/unit areas which give a higher yield under suitable

conditions. Overcrowding may cause higher fruit rot. Thus care must be taken to maintain the optimum number of plants without overcrowding.

2.6.4.2. Spaced row:

This system is suitable for cultivars that are moderate to weak in producing runners. The daughter plants are spaced at definite distances by covering the selected tips of runners with soil to induce formation of plants. This is done till the desired numbers of daughter plants are obtained for each mother plant. The runners formed later on are removed.

2.6.4.3. Hill system:

This system is followed for cultivars developing few runners. All runners are removed from the mother plants. The individual plants become large and bear more than those in matted row: The plants are planted 25-30 cm apart in twin rows 20-30 cm apart and 100-110 cm spacing between twin rows. A small tractor can be used for tillage. Where cultivation is done manually, the rows can be spaced closer at 60 cm. In some cases triple rows are set.

2.6.4.4. Plastic film:

Green or black plastic film is used as mulch for the hill system to control weeds and conserve moisture, but on hot hays, some scalding of berries occurs. In this system, berries are kept clean and rot and mold are reduced. The plants bloom ealier but are prone (http://plantsforhumanhealth.ncsu.edu/extension/overview), (http://.ncsu.edu/enterprises .ncsu.edu/enterprises strawberries/production).

2.6.5. Renovation or renewing the planting:

Matted row strawberry plantings may bear fruit for more than one season, and may be kept for two or possibly 3 to 4 fruiting seasons if properly renovated. The main purpose of renovation is to keep plants from becoming too crowded in beds. Renewal of strawberry beds infested with weeds, diseases, or insects should be

avoided.It is better to set a new planting instead (http://strawberryplants.org/wp-content/uploads);

2.6.6. Irrigation:

Strawberry plants have a shallow root system and cannot stand severe drought. Irrigation, if used properly, can also help prevent frost injury to blossoms in spring (http://strawberryplants.org/wp-content/uploads).

2.6.6.1. Overhead sprinkler irrigation:

Strawberry plasticulture requires overhead sprinkler irrigation for establishing transplants, protecting blossoms from cold injury and for evaporative cooling in the event of a spring heat wave that could cause open blossoms to abort. Water may come from wells, ponds, lakes and municipal lines. An irrigation pond would need to hold about 150,000 gallons of water for each acre of plasticulture production to provide protection for three consecutive frosts or freeze nights (http://www.strawberry.dreab.com)

2.6.6.2. Drip irrigation:

Drip irrigation provides the most efficient use of water and fertilizer. Many deep wells are fairly clean and require only a screen filter to remove particles. However, the presence of precipitates or other contaminants in the water should be determined by a water-quality test before considering the well for a drip system. Any surface water source, such as a stream, pond, pit or river, will contain bacteria, algae or other aquatic life, making sand filters or other special filters a necessity.

For strawberries, drip tape is used to wet a continuous strip along the center of the row. A 12-inch emitter spacing is recommended for sandy loam and clay soils. For coarse sands, 8-inch emitter spacing is recommended. Drip emitter discharge rates are generally expressed in gallons per minute (gpm) per 100 feet of length for the selected emitter spacing. A common tape selection for plasticulture strawberries on

sandy loam or clay soils uses 0.40 gpm emitters: 24 gallons per hour (gph) per 100 feet. To determine field water requirements in gpm per acre, simply multiply 24 gph times 87.12 (the number of 100-foot row units per acre on five-foot bed spacing) and divide by 60, which yields 35 gpm.

Because strawberries grown on plastic mulch are considered annuals and are grown for only one season, thin, disposable drip tape (8 mils thick) is commonly used. Once a drip irrigation system is installed, the crop can be fertilized via the drip system (fertigation) (http://www.strawberry.dreab.com).

2.6.7. Fertilization:

The strawberry plant is shallow rooted and must be fertilized during the growing season to keep it vigorous. The plants should be fertilized prior to the period of fruit-bud initiation. The percentage of humic matter and total nitrogen content give indications of overall soil fertility and this can be a useful guide to nitrogen availability. Essentially, the test recommendations for adjusting soil phosphorus (P) and potassium (K) should be checked before planting. If no soil test has been made, about 4 pounds of 10-10-10 fertilizer for each 100 feet of row should be broadcasted 2 to 3 weeks before planting. Organic fertilizers if properly used are perfectly satisfactory. Dried blood (12-14% N) is of course organic and immediately available. It leaves an acid reaction. Bone meal contains 20-24% phosphoric acid, acting slowly, while steamed bone meal acts more quickly. Wood ashes can be used for supplying potash (http://www.strawberry.dreab.com).

2. 7. Pests and Diseases:

Around 200 species of pests are known to attack strawberries both directlyor indirectly. These pests include slugs, moths, fruit flies, chafers, strawberry root weevils, strawberry thrips, strawberry sap beetles, strawberry crown moth, mites, aphids, and others. Strawberry plants are vulnerable to a number of diseases. The leaves may be infected by powdery mildew, leaf spot (caused by the fungus

Sphaerella fragariae), leaf blight (caused by the fungus *Phomopsis obscurans*), and by a variety of slime molds. The crown and roots may fall victim to red stele, verticillium wilt, black root rot, and nematodes. The fruits are subject to damage from gray mold, rhizopus rot, and leather rot. The plants can also develop disease from temperature extremes during winter. When watering your strawberries, be sure to water only the roots and not the leaves, as moisture on the leaves encourages growth of fungus. Ensure that the strawberries are grown in an open area to prevent fungal disease from occurring (http://www.strawberry.dreab. com). *Colletotrichum* fungi are the second most important organism in strawberry production after *Botrytis cinerea* (Pers.). Also known as black spot, crown rot, and anthracnose *Colletotrichum* is a significant problem worldwide.

2.8. Propagation method of strawberry:

The strawberry plant is propagated either sexually or vegetative. Sexual propagation by seeds is not commonly used commercially because the strawberry seed needs stratification through freezing for 3 months before use and is not suitable as the seedlings do not come true-to type. Due to this, old strawberry buds may have many untrue seedlings undesirable for propagation. Sexual methods are used only in breeding programs, a few seed-propagated cultivars have been developed for home use, and research on seed propagation of strawberry is ongoing. Seeds (achenes) are acquired either via commercial seed suppliers, or by collecting and saving them from the fruit. (http://www.strawberry.dreab.com); (Ragab and khidir, 2005).

2.8.1. Propagation by runners:

Strawberry is commercially propagated by runners. Generally one plant produces 7-10 runners but under proper management, it can go up to15 runner/plant. These runners produce roots at each node (commonly begins at the third node) and form new plants. A rooted runner plant can function as a root branch (Rom and Dana,

1960). Runners are the source of planting materials for establishment of new plantations. Each strawberry plant planted in spring and grown under favorable conditions will readily produce up to 25 layers of runners. Ever-bearing strawberries produce few runners; because the vegetative buds that normally grow into runners, from flower buds instead. Because of this characteristic another method of propagation than runners and is often desirable (Adriance and Brison, 1955); (http://www.strawberry.dreab.com).

2. 8.2. Propagation by crown division:

A crown is considered as a stem consisting of a short wood growth. The buds, leaves and runners arise from the crown. Strawberry varieties that produce few runners are propagated by branch crown divisions (3-5 plants/crown), (Pritts and Handley, 1998). Vigorous full-bearing plants that produce few runners usually develop into plants with several branch crowns in winter. Some varieties produce 10-15 strong branch crowns per plant. In propagation, individual branch crowns is cut or broken in a way that roots remain attached to the division. Branch crown with 5-10 roots, 1.3 cm long and 1.5 cm or more in diameter is desirable for propagation (Hoemaker, 1954).

Division of crowns of older plants is expensive compared to cultivars producing runner plants readily. Runner formation can be stimulated with the application of BA, 10 days before flowering (Pritts, *et al.* 1986). The promotive effect was proportional to the concentration of BA in the solution; however, repeated applications repress runnerring. BA could promote early runners formation and be of value for increasing numbers of daughter plants.

Where viruses and nematodes are present, primarily in commercial plantation, the growth and production of plants may be reduced by half or more. It is desirable to procure virus-free plants for commercial plantations. In addition, these plants are raised in fumigated soils to control nematodes. Planting virus and nematode-free

stock, provides protection against serious diseases. (http://www.strawberry.dreab.com).

For large scale propagation of virus-free plants, tissue culture is widely used. Under favorable conditions, one strawberry meristem can be multiplied to yield more than one million plants in a year. Plants can be regenerated from meristematic callus, anthers and immature embryos.

2. 8.3. Propagation by plant tissue culture:

Vegetative propagation has been cited as the most important application of tissue culture technique in plants. The general principles in micropropagation are the same for all crops. Details of the techniques for specific crops often differ (Vasil and Thorpe, 1994). The use of tissue culture for the clonal propagation of herbaceous and woody plant species has become an increasingly applied practice in commercial industry. The feasibility of the technique developed depends on the multiplication rate from sub-cultured shoots and the percentage of rooting of the shoots produced. While propagation by conventional means is sometimes hampered by seedling heterozygosity, space and time considerations, seed and cutting dormancy and limited propagules production, tissue culture propagation offers the advantages of rapid multiplication of pathogen-free plants on a year-round basis. Several cultural methods have been applied to strawberry and rapid clonal multiplication has been widely used (Boxus, 1974). When agricultural crops are reproduced by division after several generations, often a decline occurs in qualities such as vigor, yield, disease resistance, plant and fruit appearance and uniformity of size or shape. This condition of decline is commonly called, "run out". Strawberry plants have demonstrated this clonal decline (running out) for many years. After growing strawberry plants for five or more years, gardeners became accustomed to dividing a clump of plants that contained the mother plant (oldest plant) in the center and replant the smaller daughter plants to be used as seed plants the following season. Certain genetic, undesirable changes (mutations) were brought to the surface, as seen in daughter plants; as more and more plants were continuously grown, generation after generation. Some of these corrupting mutations may be visually observed as the plant vigor declines; the yield of strawberries is less, and sometimes the berries are misshapen; and finally, the plants become extremely susceptible to diseases caused by virus, bacteria, fungi,

insect susceptibility, and nematode victimization. Agricultural researchers advised strawberry growers to discontinue old variety lines and clones and were told to buy new, certified plants that restore the vigor needed to increase yields of future strawberry gardens.

Old "run out" become a common in agricultural crops, have been in the last decade rejuvenated to produce unprecedented yields and to restore confidence in a high quality product. The use of meristem and shoot tip culture for the recovery and establishment of pathogen-free plants has also become a common practice in the production of virus-free stock of vegetatively propagated plants in many commercial nurseries in developed world (Propagation by tissue culture run out 2010).

Boxus, (1976) obtained an increased proportion of virus free plants by using heat therapy prior to meristem tip culture. In addition, Vine (1968) reported fast growth of meristem excised from heat-treated plants. The first meristem culture of strawberry was initiated in 1962 by Belkengreen and Miller (1962).

Micropropagation of strawberry plants was introduced about thirty years ago (Boxus 1974). Immediately, the most important European nurseries producing several millions plants per year, were interested in this technique as it give a definitive answer to the problems of soil fungi, causing a lot of damage to the strawberry fields. Tissue culture plants seemed to produce more runners per mother plant in a short time (Mohan, *et al.* 2005). Micropropagation has also been widely used (Zimmerman 1981) in commercial propagation of strawberries and in breeding programs to produce many plants rapidly. Conventionally, strawberries plant propagation through runner produces a limited number of propagules and the produced plants are susceptible to several fungal diseases (Dijkstra, 1993). Micropropagated strawberry plants can be stored under refrigeration (Mullin and Schlegel, 1976), making it a valuable technique for storage of germplasm.

Complete new plants can be derived from tissue either from pre-existing buds through direct shoot proliferation, or indirectly via callus and shoot organogenesis through adventitious shoot regeneration (organogenesis) or through formation of somatic embryos (embryogenesis).

Yields and fruit quality have been found to be markedly improved by using virus-free planting material rather than standard stocks. It is possible to initiate cultures of strawberry meristem tips and obtain rooted plantlets on a single medium. However; several workers have obtained a higher proportion of plantlets from explants by using two media (Boxus, 1974; Vanhoof, 1974; Elmana, *et al.*, 2003).

The *in vitro* propagation of strawberries has advantages and disadvantages. The greatest disadvantages are the high initial laboratory establishment costs and need of highly qualified staff. Therefore, the cost of tissue culture plants establishment estimated as 4-5 times more than plants produced from runners. The advantages of micropropagation to be appreciated by grower include the ability to multiply virus-free stocks, vigorous and uniform plants, and have ability to build up new cultivars quickly. Also micropropagated plants are excellent source of healthy runners,

It is worth mentioning that three clones of strawberry were found to have normal and free from virus diseases. Substantial yield increase was obtaind in meristem derived over their source plant (Bisawas *et al.,* 2007b).

2.9.3.1. The explants:

Many factors should be considered in preparing material for strawberry *in vitro* culture, such as; the part of mother plants used and the treatment of mother plants before explants are excised. Cultures and subcultures should be initiated with clean and diseases-free materials. However, selection of the most suitable source of explants material is an essential factor to the success of *in vitro* propagation (Murashige, 1974). The totipotency exhibited by the apical meristem and the adjacent shoot tip region is the cornerstone for commercial micropropagation

(Nehara, *et al.* 1990; Kordastani and Karami, 2008; Castillo, *et al.*1998; Bisawas *et al.,* 2007b; Nagib, *et al*. 2003; Mozafari and Gerdakaneh, 2012; Faedi, *et al.* 2002; Mozafari and Govaovora, 2005; Zobrowska, *et al.* 2003;).

In this respect, Miller and Chandler, (1990) tried cotyledon explants taken from mature achenes. Laneri and Damiano, (1980), on the other hand, used anthers of F. ananassa, taken from flowers, with sepals 5-6 mm long. While Foucault and Letouze, (1987) and Sutan, *et al*. (2010) used other explants such as leaf petiole segments. Wang, *et al.* (1984) used immature zygotic embryos. Moradi, *et al.* (2011); Sakila, *et al.* (2007); Bhatt and Dhar, (2000); Adel and Sawy, (2007); Hasan, *et al.* (2010); Islam, *et al.* 2007; Miers and Thoms, (2007), used nodal segments. Nehra, *et al.* (1990) used leaf discs and obtained plant regeneration, Gerdakaneh, *et al.* (2009) used immature flower buds.

2.8.3.2. General components of tissue culture media:

Plant tissue culture media are generally made up of several components such as macro mineral elements, vitamins, amino acids, sugar, some other undefined organic supplements, solidifying agents and growth regulators (Pierik, 1989). Some media contain low concentration of macronutrient component. The total ionic concentration in Anderson (1978) medium is 44.44mM while other have a high concentration of macronutrients component, like Murashige and Skoog (1962), with ionic concentration 95.75mM (George, 1995a).

In general, growth and development of *in vitro* cultured plant tissues and organs, depends on such factors as mineral elements composition and total salt strength, an energy source and growth regulators The inorganic mineral requirements of plant tissues and organs cultured *in vitro* are basically the same as those of intact plants, at least qualitatively. The requirements for inorganic salts and total salt strength of plant tissue culture medium among diverse plant species and genotypes, organs, tissues, cells for a variety of purposes research applications is rather constant.

There are now many basic salt mixtures available that were developed to satisfy the nutritional requirements of *in vitro* cultured plant cells, tissues and organs for various purposes and served as bases for further modifications. A number of major salt formulations, adequate to satisfy the requirements of explants tissues and organs of plant, have been developed. The most important salt mixtures in plant tissue culture are White's- (White, 1943); Murashige and Skoog's- (Murashige and Skoog, 1962); Nitsch and Nitsch's- (Nitsch and Nitsch, 1969); Lepoivre's- (Quoirin, et al. 1977) and woody plant (LIoyd and McCown, 1981) salts. Each of these salt mixtures was developed using specific test cultures. For example White, (1943) used tomato root culture whereas Murashige and Skoog, (1962) used tobacco callus cultures. The most common and most frequently used is the inorganic salt mix of Murashige and Skoog, (1962). It is highly concentrated, with balanced ions and contains high levels of nitrogen, phosphorus and potassium and some of the microelement such as boron and manganese (Cohen, 1995). This salt mixture has undergone many modifications and improvement and the great majority of salt formulations used in plant tissue culture media {e.g. Linsmaier and Skoog, (LS), (Linsmaier and Skoog, 1965), Gamborg B5 (GB), (Gamborg, *et al.*1968), and Woody Plant Medium (WPM) (LIoyd and McCown, 1981)} are but derivatives of Murashige and Skoog salt mixture which is still the salt mixture of choice in most instances.

Comparative studies on the various salt mixtures used in plant tissue culture also exist. Elmana, *et al*. (2003) found MS salts best for strawberry tissue culture than Knop's salts. A comparison between MS salt and White's salt mixture (White, 1943) on date palm root culture by Dlaigan (1995) confirmed the superiority of MS salts mixture. These studies attest to the effectiveness of MS salt mixture as a source of essential mineral elements in plant tissue culture media for various plant species and for various purposes. However, similar and more recent comparison

between (MS Murashige and Skoog, 1962), B5 (Gamborg, *et al.* 1968) and NN (Nitsch and Nitsch, 1969) salt solutions revealed the superiority of the latter for strawberry tissue culture (Mozafari and Gerdakaneh, 2012).One of the major characteristics of MS salt mixture is the presence of high levels of nitrogen in the form of ammonium and nitrate ions (George *et al.* 2008). It is the most widely used salt mixture in plant tissue culture media for various plant species and for various purposes (Murashige, 1974). Nevertheless, several researchers have tried to optimize enhancement and promoting effects of this famous salt mixture by modification of the normal salt strength of the whole salt mixture or that of some of its individual components. Bougerfaoui and Zaid, (1993) attributed the high incidence of vitrification in date palm *in vitro* produced plantlets to the high concentration of NH_4NO_3 in MS salt mixture. Reduction of the concentration of NH_4NO_3 of the original MS salt resulted in high percentage of *ex-vitro* establishment of date palm plantlets (Darwesh, *et al.* 1995) Modification of MS salt mixture by reducing the concentration of NH_4NO_3 and KNO_3 for best shoot formation was reported by Economou and Read, (1984). Legrand, (1975) found that one-fifth the concentration of the chelated iron used in MS medium is best for bud formation on leaf explants cultured *in vitro*. The optimum iron concentration for tomato roots was determined eventually to be about one-third the level prescribed in the Murashige and Skoog's medium (Said and Murashige, 1979). More recently Mohamed (2012) enhanced shoot and root formation in ginger by reducing the normal nitrogenous component of MS medium to one-fourth or half strength the normal strength of the nitrate component of MS medium. The level of the concentration of both, potassium and phosphorus ions in the original MS medium is low (Troncoso *et al.,* 1999). Murashige, (1974) advocated increasing the concentration of phosphorus, higher than normal, in their famous MS medium by inclusion of 170 mg/l NaH_2PO_4.

Beech, *et al*. (1988), Wickremasinghe and Fernando, (1988) and Wang, *et al*. (1984) used MS medium in strawberry meristem culture and initiation of somatic embryogenesis from immature embryos of strawberry. Nehra, *et al*. (1990) used MS basal media with strawberry leaf discs and obtained plant regeneration. Miller and Chandler, (1990), Green *et al*, (1991), Biswas*, et al.* (2007b) and Sakila, (2007) successfully used media containing MS salts for growth and development of strawberry explants. However, some researchers used modified MS medium or media other than MS medium. For example Nehra, *et al.* (1989) used MS salts with B5 vitamins, while Atkinson, *et al.* (1986) and Katano and Kenda, (1988) tested different media formulation including MS and Gamborg (Gamborg, *et al.* 1968) B5. Others used only Gamborg B5 medium (Kondakova *et al*., 1986). Full strength of MS basal medium was found to be the best for induction of roots (Elmana, *et al.* 2003).

2.8.3.2.1-Vitamins:

The vitamins most frequently used in plant tissue culture media are thiamine (Vit. B1), nicotinic acid (niacin) and pyridoxine (Vit. B6) and apart from these three compounds, there is little common agreement about which other vitamins are really essential.

The advantage of adding thiamine was discovered almost simultaneously by Robbins and Bartley, (1937) and White, (1937). Nicotinic acid and pyridoxine appear, in addition to thiamine, in media published by Gautheret (1942) and White, (1943). This was following the findings of Bonner and Devirian, (1939) that nicotinic acid improved the growth of isolated roots of tomato, pea and radish;, and pyridoxine are ingredients of Murashige and Skoog, (1962) medium and have been used in varying proportions for the culture of tissues of many plant species. However, unless there has been research on the requirements of a particular plant

tissue or organ, it is not possible to conclude that all the vitamins which have been used in a particular experiment were essential.

The requirements of cells for added vitamins vary according to the nature of the plant and the type of culture. Welander, (1977) found that Nitsch and Nitsch (1965) vitamins were not necessary, or were even inhibitory to direct shoot formation on petiole explants of *Begonia* x *hiemalis*. Roest and Bokelmann, (1975) on the other hand, obtained increased shoot formation on Chrysanthemum pedicels when MS vitamins were present.

Inositol is a pentose sugar alcohol that is routinely added to plant tissue culture media at the concentration of 100 mg/l after the demonstration by Jacquoit, (1950) of its promoting effects on growth and development of callus tissues of *Castanea vesca* Gaertin and has been added routinely since then in plant tissue culture by many tissue culturist in many tissue culture laboratories at the concentration of 100 mg/l. It is categorized as a vitamin because its function is similar to those of vitamins and is not added in plant tissue culture as a source of carbon or energy (Loewus and Loewus, 1983). It is incorporated in MS- (Murashige and Skoog, 1962) and B5 media (Gamborg *et al.,* 1968) at a concentration of 100 mg/l. The response of *in vitro* cultured plant tissues to inositol is not absolute. Inclusion of inositol in plant tissue culture may be essential (Nesius, *et al.* 1972; Kaul and Sabharwal, 1975), beneficial (Murashige, 1974; Gamborg and Shyluk, 1981; Goforth and Torrey, 1977), inhibitory or without effect (Sharron and Liu, 1977; Said and Murashige, 1979; Zhang, *et al.* 1986; Saied, 1999).

Callus of *Pinus strobus* grew best when the level of inositol in MS medium was reduced to 50 mg/l whereas that of P. *echinata* proliferated most rapidly when no inositol was present (Kaul and Kochbar, 1985).

Research workers often tend to adopt a ‘belt and braces’ attitude to minor media components, and add unusual supplements just to ensure that there is no missing

factor which will limit the success of their experiment. Sometimes complex mixtures of as many as nine or ten vitamins have been employed.

Experimentation often shows that some vitamins can be omitted from recommended media. Although four vitamins were used in MS medium, later work at Professor Skoog's laboratory showed that the optimum rate of growth of tobacco callus tissue on MS salts required the addition of only thiamine. The level of thiamine was increased four-fold over that used by Murashige and Skoog, (1962), but nicotinic acid, pyridoxine and glycine (an amino acid) were unnecessary (Linsmaier and Skoog, 1965).

Soczck and Hempel, (1988) found that medium without thiamine, pyridoxine and inositol is best for increasing the rate of shoot multiplication of *Gerbera jamesonii* local cultivars.

Jones and Vine, (1968) used complex vitamin components and 100mg/l inositol for best growth and development of cultured plant tissues. Moreover, VanHoof, (1974) used nicotinic acid, pyridoxine, Ca pantothenate and biotin in addition of 100mg/l inositol for optimum growth response under *in vitro* conditions, while Mulilin, (1970) added thiamine only to strawberry media.

2.8.3.2.2-Carbohydrates:

Cultured plant tissues and organs are hetrotrophic, and require exogenous sugar. Sucrose is assumed to be the best carbon and energy source in plant cells and tissue culture media, because it is the main sugar found in the phloem of many plant species (Strickland *et al.*1987). It is almost universally used in plant tissue culture as it is easily utilizable by cultured plant tissues and organs. Carbohydrates, in general, serve not only as an energy and carbon source but also as an osmoticum (i.e. influence osmolarity of the culture medium). In addition, carbohydrate modulated gene expression in plants (Koch, 1996). Plant gene responses to changing carbohydrate status can vary markedly. Some genes are induced, some

are repressed, and others minimally affected. As in microorganisms, sugar sensitive plant genes are part of an ancient system of cellular adjustment to critical nutrient availability. However, there is no evidence that this role of carbohydrate is important in normal growth and organized development in cell cultures. Sugars serve as energy sources (George, *et al.* 2008).

The addition of carbohydrates to the media is necessary, because photosynthesis is not efficient for plant growth and development. The most commonly used carbohydrate is sucrose. However, sucrose is not always the most effective carbohydrate for shoot induction and other morphogenic functions. Sugars have two principal functions: one is the cell provision of energy and the other is the supply of carbon skeletons for cell components. Usually 1-5% concentration of sucrose is used (Murashige, 1974). The superiority of sucrose for plant tissue culture was ascribed to its ability to induce production of substances that are essential for growth and physiological development. Heat sterilization of culture media that contain activated charcoal (AC) results in marked reductions in sucrose concentration (Pan and VanStaden, 1999). Formation of toxic inhibitory compounds, in the culture medium, from sucrose dehydration during autoclaving has been reported by Weatherhead, *et al.* (1978).

It is to be noted, that several investigators (Boxus, PH. 1974; Rugini and Orlando, 1992; Elmana *et al.* 2003) used, the monosaccharide sugar, glucose as an alternative to sucrose as a carbon and an energy source in strawberry tissue culture media. Shoots of strawberry obtained from a glucose containing medium have a better *ex-vitro* rooting capacity and a higher photosynthetic activity than shoots obtained from a sucrose containing medium Hou, (1992) used sucrose in strawberry culture media at 3%. Rugini and Orlando, (1992) used glucose at 3%

concentration. Occasionally both sucrose and glucose are used in combination, with media of low osmotility, where the high sucrose could be expected to maintain a sufficiently low osmotic potential (George, 1995a)

2.8.3.2.3. Growth regulators:

Growth regulators are artificially produced organic substances which have the same characteristics as natural hormones. Five principal classes of plant growth regulators have been recognized. Namely; auxins, cytokinins, gibberellins, abscisic acid and ethylene. None of these is associated with a single physiological or development process (Leopold, 1987). Growth regulators interact with hormones synthesized by plant cells and organs.

2.8.3.2.3.1- Cytokinins:

Cytokinins are one of the important growth regulators in plant tissue culture. They are a group of compounds that have a role in cell division and cell growth are thus used to break apical dominance and to enhance growth and shoot elongation (Jacobs, 1979).The main function of cytokinins is induction of adventitious shoots and shoot proliferation. In some plants, direct shoot formation is induced by cytokinins alone and addition of auxins is inhibitory (Bapat and Roa, 1977). Where cytokinins alone induce the formation of shoots, their action may depend on the presence of endogenous auxins within the explants (George, 1995a). Cytokinins are often required for callus formation and other processes involving cell division (Minocha, 1987).

Benzyl adenine (BA) is one most used cytokinin particularly in commercial micropropagation establishments (Zaew and Mapes, 1982). Several reports of using BA in the medium have been presented (Kandakova, *et al.* 1986) where relatively high BA concentrations caused rapid shoot proliferation and axillary bud formation. Simpson and Bell, (1989) found that the optimum levels of BA for maximum proliferation were between 0.23-1.8mg/l. Katano and Kenda, (1988)

tested 1mg/l BA and 0.2mg/l IBA on MS medium, and they found that strawberry grew well on MS with BA. Beech, *et al.* (1988) used cultures of MS medium containing BA at 0.5, 1.0, 2.0 or 5.0 mg/l**.** Atkinson, *et al.* (1986) used BA at 0.1-1.13 mg/l.

However, high BA concentration during multiplication decreased the rooting capacity of shoots. The stimulative effect of cytokinins on root formation might be temperature dependent (Fanizza, *et al.* 1988).

2.8.3.2.3.2-Auxins:

Auxins are involved in cell expansion and elongation, cell wall synthesis and root differentiation. The wounding caused during the preparation of cutting, increases the concentration of phenolic component, which act as auxin precursors. The number of roots formed on each shoot often increases in proportion to the concentration of auxin applied, but when the concentration becomes supra-optimal, callus formation is promoted, and root have an abnormal appearance and their average length, and subsequent shoot growth may be decreased (George, 1995b).

Indole butyric acid (IBA) is a preferable auxin for tissue culture because it is partially destroyed by autoclaving (Nissen and Sutter, 1988). Another advantage of using IBA over some synthetic auxins, is that IBA is metabolized to IAA (Epstein and Lavee, 1984). IBA was also noted as being effective in root formation at 1mg/l (Damiano, *et al.* 1988) or even at 0.5mg/l (Wichremesinhe, *et al.* 1988). Rooting in strawberry can be induced readily on hormone-free medium (Sarwar, 1989 and Green, *et al.* 1991).

2.8.3.2.3.3. Gibberellins:

Gibberellins are used to stimulate normal development of plantlets *in vitro*; however, they are rarely used (Bhojwani and Razandan, 1983).

Growth and development of explants *in vitro* is often controlled by the cytokinin: auxin ratio of the basal culture medium (Murashige, 1974; Huang and Murashige, 1976; James and Newton, 1977; George, *et al*. 2008). BA was effective in axillary shoot proliferation of *in vitro* shoot tip culture (Biswas, *et al.* 2008), and bud-break lateral shoot production in intact plants (Henny, 1985; Abedrabo and Said, 2012) and promote runnering in intact strawberry plants (Pritts, *et al.*1986).

The concentration of cytokinin/auxin ratio in the basal medium for best growth and development of strawberry tissue culture ranged from 0.25-2.5 mg/l and 0.25-1.0 mg/l respectively (James and Newton, 1977) whereas Hansan, *et al.* (2010) obtained best results with a cytokinin/ auxin range of 1.0-3.0 mg/l cytokinin and 0.1-0.3 mg/l auxin. Reports on the incorporation of more than one cytokinin in the medium of strawberry tissue cultures for best growth and morphological development exist (Sakila, *et al*. 2007; Moradi, *et al*. 2011). On the other hand, best growth responses were obtained on media containing 0.5 mg BA/l alone (Biswas, *et al.* 2008) or 2.0 mg/l picloram alone (Kordestani and Karami, 2008), as the only growth regulator in the culture medium of strawberry tissues. One can only speculate on the potential carry-over effect of the endogenous growth regulators content of the tissues of the cultured explants. The importance of the presence of an auxin and a cytokinin in the basal medium of plant tissue culture has been stressed by Murashige, (1974) and by George and Sherrington, (1984). These contrasting effects of BA on *in vitro* cultured tissues of strawberry reflect differences among studies in plant genotype, concentrations of BA used, type and source of explants, media components, and incubation conditions.

2.8.3.3. Chemical compounds with growth regulators-like activity:

During the recent decade several growth-active substances such as inhibitors of seed germination, pesticide, herbicide, fungicide and acaricide have been shown to exhibit growth regulators-like activity. The motivation for using these chemicals in plant tissue culture comes from the scarcity and availability and virtually prohibitive cost of the natural and synthetic growth regulators, (i.e. auxins and cytokinins), that have been used extensively in plant tissue culture to elicit specific morphogenic response. The successful use of some of these substances as growth regulators for *in vitro* cultured plant tissues and organs has been documented by several researcher (Jansson and Svensson, 1980; Scora, *et al.* 1984; Gowda and Prakas, 1998; Werbrouch, *et al.*1999; Idris, 2002; Idris, *et al.* 2010; Mohammed, 2012) and under *in vivo* conditions (Neuman ,1959; Stebbins, 1962; Coupland and Casely, 1975; Welker, 1976; Baur, *et al.* 1977; Baur, 1979; Byers, 1978; Byers, *et al.* 1982; Rogers and Thompson, 1983; El-Khair,2013) as having growth regulators-like effect. The effects of sub-lethal levels of some of these chemicals suggest that they have growth-regulating properties exhibiting cytokinin-like effects (Welker, 1976; Scora, *et al.* 1984) such as Stroby (El-Khair, 2013) or an auxin-like effect such as Furidan (Idris *et al.* 2010) and coumarin (Neuman, 1959; Jansson and Svensson, 1980).

El-Khair, (2013) evaluated the influence of Seven, BA, Furidan and Stroby on scion graft-take percentage in mango. Seven, BA and Furidan produced positive scion graft growth responses of equivalent magnitude. Stroby significantly enhanced percentage of graft take and subsequent growth and development of scion shoots over all other treatments. The effects of BA were more or less equal to those of Stroby in most parameters measured (El-Khair, 2013). On the other hand, Mohamed, (2012) used *in vitro* tissue techniques to test the efficacy of 4 pesticides; namely, Confidor, Seven, Furidan and Stroby on shoot proliferation in

ginger *in vitro* cultured tissues. Seven proved to be the most effective in increasing shoot formation at the lowest concentration tested.

The enhancement of scion graft take reported by El-Khair, (2013) is a result of loss of apical dominance; a phenomenon that is associated with cytokinins. It appears that Stroby exhibited a cytokinin-like effect on scion graft take and subsequent scion shoot growth and development, in disagreement with Mohammed (2012) who attributed the promotive effects of stroby on growth and development of ginger tissue culture to an auxin-like effect. A good and promising chemical with cytokinins-like effect is the weed killer glyphosate (Gly)

2.8.3.3.1. Glyphosate:

Glyphosate, (Roundup), {N-(phosphono-methyl) glycine} , (Gly) a nonselective, very broad spectrum, foliar applied, systematic post-emergence herbicide which is effective in controlling deep-rooted perennial, annual and biennial species of grasses, sedges and broad-leafed weeds. The effects of sub-lethal concentrations of Gly on plant growth suggest that it has growth regulating properties. Some of these effect include increasing fruit drop in grapefruit (Klosterboer, 1974), increased tillering in quackgrass (Coupland and Casely, 1975) and in wheat and sorghum (Baur, 1979; Baur, *et al*. 1977) and stimulation of bud-break and subsequent branching in cranberry (Welker, 1976).

2.8.3.3.2. Gum arabic:

Gum arabic, a complex natural substance of undefined constituents, is used worldwide in foods and drinks as an additive and in medicine as an anti-oxidant to protect against hepatic, renal and cardiac toxicities in experimental animals (Ali, *et al.* 2009). It is a branched-chain complex polysaccharide, found as a mixture of calcium, magnesium, and potassium salts of a poly-saccharidic acid. The main

source of gum arabic is the woody tree species *Acacia senegal* L. which belongs to the Leguminosae family, hence the name acacia gum. Only very scanty information, (Mohamed, 2012), is available on the use of gum arabic in plant tissue culture.

2.8.3.3.3. Silver nitrate:

In plant tissue culture, the culture vessels are usually caped with polyethylene closures to prevent contamination of cultured explants. This leads to accumulation of gases in the head space of the culture vessel. Among these accumulated gases, ethylene is apparently the most effective (Biddington, 1992). It is a natural plant growth hormone produced by plant cells, tissues and organs under *in vivo* and *in vitro* conditions. Excessive accumulation of ethylene in closed tissue culture vessels may cause unusual tissue growth and may consequently inhibit morphogenesis and result in the formation of vitreous tissues and callus (Auboiron, *et al.*1990; Adkins, *et al*. 1993; Mayor, *et al.* 2003; Anantasaran and Kanchanapoom, 2008). The rate of ethylene production by *in vitro* cultured plant tissues increases under conditions where cultured tissues are subjected to stress in culture (George, *et al.* 2008). Incubation of cultures at 20°C for example resulted in significantly better growth and development of papaya stem nodal cultures than incubation at 30°C, hence high temperature stress (Magdalita, *et al.* (1997) denoting more ethylene production and accumulation by the *in vitro* cultured explants under the latter condition than the former. Better control and management of ethylene accumulation in plant tissue culture vessels could contribute to better growth and morphological development of cultured plant tissues (Pablito *et al.* 1997).

$AgNO_3$ is a potent inhibitor of ethylene action (Beyer, 1976; Purnhauser, *et al.* 1987; Hyde and Phillips, 1996) and an enhancer of shoot regeneration in plant tissue culture. Williams, *et al*. (1990) using callus from *Brassica oleracea*

regenerated shoots from callus by adding $AgNO_3$ to the regeneration medium. In the absence of $AgNO_3$ callus died shortly after separation from the explants. Furthermore, inclusion of $AgNO_3$ into a regeneration medium devoid of growth regulators resulted in shoot formation while $AgNO_3$-free regeneration medium failed to induce shoot regeneration (Ozudogru, *et al*. 2005).

$AgNO_3$ has been widely, and in most cases, successfully used to overcome the inhibitory effects of ethylene on *in vitro* cultured plant tissues. In a number of crops such as ginger (Mohamed, 2012), zinnia (Anantasaran and Kanchanapoom, 2008), coffee (Giridhar, *et al.* 2006), wheat (Wu, *et al.* 2006), pistachio (Ozden-Tokatli, *et al.* 2005). peanut (Ozudogru, *et al.* 2005), potato (Turhan, 2004), pomegranate (Naik and Chand, 2003), cassava (Zhang, *et al.* 2001), lemon (Kotsias and Roussos, 2001), pearl millet (Oldach, *et al.* 2001), date palm (Al-Khayri and Al-Bahrany, 2001) apple (Ma, *et al.* 1998), lime and mandarin (Perez-Molphe-Balch and Ochoa-Alejo, 1997), cucumber (Mohiuddin, *et al.* 1997) and chile pepper (Hyde and Phillips, 1996). The effect of $AgNO_3$ is, however, genotypic dependent (Biddington, *et al.* 1988; Williams, *et al.* 1990; Pua, *et al.* 1999; Mohiudddin, *et al.* 2005; Wu, *et al*. 2006; Anantasaran and Kanchanapoom, 2008).

The precise explanation of the mechanism of $AgNO_3$ action is not fully understood. Several researchers (Beyer, 1976; Lieberman, 1979; Yang, 1985; Santana-Buzzy, *et al.* 2006) attributed the beneficial effect of $AgNO_3$ to its ability to ameliorate the detrimental effect(s) of ethylene on cultured plant tissues rather than inhibiting its production by the cultured plant tissues. The latter authors, however, detected high levels of ethylene in the headspace of culture vessels when high concentrations of $AgNO_3$ were added to the culture media. Growth and morphological development of cultured plant material was not negetively affected by the accumulated ethylene indicating that $AgNO_3$ modified ethylene effect(s) on

cultured plant tissues but did not inhibit its production. Yang, (1985) postulated that the Ag2+ ions in $AgNO_3$ inhibits ethylene action, consequently reduces the receptor capacity to bind ethylene. The interference of Ag2+ with polyamines has also been implicated with ethylene mode of action (Pua, *et al*. 1999). It is worth mentioning that inclusion of $AgNO_3$ in the regeneration medium promoted *in vitro* flowering of cultured plant tissues (Chi, *et al.* 1990) which is of importance for breeding purposes as well flower morphogenesis.

2.8.3.3.4. Casein hydrolysate (CH):

CH is one of the natural complexes of undefined composition that contain all amino acids usually found in normal proteins. It is used in plant tissue culture at concentration of 100-1000 mg/l. The natural complexes of undefined composition have been used in the early tissue culture work because of their beneficial effects on growth and multiplication of yeast and bacteria. The natural complexes used as nutrient medium ingredients in plant tissue include substances, such as casein hydrolysate (CH), coconut water (the milky endosperm of *cocos nucifera*) (CW), yeast extract (YE) and malt extract that are most frequently used in plant tissue culture. Other natural complexes of less use in plant tissue culture include leaf extract, ovary extract, meat extract, fish extract, peptone, fruit juices, potato tuber and banana extracts. Certain complexes are rich in nitrogenous substances (e.g. CH), sugars, (e.g. CW) and still, others rich in vitamins (e.g.YE). These substances are used when chemically defined substances cannot satisfy the requirements for growth and development of *in vitro* cultured plants tissues as the last resort (Murashige, 1973). The addition of these substances should be considered only if growth and development are not achieved even after all definable chemical substances known to be physiologically active have been used in optimal amount.

It is worth mentioning that these natural complexes have been reported to promote growth of plant tissue culture. Murashige and Skoog (1962) reported that inclusion

of CH in the culture media of low salt content increased callus formation in tobacco tissue cultured on media of low salt concentration such as Nitsch and Nitsch (1956), but gave only marginal increments on tissues cultured on MS medium which is high in mineral salts concentration. The values of organ formation obtained by the inclusion of CH in the culture medium were significantly higher than those obtained by supplementing the culture media with a wide range of IAA and Kin concentrations. Somatic embryos of *Theobroma cacao* were enhanced by inclusion of CW in the culture medium (Pence, *et al.* 1980; Pence and Soukup, 1986; Elhag *et al.* 1988) whereas, Elhag and Butler, (1992) promoted callus formation in sorghum in medium containing 10% CW and enhanced shoot regeneration with medium containing 1000 mg/l CH. Banana extract has been added routinely to media for *in vitro* culture of orchids (Pullman, *et al.* 2003). Shilpa and Rajani (2000) promoted asexual embryogenesis of cashew nut by inclusion of 10% CW and 500 mg/l CH in the induction medium. Shoot proliferation from nodal stem segments of vanilla was enhanced by inclusion of 1000 mg CH/l in the nutrient medium (Kononowicz and Janick, 1984).

The beneficial effect of CW would appear to be due, in part, to its high content of glucose and fructose (Tuleke, *et al.* 1961). Hasan, *et al.* (2010) reported the insignificance of inclusion of CW in the culture medium of strawberry. CW is usually added to culture media in a concentration of 10-30% (by volume) as suggested by Murashige, (1973). It is, perhaps, possible that the concentration used by these authors is insignificant rather than the effects of CW on growth and development of cultured strawberry tissues.

Generally the use of complex addend in plant tissue culture media is not recommended especially for research purposes because it is difficult to quantify their contribution.The chemical composition of these compounds is undefined and varies considerably with source, age and means of preparation. Inclusion of any of

the natural complexes in plant tissue culture, make it difficult to obtain the same result at any time and at any place. These substances are rarely used nowadays because of the improvement in development of chemically defined salt formulae and the discovery of an array of growth regulators which contributed to employment of defined plant tissue culture media.

2.8.3.3.5. Activated charcoal, (AC):

AC is commonly used in plant tissue culture media at a wide range of concentration 0.1%-3%, to counteract media and explants browning during establishment stage and to improve survival, growth and development of cultured plant tissues (Reynolds and Murashige, 1979; Tisserat, 1984).

Addition of AC to culture media has been shown to either enhance or inhibit *in vitro* growth and development of various plant tissues in culture. The beneficial effects of AC on embryogenesis and plant regeneration has been reported to be due to removal of undesirable inhibitory substances such phenolic compounds and volatile substances present in the medium or originating from the cultured tissues (Fridborg and Eriksson, 1975; Wang and Huang, 1976; Drew, 1979; Weatherhead, et al. 1978; 1979; Johansson, *et al.* 1982; Peck and Cumming, 1986). Callus from adult tissues, for instance, produce somatic embryos only on medium containing AC and remained undifferentiated on medium without AC (Banks, 1979).

Absorption and/or adsorption of growth regulators and reduction of their toxic levels are also implicated in the enhancement of regeneration (Steinitz and Yahel, 1982; Zaghmout and Torella, 1988). The former authors nullified the inhibitory effect of BA and NAA on bulb formation in *Narcissus tazetta* tissue cultures by the inclusion of 5.0 g/l AC in the culture medium whereas the latter authors promoted shoot and root formation in long term callus cultures of *Festcua rubra* L. by the inclusion of AC in the maintenance medium which contains 2, 4-D, before culture on the regeneration medium. Inclusion of AC promoted plant formation by

absorbing and/or adsorbing 2, 4-D and reducing its residual activity that may be retained in callus tissue after transfer to regeneration medium.

The possibility of the absorption and/or adsorption of probable toxic contaminants found in agar by AC (Romberger and Tabor, 1971; Kohlenbach and Wernicke, 1978; George and Sherrington, 1984) or release of growth promoting substances present in or adsorbed by agar could also be a factor contributing to the beneficial effect of AC on cultured plant tissue culture. Pan and VanStaden, (1998), however, ascribed the beneficial effects of AC on plant tissue culture to its modification of light intensity in the culture vessels creating favourable illumination conditions for growth and development of cultured explants.

AC is not selective in removing only toxic metabolites and compounds from plant tissue culture. It absorbs and/or adsorbs, in addition to toxic compounds, other chemical compounds essential for growth and morphological development. AC completely inhibited shoot proliferation in concurrence with preceding studies (Nissen and Sutter, 1990; VanWinkle, *et al.* 2003), that inclusion of AC in the culture medium results in the inhibition of growth and development of *in vitro* cultured plant tissues and organs. The inhibitory effect of AC on shoot proliferation of strawberry shoot tips could be attributed to depletion of essential growth factors as a result of absorption and/or adsorption by AC. This contention confirms previous findings that indicate that AC reduces the concentration of chemical compounds necessary for growth and development of *in vitro* cultured plant tissues such as various auxins and cytokinins sources, vitamins and other critical media constituents (Constantin *et al.*1977; Weatherhead *et al.* 1978; 1979; Ebert and Tayor, 1990; Nissen and Sutter, 1990; Pan and VanStaden, 1999; VanWinkle, *et al.* 2003).

It is advisable to adjust the constituents of culture media when the addition of AC and/or agar is essential. In date palm tissue culture the inclusion of AC is a must to

counteract the detrimental effects of browning. The auxin, 2, 4-D is essential for callus induction and morphogenesis in date palm tissue culture. It is added at a high concentration of 100 mg/l to ensure its availability at concentrations optimal for growth and development of cultured plant tissues (Reynolds and Murashige, 1979, Tisserat, 1984). On the other hand, AC reduced the availability of a number of essential mineral elements (Van Winkle, *et al*. 2003) necessitating the adjustment of the elemental composition of culture media. Gawel, *et al*. (1990) added 750 mg $MgCl_2$/l to replace divalent cations adsorbed by Gelrite, whereas, Dahleen, (1995), increased the concentration of copper in the regeneration medium of barley callus to optimize the frequency of plant regeneration. Improvement of the regeneration capacity of polyembryogenic cultures of barley by increasing the concentration of media nitrogen and copper has been achieved by Nuutila, *et al.* (2000).

Despite widespread use of AC in plant tissue culture, limited information is available on the manner(s) by which it exerts its physiological effects.

2.8.3.4. Physical support:

Most plant tissue culture media are solidified with a gelling agent to support and orient the tissue. Among the gelling agents, agar has been consistently used as a tissue culture support (George and Sherrington, 1984). Agar has good gelling properties, stability and resistance to metabolism during use (Henderson and Kinnersley, (1988). It is, however, the costliest ingredient of culture media and often contains impurities that may affect growth and development of cultured plant tissues (Debergh, 1983). Wide variation in the chemical and explants responses have been reported (Singha, 1984). These observations have stimulated recently a surge of interest and excitation among researchers to search for new support matrices as well as cheaper and advantageous gelling agents. Accordingly, a number of gelling agents and support matrices have been tested including

mucilaginous tissue extract (Titel *et al.* 1987; Bhattacharya *et al.* 1994; Idris, 2002), plant starches (Henderson and Kinnersley 1988; Kinnersley and Henderson, 1988; Simonson and Baenziger, 1992). The support matrices, on the other hand, included: polymeric substances (Matsumoto and Yamaguchi, 1989), filter paper (Horsch, *et al.*, 1980; Bhattacharya *et al*. 1994; Almobasher, *et al.* 2009), glass fibre (Tabor, 1981; Sabja, *et al.* 1990; Bhattacharya, *et al*., 1994), fabric tissues (Cheng and Voqui, 1977), cotton fibre (Moraes-Cerdeira, *et al.*1995; Almobasher, *et al.,* 2009), nylon cloth and polystyrene foam (Bhattacharya, *et al.* 1994), sugarcane bagasse (Mohan, *et al.* 2005) and "Luffa" fibre (Mohamed,2012). A matrix support should be, inert, available, reusable and cost-effective compared to agar. The use of matrix supports can improve aeration of stationary liquid media and enable maximum nutritional benefits through excellent contact between the nutrient medium and the explants beside the dilution of toxic metabolites.

The growth of plant tissues on liquid medium was significantly greater than on agar-solidified medium (Snir and Erez, 1981; Hammerschlag, 1982). This is attributable to better nutrient uptake, more availability of dissolved oxygen and easier dispensing compared to agar-solidified medium. Growth and development of plant tissues on agar solidified medium is inhibited as a result of toxic contaminants in the agar (Romberger and Tabor, 1971; Kohlenbach and Wernike, 1978). A major factor involved in inhibition of growth and development of plant tissues cultured on agar medium is the accumulation of cell exudates at the cut basal end of cultured explants which react with the chemical components of the culture medium forming toxic compounds (De Fossard, 1985). The superiority of liquid medium for plant tissue culture is primarily related to the dilution of these exudates more readily compared to agar (Murashige, 1977). It is also possible that nutrients, water and other growth factors are more available in liquid medium than on agar-gelled medium (Romberger and Tabor, 1971; Stoltz, 1971).

The nutritional requirements of plant cultures can be supplied by liquid media but growth in liquid medium may be retarded and development affected by oxygen deprivation and hyperhydration. The oxygen concentration of liquid media is often insufficient to meet the respiratory requirements of submerged cells and tissues. It can be increased either by raising the oxygen concentration of the medium or placing cells or tissues in direct contact with air. (Debergh, *et al.* 1992; Gaspar, *et al.* 1987; Ziv, 1991).

Liquid medium, without supporting structures, is used for the culture of protoplasts, cells or root systems for the production of secondary metabolites, and the propagation of somatic embryos, meristematic nodules, microtubers and shoot clusters. In liquid medium, these cultures often give faster growth rates than on agar-solidifed medium. Cultures may be fully or only partially immersed in the medium. Aeration of liquid medium in stationary Petri dishes is sometimes adequate for the culture of protoplasts and cells because of the shallow depth of the medium, but may still be suboptimal. Gelled media provide semi-solid, supporting matrices that are widely used for protoplast, cell, tissue and organ culture. Agar, agarose, gellan gums and various other products have been used as gelling agents.

Media are either gelled (semi-solid) or liquid. Liquid formulations are either stationary or agitated. Iner materials to support the tissue, like filter paper bridges and glass wool can improve the aeration of stationary liquid media (Murashige, 1977). Liquid media were reported to enable maximum nutritional benefits via excellent contact between the explants and the media. Besides, toxic metabolites are easily diluted in liquid media (Murashige and Skoog, 1962).

Agar is most widely used gelling agent for tissue culture media. However, wide variation in the chemical composition and responses obtained with different agar brands have been reported (Debergh, 1983).

Between tissue and medium, a lower concentration of agar is generally used for plant cultures than for the culture of bacteria. Plant media are not firmly gelled, but only rendered semi-solid. Depending on brand, concentrations of between 0.5-1.0% agar are generally used for this purpose. Agar is thought to be composed of a complex mixture of related polysaccharides built up from galactose. These range from an uncharged neutral polymer fraction, agarose that has the capacity to form strong gels, to highly charge.

2.8.3.5. pH value:

The pH value determines the acidity and alkalinity of solutions. It is the measure of hydrogen concentration in the solution. The pH of nutrient media is usually adjusted at 5-6 (Murashige, 1974; Seabrook, 1980; George and Sherrington, 1984). A pH value range of 5.7- 5.8 for agar- solidified media was optimal for keeping the mineral elements of the nutrient media in an available form for the *in vitro* cultured plant tissues (Murashge and Skoog, 1962). A pH value of 5.0 was, however, most suited for liquid media (George and Sherrington, 1984).

Most plant tissue culture media are not buffered. AC changes media pH by 1.0 to 2.0 pH units (Pan and VanStaden, 1999). The external pH of the culture medium has a pronounced effect on growth and development of *in vitro* cultured plant tissues and influences some plant developmental processes (Zhang and Stoltz, 1989; Smith and Krikorian, 1990). The former authors, working with scarlet plum, found that optimal medium pH for shoot proliferation was 5.3, lower pH values than 5.3 promoted shoot growth. In carrot, cultures retained their morphology as proembryos at pH 4, but developed into later stages of embryo development at pH 4.5 (Smith and Krikorian, 1990).

Numerous studies have, however, reported a drop of pH related with media sterilization by autoclaving (e.g. Wetzstein, *et al.* 1994; Owen, *et al.* 1991, Sarma, *et al.* 1990). In all cases autoclaving resulted in a decrease in pH ranging from

about 0.2-0.5 pH units. Decrease in medium pH associated with agar has also been realized (Selby, *et al.* 1989) who indicated that reduction in pH of the medium increases with increasing agar concentration. They also showed that the drop in pH varied with the initial pH of the medium; 0.8 to 0.9 of a pH unit drop at pH 6.0 whereas, the drop was a 0.1 to 0.2 of pH units at pH 4. Fluctuations in pH with storage of culture medium and tissue growth on the medium have also been implicated in fluctuations of medium pH (Skirvin, *et al.*1986; Sarma, *et al.* 1990; Owen, *et al.* 1991). Cultured plant tissues tend to buffer the pH of the nutrient medium by ion exchange and respiratory CO_2.

Extremes of pH values affect the availability of growth regulators, solidification of agar, results in precipitation of phosphorus and iron, (Dalton *et al.* 1983), as well as suppression of the absorption of ammonium ions. Rapid phosphate uptake occurs at medium pH value of 4.0, declining progressively with increase in pH values of more than 4.0 (George and Sherrington, 1984). At pH values lower than neutral the uptake of inorganic nutrients is inhibited and the leakage of essential metabolites from cultured tissues into the nutrient medium is enhanced (George, et al. 2008). Excised tomato roots elongated best in a nutrient medium the initial pH of which was 5.0-5.5 (White, 1932) and root initiation in pea-root culture was maximal in a pH range of 6.0 to 6.5 (Torrey, 1956).

Various authors used to adjust the pH to 5.8 before autoclaving (Hwang, *et al.* 1984; Cronauer and Krikorian, 1984, Wang, 1986). Vyskot and Bezdek (1984) found that the buffering capacity of MS medium was increased by adding 1.25 mM sodium citrate or 1.97 mM citric acid plus 6.07 mM dibasic sodium phosphate. Citric acid and some other organic acids have been noted to enhance the growth of *Citrus* callus when added to the medium (Murashige and Tucker, 1969; Erner and Reuveni, 1981). For the propagation of various cacti from axillary buds, Vyskot

and Jara (1984) added sodium citrate to MS medium to increase its buffering capacity.

2.8.3.6. Incubation of culture:

Cultures should be maintained under a 16 h photoperiod at 25-28C with a photon flux density of 12.5-20 µm m^{-2} s^{-1}.

Light is a physical factor that has profound effects on *in vitro* cultured plant tissues via its duration (photoperiod), intensity and quality. The implication of light in plant tissue culture is primarily related to morphogenesis rather than photosynthesis. *In vitro* cultured plant tissues and organs are usually incubated under a 16/8 light/dark daily photoperiod and a light intensity of 13.51 µm m^{-2} s^{-1} for the initiation and multiplication stages and 40.54 µm m^{-2} s^{-1} light intensity for rooting, hardening and acclimatization. Light in the incubation room is provided by cool white fluorescent lamps.

Adventitious shoot regeneration from callus of Norway spruce was best under conditions of continuous light intensity of 81.08 µm m^{-2} s^{-1} than the normal 16/8 photoperiod (Jain, *et al.* 1988). Direct shoot formation on leaf segments of a *Peperomia* species was markedly enhanced obtained when cultures were incubated under 36.49 µm m^{-2} s^{-1} of continuous light (Kukulczanaka, *et al.* 1977). Mohamed, (2012) obtained better growth and development of ginger tissue culture, by incubation of cultures under very low light intensity of 1.35 µm m^{-2} s^{-1} whereas multiple shoot formation was enhanced under continuous light of 6.76 µm m^{-2} s^{-1} (Debergh and DeWael,1977). The best response for plant regeneration by direct organogenesis from internodal segments of lime and mandarin was obtained when cultures were incubated for three weeks under dark followed by four weeks on a 16/8-h light/dark cycle (Perez-Molphe-Balch and Ochoa-Alejo, 1997). Similarly, Barcelo, *et al.* (1998) incubated strawberry explants under dark conditions during the initiation stage for four weeks before transfer to 16/8-h photoperiod for

successful growth and development. The beneficial effect of incubation of cultured plant tissues immediately after culture initiation for a period of time under dark conditions would possibly be attributed to that light degrades media components essential for growth and development such as auxins. A previous report by Nissen and Sutter, (1990) showed that light promotes the degradation of IAA and IBA in liquid and agar culture media. Nehra, *et al.* (1989) maintained a similar view that light degrade essential growth factors. Rooting of apple was enhanced by incubation of cultures under dark conditions (Druart, *et al.* 1982) attributing that to the effects of light on the activity of endogenous enzymes and phenolic compounds. Bi, *et al*. (2007) successfully obtained high frequency of high quality callus induction and subsequent plant regeneration by incubating cultured mature embryos of wheat under total darkness than 16/8-h light/dark photoperiod cycle. Low light intensities during callus induction and plantlet regeneration significantly increased regeneration frequency in anther culture of bread wheat (Ekiz and Konzak, 1993). Incubation of *in vitro* cultured strawberry shoot tips under dim light intensity of 6.76 µm m^{-2} s^{-1}, significantly improved their survival rate (KO, *et al.* 2009). The beneficial effect of incubation under dark conditions could be due to a reduction in synthesis of endogenous phenols and/or activity of peroxidases which are implicated in morphogenesis of cultured plant tissues. This conclusion supports Andersen and Kasperbauer's, (1973) contention that the inhibitory effects of relatively high light intensity is attributed to increased production of phenolic compounds which interfere with the activity of growth regulators. Sharma and Singh, (2002) reduced the phenolic content and the activity of polyphenol oxidase at the pre-culture stage as well as the exudation of phenols from cultured explants by incubation under dark conditions.

The study of the reaction of *in vitro* cultured strawberry tissues to different light/dark cycles conducted by Litwinczuk and Zubel, (2005) revealed the

superiority of shortened light/dark {4/2 light/dark (4 cycles/24-h} than the normal 16/8-h cycle or the 22/2 light /dark cycle. The beneficial effects of shortened light/ dark cycles on *in vitro* cultured plant tissues have been reported (Morini, et al. 1991; 1992; Zimmermann and Scorza, 1994). AC establishes a degree of darkness in the culture vessel thus reducing light intensity (Pan and Van Staden, 1998). The effect of AC on the modification of light intensity in the culture vessel may be contributory to the remarkable effects of AC on plant tissue culture, but is unlikely to be the major causative factor in that respect.

Light in plant tissue culture is essential for morphogenesis rather than photosynthesis. Plant tissues and organs cannot synthesize their nutritional needs for growth and development under *in vitro* conditions (i.e. they are heterotrophic). The requirement for light is genotypic dependent (Murashige, 1974; George and Sherrington, 1984; George, *et al.* 2008). Adventitious shoot formation in Norway spruce was best under 81.08 µm m^{-2} s^{-1} continuous light than the normal 16/8(d/n) photoperiod (Jain, *et al.* 1988).

Direct shoot formation from leaf explants of *Peperomia scandens* was markedly enhanced under 36.49 µm m^{-2} s^{-1} of continuous light compared to the normally used 16/8 cycle photoperiod (Kukulczanaka, *et al.* 1977). Nhut, *et al.* (2006) obtained significant increase in shoot and root elongation, leaf area and plant fresh weight in strawberry incubated under continuous relatively low light intensity.

Many strawberry cultivars are sensitive to photoperiod. Litwinczuk and Zobel, (2005) studied the response of a number of strawberry cultivars to different light/dark cycles {16/8 (d/n), 22/2 (d/n) and 4/2 (d/n), 4 cycles per 24h}.The latter treatment was superior over the other two photoperiod treatments. They concluded that shortened light/dark cycles are beneficial for growth and morphological development of *in vitro* cultured plant tissues and organs. Shortened light/dark

cycles are better for propagation of some woody plant species (Zimmermann and Scorza, 1994, Morini, *et al.* 1991; 1992).

2.8.3.7. Transfer to *ex-vitro* conditions:

Acclimatization is the process of an organism adjusting to chronic change in its environment, often involving temperature, moisture, food, relating to seasonal climate changes. In laboratory condition, this process is controlled to one variable change only. Some authors have used acclimatization to describe the process of an organism being forced to adjust to change in their environment by artificial means, such as in a laboratory setting, (Wikipedia, 2008).

Micropropagated strawberry plants were hardened in polyethylene bags and plastic trays. The maximum survival during hardening was observed in polyethylene bags filled with soil and farmyard manure (1:1) (Kaur and Chopra, 2002). Some authors advocate washing agar away from the root with warm water, and potting rooted plantlets in a peat; sand mixture,(George,1995c), also it is preferred to put strawberries rooted plantlet in a well drained sandy loom soils which have a high organic matter or alkaline soils amended with soil sulfur, (Ola and Anita 2007).

In vitro cultured plantlets are heterotrophic depending entirely on nutrient elements and other factors essential contained in the culture medium for their growth and development. Besides, they grow in culture vessels under conditions of high relative humidity, wilting as a result of loss water by transpiration. These plantlets lack protective means to reduce transpiration rate and subsequently excessive water loss (Brainerd, *et al.* 1981; Martin, *et al*.1988; Sutter, 1988). A report by Zaid and Hughes, (1995) showed that the surface of leaves of *in vitro* produced date palm plantlets have lower precipitation of wax in addition to structural malformation and function of the cuticle and slow rate of root formation. On the

other hand, El-Bahr, *et al.* (2003) reported the absence of conducting tissues in the roots of produced plantlets resulting in inadequate water absorption.

The growth and development of these plantlets is adversely affected upon transfer to *ex-vitro* conditions. An important step in preparing *in vitro* produced plantlets to continue their growth and development after transfer to greenhouse is to acclimatize and harden these plantlets to *ex-vitro* conditions. Successful transfer and establishment of *in vitro* produced plantlets determine the success or failure of any tissue culture technique for the vegetative propagation. The *in vitro* attempts to improve the growth and development of *in vitro* produced plantlets under greenhouse conditions included: incubation of cultured plantlets under relatively high light intensity (Al-Jibouri, *et al.*1988); enhance *in vitro* rooting (Tisserat, 1982; Shakib, *et al.* 1994); and weaning the plantlets by culturing on medium containing the minimal requirements for growth and development (Othmani, *et al.*2010). Greenhouse cultural practices that could contribute to better establishment and high survival percentages through reducing loss water via transpiration included maintenance of relatively high humidity (Brainerd and Fuchigami, 1981; Sutter and Hutzell, 1984) and spray with anti-transpirants (Sutter and Hutzell, 1984; Voyiatzis and McGranahan, 1994; Zaid and Hughes, 1995).

2.8.3.8. Media for strawberry culture:

Boxus, (1974) experimented with BA to determine its influence on shoot and root production *in vitro*. He established that shoots would proliferate in the presence of the cytokinin but roots would not form until the explant was without cytokinin. He concluded with the statement that micro-propagated strawberry plants would replace traditional methods of propagation for the commercial trade. Further studies have shown that selection of growth regulators types and their concentrations are vital to *in vitro* propagation of strawberry. BA, IBA, 2, 4-D, CH, and KNO_3 have been reported to be used in propagation and transformation

studies of strawberries (Goffreda, 1995; Nehra *et al*, 1990). Concentrations of 0.06-0.56mg/l of BA and 0.06-0.23mg/l of IBA seems to give the best result in strawberry culture media (James and Newton, 1977).Turovskaya, *et al.* (1986) found that the regeneration of plants from strawberry meristem culture was due to the cytokinin interaction in the processes of proliferation and rooting. Also, Osipova, *et al.* (1986) examined the effect of cytokinins and auxins at different concentration on the *in vitro* development of apical meristems. Rugini and Orlando, (1992) used 2.25mg/l BA and 0.51mg/l IBA on Gamborg medium for callus induction. Sovariet, *et al.* (1993) precultured stock plants on medium with 0.5mg/l BA and 0.5mg/l IBA. Elmana, *et al.* (2003) supplemented strawberry medium with 1mg/l BA, 1mg/l IBA. Kaur and Chopra, (2002) cultured strawberry plantlets on MS basal medium supplemented with (0.5mg/l BA and 0.1 mg/l IBA) for shoot multiplication. On the other hand, rooting of the micropropagated strawberry plants was best obtained on the MS basal medium supplemented with 1mg/l IBA and 0.5mg/l BA (Kaur and Chopra, 2002).

Liu and Sanford, (1988) reported using casein hydrolysate (CH) and potassium nitrate on leaf explants of 'Allstar' strawberry. Both chemicals stimulated the production of shoots; most probably they have an additive effect. Best shoot production in their study was achieved with a combination of BA, IBA, CH, and KNO_3. Kartha et al. (1980) successfully regenerated 'Redcoat' using a combination of BA, IBA and GA_3 as a precursor to a cryopreservation study of the cultivar. Kartha, *et al.* (1980) studied the effects of light intensity and media on greenhouse-grown and *in-vitro*-grown cultures of 'Redcoat'strawberry cultivar. Calli from *in vitro* grown cultures had very poor regeneration

3-MATERIALS AND METHODS

3.1. Location of the experiments:

All of these experiments were carried out at the tissue culture laboratory of the Department of Horticulture, College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Khartoum North.

3. 2. Plant material:

The plant material was of *in vitro* stocks of strawberry plantlets of the cultivar "Festival". 1.5-2.0 cm long shoot terminals were used as sole ex1plants for culture initiation and subsequent experimentation. Multiple shoots induction and plantlets regeneration were obtained from *in vitro* cultured shoot tips. Proliferating shoot culture was established by repeatedly sub-culturing the original *in vitro* produced plantlet shoot tip on stock plant medium after each harvest of newly formed shoots. When sufficient numbers of shoot tips were obtained experimentation was started. Due to plant material limitations, all experiments were not conducted at the same time.

3.3. Chemicals tested:

The chemicals tested and the concentrations were chosen on the basis of preliminary experiments carried out in our laboratory with shoot tips of a diverse number of plant species including strawberry. The following chemical compounds were tested for their influence on shoot proliferation and subsequent growth and development of *in vitro* cultured strawberry shoot tips:

1- MS salt mixture (Murashige and Skoog medium, 1962).

2- MS- NO_3.

3- KH_2PO_4.

4. Sucrose.

5- Inositol.

6- Benzyl adenine (BA).

7- Naphthalene acetic acid (NAA).

8- Fungicides, pesticides, and herbicides (furdan, stroby, seven, glyphosate).

9-Gum arabic.

10- Silver nitrate ($AgNO_3$).

11- Casein hydrolysate (CH).

12- Activated charcoal (AC).

3.4. Sterilization:

3.4.1Medium, dishes and distilled water:

Medium, dishes and distilled water were sterilized by autoclaving for 15 minutes at 121°C and 15 psi (1.06 kg/cm²).

3.4.2. Forceps and dissecting blades:

Forceps and dissecting blades were sterilized by autoclaving for 30 minutes at 121°C and a pressure of 1.06 kg/cm² at 121 and then dipping in 95% ethanol and exposed to gas flame after every use they were again dipped and re-flamed.

3.4.3. Working surface:

The laminar air flow cabinet was surface sterilized by wiping with 90% ethanol. The fan of the cabinet was switched on, 15 minutes before use.

3.5. Basal medium:

Unless otherwise stated, the basal medium used in the first two experiments consisted of full-strength Murashige and Skoog' s (MS) (1962), inorganic salt, 30 g sucrose; 10ml/l of a vitamin stock made of (glyacine 2mg/l, thiamine hydrochloride 1mg/l pyridoxine hydrochloride 0.5 mg/l plus nicotinic acid 0.5mg/l); 100 mg/l inositol; 0.5mg /l BA; 0.01 mg/l NAA and 7g /l agar. Thereafter the salt strength was reduced to ¾X {MS (¾ salt-strength)}, instead of the full MS salt- strength, and was used throughout this study. The pH's of all media were set at 5.7 ± 0.2 with 0.1N NaOH and/ or 0.1N HCL prior to agar addition. Agar was melted by heating on a stirring hot plate and the medium was

dispensed in aliquots of 25 ml into Magenta (GA-7-3) culture vessel and sterilized by autoclaving at 1.06 kg/cm² and 121°C for 15 min.

3.6. Experimentation:

Shoot terminals of 1-2cm long were excised from the *in vitro* stock plantlet cultures of strawberry cultivar "Festival" and were transferred aseptically to the appropriate test treatments for evaluation of their shoot proliferation efficiency.

3.6.1. The effect of MS salts mixture on shoot regeneration:

MS salt concentrations of 1/4X-, 1/2X-, 3/4X-, 1 X- and 2X, MS-salt strengths were tested on shoot proliferation and subsequent growth and development.

3.6.2. The effect of MS nitrate on shoot regeneration:

MS- NO_4 concentrations at 0.0X, 1/4X-, 1/2X-, 3/4X-, 1X- and 2X were tested for their effects on shoot proliferation and subsequent growth and development.

3.6.3. Additional Phosphate:

Supplementation of culture medium with 127.5 mg/l KH_2PO_4, (an equivalent to 3/4X KH_2PO_4 stock solution strength usually used in plant tissue culture media, where 1X equals 170 mg/l), was tested on shoot proliferation and growth and development.

3.6.4. The effect of sucrose:

Sucrose concentrations of 7.5, 15, 30, 60, and 120g/l were tested on shoot proliferation and subsequent growth and development.

3.6.5. The effects of inositol:

The effects of inositol concentrations at 0.0, 25, 50, 100 and 200mg/l on shoot proliferation and subsequent growth and development were examined.

3.6.6. The effect of BA:

Natural and synthetic growth regulators have been used extensively in plant tissue culture to elicit specific morphogenic responses. BA and NAA are needed to maximize shoot proliferation from axillary buds. Neither BA alone nor NAA

alone was able to induce shoot proliferation (data not presented**).** With NAA concentration in the basal medium held constant at 0.01 mg/l, different concentrations of BA at 0.0, 0.1, 0.3, 1.0 and 3.0 mg/l were tested for shoot proliferation rates and growth and development.

3.6.7. The effect of NAA:

Similarly, the effects of different concentrations of NAA (in mg/l) at 0.0, 0.01, 0.03, 0.1and 0.3, with BA concentration in the basal medium held constant at 0.1mg/l, on shoot proliferation and subsequent growth and development were evaluated.

3.6.8. The effects of Furidan:

Furidan concentrations at 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l were added to the basal medium to test their effect on shoot regeneration proliferation and subsequent growth and development.

3.6.9. The effects of Stroby:

Stroby concentrations at 0.0, 1.5, 3.0, 6.0 and 12.0 mg/l were added to the basal medium to evaluate their influence on shoot proliferation and subsequent growth and development.

3.6.10. The effects of Seven:

Seven concentrations of 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l were added to the basal medium to determine their effects on shoot proliferation and subsequent growth and development.

3.6.11. The effects of Glyphosate:

Glyphosate concentrations of 0.0, 0.2, 0.8, 3.2 and 6.4 mg/l were added to the basal medium to test their effects on shoot proliferation and subsequent growth and development.

3.6.12. The effects of Gum arabic:

Gum arabic concentrations of 0.0, 1.0, 3.0, 6.0 and 12.0 g/l were added to the basal medium to determine their effects on shoot proliferation and subsequent growth and development.

3.6.13. The effects of silver nitrate:

Silver nitrate concentrations of 0.0, 1.0, 2.0, 4.0 and 8.0 mg/l were added to the basal medium to evaluate their influence on shoot proliferation and subsequent growth and development.

3.6.14. The effects of casein hydrolysate:

Casein hydrolysate concentrations of 0.0, 12.5, 25.0, 50.0 and 100.0 mg/l were added to the basal medium to test their effect on shoot proliferation and subsequent growth and development.

3.6.15. The effects of activated charcoal:

Concentrations of activated charcoal at 0.0, 0.5, 1.0, 1.5 and 2.0 g/l were added to the basal medium to determine their effect on shoot proliferation and subsequent growth and development.

3.6.16. The effects of pH on shoot regeneration:

The effects of pH values of4.0, 4.5, 5.0, 5.5 and 6.0 on shoot proliferation and subsequent growth and development were evaluated.

3.7 The effects of the physical status of media:

"Ashmeik", date palm's trunk fibre, filter paper and the fruit fibre of *Luffa* sp., as support matrices or agar, the commonly used gelling agent, as a control were tested as physical support on a liquid medium.

3.8 The effects of light intensity:

Cultures were incubated in different levels of light intensity: Relatively high light intensity 13.51 μm m^{-2} s^{-1}, relatively low light intensity 6.76 μm m^{-2} s^{-1}, diffused

dim room light intensity 1.35 µm m^{-2} s^{-1} or dark conditions (cultures were cover with black cloth).

Unless otherwise stated, all cultures were maintained in an incubation sterile room at 25±2°C and 16-hours light and 8 hours dark using white; cool fluorescent lamps (light intensity was 20.27 µm m^{-2} s^{-1}. Transferes were conducted every other week, and after 6 weeks the experiment was terminated.

In vitro rooted plantlets of 2-3cm long were taken to the green house. Plantlets were removed from the culture vessels, washed thoroughly to remove agar and transplanted into one of the following four types of potting media: peat moss; sand: clay 1:1; sand: clay; 1:2; sand and clay .

3.10. Parameters measured:

After 6 weeks from culturing, the following parameters were recorded:

1. Number of leaves
2. Number of shoots
3. Number of roots
4. Mean length of longest plantlets.
5. Mean length of roots**.**

3.11. Statistical analysis:

All experimental treatments were arranged in completely randomized design. Each treatment in an experiment was replicated ten times of one explant each. Each experiment was repeated at least thrice. Analysis of variances was carried out using M stat (A computer programme) and Duncan Multiple Range Test was used to separate treatment means. The *t* test was used where appropriate for mean comparisons.

4-Results

Effect of MS- salt strengths:

Significant growth differences between treatments were evident for all parameters measured (Table 4.1) the normal MS-salt strength had the greatest effect on the various growth parameters measured. The highest value for shoot number (85.5), shoot elongation (2.4cm), leaves number (9.5), root number (4), and length (1.5cm) was obtained on medium containing (1X), the normal MS- salt strength commonly used in plant tissue culture media. Higher or lower salt strengths than the (1X) treatment negatively affected all growth variables measured with significant difference with the (1X) salt strength treatment. The two lowest MS- salt strengths tested promoted root initiation with no significant difference with the (1X) treatment. The normal MS- salt strength (1X) had the greatest effects on the various growth parameters measured. Shoot number and elongation, number of leaves and number of roots and root elongation were markedly promoted at 1X MS-salt strength but not at either lower or higher MS-salts strengths where significant negative effects were obtained.

Nitrogenous component:

The data depicted in (Table 4. 2) present the responses of strawberry shoot tips explants to different concentrations of the nitrogenous component of MS medium. The highest concentration of nitrate tested significantly reduced shoot proliferation. Response differences between treatments were evident for all parameters measured. The 3/4 MS nitrate strength {MS (3/4 NO_3)} treatment had the greatest effect on the various growth parameters measured. The highest number of shoots (61.7) and of roots (3.0), the longest shoots (1.7 cm) and of roots (2.3 cm) and the largest number of leaves (8.0) was obtained on medium containing 3/4

MS nitrate strength. Higher or lower concentrations of MS nitrate than 3/4 MS nitrate strength significantly reduced the values of all parameters measured.

Potassium dihydrogen phosphate:

The data presented in (Table 4. 3) illustrate the influence of the additional phosphate in MS medium on growth and development of *in vitro* cultured strawberry shoot tips. Increasing the original phosphorus concentration in MS medium by an addition of KH_2PO_4 had a positive, marked effect on growth and development of strawberry shoot tips. There were significant differences in shoot, leaf and root numbers and root elongation between treatments. Increasing the concentration of the original MS medium, by the addition of 127.5 mg/l potassium dihydrogen phosphate, markedly increased shoot proliferation as well as number of leaves number of roots and root elongation. The greatest number of shoots (26.5) and leaves (5.7), the largest number of roots (13.8) and the longest roots (5.0 cm) were produced on medium supplemented with additional phosphate with significant difference than the mean values produced on medium without additional phosphate. The least values for shoot number (16.8), leave number (4.5), root number (1.3) and root length (1.8 cm).

Sucrose concentration:

The results of the effects of different concentrations of sucrose on growth and development of *in vitro* cultured strawberry shoot tips are shown in (Table 4. 4) the values of all growth parameters measured increased as the concentration of sucrose in the medium was increased up to a maximum of 3%. The average mean number of leaves at 3% sucrose was highest but not significantly different from that at 0.75%, 1.5%, 3% and 6% sucrose concentrations tested. The highest value for all other parameters measured were obtained on medium containing 3% sucrose compared to other treatments tested.

Inositol concentration:

All inositol concentrations tested increased significantly shoot number over the control with progressive increases in shoot number as the concentration of inositol in the medium was increased up to a maximum of 100 mg/l and then declined sharply at the higher concentration tested (Table 4.5) the greatest number of shoots, (54.2), and roots (2.0) was obtained on medium containing 100mg/l inositol. Shoot elongation, on the other hand, was non-significantly reduced over the control and was significantly inhibited by inositol concentrations higher than the lowest concentration of inositol tested. The longest shoots were obtained on medium devoid of inositol with no significant difference from the value for shoot height obtained on medium containing 25 mg/l inositol, the lowest concentration tested. Significant differences among treatments were obtained for leaves number, and root length. The highest values of leaf number and root elongation occurred at either 50 or 100 mg/l inositol. These values were non-significantly higher with 100 mg/l than with 50 mg/l inositol.

BA concentration:

The data portrayed in (Table 4.6) summerize the results of an experiment conducted to determine the effects of different concentrations of BA on *in vitro* culture of strawberry shoot tip with a 0.03 mg NAA/l held constant. The lowest concentration of BA tested increased the values of shoot number, and length, number of leaves and root number significantly over the control with significant differences between treatments. With increasing BA concentration above 0.5 mg/l shoot formation declined but was still higher than in the control. The effect of BA on shoot length, number of leaves and roots of *in vitro* cultured strawberry shoot tips was highly significant. The highest values of these growth responses occurred at 0.5 mg BA/l. Root elongation was unaffected by all BA concentrations tested.

The greatest number of shoots (68.2), the longest shoots (5.2), and the highest number of leaves (8) and roots (32.3) were obtained on medium containing 0.5 mg/l BA. The decreased values obtained for shoot proliferation and elongation were still significantly higher than those obtained with the control treatment. The least number of shoots (7.7) and the shortest shoots were obtained on medium devoid of BA whereas, the least value for leaves number (4.7) and the least value for root number (0.0) were recorded on medium containing 3.0 mg/l BA. Although BA at the lowest concentration tested promoted root initiation.

NAA concentration:

The effects of NAA concentrations on growth and development of *in vitro* cultured strawberry shoot tips are presented in (Table 4. 7). Growth differences between treatments were evident for all parameters measured. With BA held constant at 0.3 mg/l, the three lowest concentrations of NAA, (0.01 mg/l, 0.03 and 0.1 mg/l), tested promoted shoot proliferation, non-significantly different over the control with no significant difference between treatments. There were significant differences in shoot number and shoot length, root number and root length between treatments lacking and containing NAA (0.0 mg/l versus 0.01 mg/l). The highest concentration of NAA, (0.3 mg/l), tested significantly decreased shoot count denoting the failure of NAA at this concentration to induce shoot bud differentiation. This would probably be due to the augmentation of natural apical dominance by NAA, hence suppressing shoot formation. The highest number of shoots (26.5) was obtained on medium containing 0.00 mg/l NAA with no significant difference with the 0.01 mg/l and the 0.03 mg/l NAA treatments. The least number of shoots (3.3) was obtained on medium containing 0.3 mg/l NAA, the highest concentration of NAA tested. Both shoot and root lengths were suppressed by all NAA concentrations tested with significant differences over the control. The longest shoots (4.8 cm) were obtained on medium devoid of NAA and

the shortest shoots (3.0 cm) were obtained on medium containing 0.3 mg/l NAA, the highest concentration of NAA tested. The longest roots were obtained on medium devoid of NAA (5.0 cm) and the shortest roots (1.2 cm) were obtained on medium containing the highest NAA concentration tested. Leaves number was, however, unresponsive to all concentrations of NAA tested whereas root formation was promoted with the lowest concentration of NAA tested with significant difference over the control. The highest number of roots, (18.8), was obtained on medium containing 0.03 mg/l NAA and the least number (8.3) on medium devoid of NAA.

Furidan concentration:

It was apparent that all measured parameters of *in vitro* cultured strawberry shoot tips responded to Furidan concentrations tested. The magnitude of response varies with concentration and the growth variable measured (Table 4. 8) the concentration of Furidan, (1.0 mg/l), tested significantly increased shoot number with significant differences between treatments. The largest number of shoots (134.0),) was registered on medium containing (1.0 mg/l) concentration of Furdan tested. The longest shoots, (3.2 cm), the greatest number of roots (20.0) and the longest roots (3.8 cm),) were obtained on medium containing 0.5 mg/l furdan the lowest concentration of Furdan tested.

The least number of shoots, (43.5), was obtained on medium containing 4.0 mg/l furdan, the highest concentration of Furdan tested, the shortest shoots (2.0 cm), was obtained on medium containing 0.0 mg/l furdan, the control.

Stroby concentration

Table 4. 9 illustrates the results of the effects of Stroby concentrations on growth and development of strawberry shoot tip explants. All Stroby concentrations tested significantly repressed shoot elongation, leave formation, root initiation and elongation over the control. Shoot proliferation was however, non-significantly

increased by the lowest Stroby concentration tested relative to the control. The highest level of shoot number (50.2) was obtained with Stroby at 6.0 mg/l and the least number of shoots (14.2) was obtained with Stroby at 12 mg/l the highest concentration of Stroby tested.

Seven concentration:

The effects of Seven on *in vitro* growth and development of strawberry shoot tips are presented in (Table 4. 10). Leaves number, root number as well as root length were largely unresponsive to the Seven levels tested. Shoot proliferation and elongation however, responded differently. The lowest concentration of Seven tested significantly increased shoot number and reduced shoot elongation relative to the control. The highest number of shoots (37.7) was obtained on medium containing 1.0 mg/l and the least number of shoots (4.7) was recorded on medium containing 4.0 mg/l Seven.

Glyphosate concentration:

Neither root number nor root elongation differed for the Glyphosate levels examined; but these levels inhibit shoot elongation and stimulated shoot proliferation and leave formation with significant difference relative to the control (Table 4. 11) the greatest number of shoots (91.2) and of leaves (12.2), were obtained on medium containing 0.8 mg/l Glyphosate. The concentration treatment containing Glyphosate at 6.4 mg/l, the highest Glyphosate concentration tested resulted in the least value for shoot number (6.7) and number of leaves (3.8).

Gum arabic concentration:

It was apparent from the data presented in (Table 4 .12) that gum Arabic concentration treatments tested inhibited shoot elongation, root number and length relative to the control treatment. Significant differences between treatments on all measured growth parametars were noted. Shoot number was significantly increased by gum arabic concentration tested over the control with significant

difference among treatments. The longest shoots (2.5cm), the greatest number of roots (10.5) and highest root length (4.2cm) were obtained on medium devoid of gum arabic. The lowest concentration of gum arabic (1.0 g/l), however, significantly increased shoot proliferation compared with other concentrations of gum arabic tested and shoot number as high as 105 shoots were obtained on medium containing this concentration. Concentrations higher than 1.0 g/l significantly reduced shoot proliferation. The lowest number of shoots (63.0) was obtained on medium containing no gum arabic.

$AgNO_3$ concentration:

The data portrayed in (Table 4. 13) indicated that all concentrations of silver nitrate tested had no significant significantly effect on shoot elongation but promoted shoot proliferation, leaf number, root number, and root length with significant differences among treatments. The highest number of shoots (33.0), leaves (10.0), roots (4,5) and root length (2.6cm) were obtained on medium containing 4.0 mg/l $AgNO_3$ and the lowest values, 5.3, 4.8, 0.8 and 0.8 cm were registered for shoot number, leaves number, root number and root length respectively, with silver nitrate concentration at 8.0 mg/l.

Casein Hydrolysate concentration:

The data of the effect of CH on growth responses of strawberry shoot tips under *in vitro* conditions are depicted in (Table 4. 14) all CH concentrations tested increased shoot proliferation and elongation significantly over the control. Leave number followed the same trend as shoot proliferation and elongation, increased with increase in CH concentration, reaching a maximum at 50 mg/l then decreased. Neither root number nor root elongation differed for CH concentrations tested. The highest number of shoots (59), the longest shoots (2.1cm) and the greatest number of leaves (8.5) were obtained on medium containing 50 mg/l CH. With increasing CH concentration to 100 mg/l, the highest concentration tested; shoot number,

shoot length, and number of leaves declined but were still higher than in the control. The lowest values of shoot number (10.33), elongation (1.18 cm) and leaves number (5.17) were recorded on medium containing no CH.

Activated charcoal concentration:

All concentrations of activated charcoal, (AC), tested significantly reduced shoot proliferation and increased leaf number, root number, and root length with significant differences among treatments, (Table 4.15) shoot elongation, number of leaves, and root number were unaffected by the lower concentration (0.5 g/l) of AC. The effect of AC at 1.0 g /l on shoot elongation, number of leaves, root number and length was highest but not significantly different from that at 1.5 g/l AC. The longest roots (4.1 cm), the greatest number of leaves (13.0) and of roots (11.8) and longest roots (6.9 cm) were recorded with AC at 1.0 g/l. Shoot proliferation, on the other hand responded differently. The largest number of shoots (54.5) was obtained on medium containing 0.0 g/l AC and the least number of shoots (3.7) was obtained on medium containing the highest concentration of AC (2 g/l) tested. The highest concentration of AC significantly inhibited all parameters measured.

pH:

The result of the effects of pH of culture medium on growth responses of strawberry shoot cultured under *in vitro* conditions is shown in (Table 4. 16). The value of all parameters measured increased with decreasing pH value up to a maximum pH of 5.5 and then declined sharply at the pH 6.0. The highest values of shoot number (25.7) and elongation (4.5 cm), leave number (5.5), root number (12.0) and root length (4.2 cm) were obtained at pH 5.5. The least values for all parameters measured were associated with the lowest and highest pH values tested. Root number and elongation seems to be highly sensitive to pH of the culture medium.

Physical support:

Table4.17 illustrates the results of an experiment designed to examine the effects of physical medium supports on growth and development of strawberry shoot tip explants. "Ashmaik", the date fibre, gave significantly high values for most parameters measured compared to the other physical medium supports tested. The highest number of shoots (65.7), the longest shoot (3.8 cm), the largest number of leaves (12.8), and the greatest number of roots (8.0), were obtained with cultures supported by "Ashmaik". Root elongation was also highest (4.3 cm) with the "Ashmaik" treatment which was not significantly different from the value for root length (4.1 cm), recorded on the agar solidified medium treatment. The least values for all growth responses measured were associated with liquid medium and "Luffa" support treatments.

Illumiation:

The data displayed in (Table 4. 18) illustrated that shoot proliferation increased with decreasing light intensity reaching a maximum with cultures incubated under diffused dim light intensity of 1.35 µm m^{-2} s^{-1} then sharply declined with cultures incubated under dark conditions. The greatest number of shoots (85.0) was obtained with cultures incubated under diffused dim room light intensity 1.35 µm m^{-2} s^{-1} and the least value (35.0) was recorded with cultures incubated under darkness. On the other hand, shoot elongation increased with decreasing light intensity reaching a maximum of 2.2 cm with cultures incubated under darkness. The shortest shoots (1.48 cm) were obtained with cultures incubated under the relatively high light intensity 6.76 µm m^{-2} s^{-1}. Leaves formation was positively influenced by high light intensity and was significantly inhibited by dark incubation conditions. The highest number of leaves (8.67) was obtained with cultures incubated under conditions of high light intensity 13.51 µm m^{-2} s^{-1} which was not significantly different from that obtained with cultures incubated under

conditions of the lower light intensity 6.76 μm m^{-2} s^{-1}. The least value of leaves number (5.67) was recorded with cultures incubated under dark conditions. Root formation and elongation followed the same trend as leaves number. Significantly higher values of root number (3.5) and root length (4.1) were obtained with cultures incubated under the relatively high light intensity, 13.51 μm m^{-2} s^{-1}, compared with other treatments tested. Incubation under dark condition completely inhibited root initiation.

Potting media:

Peat moss planting medium had the greatest effect on the various growth parameters measured compared to other planting media tested (Table 4.19) transplanted plantlets responded better to peat moss and this response was expressed in the highest number of shoots (3.2), the longest shoots (8.2 cm), the largest number of leaves (7.2), and the longest roots (15.2cm) registered with plantlets transplanted on the peat moss potting medium. Root formation behaved differently. The highest value for root number was obtained with transplants on peat moss (10.8) which was not significantly different from transplant on 2:1 sand: clay (10.3) or clay (10.0).

Table (4.1): Effect of dilute and concentrated solutions of MS salt strengths on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

MS-salt strength (X)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
1/4X	52.50d	2.37a	6.67c	4.83a	1.24b
1/2X	64.50b	2.50a	7.50bc	4.50a	1.21b
3/4X	60.50c	1.53b	8.17b	0.83b	0.27c
1X	85.50a	2.40a	9.50a	4.00a	1.52a
2X	32.33e	1.75b	7.00c	1.67b	1.23b
CV%	0.92	9.58	11.27	25.65	15.48

Means in a column followed by the same letter are not significantly different at P= 0.05, according to Duncan's Multiple Range Test.

Table (4.2): The effect of dilute and concentrated solutions of the nitrogenous component, (NO_3), of MS salt strength on growth and development of strawberry shoot tips cultured *in vitro*, after 6 week of incubation period.

Nitrate concentration (X)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0 X	**0.00f**	**1.33d**	3.83d	1.00c	1.00b
1/4X	**24.00c**	**1.40c**	4.83c	1.00c	1.00b
1/2 X	**10.33d**	**1.50b**	3.33d	2.00b	1.00b
3/4 X	**61.67a**	**1.70a**	8.00a	3.00a	2.25a
1 X	**54.83b**	**1.50b**	6.50b	1.00c	1.00b
2 X	**2.67e**	**1.45b**	3.33d	1.00c	1.00b
C.V%	**4.82**	4.75	12.63	29.81	5.55

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.3): Effect of potassium dihydrogen phosphate (KH_2PO_4) on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Supplement(127.5 mg/l KH_2PO_4)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
With	26.50 a	4.80a	5.70a	13.80a	5.01 a
Without	16.83 b	2.40b	4.50b	1.30 b	1.80 b

Means in a column followed by the same letter are not significantly different at P = 0.05, according to the *t* test.

Table (4.4): Effect of sucrose on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Sugar concentration (%)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.75	42.17c	2.20c	5.50ab	0.05e	0.52e
1.5	51.50b	4.53b	5.50ab	6.67b	3.18b
3	106.30a	5.72a	6.50a	31.17a	5.00a
6	25.00d	2.42c	6.33a	3.33c	1.90c
12	3.50e	2.32c	5.17b	2.00d	1.37d
CV%	5.96	6.78	14.90	12.75	12.08

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.5): Effect of Inositol on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Inositol concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	14.00e	1.95a	4.83c	0.67b	0.13b
25	18.00d	1.92a	4.67c	0.83b	0.12b
50	40.00b	1.53b	8.00a	1.00b	0.48a
100	54.17a	1.50b	8.33a	2.00a	0.50a
200	19.00c	1.63b	6.50b	1.00b	0.13b
CV%	0.63	8.85	16.66	26.76	25.70

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.6): Effect of BA on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

BA concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	7.67e	2.35d	5.33c	9.50c	3.33a
0.1	23.83d	3.00c	6.67b	11.67c	3.28a
0.3	49.68 b	3.18b	5.33c	15.50b	2.68b
0.5	68.17a	5.17a	8.00a	32.33a	1.68c
1.0	47.17b	2.68c	5.67bc	0.67d	0.30d
3.0	33.00c	2.60c	4.67c	0.00d	0.00e
CV%	4.75	5.13	14.62	10.31	10.27

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.7): Effect of NAA on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

NAA concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.00	26.50a	4.75a	5.67b	8.33d	5.02a
0.01	25.83a	3.26b	5.33b	13.83b	2.25b
0.03	25.67a	3.25b	5.17b	18.83a	1.92c
0.1	23.50b	3.03c	5.83b	18.50a	1.47d
0.3	3.33c	3.03c	5.50b	11.00c	1.15e
CV%	5.60	4.35	11.66	10.66	7.29

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.8): Effect of Furidan on growth and development of strawberry shoot tips.Cultured *in vitro*, after 6 weeks of incubation period.

Furidan concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root Length (cm)
0.00	69.50c	1.98d	9.33b	3.67c	1.83c
0.5	71.50c	3.17a	8.17c	20.0a	3.82a
1.0	134.a	2.83b	9.83b	15.67b	3.08b
2.0	103.0b	2.65c	11.50a	14.17b	2.82b
4.0	43.50d	2.65c	8.11c	11.00c	2.02c
CV%	2.64	5.02	5.74	13.47	13.20

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.9): Effect of Stroby on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Stroby concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.00	23.67c	2.00a	8.167a	2.17a	1.97a
1.5	25.50c	1.33c	6.83b	1.33b	0.97b
3.0	36.17b	1.83c	5.50c	1.33b	0.60c
6.0	50.17a	1.58c	6.50bc	1.00b	0.57c
12.0	14.17d	1.20c	5.50c	1.00b	0.47c
CV%	7.54	9.39	12.72	27.38	27.12

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.10): Effect of Seven on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Seven concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root Length (cm)
0.00	16.50d	2.40a	7.67a	2.00a	0.23a
0.5	22.00c	1.48c	7.33a	2.00a	0.20a
1.0	37.67a	1.50c	8.17a	2.17a	0.22a
2.0	29.50b	1.70b	8.00a	2.00a	0.22a
4.0	4.67e	1.38d	7.33a	2.00a	0.22a
CV%	5.85	4.37	9.02	8.98	18.07

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.11): Effect of Glyphosate (Gly) on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

(Gly) concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	10.83d	2.03a	7.00b	1.33a	0.47a
0.2	30.83c	1.63b	7.00b	1.33a	0.50a
0.8	91.17a	1.71b	12.17a	1.33a	0.45a
3.2	39.33b	1.27c	5.00c	1.33a	0.45a
6.4	6.67e	1.17c	3.83c	1.33a	0.45a
CV%	4.15	6.64	16.08	30.41	10.43

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.12): Effect of Gum Arabic on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Gum arabic concentrations (g/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	63.00d	2.53a	9.43b	10.50a	4.15a
1	105.0a	1.42b	10.67a	8.90 b	3.67b
3	89.71b	1.75b	10.57a	6.43b	1.34c
6	80.83c	1.70b	9.35b	1.17c	0.30d
12	78.00c	1.66b	6.17c	0.80c	0.30d
CV%	5.42	11.47	9.23	25.71	18.89

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.13): Effect of Silver nitrate on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Silver nitrate concentrations (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	5.37d	1.97a	5.00c	1.17c	1.05c
1	6.50c	2.20a	5.67c	1.00c	1.08c
2	22.50b	2.03a	7.50b	3.17b	1.67b
4	33.00a	2.17a	10.00a	4.50a	2.57a
8	5.28d	2.25a	4.83c	0.83c	0.83c
CV%	5.42	12.19	14.11	22.96	14.85

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test

Table (4.14): Effect of Casein hydrolysate (CH) on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Casein hydrolysate concentrations(mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.00	10.33e	1.18c	5.17c	1.00a	1.02a
12.5	20.00d	1.67b	7.17b	1.33a	1.07a
25	23.50b	1.80b	6.33b	1.17a	1.03a
50	59.00a	2.05a	8.50a	1.10a	1.01a
100	21.50c	1.37c	6.33b	1.00a	1.02a
CV%	1.55	10.45	13.95	26.76	10.90

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.15): Effect of activated charcoal (AC) on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Concentrations of activated charcoal (g/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	54.50a	2.45c	5.67b	5.67b	1.85d
0.5	8.33b	2.56c	9.17b	5.83b	6.13b
1.0	7.50b	4.05a	13.00a	11.80a	6.93a
1.5	6.00c	3.95a	12.17a	11.50a	6.87a
2.0	3.67d	2.83b	7.83b	3.50c	4.70c
CV%	6.57	8.80	12.16	13.07	4.59

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.16): Effect of pH on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

pH	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
4.0	2.50e	1.25d	3.47c	0.00c	0.00d
4.5	6.67 c	2.22c	3.83c	2.00b	0.80c
5.0	9.00 b	4.32a	4.33b	1.17b	0.93b
5.5	25.67a	4.48a	5.50a	12.00a	4.22a
6.0	4.83d	1.25d	3.67c	0.00c	0.00d
CV%	11.07	4.55	17.68	27.05	6.33

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table (4.17): Effect of the physical state of the culture medium on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Treatments	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
Liquid media	6.00d	1.67d	7.50c	0.00c	0.00c
"Luffa"	8.00d	1.67d	6.33c	0.50c	0.25c
Filter paper	26.33c	2.65b	8.62b	2.83b	1.30b
"Ashmaik"	65.67a	3.78a	12.83a	8.00a	4.28a
Solid media	61.00b	1.93c	8.67b	3.50b	4.10a
CV%	7.15	5.56	8.04	30.02	10.86

Means in a column followed by the same letter are not significantly different at P =0.05, according to Duncan's Multiple Range Test.

Table (4.18): Effect of illumination on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period.

Treatment (Lux)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
1000	54.00c	1.57c	8.67a	3.50a	4.10a
500	77.00b	1.48c	8.50a	1.50b	1.65b
100	85.00a	1.93b	6.00b	1.00c	0.45c
0.0	35.00d	2.18a	5.67b	0.00d	0.00d
CV%	0.44	6.87	7.7	25.82	13.91

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table(4.19): Effect of planting substrates on growth and development of strawberry transplants plantlets under *ex-vitro* conditions.

Treatment substrates	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
Clay	1.00c	6.40c	3.60c	10.00a	9.20b
Sand: Clay 1:1	1.00c	6.00c	3.80c	7.00b	7.68c
Sand: Clay 2:1	2.00b	7.20b	5.00b	10.30a	9.60b
Peat moss	3.20a	8.20a	7.20a	10.80a	15.20a
CV%	30.43	13.82	15.95	11.47	9.37

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

5 Discussion

The selection of a suitable salt formulae and salt-strength for culture and shoot proliferation is a crucial step for propagation of plants by tissue culture.

The data of the effect of MS salt- strengths on the growth and development of shoots tip of strawberry revealed the superiority of the normal MS-salts strength for growth and development of strawberry shoot tip explants. Murashige and Skoog salts were most effective when used at the standard strength; dilutions as well as higher dosages only reduced growth and development of a wide range of plant species (Murashige, 1974) and strawberry is an exception. Numerous workers in the field of strawberry tissue culture employed full MS salt-strength as their basal media (Boxus, 1974; Mullin, *et al.* 1974; Miller and Chandler, 1990; Nehra *et al.*, 1990; Elmana, *et al.*; 2003; Gerdakaneh, *et al.* 2007; Biswas *et al.*, 2008; Hasan *et al.,* 2010; Mir *et al.*2010). The results of these studies indicate that media containing MS normal salt-strength are most effective in inducing growth and development of plant tissue and organs culture for various purposes. This could be attributed to its high concentration of N and/or K, or of its total salts. MS-salt strength lower- (Lauzer and Vieth, 1990, Tessumura, *et al*. 2008; Mohamed, 2012) and higher than normal (Saadalla and Said, 2012) have been, however, used successfully for *in vitro* establishment and shoot proliferation of a number of woody plant species.

MS salt mixture is reputed for its high salt content and its high total salt strength in comparison to many other salt mixtures. Nevertheless, several researchers have tried to optimize enhancement and promoting effects of this famous salt mixture by modification of the normal salt strength of the whole salt mixture or that of some of its individual components. Modification of MS salt mixture by reducing the concentration of $NHNO_3$ and KNO_3 for best shoot formation was reported by

Economou and Read, (1984). The result of this study revealed the superiority of reduced nitrogenous component of MS over the normal strength. Best establishment and growth and morphological development were obtained on medium with the nitrate reduced to 3/4 strength, {MS (3/4 NO_3)}. The positive effects of reduced nitrogen in MS medium reported herein corroborated earlier findings by several workers (Lunderg and Janick, 1980; Sugiura *et al.* 1986 and Igarashi and Yuasa 1994) that indicate that establishment and shoot regeneration in cultured plant tissues and organs is improved by reducing the total nitrogen in the culture medium. The results are also comparable to findings of others (Sing, 1978; Asokan *et al.* (1983) who reduced nitrogen in MS medium by the inclusion of 500 mg/l $NH4NO_3$ as the only nitrogen source. The beneficial effects of 3/4 MS nitrate is related to an alteration in nitrate: ammonia ratio. Nuutila, *et al.* (2000) stressed the vitality of a balanced nitrate: ammonia ratio for growth and physiological development of *in vitro* cultured plant tissues and organs. Nonetheless, reports on the suitability of the normal nitrogenous component of MS medium (Villamor and Birnbaum, 1999; Kamaska and Santilata, 2009; Cecilia, 2010; Mohamed, 2012) exist. A contradictory report by Liu and Sanford, (1988) advocated supplementing the culture medium with 2000 mg KNO_3 to enhance regeneration efficiency of cultured strawberry leaf tissues. These inconsistencies in results could be due to differences in plant species and varieties, type of explants, purpose of culture, media composition or incubation conditions.

The depletion of phosphorous early during the initiation and establishment stage has a major effect on the pH value of the medium in which phosphorous is an important buffering component (George *et al.,* 2008). High and low pH values cause the precipitation of phosphorous together with iron (Dalton *et al.*1983). The results of this study are consistent with those reported by others (Smith and Murashige, 1970; Idris *et al.*2006; Momamed, 2012; Osman *et al.,* 2013) that

indicate that increasing the concentration of phosphorus in MS-salt based medium improved growth and development of *in vitro* culture of tissues and organs of various plant species for various purposes. The beneficial responses of strawberry shoot tips to the increase in phosphorus concentration in the original MS medium merits further studies.

Inclusion of sucrose in culture medium is essential for shoot proliferation of many plant species including strawberry (Huang and Murashige, 1976; George and Sherrington, 1984; Gerdakaneh *et al.* 2009). Sucrose at 3% concentration is the standard carbon and energy source in tissue culture of many plant species (Murashge, 1974) and strawberry is not an exception (Hou, 1992; Kordestani and Karami, 2008; Biswas *et al.* 2010).Sucrose at 12% in the medium negatively affected shoot proliferation capability as well as shoots elongation, leave and root number and elongation. Leave number seems to be less sensitive to sucrose concentrations compared to the other growth responses measured. Inhibition of leave formation occurred at 12% sucrose concentration. Relatively high concentrations of sucrose (6%-10%), however, enhanced the induction and development of somatic embryogenesis in strawberry (Kordestani and Karami, 2008; Gerdakaneh *et al.* 2009) and in other plant species (Ricci *et al.* 2002; Karami *et al.* 2006). The effect of sucrose on *in vitro* cultured plant tissues is apparently osmotic rather than nutritional. The osmotic effect of sucrose is what triggers growth and development of cultured tissues in general agreement with Shahnewaz and Bari, (2004) contention that the effects of sucrose on callus induction and plant regeneration in rice tissue culture are due to its contribution to the osmotic potential of the culture medium rather than its utilization as an energy source. The beneficial effect of inositol on strawberry shoot tip cultures has been consistent with previous findings by Goforth and Torrey, (1977) of the promoting effect of inositol on *Comptonia peregrina* (L.) Coult. root cultures and by Said and

Murashge, (1979) with root cultures of citron. Additionally Cronauer and Krikorian, (1984) reported beneficial effects of inositol on *in vitro* growth and morphological development of banana shoot tip culture. Beneficial responses of higher- (Said and Murashige, 1979) and lower- concentrations (Matsubara and Hegazi, 1990; Dlaigan, 1995) than 100 mg/l inositol have been reported. The former authors found inositol at 5000 mg/l optimum for citron root culture whereas the latter author found 50 mg/l inositol optimal for date palm root culture. Matsubara and Hegazi, (1990) promoted plantlet regeneration from radish callus by supplementing the regeneration medium with 2.0 mg/l inositol. Inositol at the standard concentration (100 mg/l) in MS medium, however, repressed tomato root cultures noticeably and lower concentrations were without effect (Said and Murashige, 1979). The manner in which inositol exerts its physiological effects on *in vitro* cultured plant tissues, organs and cells, remains unexplainable.

Shoot proliferation was the principal morphogenic pattern in the experiment where different concentrations of BA were tested. Best shoot proliferation and elongation, and leaf and root formation occurred on medium containing 0.5 mg/l BA. The results were close to the findings of a number of workers (Ko, *et al.* 2009; Biswas, *et al.* 2008; Moradi, *et al.* 2011) who successfully used and recommended the use of relatively low concentrations of BA, (0.5 mg/l BA), in media for *in vitro* culture of strawberry tissues. BA permits the development of shoots from axillary buds which normally would remain dormant. The significantly high increase in shoot number on medium containing 0.5 mg/l BA would appear to be the result of a loss of apical dominance. The potential number of shoots formed is dependent on cultured shoot tip size and the number of axillary buds it contains. Concentrations of BA higher than 0.5 mg/l repressed shoot number, elongation, leaf number and root initiation. The results of this experiment deviate from those reported by other workers (Elmana, *et al*. 2003; Sakila, *et al.* 2007; Gerdakaneh, *et al.* 2009; Ko, *et*

al. 2009; Biswas, *et al.* 2010; Mir, *et al.* 2010; Mozafari and Gerdakaneh, 2012) who employed relatively high concentration of BA (higher than or equal to 1.0 mg/l) for best growth and development of *in vitro* cultured strawberry tissues. The promotion of axillary shoot proliferation in cultured strawberry shoot tips by exogenously applied BA is thought to be a consequence of loss of apical dominance as a result of alterations in the endogenous balance between naturally occurring hormones (Phillips, 1975). It is also possible that BA was implicated in the availability of optimum nutrients and growth factors essential for shoot proliferation and subsequent growth. This contention concurs with the speculation of Shindy and Weaver, (1967) that exogenously applied BA increased the translocation of assimilates to the meristematic regions of axillary buds.

Neither NAA nor BA alone was able to induce multiple shoot proliferation (data not shown). This would be a result of the possible implication of a synergistic effect between NAA and BA on *in vitro* growth and development of shoot tip explants of strawberry. NAA and BA are both needed to maximize shoot proliferation and root formation. The highest shoot and root numbers were produced at 0.03 mg/l NAA and 0.3 mg/l BA. Shoot and root lengths were highest at 0.0 mg/l NAA with 0.3 mg/l BA. The results are parallel to those reported by several investigators (i.e. Elmana, *et al.* 2003, Sakila, *et al.* 2007; Gerdakaneh, *et al*. 2009; Ko, *et al.* 2009; Mir, *et al.* 2010; Mozafari and Gerdakaneh, 2012) who added a cytokinin and an auxin to culture media of strawberry for best growth and development. Different types and concentrations of cytokinins and auxins were used. Discrepancies in chemicals and concentrations used depend on availability, plant genotype, purpose of culture and media components.

It is worth mentioning that Biswas, *et al.* (2008) obtained better multiple shoot regeneration on medium supplemented with 0.5 mg/ l BA only whereas

Kordestani and Karami, (2008) induced somatic embryos only on medium containing picloram at 2.0 mg /l.

Furidan, (Carbo-furan), is an important chemical pesticide. It has been found to exhibit growth regulator-like effect at sub-lethal concentrations (Idris, *et al.* 2010).The current results disclosed that the concentration of Furidan tested (1.0 mg/l) significantly improved growth and development of *in vitro* cultured strawberry shoot tips. The results were in accordance with those of Idris, (2002) who accelerated embryogenesis and plantlets regeneration in pineapple by adding 0.5 mg/l Furidan in the culture medium. Inclusion of 2.0 mg/l Furidan in the culture medium of ginger markedly enhanced shoot proliferation (Idris, *et al*. 2010). Comparable results were also obtained by (Mohamed, 2012) working with ginger where Furidan at 1.0 mg/l increased shoots formation in ginger. The results of this showed that Furidan exhibited cytokinin-like stimulation of shoot proliferation in shoot tips of strawberry. Idris, *et al.* (2010), working with ginger tissue culture maintained the same view.

Stroby, a fungicide/acaricide has a profound effect on shoot number when added to culture medium at a concentration of 6.0 mg/l. The results corroborate those of Mohamed, (2012) where best responses for shoot proliferation in ginger tissue culture was obtained on medium containing 5.0 mg/l Stroby. This promoting effect on shoot proliferation and the inhibitory effect on shoot elongation, leaf formation, root initiation and elongation testify to the cytokinin-like effect of Stroby. The beneficial effect of Stroby on strawberry shoot prolieration would appear to a result of loss of apical dominance. Similar results were obtained and similar conclusions were reached by El-Khair, (2013), attesting to the potency of Stroby as a chemical compound with high cytokinin-like activity. The author evaluated the influence of BA, Furidan, Stroby and Seven on the percentage of scion graft success in mango (*Mangifera indica* L.). The results revealed the superiority of

Stroby over the other chemicals tested for high percentage of scion graft-take. Stroby gave the highest percentage of scion graft success compared to the other chemicals tested. Mohamed, (2012) obtained best shoot formation in ginger explants cultured on medium containing 5.0 mg Stroby/l. In contrast to our contention and that of El-Khair, (2013) Mohamed (2012), attributed the beneficial effects of Stroby to an auxin-like activity.

Seven (carbary: 1-naphyl-N-methyl carbamate), a pesticide, has been shown to exihibit growth- regulator like effects on intact plants (Stebbins, 1962; Southwick, *et al.*1964; Byers, 1978; Byers, *et al.* 1982; Rogers and Thompson, 1983).The current study showed that Seven has a pronounced effect on shoot proliferation; doubling the number of shoots of strawberry shoot tips over the control at a concentration of 1.0 mg/l. Shoot elongation was, however, inhibited by the all concentration tested. The result supported the finding of Mohamed (2012) working with ginger, that Seven at 1.0 mg/l significantly enhanced shoot proliferation.The effect of Seven on strawberry tissue culture, under the condition of this experiment, indicate that Seven has cytokinin-like effects. The influence of Seven on *in vitro* cultured strawberry shoots are possibly due to a cytokinin-like effect.

Addition of 0.8 mg/l Gly in the culture medium resulted in a marked increase in shoot and leaf numbers while inhibiting shoot elongation. It seems that Gly antagonizes apical dominance, resulting in axillary bud-break. Root initiation and elongation were largely unresponsive to Gly levels tested. Similar results on the beneficial effects of Gly were reported by Scora, *et al.* (1984); Gowda and Prakas, (1998).The addition of Gly to the culture medium by the former authors resulted in better growth and development of *in vitro* cultured cranberry node explants; while the latter authors enhanced somatic embryo development in *Ipomea batatus* by supplementing the nutrient medium with Gly. The current results indicate that Gly effects on strawberry shoot tips are cytokinin-like effects a conclusion that is

supported by the finding by others (Klosterboer, 1974; Coupland and Casely, 1975; Welker, 1976; Bauer, 1979) that application of Gly to intact plants result in a loss of apical dominance and release of quiescent buds enhancing the formation of shoots. Cytokinins release quiescent buds and promote branching in intact plants (Abedrabo and Said, 2012).

Gum arabic or acacia gum, effectively increased shoot proliferation, as well as leaf formation and had a suppressive effect on shoot elongation, root formation and elongation. This result is in line with the finding of Mohamed, (2012) where inclusion of 3.2 g/l gum arabic improved growth and development of cultured ginger tissues attributing that to an antioxidant effect. The beneficial and the repressing effects of gum arabic on strawberry shoot tips growth and development could perhaps be due to its antioxidant effects. Antioxidants such as citric acid and polyvinylpyrrolidone (PVP) have been used in tissue to modify the regeneration capacity of culture media for root formation (Standardi and Romani, 1990). It seemed that gum arabic interfered with the activity of endogenous peroxidases and phenols and, consequently, with morphogenesis of cultured strawberry shoot tips.

The results of this study showed that MS basal medium supplemented with 4.0 mg/l $AgNO_3$, not only increased shoot proliferation of *in vitro* cultured strawberry shoot tips but also improved shoot elongation, leaf formation as well as root initiation and elongation. The results are similar to those of Qin, *et al.* (2005) who enhanced shoot regeneration efficiency and adventitious bud formation in strawberry by the inclusion of 1.0 mg/l $AgNO_3$ in the culture medium. $AgNO_3$ has been widely, and in most cases, successfully used to overcome the inhibitory effects of ethylene on *in vitro* cultured plant tissues. In this study, supplementation of the culture medium with 4.0 mg/l $AgNO_3$ enhanced growth and morphological development in strawberry shoot tip culture. Similar results were reported in a number of crops such as, pistachio (Ozden-Tokatli, *et al.* 2005), pomegranate

(Naik and Chand, 2003), lemon (Kotsias and Roussos, 2001), date palm (Al-Khayri and Al-Bahrany, 2001) apple (Ma, *et al.* 1998), lime and mandarin (Perez-Molphe-Balch and Ochoa-Alejo, 1997). The effect of $AgNO_3$ is, however, genotypic dependent (Williams, *et al.* 1990; Wu, *et al*. 2006; Anantasaran and Kanchanapoom, 2008). A contradictory report on the inhibition of embryogenesis, by inclusion of $AgNO_3$ in the culture medium of anther culture of Brussels sprouts, was published by Biddington, *et al*. (1988). The decreased values for shoot, leaf, root number and root elongation obtained with higher and lower concentration than 4.0 mg/l $AgNO_3$ was perhaps due to the addition of supra- or sub-optimal concentration of $AgNO_3$ for maximum growth and development. The precise explanation of the mechanism of $AgNO_3$ action is not fully understood. Several researchers (Beyer, 1976; Lieberman, 1979; Yang, 1985; Santana-Buzzy, *et al.* 2006) attributed the beneficial effect of AgNO3 to its ability to ameliorate the detrimental effect(s) of ethylene on cultured plant tissues rather than inhibiting its production by the cultured plant tissues.The ability of $AgNO_3$ to minimize callus formation at the basal cut end of cultured explants has been recognized (Ozden-Tokatli, *et al.* 2005; Ozudogru, *et al.* 2005). This has an important impact on clonal propagation by tissue culture where it is highly desirable to avoid callus formation because of the possibility of shoots originating from the callus tissue with a subsequent change in clonal characteristics.

In strawberry, Wang, *et al.* (1984) increased the frequency for initiating embryogenic callus by inclusion of 500 mg/l CH in the culture medium and Liu and Sanford, (1988) stimulated shoot production on leaf explants of “Allstar” strawberry cultivar by adding 400-600 mg /l CH to the regeneration medium.

The promoting effect of CH recorded in this study and earlier studies with strawberry, (Wang, *et al.* 1984 and Liu and Sanford, 1988), could, perhaps, be a

reflection of the beneficial effects of its amino acids content. The beneficial effect of amino acids on plant tissue culture has been reported (Kamada and Harada, 1977). Amino acids provide plant tissues organs and cells culture *in vitro* with an immediately available source of nitrogen and uptake can be much more rapid than that of inorganic nitrogen in the same medium (Thom *et al.* 1981). Only the L-forms of amino acids are biologically active. The beneficial effects of amino acids on plant tissue culture have been documented (Green and Phillips, 1975; Kamada and Harada, 1977; Ishii, 1986) but they are not essential components of plant tissue culture media. Their addition in plant tissue culture media, as identified pure compounds, was limited because of scarcity and virtually prohibitive cost. The satisfaction of possible beneficial effects of amino acids can be achieved through the addition of an inexpensive natural complex of undefined composition known to contain protein complexes such as protein hydrolysates. The hydrolysate of the protein of milk, CH, is an excellent candidate for this purpose. Its major constituent are amino acids and is an inexpensive chemical compound that can be used to ensure against media deficiency of possible beneficial effects of amino acid(s) as well as providing an organic source of nitrogen that is readily available to explanted tissues.

The beneficial effects of AC on shoot elongation, number of leaves, root formation and elongation of strawberry shoot tips would appear to be a result of its ability to absorb and/or adsorb exudates that leach from cut basal end of cultured shoot tips. The interaction of these exudates with the chemical components of the culture medium may result in the formation of toxic substances that may repress growth and development of cultured plant tissues. Similar results were obtained and similar conclusions were reached (Fridborg and Eriksson, 1975; Wang and Huang, 1976; Fridborg *et al.* 1978; Banks 1979; Drew, 1979; Weatherhead *et al.* 1979; 1978; Peck and Cumming, 1986; Zaghmout and Torella, 1988; Dlaigan, 1995) who

indicate that AC removes substances which seem to be deleterious to morphogenesis in plant tissue cultures.

On the other hand, AC completely inhibited shoot proliferation in concurrence with preceding studies (Constantin, *et al.* 1977; Lashermes, 1992; Chrystiane, *et al.* 2004), that inclusion of AC in the culture medium results in the inhibition of growth and development of *in vitro* cultured plant tissues and organs. The inhibitory effect of AC on shoot proliferation of strawberry shoot tips could be attributed to depletion of essential growth factors as a result of absorption and/or adsorption by AC. This contention confirm previous findings that indicate that AC reduces the concentration of chemical compounds necessary for growth and development of *in vitro* cultured plant tissues such as various auxins and cytokinins sources, vitamins and other media constituents (Constantin *et al.*1977; Weatherhead *et al.* 1978; 1979; Ebert and Tayor, 1990; Nissen and Sutter, 1990; Pan and Vans staden, 1999; VanWinkle *et al.* 2003).

In this study, an optimal growth and development of strawberry shoot tips was obtained at pH 5.5. Strawberry shoot tips appeared sensitive, and a pH of 5.5 appeared critical. Growth and development decrease towards pH 4.0 or 7.0. This could be due to disturbance in nutrient uptake; in accordance with the findings of others (Dalton, *et al*. 1983, George, *et al*. 2008) which indicate that extremes of pH values affect the availability of growth regulators, solidification of agar, results in precipitation of phosphorus and iron, as well as suppression of the absorption of ammonium ions. The requirements for inorganic salts and total salt according to Jay, *et al.* (1994), a rapid drop in pH to 4.0-4.5 occurs within 24 to 48-h from culture initiation.

"Ashmaik" was found to be the best support matrix because it had the greatest effect on the various growth parameters measured. The largest number of shoots was produced on "Asmaik" matrix support, a lesser on the agar-gelled medium and

the least on the liquid medium. A matrix support should be, the beneficial effect of "Ashmaik" was ascribed primarily to an improved aeration of stationary liquid media and better availability of nutrient via direct contact between the nutrient medium and the cultured tissues. Besides, it is inert, available, reusable and cost-effective compared to agar. Matrix support should be, inert, available, reusable and cost-effective compared to agar.

Other studies, however, indicate that the performances of cotton fibre (Cheng and Voqui, 1977; Moraes-Cedeira *et al.*1995), filter paper discs (Horsch *et al.* 1980), glass wool (Tabor, 1981; Bhattacharya *et al.*1994) and "Luffa" (Mohamed, 2012) were found satisfactory and could compare well with that of agar.

The beneficial effect of very low light intensity on shoot proliferation reported herein parallel the results reported by Mohamed, (2012) on ginger where best growth and morphological development was obtained by incubation of cultures under very low light intensity of about 1.35 µm m^{-2} s^{-1}. The results also were comparable to the findings of Ekiz and Konzak, (1993) that indicate that low light intensities during callus induction and plantlet regeneration significantly increased regeneration frequency in anther culture of bread wheat. Similarly, Ko, *et al.* (2009) significantly improved the survival of *in vitro* cultured strawberry shoot tips by incubation under dim light intensity. The beneficial effects of incubation of plant tissues and organs under low light intensity would perhaps be attributed to a reduction in the formation of inhibitory substances as a result of photochemical reactions. This conclusion is accordance with the contention of Andersen and Kasperbauer, (1973) and Druart, *et al*. (1982) that the inhibitory effects of relatively high light intensity to increased production of endogenous peroxidases and phenols which interfere with the activity of growth regulators. Sharma and Singh, (2002) reduced the phenolic content and the activity of polyphenol oxidase at the pre-culture stage as well as the exudation of phenols from cultured explants

by etiolation. The suppression of initial cell divisions in explants of artichoke by light has been reported by Fraser, *et al.* (1967) The results, however, contrasted with those of Jain, *et al.* 1988; Debergh and DeWael,1977; Kukulczanaka, *et al.* (1977) who obtained best adventitious shoot regeneration under conditions of continuous relatively high light intensity than the normal 16/8 photoperiod.

The critical test for any propagation technique is the ability of the propagated material to survive under field environment. The potting medium is an important determinant in successful propagation by tissue culture. The growth responses of transplanted strawberry plantlets vary with type of potting medium. Peatmoss resulted in the highest values for all measured parameters compared to the other potting media tested; giving the best growth and morphological development of strawberry plantlets. Shoot number and elongation, number of leaves and root length of transplants on peatmoss were significantly increased over the all other potting media with significant differences between treatments. Growth and development decreased significantly when strawberry plantlets were transplanted on clay alone or clay and sand mixtures. The results were in concurrence with preceding reports (George, 1995b; Hassan, 2002; Ahmed, 2009). The latter author evaluated the influence of four potting media namely, peatmoss, clay, sand and clay: sand (1:1) mixture on survival percentage, shoot number and elongation leaf number of transplanted strawberry plantlets. Peatmoss excelled all other potting media tested. The superiority of peatmoss over the other potting media tested in this study, was attributed to its high water holding capacity, lower bulk density and increased porosity and hence soil infiltration. It is relatively firm and dense to hold the plantlets in place during establishment. The low values obtained for shoot number and elongation, number of leaves and root elongation on the other planting media could probably be due, in part, to poor drainage and/or poor aeration. Well

rooted strawberry plantlets were successfully acclimatized and eventually transferred to soil, planted in the lath house and 90% of them showed rapid growth.

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Propagation by runners 2010 http://www.strawberry.dreab.com.

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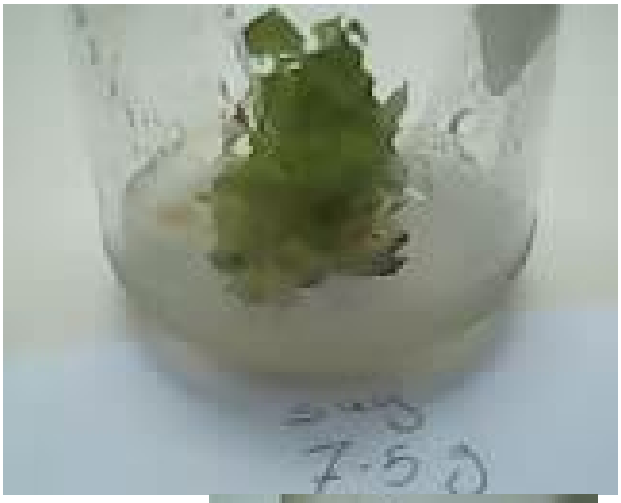
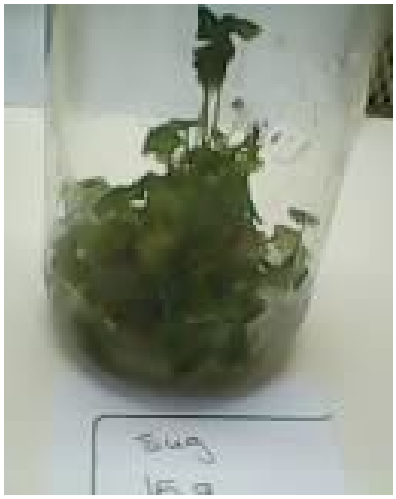
7-Appendices

Appendix1Effect ofMS

Appendix2 Effect of MS nitrate





Appendix3 Effect of sugar

Appendix4 Effect of instol

Appendix5 Effect of BA

Appendix6 Effect of Furidan

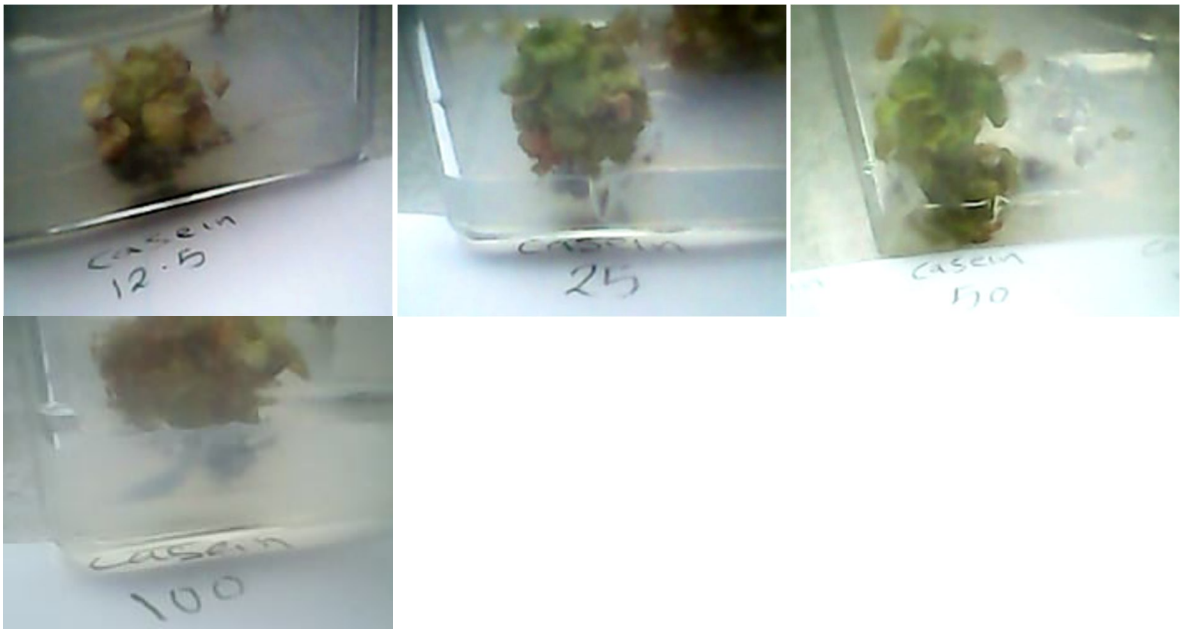
Appendix 7 Effect of Stroby

Appendix 8 Effect of Seven

Appendix 9 Effect of Glyphosate

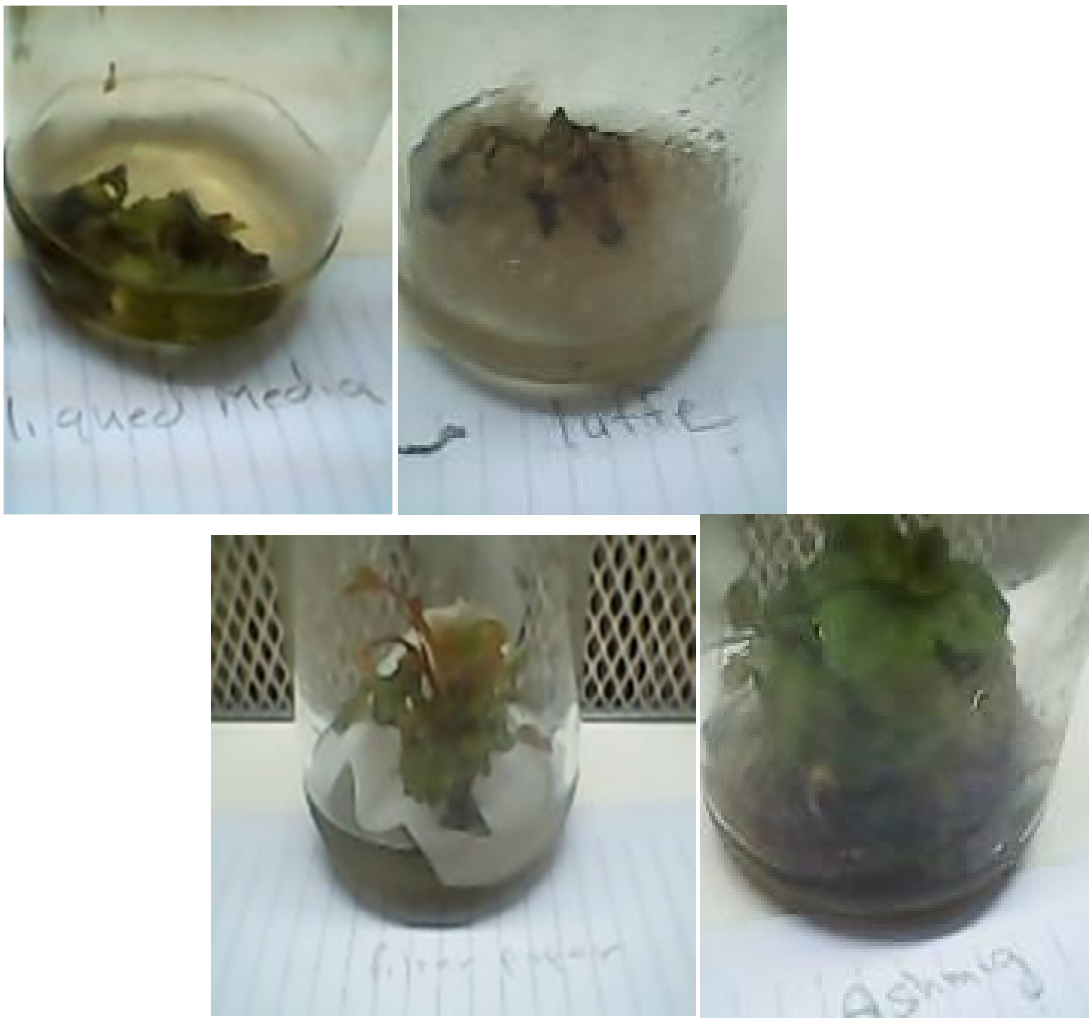
Appendix 10 Effect of Gum arabic

Appendix 11 Effect of Silver nitrate



Appendix 12 Effect of Casein hydrolysate

Appendix 13 Effect of Activated charcoal



Appendix 14 Effect of physical status of media

Appendix 15 Effect of Light intensity

a

Appendix 16 Effect of ex-vitro transplanting

A N A L Y S I S O F V A R I A N C E T A B L E

Table (4.1)

Degrees of Freedom	Sum of Squares	Mean Square
25	7.333	0.293
25	0.755	0.030
25	19.167	0.767
25	16.667	0.667
25	0.683	0.027

Table (4.2)

Degrees of Freedom	Sum of Squares	Mean Square
30	45.667	1.522
30	0.148	0.005
30	11.833	0.394
30	6.000	0.200
30	0.135	0.004

Table (4.3)

Degrees of Freedom	Sum of Squares	Mean Square
10	280.34	28.03

10	82.62	8.2
10	868.34	86.83
10	579.41	57.94
10	85.22	8.52

Table(4.4)

Degrees of Freedom	Sum of Squares	Mean Square
25	185.167	7.407
25	1.358	0.054
25	18.667	0.747
25	31.000	1.240
25	2.090	0.084

Table(4.5)

Degrees of Freedom	Sum of Squares	Mean Square
25	0.833	0.033
25	0.570	0.023
25	29.000	1.160
25	2.167	0.087
25	2.167	0.08

Table(4.6)

Degrees of Freedom	Sum of Squares	Mean Square
30	99.167	3.306
30	0.625	0.021
30	22.667	0.756
30	43.000	1.433
30	1.118	0.037

Table(4.7)

Degrees of Freedom	Sum of Squares	Mean Square
25	37.833	1.513
25	0.568	0.023
25	11.833	0.473
25	56.500	2.260
25	0.633	0.025

Table (4.8)

Degrees of Freedom	Sum of Squares	Mean Square
25	123.833	4.95
25	0.445	0.018
25	7.333	0.293
25	75.500	3.020
25	3.207	0.128

Table (4.9)

Degrees of Freedom	Sum of Squares	Mean Square
25	41.667	1.667
25	0.137	0.005
25	10.833	0.433
25	0.833	0.033
25	0.038	0.002

Table (4.10)

Degrees of Freedom	Sum of Squares	Mean Square
25	55.167	2.207

25	0.287	0.011
25	31.667	1.267
25	6.167	0.247
25	0.058	0.002

Table (4.11)

Degrees of Freedom	Sum of Squares	Mean Square
25	127.333	5.093
25	0.470	0.019
25	18.167	0.727
25	3.500	0.140
25	1.533	0.061

Table (4.12)

Degrees of Freedom	Sum of Squares	Mean Square
25	514.262	20.570
25	1.505	0.060
25	18.414	0.737
Degrees of Freedom	Sum of Squares	Mean Square
25	62.848	2.514

25	3.885	0.155

Table (4.14)

Degrees of Freedom	Sum of Squares	Mean Square
25	15.667	0.627
25	1.675	0.067
25	21.667	0.867
25	6.000	0.240
25	1.143	0.046

Table(4.14)

Degrees of Freedom	Sum of Squares	Mean Square
25	4.333	0.173
25	0.710	0.028
25	21.833	0.873
25	2.167	0.087
25	0.087	0.003

Table(3.15)

Degrees of Freedom	Sum of Squares	Mean Square
25	27.667	1.107
25	1.972	0.079
25	33.833	1.353
25	24.667	0.98
25	1.475	0.059

Table (4-16)

Degrees of Freedom	Sum of Squares	Mean Square
25	29.000	1.160
25	0.328	0.013
25	15.833	0.633
25	16.833	0.673
25	0.142	0.006

Table (4-17)

Degrees of Freedom	Sum of Squares	Mean Square
25	142.667	5.707
25	0.423	0.017

25	10.333	0.413
25	19.833	0.793
25	1.163	0.047

Table (4-18)

Degrees of Freedom	Sum of Squares	Mean Square
20	1.500	0.075
20	0.303	0.015
20	6.167	0.308
20	4.333	0.217
20	3.323	0.166

Table(19)

Degrees of Freedom	Sum of Squares	Mean Square
16	1.200	0.075
16	15.300	0.956
16	10.800	0.675
16	19.600	1.225
16	15.268	0.954