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Induction of Somaclonal Variation in Strawberry and Evaluation of Variants to Different Biotic and Abiotic Stresses

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INDUCTION OF SOMACLONAL VARIATION IN STRAWBERRY AND EVALUATION OF VARIANTS TO DIFFERENT BIOTIC AND ABIOTIC STRESSES

M. Phil. Thesis

A Thesis
Submitted to the Department of Botany, University of Rajshahi in Partial Fulfilment of the Requirements for the Degree of Master of Philosophy in Botany

SUBMITTED
BY

Most. Tanziman Ara

July 2013

Plant Breeding and Gene Engineering Laboratory
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Dedicated
To My
Beloved Parents

DECLARATION

I do hereby declare that the whole work submitted as a thesis entitled ***"INDUCTION OF SOMACLONAL VARIATION IN STRAWBERRY AND EVALUATION OF VARIANTS TO DIFFERENT BIOTIC AND ABIOTIC STRESSES"*** *in the Plant Breeding and Gene Engineering Laboratory, Department of Botany, Rajshahi University, Rajshahi, Bangladesh for the degree of* ***MASTER OF PHILOSOPHY*** *in* ***BOTANY*** *is the result of my own investigation and was carried out under the supervision of Professor* ***DR. A. K. M. RAFIUL ISLAM****, Department of Botany, Rajshahi University, Rajshahi 6205, Bangladesh. The thesis has not already been submitted in the substance for any degree or qualification of this or any other university or other institutions of learning.*

Date........................

*(**Most. Tanziman Ara**)*

Candidate

CERTIFICATE

I do hereby certify that ***MOST. TANZIMAN ARA*** *has been working on her thesis entitled* ***"INDUCTION OF SOMACLONAL VARIATION IN STRAWBERRY AND EVALUATION OF VARIANTS TO DIFFERENT BIOTIC AND ABIOTIC STRESSES"*** *under my supervision, which is the record of bonafide research carried out at the Plant Breeding and Gene Engineering Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh. The results of the investigation, which are embodied are original and have not been submitted before in substance for any degree or diploma of this University. She has fulfilled all the requirements of regulations relating to the nature and prescribed period of research for submission of thesis for the partial fulfillment of M.Phil degree. I was pleased to forward her for getting fund for this research from the* ***Ministry of Science, Information and Communication Technology of the People's Republic of Bangladesh****.*

Date**....................**

(Professor Dr. A. K. M. Rafiul Islam)
Supervisor

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Most. Tanziman Ara

ABBREVIATIONS

The following abbreviations have been used throughout the text:

%	Percentage
ºC	Degree Celsius
0.1N	0.1 Normal solution
½ MS	MS with half strength of major salts only
2,4-D	2, 4-dichlorophenoxy acetic acid
BA	6-benzyl adenine
CH	Casein hydrolysate
cm	Centimeter (s)
e.g.	Example gratia = For example
et al.	et alile = Other people
etc.	Etcetera = And others
Fig.	Figure (s)
g	Gram (s)
g/l	Gram per liter
GA3	Gibberellic acid grade 3
h	Hour
IBA	Indol-3-butyric acid
kg	Kilogram (s)
Kin	6-furfuryl amino purin (Kinetin)
lb	Pound (s)
mg	Milligram
mg/l	Milligram per liter
mg/ml	Milligram per milliliter
ml	Milliliter
Mm	Millimeter (s)
MS	Murashige and Skoog (1962) medium
MS0	Growth regulator free MS medium
NAA	α-napthalene acetic acid
No.	Number(s)
PGR	Plant growth regulator
pH	Negative logarithm of hydrogen ion (H) concentration
R0	Regenerated plants
var.	Variety
wt.	Weight

ABSTRACT

The cultivated strawberry (*Fragaria* × *ananassa* Duch.), a member of the Rosaceae is the most important soft fruit world wide. Commercial cultivation of strawberry in Bangladesh has been initiated since 2008. However, its productivity seriously hampered due to different diseases, due to crown rot and verticillium wilt, which is proven difficult to control through fungicides. On the other hand, hot and dry weather and soil pH are the major abiotic factors affect strawberry cultivation in Bangladesh. In this context, induction of somaclonal variation can be used to create new genetic variability for improvement of the strawberry.

The present study was conducted to standardize a suitable protocol of *in vitro* plant regeneration potentiality of seven strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival. Young leaves, mature leaves and nodes of seven strawberry varieties were used as explants. To collect leaf segments and node, surface sterilization and initial culture establishment of runner tips and nodal segments of field grown strawberry plants were adopted. The nodes and leaf segments were collected from the *in vitro* runner tip derived shoots and cultured in MS medium supplemented with different concentrations of 2,4-D or NAA alone or in combination with BA and the cultures were incubated in dark for four weeks for callus induction. The highest degree of percentage of callus was observed in some formulations such as 2.0 mg/l 2,4-D, 2.0 mg/l NAA and 0.5 mg/l NAA +1.5 mg/l BA. Calli depeloped from all types of explants were subcultured onto MS medium supplemented with different concentration and combination of BA + NAA for shoot regeneration. The medium with 1.5 mg/l BA + 0.5 mg/l NAA showed the best respons to indirect shoot regeneration. From the above description it is clear that NAA, BA combination was found to be the most suitable for callus induction and regeneration. Mature leaves were favorable for callus induction and shoot regeneration more than young leaves and nodes in all case. Successful shoot formation became evident when small green fresh leaves began to emerge. These leaves when developed in their actual shape were transferred into fresh medium containing the same hormonal combination or best one among them for further proliferation and development. Sub culture was carried out regularly at an interval of

3-4 weeks. After shoot proliferation, the mini regenerated shoots were separated aseptically and cultured in MS medium and ½ MS medium containing different concentrations of NAA, IBA alone and MS and ½ MS medium without growth regulators (MS0). Cent percent cultured shoots induced to develop roots when cultured in MS0 rooting medium. Among the seven strawberry varieties AOG was found to be the most responsive genotype in terms of different parameters such as ability to primary culture establishment, percentage to callus induction, shoot regeneration and rooting response.

After sufficient root formation, the *in vitro* regenerated plantlets were acclimatized gradually. Then successfully acclimatized plantlets were transplanted in to field. A total of 30- 50 somaclones from each of the tested varieties were established and maintained in the field and were considered as Ro plants. Four somaclones from AOG and two somaclones from other six strawberry varieties viz. JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival were selected from Ro plants. The seven strawberry varieties were also grown in the field to compare with the somaclones. Eight morpgological and eight fruit yield and yield contributing characters were analyzed to find out better strawberry varieties for Bangladesh environment condition. In the analysis of variance the main item genotype was highly significant for all characters. In the analysis of components of variation it was found that phenotypic variation was greater than genotypic variation.

In the second part of this research was field evaluation of the seven strawberry varieties and their somaclones under biotic and abiotic stress condition. Verticillium wilt, phytophthora crown rot, leaf scotch, leaf spot, leaf blight and botrytis fruit rot diseases were found in the strawberry field but disease incidences (%) of verticillium wilt and phytophthora crown rot were high. There were no plants found resistance to fungal diseases but somaclones showed better performance than the donor plants. Majority of plants were found heat sensitive in donor plants but somaclone AOG SC 3 showed better performance than other somaclones and donor parents in terms of summer overcoming capacity. These somaclones can be acceptable commercially if the good characters exhibited are transmitted through generations or could be used in future breeding programme for the improvement of strawberry varieties in Bangladesh.

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I. INTRODUCTION

1.1. SOMACLONAL VARIATION

Somaclonal variation used to describe the variation seen in plants that have been produced by plant tissue culture. Somaclonal variation may be used as a source of variation to get superior clones. In addition to the variants/mutants (cell lines and plants) obtained as a result of the application of a selective agent in the presence or absence of a mutagen, many variants have been obtained through the tissue culture cycle itself. These somaclonal variants, which are dependent on the natural variation in a population of cells, may be genetic or epigenetic, and are usually observed in the regenerated plantlets. Somaclonal variation itself does not appear to be a simple phenomenon, and may reflect pre-existing cellular genetic differences or tissue culture-induced variability. The variation may be generated through several types of nuclear chromosomal rearrangements and losses, gene amplification or de amplification: non-reciprocal mitotic recombination events, transposable element activation, apparent point mutations or re-activation of silent genes in multigene families, as well as alternations in maternally inherited characteristics (Karp 1993).

Many of the changes observed in plants regenerated *in vitro* have potential agricultural and horticultural significance. These include alterations in plant pigmentation, seed yield, plant vigour and size, leaf and flower morphology, essential oils, fruit solids, and disease tolerance or resistance. The same types of variation obtained from somatic cells and protoplasts can also be obtained from gametic tissue (Karp 1993). One of the major potential benefits of somaclonal variation is the creation of additional genetic variability in coadapted, agronomically useful cultivars, without the need to resort to hybridization. This method could be valuable if selection is possible *in vitro* or if rapid plant-screening methods are available. It is believed that somaclonal variants can be enhanced for some characters during culture *in vitro*, including resistance to disease pathotoxins and herbicides and tolerance to environmental or chemical stress. However, at present few

cultivars of any agronomically important crop have been produced through the exploitation of somaclonal variation (Karp 1993). Although somaclonal variation can be used as a source for variation to obtain superior clones, it can be also a very serious problem in the plant tissue culture industry resulting in the production of undersirable plant off- types (Karp 1993).

1.2. STRAWBERRY PLANT

1.2.1. Taxonomy and Origin of Strawberry

Strawberries are members of the family Rosaceae, sub-family Rosoideae and genus *Fragaria*. Closely related genera include Duchesnea, the mock strawberry, and Potentilla, the cinequefoils. *Fragaria* species can be grouped by ploidy: there are nine diploids, two tetraploids, one hexaploid, and four octoploids. Diploids (2n=14) include *F. vesca* Duch., *F. viridis* Duch., *F. nilgerrensis* Schlect., *F. daltoniana.* J. Gray, *F. nubicola* Lindl. Ex. Lucaita. *F. iinumae* Makino, *F. yezoensis* Hara, *F. nipponica* Makino and *F. mandschurica* Staudt (Staudt 1989).

The alpine strawberry *F. vesca* is the most geographically widespread. Cytogenetic studies indicate that this species may be a diploid progenitor of the octoploid strawberries. The two tetraploids (2n=28) are *F. orientalis* Losinsk and *F. moupinensis* (Franch.) Card. The lone hexaploid (2n=42), *F. moschata* Duch. Or musky strawberry is found in northern and central Europe into eastern Russia. This species was domesticated in the early 1600’s and fruit were commonly known as ‘Hautbois’ or ‘Hautboy’. Cultivated plantings still exist in Europe.

Four octoploids (2n=56) are known *F. iturupensis* Staudt, *F. chiloensis* (L.) Duch., *F. virginiana* Duch., and *F. × ananassa* Duch. *F. iturupensis* is found in Iturup Island of the Kuril Islands (northeast of Japan) (Staudt 1973). Taxonomic characteristics include obovate sub-glaucous leaves (similar to *F. iinumae*), hermaphroditic flowers, and almost spherical fruit. The beach or Chilean strawberry, *F. chiloensis*, is found along the Pacific coast from Alaska down through central California, along the beaches of Chile and inland to the Andes Mountans, and on top of mountains in Hawaii. They were once extensively cultivated in Chile, Peru, and possibly Ecuador. Wild populations are primarily dioecious although hermaphrodites have been found in

California (Hancock and Bringhusrst 1979; Darrow 1966). Plants are low-spreading, vigorous, and produce many runners. Leaves are thick, dark-green and very glossy. Fruit are dull to bright red, firm, white fleshed, pungent, and large. Four subspecies are recognized based on morphology and distribution (Staudt 1962): ssp. *chiloensis* (South America), ssp. *lucida* (Washington to California), ssp. *pacifica* (California to Aleutian Islands), and ssp. *sandwicensis* (Hawaii).

The scarlet or Virginia strawberry *F. virginiana* is found North America from the Southeastern U.S. north to Newfoundland and as far west as the Yukon Territory into Alaska, oftentimes in meadows. Plants are slender, tall, and have many runners. Only females and hermaphrodites are observed in the eastern U.S. while all three sexes are equally found in western populations (Luby and Stahler 1993; Stadler *et al.* 1988). Fruit are soft, round, up to 1.5 cm diam, light red, aromatic, with deeply embedded seeds, and white flesh. Both plant and fruit characters are highly variable. Four subspecies are recognized by Staudt (1989) ssp. *virginiana* Duch. (Eastern U.S. to Newfoundland and west to Yukon Territory); ssp. *glauca* (Wats.) Staudt (southern Arizona through the Rocky mountains into northwest Canada and central Alaska, probably equivalent to *F. ovalis* (Darrow 1966) due to lack of hybridization barriers and intermediate characters); ssp. *platypetala* (Rydb.) Staudt (California to British Columbia and in the Rocky mountains in Colorado and Wyoming); and ssp. *grayana* (E. Vilmorin ex Gay) Staudt (Texas through Louisiana, Alabama and north to New York). However, this classification scheme by Staudt has undergone considerable debate. New evidence suggests that these four subspecies are too closely related to be considered intraspecific taxa. Welsh *et al*. (1987) suggested that ssp. *glauca* and ssp. *platypetala* completely intergrade and should be referred to as a single taxa var. *glauca*. Hokanson *et al.* (1993) suggested that strawberries in the Black Hills and eastern front ranges of the Rocky Mountains may be introgressive swarms between ssp. *glauca* and ssp. *grayana*. Finally, Harrison *et al.* (1997) using multivariate analysis, found that these strawberries from the Black Hills were morphologically intermediate between collections of eastern ssp. *virginiana* and western ssp. *glauca*. Furthermore, when multivariate analysis was done using RAPD data, this population of strawberries from the Black Hills was part of a large cluster group that encompassed the eastern ssp. *virginiana* and western ssp. *glauca*. The dessert or

pineapple (Ananas) strawberry, *F.* × *ananassa* is the most important cultivated strawberry worldwide. It arose as a chance hybrid of *F. chiloensis* x *F. virginiana* within European gardens during the mid 1700's. Naturally occurring hybrids have been found in coastal areas of southwest British Columbia, Washington, Oregon, and northern California. Staudt (1962) recognized these hybrids as *F.* × *ananassa* nm. cuneifolia (Nutt. Ex. Howell). Many of the dessert strawberry's traits are intermediate to its parents. Plants have large fruit, high yields and vigor.

1.2.2. Description of *Fragaria* x *ananassa* Duch.

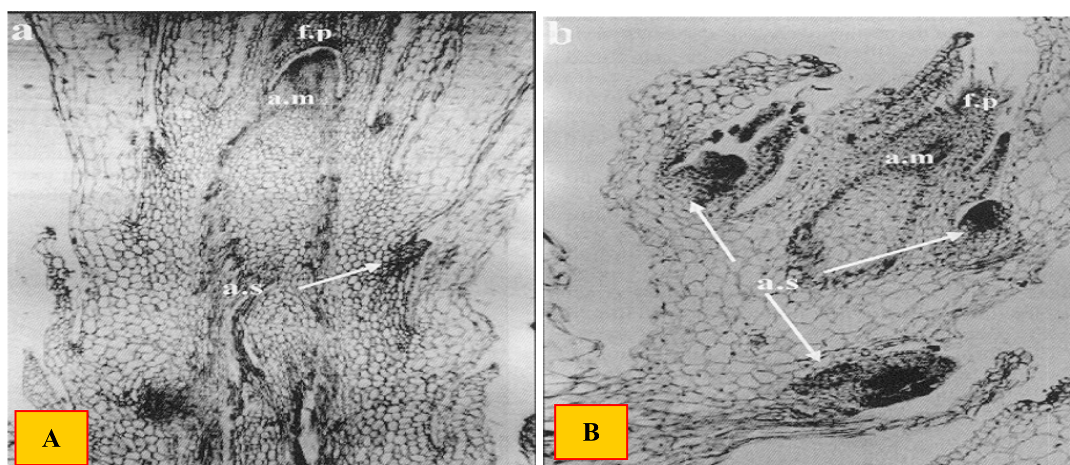
The *F.* × *ananassa* is a perennial which arises from a crown of meristematic tissue or compressed stem tissue. Leaves, stems, runners, axillary crowns, inflorescences and roots all arise from the crown. The plant has trifoliate leaves which spiral around the crown, with buds in the leaf axils giving rise to the runners. Runners have two nodes with a plant produced at the distal node. Strawberry blossoms contain many pistils, each with its own style and stigma attached to the receptacle. When fertilization occurs the receptacle develops into a fleshy fruit (Darnell 2003).

The fruit is called an achene which contains the seeds. The edible part is an accessory type fruit. The seeds are arranged on the outside of the receptacle tissue. 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). Following further hybridizations, especially since 1850, *Fragaria* × *ananassa* has developed into the large, fragrant, tasty red fruit that is now cultivated worldwide. The high degree of genetic heterozygosity present in *Fragaria* spp. enabled the development of strawberry cultivars adapted to widely varying environment conditions and resistant to several diseases and pests. Not only the genetic variability, but also a high adaptability and plasticity of the strawberry plant itself give this crop such a remarkable range of adaptation (Darrow 1966). That heterozygosity was explained by (Gaafar and Saker 2006) as there are more than 20 *Fragaria* species worldwide, there are seven basic types of chromosomes that they all have in common. However, they exhibit different polyploidy. Some species are

diploid, having two sets of the seven chromosomes (14 chromosomes total). Others are tetraploid (4x = 28), hexaploid (6x = 42), octoploid (8x = 56) or decaploid (10x = 70) (Emarah 2008).

1.2.3. Histological Characteristics of Strawberry

Microscopic observations (Figure 1. a, b) showed that the apex of stipular shoots presented more sites of higher cell activity in comparison to axillary shoots. In the former, one axillary meristem was developed at nearly each foliar premordium. Moreover, the number of primordia on the apical meristem is more important in stipular than in axillary shoots (Jemmali *et al.* 2002).



(a.m = apical meristem, a.s = axillary shoot, f.p = foliar primordium. G × 100)

Figure 1. Longtudinal section of (a) axillary shoot apex and (b) stipular shoot apex of strawberry cv. Elsanta (Jemmali *et al.* 2002).

1.2.4. Strawberry Nutritional Value

For many centuries before, strawberries had been a favorite among the fruits of the temperate world. They were valued for delicious flavor and fragrance, for health restoring qualities and as harbinger of spring (Wilhelm and Sagen 1974). Flavourful and nutritious, strawberries are enjoyed by millions of people in all climates, including and are predominantly used as fresh fruit. Their use in processed forms such as cooked and sweetened preserves, jams or jellies and frozen whole berries or sweetened juice extracts or flavorings, and their use in making a variety of other processed products made them one of the most popular berry crops, more

widely distributed than any other fruit (Samir *et al.* 2007). Of its many positive characteristics, the nutritional value of strawberries is nearly perfect. Eight medium strawberries contain more vitamin C than an orange, 20% of the recommended daily allowance for folic acid, no fat, no cholesterol and are considered high in fiber (Driscoll's 2004).

Strawberry is cultivated all around the world, not only for its digestive and tonic properties, but because of the nutritional value of its fruits, important source of folate, vitamin C, fiber, potassium, flavonoids, autocianidin, phytochemicals and antioxidants.

Table 1.1. Nutritional values for 100 grams edible portion of strawberry (USDA 2003).

Nutrients	Amounts	Nutrients	Amounts
Protein	0.61 g	Sodium	1 mg
Fat	0.37 mg	Zinc	0.13 mg
Fiber	2.3 g	Copper	0.049 mg
Carbohydrates	7.02 g	Manganese	0.29 mg
Calcium	14 mg	Selenium	0.7 µg
Iron	0.38 g	Vitamin B-6	0.059 mg
Mangnesium	10 mg	Folate	17.7µg
Phosphorus	19 mg	Vitamin A, IU	3 µg
Potassium	166 mg	Vitamin A, RE	0.14 mg

1.2.5. Strawberry Phytochemicals and Human Health

Although strawberry is not an essential component of the diet, its delicious flavor and taste, attractive appearance and seasonal availability make this fruit an excellent crop. Even more, strawberries are rich in phytochemical compounds with potential antioxidant compounds, mainly ellagic acid and flavonoids, which can lower the risk of cardiovascular events and tumorogenesis (Hannum 2004).

These qualities have ensured that the economic importance of this crop has increased throughout the world and, nowadays, it remains as a crop of primary interest for both research and fruit production (Mercado 2007). A growing body of data suggests that consumption of a phytochemical rich diet reduces the risk of certain chronic human illnesses such as cancer, heart and neurodegenerative diseases. Strawberry (*Fragaria* × *ananassa* Duch.) fruits are a rich source of phytochemicals (plant chemicals) of which phenolic compounds predominate. Berry fruits are reported to contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins) and hydrolyzable tannins (Machiex 1990). Studies conducted *in vitro* indicate that berry phenolics have a wide range of biological properties such as anti-cancer, antioxidant, anti-inflammatory, and cell regulatory effects (Seeram 2006a, Seeram and Heber 2006b).

1.2.6. Anticancer Effects

The anticancer effects of individual phytochemical constituents of strawberries, as well as whole strawberry extracts, have been demonstrated (Seeram 2006a). These anticancer effects are exerted through multi-mechanistic means of action including the antioxidant actions of the berry's phenolic constituents by protecting DNA from damage, and also through effects exerted beyond antioxidation (Seeram and Heber 2006b). The biological activities of strawberry phytochemicals include the regulation of phase-II enzymes and the modulation of gene expression and sub-cellular signaling pathways of cell proliferation, angiogenesis and apoptosis (programmed cell death). Although there have been many published reports on the anticancer effects of individual phenolics known to be present in the strawberry fruit (Seeram 2006). Strawberry extracts have also been evaluated for their ability to inhibit mutation by the direct-acting mutagen methyl methanesulfonate and the metabolically activated carcinogen, benzopyrene. Ethanol extracts from freeze-dried fruits of several strawberry cultivars were also evaluated and hydrolyzable tannin containing fractions from strawberries were found to be most effective at inhibiting mutations (Hope *et al.* 2004).

1.2.7. Anthocyanins

Anthocyanins are natural pigments providing scarlet to blue colors in flowers, fruits, leaves and storage organs. The recent interest in the field of anthocyanin chemistry has been generated by restriction and limitation of the use of synthetic dyes as food ingredients. Because of low toxicity of anthocyanins, they have a high potential as a food colorant as the substitute of synthetic red dyes. Recently, these anthocyanins have been thought to have pharmacological effects, such as lowering the atherogenic index (Igarashi *et al.* 1990) and decreasing triglyceride and free fatty acid levels (Igarashi and Inagaki 1991). Moreover, Kamei *et al*. (1995) reported that anthocyanin was more effective to inhibit the growth of tumor cells than other flavonoids. Studies concerned with anthocyanin production using plant tissue cultures have therefore become very important. Masayuki *et al*. (1999) demonstrated that *Fragaria* × *ananassa* (strawberry) callus, which produced high amounts of anthocyanin in the dark which accumulated more than 1000 µg of anthocyanin per g fresh cell. And Sato *et al.* (1996) reported that in the suspension cultures of *F.* × *ananassa* cells, anthocyanin content increased with the intensity of light irradiation from 2500 lx to 8000 lx. They also measured the ratio of pigmented cells in total cells at 8000 lx and found that the ratios were 26% in MS medium.

1.2.8. The Strawberry Economic in the World

Strawberry world production has shown a significant increase in the last 25 years. From 1980- 2004 the land cultivated with strawberry increased by 25% and fruit production by 73%. In 2004, strawberry was cultivated on 214,118 ha, reaching a production of 3.1 million tones. Fifty seven countries contributed to this production, though only nine of them accounted for more than 72% of the total. During the period 2000- 2004, average world production was 3.17 million tonnes, the major strawberry producer nations being the USA. The total value of USA production was 1.22 billion dollars in 2002, being the third fruit in value in the non citrus category behind grapes and apples. It is noteworthy that most strawberry production is localized in areas with mild winter climates (Mercado *et al.* 2007). Approximately, 15% of world strawberry production is exported as fresh fruit and, at least in the USA, more than 25% is processed. The major fresh strawberry exporters are Spain (212,300 t in 2003, 81% of its strawberry production) and the USA (94,600 t,

11.2%). Spain is the main supplier for the European market during the winter period (February and March) and Germany, France and the UK are the major consumers of this production. In Spain, as well as other Mediterranean nations, the concentration of strawberry production over short periods often creates a product excess, reducing retail prices (Faedi *et al.* 2002).

In the case of the USA, exported fruits are destined mainly for Canada and a small quantity for Japan and Mexico. California has more than 85% of the USA strawberry production. This region also supports the most active and successful strawberry breeding programs. In fact, the program of the University of California has released very popular varieties (e.g. Camarosa, Chandler, Seascape, Selva, Pajaro, Oso Grande), accounting for more than 50% of the world's strawberry production (Mercado *et al.* 2007).

1.2.9. Biotic and Abiotic Stresses in Strawberry

Biotic stress is a stress that occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment (Hamish 1998). Abiotic stress affects animals, but plants are especially dependent on environmental factors, so it is particularly constraining. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide (Chawla 2002).

Biotic factors

Diseases and pests: Growth can be affected by different factors such as soil fertility, lack of moisture, weeds, insects and diseases. Strawberries are attacked by many diseases and pests that vary widely in their destructiveness and distribution. The following diseases and pests are available in strawberry.

Foliage disease: Powdery mildew (*Sphaerotheca humuli*, *Phytophthora parasitica*), Leaf spots (*Alternaria alternate f.* sp. *Fragariae, Colletotrichum fragariae*), Slime molds (*Selerotinia sclerotiorum, Pythium* spp.).

Root and crown diseases: Red stele (*Phytophthora fragariae*), Verticillium wilt (*Verticillium albo-atrum*, *Verticillium dahliae*), Black root rot (*Rhizoctonia fragariae, Ceratobasidium, Pythium* spp. etc.), Nematodes.

Fruit diseases: Gray mold (*Botrytis cinerae, Botryotinia fuckelian*), Rhizopus rot (*Rhizopus stolonifer*), Leather rot (*Phytophthora parasitica, Phytophthora citricola* etc.).

Abiotic factors

Hot and dry weather (Above 38°C) and soil pH are major abiotic factor affect strawberry cultivation.

1.3. STRAWBERRY CULTIVAR IMPROVEMENT THROUGH SOMACLONAL VARIATION

Plant tissue cultures isolated from even a single cell can show a variation after repeated subculture. Distinct lines can be selected with their own particular morphology and physiology. It suggests that the tissue culture contains a population of genotypes whose proportion can be altered by imposing an appropriate selection pressure. This variation can be transmitted to plants regenerated from the tissue cultures. It provides an additional source of novel variation for exploitation by plant breeders. Somaclonal variation is the term used to describe the variation seen in plants that have been produced by plant tissue culture. Larkin and Scowcroft (1981) termed the variation in the tissue culture-derived plants as ‘somaclonal variation’. Later on it became known that a wide array of alterations in nuclear and cytoplasmic genetic elements contributed to the observed phenotypic variation, and that many of them were of epigenetic nature (Micke 1999). Somaclonal variation is a wide spread among tissue culture derived regeneration (Nehra *et al*. 1992; Jain 1993 a, b; 1997a, b; Jain *et al.* 1997, 1998). It is generally attributed to pre-existing genetic variation in somatic cells (Walbot 1985), single gene mutation, aneuploidy and transposable elements (Jain *et al.* 1998). The extent of variation depends on genotype, age of the donor plant, cytogenetic changes, DNA methylation, explant type and plant hormones in the culture medium (Evans and Sharps 1983; Jain and Newton 1990; Jain and Pehu 1992; Poulimatka 1993; Arnholdt-Schmitt 1995; Gupta 1997, Jain *et al*. 1997). Unlike epigenetic changes, somaclonal variation which results from altered gene expression

is usually irreversible (Karp 1991, 1995). The segregation pattern of mutations in the progeny is mostly Mendelian (Larkin *et al*. 1984). Only alteration in the genetic information would give rise to genetically stable lines (Jain 1997a). Non genetic changes (epigenetic) appear more frequently, and are reversible and predictable (De Klerk *et al*. 1990; Jain 1998).

1.4. SIGNIFICANCE OF STRAWBERRY CULTIVAR IMPROVEMENT

Plants regenerated from calli exhibit great genetic variability in agronomic traits that is known as somaclonal variation (Larkin and Scowcroft 1981). Somaclonal variation can broaden the genetic variation in strawberry plants; many plant characters can be altered, including plant height, yield, numbers of flower per plant, early flowering, grain quality, resistant to disease, insects and pests, cold, draught and salt (Jain *et al*. 1998 and Patnaik *et al*. 1999). These somaclonal variations have been successfully employed in strawberry improvement. Reproducible protocol for the regeneration of complete plantlets from callus cultures using leaf and petiole explants was standardized for strawberry cv. Chandler (Kaushal *et al*. 2004). Tissue culture induced (somaclonal) variation generate strawberry germplasm such as cultivars, Chandler, Delmarvel, Honeoye, Latestar, Pelican and Sweet Charlie to produce somaclones with increased levels of resistance to anthracnose (caused by the fungus *Colletotrichum acuatatum*). The greatest increased in disease resistant were observed for somaclones of cultivars Pelican and Chandler that exhibited 17.5-fold and 6.2-fold increases in resistance, respectively. The highest levels of anthracnose resistance (2 to 6 % leaf necrosis) were exhibited by somaclones of cultivars Pelican and Sweet Charlie (Hammerschlag *et al.* 2006). The major reason is that somaclonal variation has not been incorporated routinely in plant breeding programs.

1.5. REVIEW OF LITERATURE

Regeneration through callus culture using different explants of strawberry has been reported. Leaf tissue has been studied and shown to have the greatest regeneration capacity of strawberry plant tissues (Jones *et al.* 1988; Liu *et al.* 1988; Nehra and Stushnoff 1989; Nehra *et al*. 1990; Jelenkovic *et al*. 1991; Popescu *et al*. 1997 and Passey *et al*. 2003). Callus production is also more prolific from the leaf tissue. In addition, leaf-derived callus produces more shoots than petiole-derived callus

(Popescu *et al*. 1997). In 1989, Nehra and Stushnoff reported successful regeneration using greenhouse grown stock plants with surface sterilized leaves excised and cut into strips avoiding the midrib and placed adaxial side down onto the media. Nehra and Stushnoff (1989) discovered that leaf disks from young plants had a higher regeneration rate than the older plants. A study of the regeneration and transformation of the cultivar 'Rapella' showed higher regeneration rates when the youngest leaves were used (James *et al*. 1990). Passey *et al*. (2003) studied seven commercial cultivars of strawberry using leaf disks, petioles, roots and stipules as explants material. They started out by establishing and growing runner tips in vitro and sub culturing them every three weeks until enough material was produced to being the experiments. The leaf disks had the highest regeneration rates for all cultivars with greater than 90% of explants producing shoots (Passey *et al*. 2003). Graham et al. (1995) used stem tissue for their regeneration and transformation studies of 'Melody', 'Rhapsody' and 'Symphony'.

The reduction in regeneration capacity was offset by the ability of the stem tissue to resist destruction by the kanamycin used as a selecting agent in the screening process post transformation. Other regeneration methods tested include use of embryos. Wang *et al.* (1984) used embryos excised from strawberry achenes. The tiny strawberry embryos were excised from surface sterile achenes under a microscope and placed on hormone modified regeneration media.

Strawberry is high valued fruit mainly grown in temperate and sub temperate region. There are many strawberry genotypes grown in tropical and sub-tropical environment but fruit of these genotypes are mostly unpalatable. For commercial planting, selection of economically important clones are needed freeing these of virus by a combination of heat therapy and meristem culture. Tissue culture aimed particularly at the rapid propagation of breeding material as briefly described with reference to strawberry by Zimmerman (1981). However, through micro-propagation of strawberries (*Fragaria* × *ananassa* Duch.), several morphological abnormalities were detected as somaclonal variation. Changes include leaf colour variants and dwarf plants, among other (Swartz *et al*. 1981; Sansavini *et al*. 1990; Irkaeva & Matveena 1997; Morozova 2003). Callus derived "Redcoat" strawberry plants differ

significantly from standard runner plant for several vegetative characteristics (Nehra *et al*. 1992). These variants pose a problem for production of uniform, true to-type plants. Nehra *et al*. (1992) found that two cultivars of strawberry responded differently to various forms of in vitro propagation and in both cases variants were found were in callus derived plantlets, but not those derived from meristems or via direct leaf regeneration. Some studies have shown that modified characteristics are epigenic and disappear over time (Koruza and Jeleska 1993). Numerous authors have reported that genetic changes including insertion, deletions, point mutations and rearrangements occur during tissue culture (Kane *et al*. 1992), but few of the phenotypic symptoms found are heritable (Karp 1995; Kumar *et al*. 1999). Most somaclonal variations occur in plants regenerated from cultures that have under grown a differentiation phase. Temporary variation may be due to methylation changes in the DNA. Methylation pattern changes have been detected in suspension cultures of soyabean [*Glycine max* (L) Merr.] and maize (*Zea mays* L.) (Kaeppler and Philips 1993).

1.6. OBJECTIVES

The present investigation was undertaken for establishment of new strawberry cultivars adaptive to local agro-climatic condition of Bangladesh. *In vitro* culture of strawberry is well applied but somaclonal variation has not been explored for strawberry improvement. Strawberry is very recently being introduced in Bangladesh; therefore, existence natural genetic variability is very limited. In the pretext of previous discussion creation of genetic variation through *in vitro* technology would be an alternative approach for the development of strawberry cv. suitable for Bangladesh condition.

Strawberries are getting popularity in Bangladesh. Strawberry is a highly priced fruit in all over the world. Development of strawberry varieties suitable for Bangladesh not only save our foreign currency reserve but will generate revenue for local farmer. Therefore, development of new cv. suitable for Bangladesh agro-climatic condition is an urgent need. In the context of above discussion the present study was initiated in order to accentuate genetic variation in AOG, JP-2, JP-3, Camarosa, Sweet Charly,

Giant Mountain and Festival through the induction of somaclonal variation that can be used to develop more superior strawberry cultivars.

Considering the problems of strawberry cultivation in Bangladesh the present research is designed with the following specific objectives:

1. *In vitro* shoot proliferation from the runner tips and nodal explants for rapid multiplication for collection of leaves and nodes for callus induction and shoot regeneration.
2. Standardization of suitable culture media composition for rapid callus induction from different parts of strawberry.
3. Plant regeneration from induced callus.
4. Optimization of root induction from regenerated shoots.
5. Acclimatization and field evaluation of somaclonal variants.
6. Selection of suitable somaclonal variants and evaluation of their performances under biotic and abiotic stress condition.

II. MATERIALS AND METHODS

Part A: Induction of Somaclonal Variation and Field Evaluation of Strawberry

2.1. MATERIALS

2.1.1. Plant Materials

In the present investigation leaf segments and node of *in vitro* grown strawberry shoots were used as experimental material for callus induction. To collect leaf segments and node, primary establishment of shoot cultures from runner tips and nodal segments of strawberry is a prerequisite. For this purpose, runner tips and nodal segments were collected from field grown plants of strawberry to establish under *in vitro* condition. The following parts of the experimental plants were used as explants:

a. Runner tips from field grown plants.
b. Nodal segments from field grown plants.
c. Leaf segments from *in vitro* raised shoots. Two types of leaf were used in this experiment, young and mature leaf. *In vitro* grown 14 days old light green coloured leaves were used as young leaf and *in vitro* grown 30 days old dark green coloured leaves were used as mature leaf.
d. Node from *in vitro* raised shoots.

The explants of AOG (Japanese variety) were collected from the field grown stocks of Akafuzi Agrotechnologis, Rajshahi, Bangladesh. JP-2, JP-3 are Japanese varieties and Camarosa, Sweet Charly, Giant Mountain, Festival are American varieties were collected from strawberry germplasm stocks maintained in Plant Breeding and Gene Engineering Laboratory, Department of Botany, Rajshahi University.

2.1.2. Chemicals

The following chemical compounds were used in the present investigation:

(1) Plant growth regulators (PGRs)

The following PGRs (Table 2.1) were used either individually or in various combinations to the nutrient media. Auxins and cytokinin were added to the nutrient

media before autoclaving. However, GA_3 was used in filter-sterilized condition after autoclaving the nutrient medium.

(2) Sterilant and surfactant

In this experiment, mercuric chloride ($HgCl_2$) was used as surface sterilizing agent and Tween-80 and Savlon (ACI Pharma, Bangladesh) were used as surfactant cum detergent.

(3) Nutrient basal salts

For *in vitro* culture nutrient basal salts were used which contain macronutrients, micronutrients and vitamins.

Macronutrients: $MgSO_4.7H_2O$, KH_2PO_4, $NaH_2PO_4.H_2O$, KNO_3, NH_4NO_3, $CaCl_2.2H_2O$, $(NH_4)_2SO_4$.

Micronutrients: H_3BO_3, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, $NaMo_4.2H_2O$, $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, KI, $FeSO_4.7H_2O$, Na_2.EDTA.

Vitamins: Thiamine HCL, Pyridoxine HCL, Nicotinic acid, Myo-Inositol.

All chemical compounds including macro and micro nutrients, organic acids and inorganic acids, sugar, agar, KOH, $HgCl_2$, ethanol etc. used in the present study were the reagent grade products of either BDH, England or MERCK, India. The vitamins, amino acids (Glycin), growth regulators were mostly products of Sigma Chemical Company; USA and Phytotec (USA) and a small portion of thiamine was a product of BDH, England.

2.1.3. Carbon Sources

Plant cells and tissues in the culture medium lack autotrophic ability and therefore, need external carbon for energy. The most preferred carbon source in the plant tissue culture is sucrose. In present investigation, sugar was used for the sources of carbon. Normally 3% sucrose was used in culture media.

2.1.4. Gelling and Solidifying Agents

Gelling and solidifying agents are commonly used for preparing semisolid or solid tissue culture media. Agar, a polysaccharide obtained from seaweed can be used as a

gelling agent in the most nutrient media. In present investigation, 0.8% agar (BDH, England) was used as a gelling agent.

Table 2.1. Various types of plant growth regulators with chemical name.

Growth regulators	Types	Short name
Auxins:		
Auxin promotes cell enlargement and root initiation (Kyte 1987)	α-Napthalene Acetic Acid	NAA
	2,4-DichlorophenoxyAcetic Acid	2,4-D
	Indole-3-butyric acid	IBA
Cytokinins:		
Cytokinins actives on cell division (Jablonski and Skoog 1954)	6-Benzyladenine	BA
	6-Furfuryl amino purine	KIN
Gibberellins:		
Gibberellins actives on cell division	Gibberellic acid	GA_3

2.1.5. Laboratory Equipments

(i) For media preparation

Different types of glass vessels including test tubes, culture bottles, conical flasks, measuring cylinders, separating funnels, pipettes, micro-pipetter, magnetic stirrer, micro wave oven, pH meter, electronic balance, squijar etc.

(ii) For aseptic transfer

Autoclave, laminar air flow machine, spirit lamp, filter paper disc, marker pens, forceps of various sizes, fire box, needles, scissors, surgical scalpel holder with disposable blades, filter paper disc, petridishes etc.

(iii) For incubation

Racks with light arrangement (16h light and 8h dark) and controlled temperature 25±1° C maintained with air cooler.

2.2. METHODS

Experimental methods used for conducting experiments are discussed under the following heads:

2.2.1. Plant Tissue Culture Media

MS (Murashige and Skoog 1962) medium was used in the present investigation. The compositions of MS medium are given in Appendix I. In the different experiments MS medium supplemented with different growth regulators were used for various purposes. A control experiment using MS medium supplemented with 3% sucrose without any growth regulators was also included whenever necessary.

For establishment of primary aseptic cultures MS semisolid medium containing different concentrations of GA_3 was used. For shoot differentiation MS semisolid medium contain different concentrations of BA and KIN was used. For callus induction and maintenance MS semisolid medium containing different concentrations of 2,4-D alone and combination with BA or different concentrations of NAA alone and combination with BA was used. For multiple shoots regeneration MS semisolid medium containing different concentrations of NAA and BA was used. For root formation and elongation ½ MS or MS semisolid medium with any growth regulators (IBA or NAA) was used.

2.2.2. Preparation of Stock Solutions for Culture Media

In the first step of the preparation of MS culture medium, stock solutions were made. Various constituents of the respective nutrient medium were prepared into stock solutions for ready use during the preparation of media for different experiments. As different constituents were required in different concentrations, stock solutions for macronutrients, micronutrients, plant growth regulators (PGRs), organic compound (vitamins and amino acids) etc. were prepared separately.

2.2.2.1. *Stock solution of macronutrients*

This stock solution was made in such a way that its strength was 10 times more than the final strength of the medium in 500 ml distilled water. For this purpose, 10 times of the weight of different salts required for 1 liter of medium were weighted accurately. Then the salts were sequentially dissolved one after another in a 500 ml

volumetric flask with 350 ml of distilled water. The final volume of the solution was made up to 500 ml by further addition of distilled water. The solution was filtered with Whatman`s No.1 filter paper to remove all the solid contaminants like the dust, cotton etc. and was poured into a clean plastic container. After labeling, the solution was stored in a freeze at 4° C for several weeks.

2.2.2.2 *Stock solution of micronutrients*

For these constituents of the medium, two separate stock solutions were prepared: The first stock solution was made with the micronutrients except $FeSO_4.7H_2O$ and Na_2-EDTA. It was made 100 times the final strength of necessary components in 500 ml of distilled water as described for the stock solution A. The solution was filtered and stored at 4^oC for several weeks. The second solution was also made 100 times the final strength of $FeSO_4.7H_2O$ and Na_2-EDTA in 500 ml distilled water in conical flask and heated slowly at low temperature until the salts dissolved completely. Finally, the solution was filtered and stored in a fridge at 4^oC for several weeks.

2.2.2.3 *Stock solution of vitamins and amino acids*

This stock solution was also made 100 times the final strength of the medium in 500 ml of distilled water as described for stock solution of macronurtients. The solution was also filtered and stored at 4^oC for several weeks.

2.2.2.4. *Stock solution for plant growth regulators (PGRs)*

The following growth regulators were used in the present investigation. Stock solution of these regulators was prepared separately.

Details of the preparation method of stock solution are given in Table 2.2. To prepare any one of the previously mentioned PGR stock solution, 10 ml of the respective PGR was placed on clean plastic weighing boat and dissolved in 1 or 2 ml of respective solvent. The mixture was then washed off with distilled water and collected in 100 ml measuring cylinder. It was then made up to 100 ml with the addition of distilled water. The solution was then filtered, poured into a clean plastic container and stored in a fridge at 4^oC for several weeks.

2.2.2.5. *Surface sterilant solution*

$HgCl_2$ solution 0.1% was used for surface sterilization of plant materials. To prepare 0.1% solution, 0.1 gm of $HgCl_2$ was taken in a bottle and dissolve in 100 ml distilled water. Generally $HgCl_2$ solution was prepared 10-15 minutes before use.

Table 2.2. Preparation of stock solution for growth regulators.

Growth regulators	**Amount Taken (mg)**	**Appropriate solvents**	**Final volume with distilled water (ml)**	**Strength of the stock solution ($mg.ml^{-1}$)**
Auxin				
NAA	10	0.1 N KOH	100	1
2,4-D	10	0.1 N KOH	100	1
IBA	10	70% ETOH	100	1
Cytokinin				
BA	10	0.1 N KOH	100	1
KIN	10	0.1 N KOH	100	1
Gibberelline				
GA_3	10	70% ETOH	100	1

Growth regulators were dissolved in appropriate solvent as shown against each of them (Plant Cell Culture Catalogue 1992).

2.2.3. Preparation of 1 Liter Culture Medium

Following steps were followed to prepare 1 liter MS medium:

2.2.3.1. *Assembling of the medium components*

For preparing 1 liter MS medium, 20 ml of stock solution-I, 20 ml of stock solution-II, 20 ml of stock solution-III, 20 ml of stock solution-IV, 20 ml of stock solution-V, 2 ml of stock solution-VI, 2 ml of stock solution-VII were added in another 1 liter flask containing 200 ml distilled water and were mixed well. 30 g of sucrose was dissolved well in 500 ml of distilled water and filtered in a 1000 ml volumetric flask. The

sucrose solution was added to the stock solution and the final volume of the mixture was then made up to 1000 ml (1 liter) with further addition of distilled water.

2.2.3.2. *Addition of sucrose*

Sucrose 30 g of was dissolved well in 500 ml of distilled water and filtered in a 1000 ml volumetric flask. The sucrose solution was added to the stock solution and the final volume of the mixture was then made up to 1000 ml (1 liter) with further addition of distilled water.

2.2.3.3. *Addition of growth regulators*

Stock solution of growth regulators was added in appropriate concentrations and combinations in above solutions and was mixed. For preparing MS0 medium, no growth regulators were added.

2.2.3.4. *pH of the medium*

The pH of the medium was adjusted to 5.8 using pH meter (TOA, Japan) with the help of 0.1N HCl or 0.1N NaOH (whichever as necessary) before addition of agar.

2.2.3.5. *Addition of agar*

The semi-solidified medium was prepared with agar. In all cases 8-10 g of agar (BDH chemical Ltd.) was added to 1 liter of medium on the basis of its quality and the whole mixture was then gently heated in a microwave oven (National, Japan) till complete dissolution of agar.

2.2.3.6. *Medium dispensing to culture vessels*

Ten to twenty ml of the prepared melted medium was disposed into culture vessels like test tubes or culture bottle through separating funnel. The culture vessels were plugged with absorbent cotton plugs, or with plastic caps, which were inserted tightly at the mouth of culture vessels.

2.2.3.7. *Sterilization*

Finally, the culture vessels with medium were autoclaved at 15 lb/inch pressure and at the temperature of 120-121°C for 20 minutes to ensure sterilization. Then the culture vessels with the medium were allowed to cool and then marked with a glass marker to indicate specific hormone supplements and stored in the culture room for ready use.

2.2.4. Culture Techniques

The following techniques that were followed for the regeneration of complete plantlets of the experimental plants are discussed below:

2.2.4.1. *Collection of the field grown explants and surface sterilization of the explants*

Different explants (runner tips and nodal segments of runner) of the healthy disease free and young strawberry plants were collected in a conical flask from the 30-45 days old field grown plants. Then, those were washed thoroughly under tap water to reduce dust and surface contaminants. The runner tips and nodal segments were cut into convenient sizes and taken in a conical flasks containing distilled water. The materials were then surface sterilized with 2-3 drops of Tween-80 and a few drop of Savlon for about 5-9 minutes with constant shacking. The materials were then washed 4-5 times with distilled water for complete removal of sterilizing agents and taken under running laminar air-flow cabinet and transferred to 500 ml sterilized conical flask. Surface sterilization was carried out in the laminar air-flow cabinet. The explants were taken into one, two or more sterile conical flaks and suspended into 0.1% $HgCl_2$ solution by gently shaking for 5-6 minutes. To remove $HgCl_2$, the materials were washed 3-4 times with sterilized distilled water.

2.2.4.2. *Inoculation techniques*

Sterilized plant materials were dissected and cultured on MS medium supplemented with GA_3 (0.5 mg/l). During inoculation special care was taken that the explants much touch the medium equally and do not dip into the medium. All inoculation and aseptic manipulations were carried out in a running laminar airflow cabinet. The cabinet was switched on half an hour before use and cleaned with 90% ethyl alcohol to reduce the chances of contamination. The instruments like forceps, scalpels, and requirements like petridishes, filter paper, empty beaker or conical flasks, cotton, distilled water were covered with brown-paper and autoclaved by steam sterilization method. These were brought to the laminar airflow cabinet. During working time, the dissecting instruments like forceps, scalpels, etc. were again sterilized by an alcoholic dip and flamed over a spirit lamp for several times. Before starting inoculation, hands were also made sterile so far it was possible by spraying rectified spirit.

2.2.4.3. *Incubation*

The inoculated culture tubes or bottles were incubated in a growth chamber providing a special culture environment. The tubes were placed on the shelves of a culture rack in the growth chamber. It may be mentioned specially that, all cultures were grown in the growth chamber illuminated by 40 watts cool–white fluorescent tubes fitted at a distance of 30-40 cm from the culture shelves. The cultures were maintained at 25±1° C with light intensity varied from 2000-3000 lux (50-70µ E.m–2s-1). The photoperiod was maintained generally 16 hours light and 8 hours dark. For the induction of callus, the culture vessels were incubated in dark chamber but for organogenesis, the culture vessels were kept in above photoperiod.

2.2.4.4. *Subculture of primary established explants for shoot multiplication*

After few days the inoculated explants developed 2–3 leaf containing plantlets, and then the plantlets were rescued aseptically from culture vessels and transferred into a freshly prepared semi-solid medium supplemented with the same or different growth regulator combinations for shoot multiplication. After 30-35 days of culture, inoculated shoots produced multiple shoots.

2.2.4.5. *Callus induction, sub-culture of callus for maintenance and organogenesis*

Leaf segments (mature and young) and node of *in vitro* grown plantlets measuring 2-3 mm excised aseptically were cultured in test tubes containing 20 ml medium supplemented with various concentrations of 2,4 D, NAA and BA alone or in combination for callus induction. When the calli attained a size of about 10-15 mm in diameter, these were rescued aseptically on a sterile petridish and were cut into convenient sizes by a sterile scalpel and again set on same or different PGRs supplemented media for maintenance of callus. The calli were placed on shoot regeneration medium supplemented with various concentrations and combinations of PGRs for enhancing organogenesis.

2.2.4.6. *Rooting and plantlet formation*

When the regenerated shoot apices reached 4-5 cm in length with 5-6 well developed leaves, they were rescued aseptically from the culture vessels and were separated from each other. Micro-cuttings were prepared from these shoots by snapping off the

basal leaves and cultured them individually in tubes containing 20 ml of rooting medium with different combinations of auxins.

2.2.5. Acclimatization of *In Vitro* Grown Plants

After sufficient growth of plantlets with good roots and shoots they were considered ready to transfer soil. Prior to transfer, acclimatization is necessary for *in vitro* plantlets. At first the culture tube caps were removed and open culture vessels were kept inside the growth chamber. Then they were taken out from the controlled environment of growth chamber and kept in room temperature to bring them contact with normal temperature for acclimatization. After hardening, the plantlets were taken out of the culture vessels, washed thoroughly under running tap water to remove medium. The plantlets were dipped in 0.1% Bavistin solution, a fungicide, (BASF Aktiengesellschaft, Germany) for ten to fifteen minutes to kill any microbes attached to the roots and transferred to plastic pots filled with 3 types of soil formulation such as:

i. Sand
ii. Sand + cow dung + ash
iii. Coir dust.

Initially, the pots were placed under shady place and covered with polythene sheet to maintain high humidity around the juvenile plants. The potten plants were watered regularly and upper layer of the potting mix was mulched occasionally. Finally the seven varieties were transplanted in separate plot.

2.2.6. Field Evaluation of Somaclonal Variants

Field was pulverized and 12´ X 3´ size and 6´ raised beds were prepared.

2.2.6.1. *Experimental site*

The experimental site is located in Namo Bhadra, Rajshahi, Bangladesh.

2.2.6.2. *Climate and soil*

The experimental site was situated in the tropical climate zone. The soil of the experimental field was sandy loam in texture having a pH around 5-7. The land was medium high with uniform topography and almost homogenous with respect to soil fertility.

2.2.6.3. *Field preparation*

The field was thoroughly prepared by ploughing and harrowing followed by laddering. All the stubbles and weeds were removed from the field. Then well-decomposed cow dung was applied (5 ton/acre). Weeds were removed completely from the soil. The beds were treated with 1% formaldehyde solution to prevent the soil borne pathogen and covered with polythene sheets for 3-5 days.

2.2.6.4. *Design of the experimental field*

Plantlets of seven strawberry genotypes and their somaclones were planted at the research field in a Randomized Block Design with three replications.

2.2.6.5. *Plantlets transplantation*

The potted plants were transplanted on the raised beds during the first week of November 2011. The planting was done within the first week of January, 2012. Necessary intercultural operations i.e. irrigation followed by mulching, spading and weeding were done.

2.2.7. Data Recording

Data on different parameters from different treatments were recorded as follows:

2.2.7.1. *Data recording on in vitro parameters*

(i) Data recording on surface sterilization

The percentage (%) of contamination free explants was determined by the following formula:

$$\text{\% of contamination free explant} = \frac{Number\ of\ contamiation\ free\ \exp lants}{Number\ of\ \exp lants\ inoculated} \times 100$$

(ii) Percentage of explants showing proliferation

Percentage of explants showing proliferation was calculated by using following formula:

$$\text{Percentage of explants showing proliferation} = \frac{\text{No. of explants formed shoots}}{\text{No. of explants cultured}} \times 100$$

(Excluding contamination & dead explants)

(iii) Percentage of explants induced callus

Explants were cultured in test tubes containing medium with different concentrations of growth regulators for callus induction. Among the culture tubes, in which explants remained fresh and resumed new growth were counted after required days of culture. Percentage of explants induced callus was calculated using following formula:

$$\text{Percentage of explants induced callus} = \frac{\text{Number of explants induced callus}}{\text{Total number of cultured explants}} \times 100$$

(iv) Symbols used for callus induction

Different symbols were used to denote the different colour and degree of callus formation as given below:

Callus colour	**Symbol**
Light creamy	L Cr
Brownish	Br
White to creamy	W Cr
Creamy	Cr
White	W
Brown to creamy	Cr B

Degree of callus formation was marked in the following way:

Index	**Description of callus formation**
—	No callusing
+	Little callusing
++	Moderate callusing
+++	Highly callusing

In the present investigation, induction and growth of callus varied on the basis of media formulation, so degree of callus growth was denoted after 6 weeks of culture.

(v) Percentage of calli induced shoots

The number of calli that produced shoots was expressed as % and data were recorded after 2-5 weeks of sub-culture.

$$\text{Percentage of calli formed shoots} = \frac{\text{Number of calli formed shoot}}{\text{Total number of cultured calli}} \times 100$$

(vi) Percentage of shoots induced to develop roots

Percentage of shoots induced to develop roots was calculated by using the following formula:

$$\text{Percentage of shoots developed roots} = \frac{\text{Number of cultures induced root}}{\text{Total number of shoots cultured}} \times 100$$

(vii) Mean numbers of roots per shoot

Number of roots per shoot was calculated after required days of culture. Mean number of roots per shoot was calculated using the following formula:

$$\text{Mean } \overline{X} = \frac{\sum X}{n}$$

Where,

X = Average number of root

Σ = Summation

X = Total number of root

N = Number of observation

2.2.7.2. *Data recording on field grown plants*

To evaluate field performance and somaclonal variation among the callus derived plants data on various morphological and agronomical characters were recorded at different stages of plant growth.

A. Data on different qualitative characters viz. flowering habit, stolon habit, crown formation, fruit shape, fruit colour, degree of attractiveness of the fruits, test, summer overcoming potential and disease susceptibility were recorded on different duration after planting.

B. Data on following quantitative characters were recorded after 1, 2 and 4 months of transplantation.

Plant height	Crown height was measured from the bottom to the top using a meter scale.
No. of leaves/plant	No. of leaves was counted on individual plant basis.
Petiole length	Petiole length of at least five leaves/ plant was measured.
No. of stolon/plant	No. of stolon was counted on individual plant basis.
No. of nodes/stolon	No. of nodes/stolon was counted from at least three stolon of individual plant.
No. of crowns/plant	No. of crowns/plant was counted on individual plant basis.
No. of flower clusters/ plant	No. of flower clusters/plant was counted on individual plant basis.
Stolon length	Stolon length was measured from base of the stolon to the tip using a meter scale.
No. of flowers/cluster	No. of flowers was counted from three selected flowers/ plant.
Canopy size	Canopy size was determined by measuring the length and the breadth of individual plant basis.
No. of fruits/plant	No. of fruits/plant was counted at different time interval.
No. of fruits/cluster	No. of fruits/cluster was counted from three flower cluster/plant.
Days to flowering	Days to flowering was counted from the plantation date to first flower opening.
Days to fruit harvest	Days to first fruit harvest was counted from the plantation date to first fruit harvest.
Average fruit wt.	At least 10 fruits/plant was weighted and mean value was calculated.
Fruit wt./ plant	All fruits of the selected plants were sequentially harvested and weighted.

2.2.8. Data Analysis

Variant characters for plant characteristics, such as: Plant height, No. of eaves/plant, Petiole length, No. of stolon/plant, Nos. nodes/stolon, No. of crowns/plant, No. of flower clusters/plant, Stolon length, No. of flowers/cluster, Canopy size, No. of fruits/plant, No. of fruits/cluster, Days to flowering, Days to fruit harvest, Average fruit wt., Fruit wt./plant were recorded by quantitative measurement. The collected data were analyzed following biometrical technique developed by Mather (1949)

based on the mathematical models of Fisher *et al.* (1932), and those of Hayman (1958), Allard (1960) and De-wey, Lu (1959) and Kempthorne (1957). The techniques used are described under the following subheads:

2.2.8.1. *Mean*

Data individual plants were added together and divide by the total number of observations and the mean was obtained as follows-

$$\text{Mean } \overline{X} = \frac{\sum X}{n}$$

Here,

$\overline{X}$ = Mean

X = The individual reading recorded on each plant

n = Number of observation

$\sum$ = Summation

2.2.8.2. *Standard error of mean*

Instead of taking one sample of several samples if several samples are considered standard deviation of the different samples varies. The variation was measured by the standard error, which was calculated as follows:

$$\text{S. E.} = \frac{S.D}{\sqrt{n}}$$

Where,

S. E = Standard error of mean

S. D. = Standard deviation

N = Total number of individuals

2.2.8.3. *Co-efficient of variation (CV %)*

This was calculated by using the following formula:

$$\text{CV \%} = \frac{S.D.}{\overline{X}} \times 100$$

Where,

CV = Coefficient of variation

$\overline{X}$ = Mean

S.D. = Standard deviation

2.2.8.4. *Analysis of variance*

Analysis of variance is a measurement of a population according to components. The analysis of variance was done for testing the significance of differences among the populations. Variance analysis for each of the character was carried out separately with raw data taken from individual plants. The sum of square for different sources was calculated by the following formula. The following table of expectations mean square (EMS) used to calculate the analysis of variance:

Item	MS	Expectation of MS
Variety (v)	Mv	$\delta^2e + v\delta^2r + r\delta^2v$
Replication (r)	Mr	$\delta^2e + v\delta^2r$
Error (e)	Me	δ^2e

Here,

Mv = Mean square of variety (v)

Mr = Mean square of replication (r)

Me = Mean square of error (e)

and

δ^2e = Environmental variance

$v\delta^2r$ = Variance due to replication

$r\delta^2v$ = Variance due to variety (genetical variance)

The phenotypic, genotypic and environmental variance (δ^2p, δ^2g and δ^2e) were calculated as follows:

Step 1

$\delta^2e = Me$

$\delta^2v = (Mv - Me)/r$

Step 2

$\delta^2p = \delta^2e + \delta^2v$

$\delta^2g = \delta^2v$

Where, r = Number of replication

2.2.8.5. *F- test*

Variance analysis is the basis for test of significance. Significance differences among population were worked out by F-test (variance ratio) as follows:

$$F = \frac{MS}{MSe}$$

Where,

MS = Mean square

MSe = Mean square of error

2.2.8.6. *Test of least significant difference (LSD)*

To test least significant difference among seven varieties the following formula was used

$$LSD = \sqrt{\frac{2 \times MSe}{r}} \times \text{ t at 5\% level or 1\% level}$$

Here,

MSe = Error mean square

r = Number of replication

5% level t = value t 5% significant level from tabulated t table

1% level t = value t 1% significant level from tabulated t table

2.2.8.7. *Genotypic variance*

Genotypic variances (δ^2_g) were derived by subtracting error MS from the genotype MS and dividing by the number of replication as shown bellow:

$$\text{Genotypic variance } (\delta^2_g) = \frac{GMS - EMS}{r}$$

Here,

GMS = Genotypic mean square

EMS = Error mean square

r = Number of replication

2.2.8.8. *Phenotypic variance*

The phenotypic variances (δ^2_p) were derived by adding genotypic variances (δ^2_g) with environmental variances (δ^2_e) as given by the following formula.

Phenotypic variance (δ^2_p) = $\delta^2_g + \delta^2_e$

Here,

Genotypic variance = δ^2_g

Environmental variance = δ^2_e

2.2.8.9. *Environmental variance*

The expected mean sum of squares for error (EMs) is environmental variance.

Environmental variance (δ^2_e) = EMs

Where,

EMs = Mean sum of squares for error

2.2.8.10. *Genotypic coefficient of variability*

Genotypic coefficient of variability was calculated as proposed by Burton (1952).

$$GCV = \frac{\delta g}{\bar{x}} \times 100$$

Here,

δg = Genotypic standard deviation

$\bar{x}$ = Grand mean for a particular character

2.2.8.11. *Phenotypic coefficient of variability*

Phenotypic coefficient of variability was calculated as proposed by Burton (1952).

$$PCV = \frac{\delta p}{\bar{x}} \times 100$$

Here,

δp = Phenotypic standard deviation

$\bar{x}$ = Grand mean for a particular character

2.2.8.12. *Environmental coefficient of variability*

Environmental coefficient of variability was calculated from the following formula:

$$ECV = \frac{\delta e}{\bar{x}} \times 100$$

Here,

δe = Environmental standard deviation

$\bar{x}$ = Grand mean for a particular character

Part B: Evaluation of Variants to Different Biotic and Abiotic Stress Condition

2.1. MATERIALS AND METHODS

2.1.1. Materials

Plant materials for the present study comprised seven strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival and their somaclones.

2.1.2. Methods

The method followed to conduct the experiments and analyses of data aere described below.

2.1.2.1. *Experimental site and period*

The present research work was conducted at the research field of Plant Breeding and Gene Engineering Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh during the period January 2012- May 2012.

2.1.2.2. *Disease identification*

Identification of the diseases found in the field grown strawberry was conducted by Plant Pathology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh.

III. RESULTS

Part A: Induction of Somaclonal Variation and Field Evaluation of Strawberry

The present investigation was carried out to the study the induction of somaclonal variation in strawberry and evaluation of new cultivars adaptive to agro climatic condition of Bangladesh. To achieve the objective the young and mature leaf segments and nodes were cultured stepwise for callus induction and subsequent plant regeneration. Finally, the regenerated plantlets were transplanted in the field for evaluation of different agronomic characters.

The results of different experiments of this part of investigation are described under the following major heads.

3.1. SURFACE STERILIZATION AND INITIAL ESTABLISHMENT OF THE EXPLANTS

Successful establishment of aseptic culture under *in vitro* condition needed surface sterilization of explants. Because, over growth of microbial contamination inhibits the resumption of growth of the excised tissue (explants) under *in vitro* culture system. Consequently, surface sterilization of field grown explants was necessary for establishing the aseptic cultures. Standardization for surface sterilization was carried out by using trial and error experiment.

In the present investigation, the runner tip (RT) and nodal segment (NS) from the field grown plants of seven strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival were treated with $HgCl_2$ for raising aseptic culture. In doing so, the collected plant materials from the field grown plants were washed thoroughly under running tap water and then washed with savlon water for 10-12 minute. Then the explants (runner tips and nodal segments) were treated with 0.1% $HgCl_2$ for different duration of time viz. 4, 6, 7 and 8 min to surmount the contamination difficulty and to increase viability rate of the explant types used for direct regeneration systems.

The explants of strawberry were very sensitive to $HgCl_2$ treatment. 85% runner tips and 80% nodal segments were found contamination free when the two type of explants were treated with 0.1% $HgCl_2$ for 6 min. On the other hand 15% runner tips and 25% nodal segments were found contamination free when the explants were treated with 0.1% $HgCl_2$ for 4 min. In this experiment, 100% contamination free explants were found when the explants of the selected seven strawberry varieties were treated with 0.1% $HgCl_2$ for 8 min. However, incase of 7 and 8 min duration the explants died due to tissue killing. The most of the cultured explants showed fungal and bacterial contamination within 3-8 days of inoculation.

Considering the sensitivity of the two types of explants of the selected seven strawberry varieties (AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival), treatment with 0.1% $HgCl_2$ for 6 min duration was considered to be the most suitable for runner tip and nodal explants.

3.2. PRIMARY CULTURE ESTABLISHMENT AND SHOOT MULTIPLICATION

3.2.1. Effect of Different Concentrations of GA_3 on *in vitro* Culture Establishment of Seven Strawberry Verities in MS Medium

In this experiment, the results regarding shoot development of seven strawberry varieties viz. AOG, JP-2 JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival were recorded after 4 weeks of culture on MS medium supplemented with GA_3 at different concentration viz. 0.1, 0.5 and 1.5 mg/1 are shown in Table 3.1. Among different concentrations of GA_3 0.5 mg/l showed best performance on shoot development. Maximum shoot induction (75% from runner tip and 70% from nodal segment) was observed within 6-8 days for runner tips and 7-9 days for nodal segments in this medium. The length of shoots 4.0 cm from runner tip and 3.5 cm from nodal segments was recorded on MS medium with 0.5 mg/l GA_3.

3.2.2. Effect of Different Concentrations of Kinetine (KIN) on *in vitro* Culture Establishment of Seven Strawberry Varieties in MS Medium

The runner tip and nodal explants were also cultured on to MS medium supplemented with various concentrations of KIN. The explants were cultured on MS medium with 0.1, 0.2 and 1.0 mg/l of KIN. Data recorded on percentage of explants responded,

days to shoot formation, no. of microshoots/ explant and average length of shoot and the results are shown in Table 3.2. Regeneration of axillary shoot from runner tip and nodal segment explants was influenced by the different concentrations of KIN used. The highest percentage of shoot multiplication (70% from runner tips and 65% from nodal segments) was noted in MS + 0.2 mg/l KIN.

3.2.3. Effect of Different Concentrations of BA on *in vitro* Culture Establishment of Seven Strawberry Variety in MS Medium

In this experiment, runner tips and nodal segments of seven strawberry varieties were cultured on MS medium supplemented with different concentration of BA (0.5, 1.0, 1.5 mg/l) and the results on the effect on primary establishment of the explants are shown in Table 3.3. The highest percentage of explant responds 90% from runner tip and 80% from nodal segment was noted in the 1.0 mg/l BA concentration. Generally, shoot formation was noticed after 12-14 days of culture. However in 1.0 mg/l BA concentration, shoot formation was observed after 7-10 days of culture (Fig. 1 C, D).

Through these experiments it was concluded that BA was more effective growth regulator and the most preferred concentration of BA was 1.0 mg/l for the primary culture establishment of the selected seven strawberry varieties.

3.2.4. Effect of Different Concentrations and Combinations of BA and KIN on Multiple Shoot Proliferation of Seven Strawberry Varieties in MS Medium

In this experiment excised shoots from *in vitro* grown primary cultures were cultured on MS medium supplemented with different concentrations of BA viz. 0.5, 1.5 and 2 mg/l in combination with KIN viz. 0.1 and 0.5 mg/l. Results on the effect of the different combinations of these growth regulators are summarized and presented in the Table 3.4. Data on the percentage of explants showing shoot proliferation, days to shoot formation, number of total shoots/explant and number of useable shoots per culture and average length of shoots per culture reveal that the specific combinations of BA and KIN produced increased number of axillary shoot than rest of the treatments. In this regard, among the various combinations of BA and KIN, best result was observed on medium supplemented with 1.5 mg/l BA + 0.5 mg/l KIN in seven strawberry varieties. On this growth regulator combination shoots derived from both

type of explants showed 100% multiple shoot proliferation in AOG within 8-10 days of culture. The highest number of shoots/culture produced from both type of explants derived culture were 12 shoot among which 10 were useable for both type of explants. The maximum length of the shoots was 3.2 cm and it was observed in 1.5 mg/l BA + 0.5 mg/l KIN combination.

In these experiments it was observed that the combination 1.5 mg/l BA + 0.5 mg/l KIN was found to be the most suitable for multiplication of shoots from *in vitro* grown explants for seven strawberry varieties.

Table 3.1. Effect of different concentrations of gibberellic acid (GA_3) on *in vitro* culture establishment from runner tips and nodal explants of seven strawberry varieties in MS medium. There were 20 explants in each treatment. Data were collected after 4 weeks of culture.

Varieties	Concentrations of growth regulator GA_3	Type of explant	% of explants responded	Days to shoot formation	No. of micro-shoots/ explant	Average length of shoot (cm)
AOG	0.1	RT	55	10-12	1	2.1
		NS	50	10-14	1	2.5
	0.5	RT	75	6-8	2	4.0
		NS	70	7-9	2	3.5
	1.5	RT	55	10-12	1	2.0
		NS	50	10-12	1	2.5
JP-2	0.1	RT	51	10-12	1	2.0
		NS	50	10-12	1	2.5
	0.5	RT	63	7-8	2	4.1
		NS	61	7-9	2	3.5
	1.5	RT	56	10-14	1	2.0
		NS	50	10-14	1	2.5
JP-3	0.1	RT	55	11-14	1	1.8
		NS	52	10-14	1	1.6
	0.5	RT	62	7-10	2	4.0
		NS	60	6-10	2	3.5
	1.5	RT	52	10-12	1	2.1
		NS	49	10-12	1	2.0
Camarosa	0.1	RT	48	10-12	1	2.0
		NS	46	10-12	1	2.3
	0.5	RT	63	7-10	2	4.2
		NS	60	7-10	2	4.0
	1.5	RT	56	10-12	1	2.1
		NS	51	10-14	1	2.0
Sweet Charly	0.1	RT	29	10-12	1	2.2
		NS	49	10-12	1	2.0
	0.5	RT	60	7-10	2	4.1
		NS	60	7-12	2	3.9
	1.5	RT	51	10-12	1	1.9
		NS	49	10-12	1	1.7
Giant Mountain	0.1	RT	49	10-14	1	2.1
		NS	47	10-14	1	2.0
	0.5	RT	64	9-12	2	4.1
		NS	61	10-12	2	3.9
	1.5	RT	49	10-12	1	2.0
		NS	48	10-12	1	2.0
Festival	0.1	RT	49	10-14	1	2.2
		NS	49	10-12	1	2.0
	0.5	RT	63	9-12	2	4.0
		NS	62	9-12	2	3.5
	1.5	RT	45	10-14	1	2.1
		NS	41	10-14	1	2.0

Table 3.2. Effect of different concentrations of kinetine (KIN) on *in vitro* culture establishment from runner tips and nodal explants of seven strawberry varieties in MS medium. There were 20 explants in each treatment. Data were collected after 4 weeks of culture.

Varieties	Concentrations of growth regulator KIN mg/l	Type of explant	% of explants responded	Days to shoot formation	No. of micro-shoots/ explant	Average length of shoot (cm)
AOG	0.1	RT	45	12-14	3	1.3
		NS	40	12-14	4	1.2
	0.2	RT	70	7-9	5	2.0
		NS	65	8-10	5	2.0
	1.0	RT	37	10-11	3	1.6
		NS	32	10-11	4	1.6
JP-2	0.1	RT	40	12-14	2	1.1
		NS	35	12-14	2	1.1
	0.2	RT	65	7-9	4	1.9
		NS	60	8-10	4	1.8
	1.0	RT	36	10-12	3	1.6
		NS	31	10-12	3	1.5
JP-3	0.1	RT	35	12-14	2	1.1
		NS	30	12-14	2	1.0
	0.2	RT	60	7-10	4	1.8
		NS	55	8-10	4	1.8
	1.0	RT	35	10-12	3	1.6
		NS	30	10-12	3	1.5
Camarosa	0.1	RT	40	10-12	2	1.1
		NS	35	10-12	2	1.1
	0.2	RT	62	7-10	4	1.9
		NS	56	8-10	4	1.8
	1.0	RT	35	10-12	3	1.6
		NS	32	10-12	3	1.5
Sweet Charly	0.1	RT	40	10-14	2	1.1
		NS	35	10-14	2	1.1
	0.2	RT	60	7-10	4	1.7
		NS	60	8-10	4	1.6
	1.0	RT	31	10-12	2	1.6
		NS	30	10-12	3	1.6
Giant Mountain	0.1	RT	40	12-14	2	1.2
		NS	35	12-14	2	1.1
	0.2	RT	61	7-10	4	1.9
		NS	60	8-10	4	1.9
	1.0	RT	35	10-12	3	1.6
		NS	30	10-12	3	1.6
Festival	0.1	RT	35	10-14	2	1.0
		NS	30	10-14	2	1.0
	0.2	RT	60	7-11	3	1.8
		NS	59	8-12	3	1.6
	1.0	RT	32	10-12	2	1.6
		NS	31	10-12	3	1.5

Table 3.3. Effect of different concentrations of BA on *in vitro* culture establishment from runner tips and nodal explants of seven strawberry varieties in MS medium. There were 20 explants in each treatment. Data were collected after 5 weeks of culture.

Varieties	Concentrations of growth regulator BA mg/l	Type of explant	% of explants responded	Days to shoot formation	No. of micro-shoots/ explant	No. of usable shoots/ culture	Average length of shoot (cm)
AOG	0.5	RT	55	10-12	8	4	0.9
		NS	45	10-12	8	4	0.9
	1.0	RT	90	7-9	12	10	2.5
		NS	80	7-9	11	9	2.5
	1.5	RT	50	10-12	9	2	1.2
		NS	40	10-12	10	3	1.1
JP-2	0.5	RT	50	12-14	7	2	0.8
		NS	40	12-14	6	2	0.8
	1.0	RT	80	7-9	10	5	1.9
		NS	75	7-10	9	4	1.9
	1.5	RT	50	12-14	8	2	1.0
		NS	45	12-14	8	2	1.0
JP-3	0.5	RT	50	12-14	5	2	0.6
		NS	40	12-14	5	2	0.6
	1.0	RT	75	7-10	9	4	1.8
		NS	70	8-10	9	4	1.8
	1.5	RT	50	12-14	8	2	1.0
		NS	40	12-14	8	2	0.9
Camarosa	0.5	RT	50	11-14	5	2	0.8
		NS	45	11-14	5	2	0.7
	1.0	RT	81	7-9	10	5	1.9
		NS	75	8-10	10	5	1.0
	1.5	RT	50	12-14	8	2	1.9
		NS	40	12-14	8	2	1.0
Sweet Charly	0.5	RT	50	11-14	5	2	0.8
		NS	45	11-14	5	2	0.8
	1.0	RT	80	8-10	9	4	1.9
		NS	71	7-10	9	4	1.9
	1.5	RT	50	10-12	8	2	1.0
		NS	40	10-12	8	2	1.0
Giant Mountain	0.5	RT	54	12-14	6	3	0.9
		NS	45	12-14	5	2	0.8
	1.0	RT	80	8-10	9	4	2.0
		NS	75	7-10	8	4	2.0
	1.5	RT	45	12-14	7	2	1.5
		NS	40	12-14	7	2	1.0
Festival	0.5	RT	50	13-15	5	2	0.6
		NS	46	13-15	4	1	0.6
	1.0	RT	80	8-10	8	4	1.8
		NS	79	7-10	8	3	1.8
	1.5	RT	45	13-15	6	1	9.9
		NS	40	13-15	6	1	0.9

Table 3.4. Effect of different concentrations and combinations of BA and KIN on multiple shoot proliferation from excised shoots of seven strawberry varieties in MS medium. Data were collected after 5 weeks of culture.

Varieties	Growth regulator BA+KIN	Type of explant	% of explant showing proliteration	Days of shoot formation	No. of total shoots/ explant	No. of usable shoots / culture	Average length of shoot / culture (cm)
AOG	1.5+0.1	RT	76	10-12	8	3	2.2
		NS	75	10-12	7	3	2.3
	1.5+0.5	RT	100	8-10	12	10	3.2
		NS	100	8-10	12	10	3.2
	2+0.5	RT	85	10-12	6	2	1.3
		NS	80	10-12	7	3	1.2
JP-2	1.5+0.1	RT	73	10-12	7	3	2.0
		NS	72	10-12	7	3	2.0
	1.5+0.5	RT	96	8-10	11	9	3.0
		NS	95	8-10	10	9	3.0
	2+0.5	RT	82	10-12	6	2	1.2
		NS	75	10-12	6	2	1.2
JP-3	1.5+0.1	RT	70	10-12	7	3	2.0
		NS	70	10-12	7	3	2.0
	1.5+0.5	RT	95	8-10	10	9	3.0
		NS	90	8-10	10	9	3.0
	2+0.5	RT	80	10-12	6	2	1.2
		NS	75	10-12	6	2	1.2
Camarosa	1.5+0.1	RT	74	10-12	8	3	2.2
		NS	72	10-12	7	3	2.2
	1.5+0.5	RT	97	8-10	11	9	3.1
		NS	96	8-10	10	9	3.0
	2+0.5	RT	83	10-12	6	2	1.2
		NS	80	10-12	6	2	1.2
Sweet Charly	1.5+0.1	RT	72	10-12	7	3	2.0
		NS	71	10-12	7	3	2.0
	1.5+0.5	RT	96	8-10	10	9	3.0
		NS	95	8-10	10	9	3.0
	2+0.5	RT	81	10-12	6	2	1.2
		NS	80	10-12	6	2	1.2
Giant Mountain	1.5+0.1	RT	71	10-12	7	3	2.1
		NS	70	10-12	7	3	2.0
	1.5+0.5	RT	95	8-10	10	9	3.1
		NS	95	8-10	10	8	3.1
	2+0.5	RT	81	10-12	6	2	1.2
		NS	80	10-12	6	2	1.2
Festival	1.5+0.1	RT	70	10-12	7	3	2.0
		NS	70	10-12	7	3	2.0
	1.5+0.5	RT	95	8-10	10	9	3.0
		NS	90	8-10	10	8	3.0
	2+0.5	RT	80	10-12	6	2	1.2
		NS	80	10-12	6	2	1.2

Plate 1

3.3. INDUCTION OF CALLUS FROM DIFFERENT PARTS OF PLANT

After 5-7 days of culture, callus started to appear at the cut surface of the cultured explants. The formation of callus, however, is influenced by many factors including plant growth regulators, genotypes, types of explants and the component of medium. Different concentrations and combination of auxin and cytokinin were used with MS (Murashige and Skoog 1962) medium. All the cultures were incubated in dark growth cabinet at 25^0 C for 4 weeks. Different parameters were taken in to consideration for finding out the most effective culture media formulation for the development of callus with high potential for plant regeneration. The results of different experiments on induction and development of callus are discussed below on the basis of PGR type.

3.3.1. Effect of 2, 4-D in MS Medium on Callus Induction

Leaf explants (matured and young) and nodes of strawberry were incubated onto MS culture medium containing different concentration (1.0, 2.0, 2.5) of 2, 4-D alone for callus induction. The effect of 2, 4-D on callus development from leaf explants was evaluated by recording data on different parameters such as % of explants induced callus, degree of callus development, colour and nature of calli, adventitious shoot regeneration and the obtained results are presented in Tables 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 and 3.11.

In almost all the concentrations of 2, 4-D used in this experiment induced the explants to develop callus. However, the callogenic responses of the explants were found to vary with doses of 2, 4-D. Among the different doses of 2, 4-D, the highest percentage of callus induction from mature, young leaf (90%) and node (80%) were noted in medium supplemented with 2.0 mg/l 2, 4-D in AOG (Table 3.5.). Whereas, the lowest percentage of explants showed callus formation from node (10%) was noted in medium supplement with 1.0 mg/l 2, 4-D in AOG (Figure 2). Moderate callusing response was recorded in media having 2.5 mg/l 2, 4-D in AOG from leaf explants. The calli induced in 2, 4-D fortified media were prolific in growth, creamy in colour and almost all calli were loosely compact in nature, few were soft. Adventitious shoot formation was not found in this media formulation.

In other varieties (JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Featival), the highest percentage of callus induction from matured and young leaf (85%) was

noted in medium with supplemented 2.0 mg/l 2, 4-D in Camarosa (Table 3.8) and Sweet Charly (Table 3.9). Lowest percentage of callus induction from node (10%) was also observed in media supplemented with 1.0 mg/l 2, 4-D and moderate callusing response was recorded in media having 2.5 mg/l 2, 4-D in other varieties (JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Featival) (Table 3.6, 3.7, 3.8, 3.9, 3.10 and 3.11). The calli induced in 2, 4-D fortified media were prolific in growth, creamy, white and few are creamy brownish in colour and almost all calli were loosely compact in nature, few were soft and compact. Adventitious shoot formation was not noticed in these varieties. The calli induced in 2, 4-D fortified media were prolific in growth, creamy and white in colour and almost all calli were loosely compact in nature, few were soft and compact.

3.3.2. Effect of NAA in MS Medium on Callus Induction

MS medium supplemented with different concentration (1.0, 2.0, and 2.5 mg/l) of NAA were also tested to induce callus development from leaf segments and nodes of strawberry. The results on effect of different concentration of NAA on % of explants induced callus, degree of callus development, colour and nature of calli and adventitious shoot regeneration are shown in Tables 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 and 3.11.

All explants such as leaf segments (young and mature) and node was induced callus in all NAA formulations; but their effect was not same on the degree of explants induced callus formulation and the degree of callus development. The maximum 90% callus induced from matured leaf and young leaf in AOG, Camarosa, Sweet Charly, Giant Mountain and Festival (Table 3.5, 3.8, 3.9, 3.10 and 3.11). This result showed when callus proliferation in MS medium supplemented with 2.0 mg/l NAA. Whereas, the lowest 15% explants from node 25% explants from young leaf and 30% explants from matured leaf responded to develop callus in medium supplemented with 1.0 mg/l of NAA in JP-2 and JP-3 (Table 3.6 and 3.7). Moderate callusing response was recorded in media having with 2.5 mg/l NAA in all varieties of strawberry. The calli proliferated in NAA supplemented culture media from each sources of explants also showed prolific and massive growth. Almost all calli were light creamy, cream and white in colour. And their nature is loosely compact, few were compact and few are soft. Like 2, 4-D, adventitious shoot formation was not noticed in this media formulation.

Table 3.5

Table 3.6

Table 3.7

Table 3.8

Table 3.9

Table 3.10

Table 3.11

Plate 2

Plate 3

3.3.3. Effect of NAA in Combination with BA in MS Medium on Callus Induction

The segments of both young and matured leaves and nodes of strawberry were also cultured onto MS medium supplemented with different concentration of NAA in combination with BA for callus induction. The combined effect of NAA and BA on callus formation from different explants in terms of % explants induced callus, degree of callus growth, colour and nature of calli and adventitious shoot regeneration are presented in Tables 3.12, 3.13, 3.14, 3.15, 3.16, 3.17 and 3.18. Adventitious shoot regeneration was not noticed from node derived callus.

Callus proliferation from both leaf explants was noticed in all NAA+BA formulations. Among the different NAA+BA formulations the highest 90% from matured leaf and 90% from young leaf and 80% from node induced to develop callus in media having 0.5 mg/l NAA + 1.5 mg/l BA in AOG. Whereas, the lowest 10% callus was induced from matured leaf, node, when the explants were cultured in media having 0.5 mg/l NAA + 0.5 mg/l BA in AOG. Moderate callusing was recorded in media having 1.0 mg/l NAA + 1.0 mg/l BA and 0.5 mg/l NAA + 1.0 mg/l BA in AOG. The calli developed in NAA+BA media formulations were light cream in colour and their nature was different such as compact, loose compact, loose and some were soft. The results indicate that NAA+BA combinations had noticeable effect on callus proliferation from all sources of explants. Adventitious shoot formation from leaf segment derived callus was not observed in all the NAA + BA combination tested in primary culture but positive result was found in 0.5 mg/l NAA+ 1.5 mg/l BA in AOG.

Same combinations of NAA and BA were also used in JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival. Best result was found in 0.5 mg/l NAA and 1.5 mg/l BA. In this combination 70% callus was recorded from young leaf, 90% from matured leaf, 80% from node in JP-2 and JP-3 (Table 3.13 and 3.14). Moderate calli were derived in 1.0 mg/l NAA + 0.5 mg/l BA concentrations and lowest calli were derived in 0.5 mg/l NAA + 0.5 mg/l BA and 0.5 mg/l NAA +1.0 mg/l BA concentrations in two Japanese vatieties JP-2 and JP-3. Adventitious shoot formation from leaf segment derived callus was not observed in all the NAA+BA combination tested in primary culture. The calli developed in NAA+BA media formulations were light cream and cream in colour and their nature was different such as compact, loose compact, loose and some were soft (Figure 4).

Table 3.12

Table 3.13

Table 3.14

Table 3.15

Table 3.16

Table 3.17

Table 3.18

Plate 4

In NAA+BA formulations the highest 90% from matured leaf and 70% from young leaf and node were found to induce callus in media having 0.5 mg/l NAA + 1.5 mg/l BA in American strawberry varieties Camarosa, Sweet Charly, Giant Mountain and Festival (Table 3.15, 3.16, 3.17 and 3.18). Whereas, the lowest 10% callus was induced from matured leaf, node, when the explants were cultured in media having 0.5 mg/l NAA + 0.5 mg/l BA. Moderate callusing was recorded in media having 1.0 mg/l NAA + 1.0 mg/l BA and 0.5 mg/l NAA + 1.0 mg/l BA in Camarosa, Sweet Charly, Giant Mountain and Festival. The calli developed in NAA+BA media formulations were cream in colour and their nature was different such as compact, loose compact, loose and some are soft. The results indicate that NAA+BA combinations had noticeable effect on callus proliferation from all sources of explants. Adventitious shoot formation from leaf segment derived callus was not observed in all the NAA + BA combination tested in primary culture but high callusing response was found in 0.5 mg/l NAA+ 1.5 mg/l BA in Camarosa, Sweet Charly, Giant Mountain and Festival.

3.3.4. Effect of 2, 4-D in Combination with BA in MS Medium on Callus Induction

The leaf segments of both young and matured leaf, node of strawberry were cultured on to MS medium supplemented with different concentration 2, 4-D in combination with BA for callus induction. The combined effect of 2, 4-D and BA on callus formation from different explants in terms of % of explants induced callus, degree of callus growth, colour and nature of calli and adventitious shoot regeneration are presented in Table 3.19, 3.20, 3.21, 3.22, 3.23, 3.24 and 3.25.

Callus proliferation from young leaf, matured leaf and nodes were also noticed in all 2,4-D+BA formulation in AOG. Among the different 2, 4-D+BA formulation the highest 90% from matured and young leaf explants (Table 3.19) was recorded in media having 4.0 mg/l 2, 4-D+1.0 mg/l BA. High callusing response was also recorded in media having 3.0 mg/l 2, 4-D and 1.0 mg/l BA. Moderate callusing was recorded in media having 3.0 mg/l 2, 4-D+1.5 mg/l BA, 4.0 mg/l NAA+1.5 mg/l BA and 5.0 mg/l NAA+1.0 mg/l BA formulation from different explants. The calli developed in 2, 4-D+BA media formulations were creamy and light creamy in colour and loosely compact in nature. The results indicate that 2, 4-D+BA combinations had

noticeable effect on callus proliferation from both sources of explants and a few instances better effect was obtained than 2, 4-D alone. Adventitious shoot formation from leaf segment derived callus was not observed in all the 2, 4-D+BA combination tested in primary culture. In JP-2 and JP-3 among the different 2, 4-D+BA formulations, the highest 90% from young leaf, matured leaf induced to develop callus in media having 3.0 mg/l 2, 4-D+1.0 mg/l BA. High callusing response was also recorded in media having 4.0 mg/l 2, 4-D+1.0 mg/l BA for different part of explants. Whereas, the lowest 40% explants from different parts of strawberry showed callus induction when the explants were cultured in media having 5.0 mg/l 2,4-D+ 1.0 mg/l BA in JP-2. Moderate callusing was recorded in media having 3.0 mg/l 2, 4-D+1.5 mg/l BA and 4.0 mg/l 2, 4-D and 1.5 mg/l BA formulations for different explants in JP-2 and JP-3 (Table 3.20 and 3.21). The calli developed in 2, 4-D and BA media formulations were creamy in colour and loosely compact in nature. Here adventitious shoot formation was observed in all the 2, 4-D+BA combination. Same experiment was tested for Camarosa, Sweet Charly, Giant Mountain and Festival. Among the different 2, 4-D+BA formulations the highest 90% from matured leaf and node induced to develop callus in media having 3.0 mg/l 2, 4-D+ 1.0 mg/l BA. The highest value also found in 4.0 mg/l+1.0 mg/l BA. Moderate callusing was recorded in media having 3.0 mg/l 2, 4-D+ 1.5 mg/l BA and 4.0 mg/l 2, 4-D+1.5 mg/l BA (Table 3.22, 3.23, 3.24 and 3.25). Whereas, lowest 35% explants from node showed callus induction when the explants were cultured in media having 5.0 mg/l 2, 4-D + 1.0 mg/l BA. The calli developed in 2, 4-D+BA media formulations were light creamy in colour and loosely compact in nature. The results indicate that 2, 4-D+BA combinations had noticeable effect on callus proliferation from all sources of explants. Adventitious shoot formation from leaf segment and node derived callus was not observed in all 2, 4-D+BA combination tested in primary culture (Figure 5).

Table 3.19

Table 3.20

Table 3.21

Table 3.22

Table 3.23

Table 3.24

Table 3.25

Plate 5

3.4. PLANT REGENERATION FROM LEAF AND NODE DERIVED CALLI

The calli developed from young and mature leaves and node segments in different culture media formulations were subcultured for plant regeneration. Different PGRs formulations such as BA and BA + NAA were used for shoot regeneration from calli derived from leaf (both young and matured) and node of strawberry. The calli were rescued aseptically on a sterile petridish and cut into convenient sizes by sterile scalpel. The callus pieces were sub-cultured onto MS medium supplemented with different concentrations of BA either singly or in combination with NAA (considered as regeneration media) and the cultures were incubated in light for 8 weeks. The calli proliferated in different callus induction media were subcultured separately on to regeneration media. Data on morphogenic differentiation of calli in regeneration media were recorded eight weeks after sub-culture. The results on the effect of both callus induction and shoot regeneration media on % of calli induced shoot regeneration and no. of multiple shoots/callus are presented in following paragraphs.

3.4.1. Effect of BA on Shoot Regeneration

The calli derived from different explants in different PGRs supplemented callus induction media were separately sub cultured onto regeneration media having different concentrations of BA only. The results are shown in Tables 3.26, 3.27, 3.28, 3.29, 3.30, 3.31 and 3.32. Regeneration media supplemented with four different concentrations of BA (0.1, 0.5, 1.0 and 1.5 mg/l) were tested to observe the effect on regeneration potentiality. The calli induced in 2, 4–D, NAA and 2, 4-D + BA supplemented callus induction media all sources of explants failed to survive and did not show any morphogenic response. The calli in BA supplemented regeneration medium were gradually became brownish in colour and finally necrotic and died. Adventitious shoot formation was observed only in regeneration media having 0.1 and 0.5 mg/l BA. Higher concentrations of BA (1.5 mg/l) were found unsuitable for induction of adventitious shoots. In JP-2 and JP-3, calli induced in this callusing media formulation showed the highest 6% shoot regeneration and average shoot/callus was 1-2 (Tables 3.27, 3.28).

In case of Camarosa, Sweet Charly, Giant Mountain and Festival, the cultured calli survived and continued to proliferate in the shoot induction medium contained with

both BA–NAA (1.5+0.5 mg/l). Adventitious shoot formation was only observed in regeneration media having 0.1, 0.5 mg/l BA. The calli in this media formulation showed 5% shoot regeneration (Tables 3.29, 3.30, 3.31 and 3.32). The range of average shoots/callus was very low (0-1).

It was also observed that the calli of AOG cultured in BA–NAA (1.5+0.5 mg/l) medium showed proliferation of new tissue (Table 3.26). Adventitious shoot formation was only observed in regeneration media having 0.1 and 0.5 mg/l BA. The calli in this callusing media formulation showed the highest 9% shoot regeneration. The average shoots/callus was moderate (1-4). In this cv. higher concentrations of BA (1.5 mg/l) were also found unsuitable for induction of adventitious shoots. From this experiment it is observed that adventitious shoot formation and the rate of average shoots/callus was higher in AOG than other six strawberry varieties.

Table 3.26. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. AOG. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	6	2
		Mature leaf	8	4
		Node	-	-
	0.5	Young leaf	8	3
		Mature leaf	9	4
		Node	-	-
	1.0	Young leaf	3	1
		Mature leaf	5	1
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.27. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. JP-2. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	4	1
		Mature leaf	6	2
		Node	-	-
	0.5	Young leaf	3	1
		Mature leaf	4	1
		Node	-	-
	1.0	Young leaf	1	1
		Mature leaf	2	1
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.28. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. JP-3. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	4	1
		Mature leaf	6	2
		Node	-	-
	0.5	Young leaf	3	1
		Mature leaf	4	1
		Node	-	-
	1.0	Young leaf	1	1
		Mature leaf	2	1
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.29. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. Camarosa. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	3	1
		Mature leaf	5	1
		Node	-	-
	0.5	Young leaf	3	1
		Mature leaf	4	1
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.30. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. Sweet Charly. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	3	1
		Mature leaf	5	1
		Node	-	-
	0.5	Young leaf	2	1
		Mature leaf	3	1
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.31. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. Giant Mountain. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	3	1
		Mature leaf	4	1
		Node	-	-
	0.5	Young leaf	2	1
		Mature leaf	3	1
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Table 3.32. Effect of different concentrations of BA in MS medium on shoot regeneration from young leaf, mature leaf and node derived strawberry calli cv. Festival. At least 10 calli were rescued and sub cultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA (2.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

PGR supplements in callus induction medium (mg/l)	BA supplements in shoot regeneration medium (mg/l)	Source of calli	Morphogenic response after 5 weeks of subculture	
			Percentage of calli induced shoot regeneration	No. of multiple shoots/callus
2, 4-D+BA (4.0+1.0)	0.1	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	0.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
NAA+BA (0.5+1.0)	0.1	Young leaf	3	-
		Mature leaf	5	1
		Node	-	-
	0.5	Young leaf	2	-
		Mature leaf	3	1
		Node	-	-
	1.0	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-
	1.5	Young leaf	-	-
		Mature leaf	-	-
		Node	-	-

- = No response

Plate 6

3.4.2. Effect of BA + NAA on Shoot Regeneration

The calli proliferated from different sources of explants in callusing media having NAA, NAA+BA and 2, 4-D+BA formulations were also sub cultured to observe the effect of different concentrations and combinations of BA+NAA formulations on shoot regeneration. Data were recorded on morphogenic responses of the calli in regeneration media after 5 weeks of culture. The results of this experiment are shown in Table 3.3. Shoot regeneration from node derived calli was not noticed in any of the growth regulator formulations. Therefore, effect of growth regulator formulations on shoot formation from node derived calli are not presented here.

PGR formulations in both preculture and subculture media showed pronounced effect on shoot organogenesis from leaf derived callus. The calli developed from leaf tissues in 2, 4-D supplemented callus induction media showed very little organogenic response (Table 3.33.). It was observed that the cultured calli survived and continued to produce new tissue on regeneration medium which contained both BA–NAA in AOG. Adventitious shoot formation was only observed in regeneration media having 1.5 mg/l BA with 0.1 mg/l NAA. In regeneration media containing 1.5 mg/l BA with 0.5 mg/l NAA, 8% of the subcultured calli induced to develop adventitious shoots. In rest of the BA-NAA supplemented regeneration media, no shoot formation was observed other than new callus tissue development. This result is followed by JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival.

The effect of BA + NAA shoot regeneration varied due to the different formulations of callus induction media of each sources of explants of seven strawberry varieties. The calli proliferated in NAA supplemented media, when subculture on to BA + NAA supplemented regeneration media continued new tissue proliferation along with organogenic differentiation (Table 3.33). It was observed that the calli under went shoot organogenesis in the regeneration media containing higher amount of BA and NAA. Regeneration media which contained equal concentrations of BA and NAA or higher concentration NAA induced the calli to proliferate only new callus tissues without any organogenesis. Among the BA + NAA combinations, adventitious shoot proliferation was noticed in 0.5 + 0.5, 1.0 + 0.5, 1.0 + 1.0, 1.0 + 1.5, 2.0 + 1.5 and 2.0 + 2.0 mg/l BA + NAA formulations from calli derived from different sources of explants. The highest 8% from mature leaf derived sub cultured calli underwent shoot organogenesis in regeneration media having 1.5 mg/l BA + 0.5 mg/l NAA (Figure 3.17). Average number of shoots/callus ranged from 1-4. Same result was observed in

AOG and followed by other six strawberry varieties viz. JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival. The calli proliferated in 2, 4-D + BA supplemented callus induction medium were also sub cultured in BA + NAA containing regeneration media (Table 3.33). It was noticed that regeneration potential of calli proliferated in 2, 4-D + BA supplemented callusing media was less than NAA supplemented callusing media different explants. In regeneration media having either equal or higher concentration of NAA than BA, the cultured calli induced to proliferate only new callus tissues without showing any sign of organogenesis in AOG. The calli derived from in 2, 4-D + BA supplemented callusing media showed organogenic response and developed adventitious shoots when cultured on to regeneration media supplemented with 0.5+0.5, 1.0 + 0.5, 1.0 + 1.0, 1.0 + 1.5, 1.5 + 1.0, 1.5 + 1.5, 1.5 +2.0, 2.0 + 1.5 and 2.0 + 2.0 mg/l BA + NAA. Among the BA + NAA formulations the highest 8% shoot regeneration was observed from mature leaf derived calli in AOG. Similar results were observed for other six strawberry varieties viz. JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival.

The calli derived from NAA + BA supplemented callusing media were also transferred onto same regeneration media for plant regeneration (Table 3.33). The calli developed in NAA + BA supplemented callusing media showed higher degree of organogenic potential than those developed in other callusing media formulations. Adventitious shoot regeneration was noticed in regeneration media containing both BA and NAA but the ratio of BA-NAA was found to be critical for organogenic response. The regeneration media contained higher concentration of BA than NAA induced the calli to develop adventitious shoots, whereas, the cultured calli produced only new callus tissues without any shoot organogenesis when the regeneration media contained higher concentrations of NAA than BA. Among the different regeneration media formulation tested 1.5 mg/lBA with 1.5 mg/l NAA was observed to be the most effective formulation where the highest 8% adventitious shoot proliferation was achieved in AOG. The regeneration medium having 1.0 + 0.5, 1.0 + 1.0, 1.5 + 0.5, 1.5 + 1.0 mg/l BA + NAA combination was also observed effective in shoot regeneration from the calli developed in NAA-BA supplemented callusing media in AOG. Similar results were also observed in other six strawberry varieties viz. JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival.

Table 3.33. Effect of different concentrations of BA + NAA in MS medium on shoot regeneration from young and mature leaf derive calli. At list 10 calli were rescued and subcultured. Data were recorded after 5 weeks of subculture.

PGR supplements in callus induction medium (mg/l)	BA+ NAA supplements in shoot regeneration medium (mg/l)	Morphogenic response after 5 weeks of subculture			
		% of calli induced shoot regeneration		No. of multiple shoots/ callus	
		Young leaves	Mature leaves	Young leaves	Mature leaves
2, 4-D (2.0)	0.5+0.5	-	-	-	-
	0.5+1.0	-	-	-	-
	0.5+1.5	-	-	-	-
	0.5+2.0	-	-	-	-
	1.0+0.5	-	-	-	-
	1.0+1.0	-	-	-	-
	1.0+1.5	-	-	-	-
	1.0+2.0	-	-	-	-
	1.5+0.5	-	8	-	2
	1.5+1.0	-	-	-	-
	1.5+1.5	-	-	-	-
	1.5+2.0	-	-	-	-
	2.0+0.5	-	-	-	-
	2.0+1.0	-	-	-	-
	2.0+1.5	-	-	-	-
	2.0+2.0	-	-	-	-
NAA (2.0)	0.5+0.5	3	7	1	2
	0.5+1.0	-	-	-	-
	0.5+1.5	-	-	-	-
	0.5+2.0	-	-	-	-
	1.0+0.5	3	5	1	1
	1.0+1.0	5	6	1	2
	1.0+1.5	4	5	1	1
	1.0+2.0	-	-	-	-
	1.5+0.5	7	8	3	4
	1.5+1.0	6	6	2	2
	1.5+1.5	4	5	1	3
	1.5+2.0	-	-	-	-
	2.0+0.5	-	-	-	-
	2.0+1.0	-	-	-	-
	2.0+1.5	3	4	1	2
	2.0+2.0	-	-	-	-

PGR supplements in callus induction medium (mg/l)	BA+ NAA supplements in shoot regeneration medium (mg/l)	Morphogenic response after 5 weeks of subculture			
		% of calli induced shoot regeneration		No. of multiple shoots/ callus	
		Young leaves	Mature leaves	Young leaves	Mature leaves
2, 4-D+ BA (4.0+ 1.0)	0.5+0.5	2	3	1	1
	0.5+1.0	-	-	-	-
	0.5+1.5	-	-	-	-
	0.5+2.0	-	-	-	-
	1.0+0.5	2	5	1	3
	1.0+1.0	4	5	2	2
	1.0+1.5	3	4	1	2
	1.0+2.0	-	-	-	-
	1.5+0.5	-	-	-	-
	1.5+1.0	4	6	2	2
	1.5+1.5	6	8	1	3
	1.5+2.0	1	2	1	1
	2.0+0.5	-	-	-	-
	2.0+1.0	-	-	-	-
	2.0+1.5	3	4	1	2
	2.0+2.0	-	-	-	-
NAA+ BA (0.5+ 1.5)	0.5+0.5	5	7	1	2
	0.5+1.0	-	-	-	-
	0.5+1.5	-	-	-	-
	0.5+2.0	-	-	-	-
	1.0+0.5	7	8	4	6
	1.0+1.0	6	8	1	3
	1.0+1.5	4	6	1	1
	1.0+2.0	-	-	-	-
	1.5+0.5	7	9	3	4
	1.5+1.0	6	6	2	2
	1.5+1.5	4	5	1	2
	1.5+2.0	-	-	-	-
	2.0+0.5	2	2	1	1
	2.0+1.0	3	4	1	3
	2.0+1.5	-	-	-	-
	2.0+2.0	-	-	-	-

- = No response

Plate 7

3.5. ROOT INDUCTION

Regenerated shoots were needed root induction to grow into complete plantlets and to establish them into soil. To induce roots, individual shoots proliferated in regeneration media from the calli developed in different callusing media, were excised and cultured in full strength MS and half strength MS (½MS) media with or without different PGR formulation (IBA and NAA). Data were recorded on percentage (%) of shoots induced roots and callus development and number of roots/shoot after 4 weeks of culture, and the results are presented in Table 3.34. In all cases 3-4 cm long shoots obtained from various experiments were used for in vitro rooting experiments. In vitro regenerated micro-shoots of strawberry were also inoculated in MS and ½MS media without plant growth regulators (MS_0 and ½MS_0). Cent percent cultured shoots induced to develop roots when cultured in MS_0 rooting medium within 7-12 days of inoculation. Whereas, 96% shoots were induced to root development in ½MS_0 rooting medium. No callus initiation with root was observed in these rooting media formulation. Micro-shoots were also inoculated in full strength MS supplemented with NAA and IBA alone (0.1, 0.5, 1.0 and 2.0 mg/l). Among the different formulations of NAA and IBA tested, 95% cultured shoots induced to develop roots when inoculated in full strength MS containing 1.0 mg/l NAA in AOG.

Among the different NAA and IBA formulation the highest 85% of shoots induced to develop roots with 1.0 mg/l NAA in AOG, Camarosa, Sweet Charly and Giant Mountain. It is observed that NAA showed better performance on rooting of micro-shoots than IBA. From the rooting experiment, it was noted that in all the tested media rooting response was satisfactory, but highest result was observed in growth regulator free full strength MS medium and 100% micro-shoots of all varieties were found to produce roots in growth regulator free MS medium. Highest no. roots/shoot (28.5±0.58**)** was also obtained in this media in AOG variety.

Table 3.34

Plate 8

3.6. ACCLIMATIZATION AND TRANSPLANTATION

When the *in vitro* regenerated shoots formed well developed root system, they were gradually transplanted into *ex vitro* condition. The plantlets were initially taken out and transplanted on thumb pots containing three types of soil formulations such as 1) sand, 2) sand + cow dung (1:1), 3) sand + cow dung + ash (1:1:1), 4) coir dust. The pots were then placed under a polythene shade to protect over desiccation and the plantlets were sprayed with Hogland solution (composition of this solution is given in **Appendix 5**) with hand spray regularly. Data on % of survibility of the planlets were recorded as per their origin after 7 days, 15 days and 30 days from transplantation date and the results are shown in the Table 3.35 and Figure 3.16.

When the plantlets of seven varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival of strawberry were transplanted on to only sand containing thump pots, it was observe that 50-66% plantlets after 7 days, 46-56% plantlets after 15 days and 44-54% plantlets after 30 days survived. However in sand + cow dung + ash (l:l:l) soil formulation, 49-70% plantlets after 7 days, 47-65% plantlets after 15 days and 45-55% plantlets after 30 days survived. In coir dust, 80-90% plantlets after 7 days, 76-85% plantlets after 15 days and 65- 82% plantlets after 30 days survived. Therefore, among these three formulations, coir dust was found to be the best for transplantation. In this soil formulation AOG showed highest % of survibility (82% after 30 days of plantation) of plantlets.

Table 3.35. Percentage of survibility of *in vitro* regenerated plantlets of seven strawberry varieties under *ex vitro* condition. Data were recorded after 7 days, 15 days and 30 days interval from transplantation date.

Treatment	Varieties	No. of plantlets transplanted	No. of plantlets survived			% of plantlets survived		
			After 7 days	After 15 days	After 30 days	After 7 days	After 15 days	After 30 days
Sand	AOG	150	100	85	82	66.6	56.6	54.6
	JP-2	150	98	82	79	65.3	54.6	52.6
	JP-3	150	95	74	70	63.3	49.3	46.6
	Camarosa	150	99	83	80	66	55.3	53.3
	Sweet Charly	150	98	83	78	65.3	55.3	52
	Giant Mountain	150	79	75	69	52.6	50	46
	Festival	150	75	70	66	50	46.6	44
Sand + Cowdung + Ash	AOG	160	113	105	96	70.6	65.6	60
	JP-2	160	110	94	88	66.7	58.7	55
	JP-3	160	109	90	85	68.1	56.2	53.
	Camarosa	160	111	102	87	69.4	63.7	54.4
	Sweet Charly	160	100	93	85	62.5	58.1	53.1
	Giant Mountain	160	85	82	76	53.7	51.2	47.5
	Festival	160	79	76	73	49.4	47.5	45.6
Coir dust	AOG	200	180	171	165	90	85.5	82.5
	JP-2	200	175	161	160	87.5	80.5	80
	JP-3	200	163	160	140	81.5	80	70
	Camarosa	200	177	168	162	88.5	84	81
	Sweet Charly	200	163	159	156	81.5	79.5	78
	Giant Mountain	200	161	154	146	80.5	77	73
	Festival	200	160	152	131	80	76	65.5

Plate 9

3.7. FIELD EVALUATION OF STRAWBERRY AND THEIR SOMACLONES

After acclimatization of *in vitro* regenerated plantlets, the successfully survived plants were then transferred to natural environment to evaluate their field performance. Among the seven strawberry varieties, 30-50 somaclones from each of the varieties were transplanted to the field. These plants were maintained properly and considered as R_0 plants. From R_0 plants four somaclones from AOG, four from JP-2, three from JP-3, two from Camarosa, two from Sweet Charly, two from Giant Mountain and two from Festival were selected.

Data were collected from 10 randomly selected plants on different morphological and agronomical characters such as plant height, no. of leaves/plant, petiole length, no. of stolons/plant, no. of nodes/stolon, stolon length, no. of crowns/plant, canopy size (cm^2), days to flowering, no. of flowers/cluster, no. of flowers/plant no. of fruits/plant, no. of fruits/cluster, days to fruit harvest, average fruit weight (g) and fruit weight/plant (g). The Results of these data are summarized in Tables 3.36 and 3.37.

3.7.1. Morphological Characters of Selected Somaclones and Comparison with their Respective Seven Strawberry Parents

Data on eight morphological chacracters were recorded from different selected somaclones and their donor parents. Out of eight morphological characters three characters were recorded after 60 days of plantation in the field and other five characters were recorded after 70 days after plantation in the field. These data were analyzed to show variation between different somaclones and their donor parents.

3.7.1.1. *Plant height of seven strawberry varieties and their somaclones*

The mean performances of plant height (cm) of seven strawberry varieties and their somaclones are showh in Table 3.36.

The mean performance of plant height of seven strawberry genotypes and their somaclones showed significant differences among the somaclones and variety AOG as shown in Appendix 6. The highest plant height was recorded in somaclone AOG SC 3 (31.97±0.32) followed by AOG SC 2 (28.00±0.29), AOG SC 1 (21.47±0.58) and AOG SC 4 (17.40±0.21). The lowest plant height was recorded in donor plant AOG (16.40±0.21). LSD and CV% of plant height were analyzed and these were 2.2250 and 1.8763 respectively.

Table 3.36

Plant height of JP-2 and its somaclones showed significant variation as shown in Appendix 7. The highest plant height was recorded in somaclone JP-2 SC 1 (24.00±0.29) followed by JP-2 SC 2 (22.23±0.21) and lowest plant height was recorded in donor JP-2 (15.47±0.20). LSD and CV% of plant height were analyzed and these were 0.5941 and 0.7940 respectively.

Plant height of JP-3 and its somaclones showed significant variation as shown in Appendix 8. The highest plant height was recorded in somaclone JP-3 SC 1 (25.03±0.26) followed by JP-3 SC 2 (22.00±0.23) and lowest plant height was recorded in donor JP-3 (16.13±0.18). LSD and CV% of plant height were analyzed and these were 0.3536 and 0.4616 respectively.

Plant height of Camarosa and its somaclones showed significant variation as shown in Appendix 9. The highest plant height was recorded in somaclone Camarosa SC 2 (24.43 ±0.12) followed by Camarosa SC 1 (22.27 ±0.15) and lowest plant height was recorded in donor Camarosa (15.43±0.23). LSD and CV% of plant height were analyzed and these were 1.3174 and 1.7483 respectively.

Plant height of Sweet Charly and its somaclones showed significant variation as shown in Appendix 10. The highest plant height was recorded in somaclone Sweet Charly SC 2 (24.03 ±0.29) followed by Sweet Charly SC 1 (22.23 ±0.15) and lowest plant height was recorded in donor Sweet Charly (15.33±0.17). LSD and CV% of plant height were analyzed and these were 1.4779 and 1.9783 respectively.

Plant height of Giant Mountain and its somaclones showed significant variation as shown in Appendix 11. The highest plant height was recorded in somaclone Giant Mountain SC 1 (22.30 ±0.15) followed by Giant Mountain SC 2 (22.13 ±0.32) and lowest plant height was recorded in donor Giant Mountain (16.47±0.15). LSD and CV% of plant height were analyzed and these were 1.4247 and 1.9294 respectively.

Plant height of Festival and its somaclones showed significant variation as shown in Appendix 12. The highest plant height was recorded in somaclone Festival SC 1 (25.30 ±0.41) followed by Festival SC 2 (22.53 ±0.14) and lowest plant height was

recorded in donor Festival (15.57±0.23). LSD and CV% of plant height were analyzed and these were 1.4169 and 1.8428 respectively.

3.7.1.2. *No. of leaves/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of leaves/plant showed significant differences among the somaclones and the variety AOG as shown in Appendix 13. The highest number of leaves/plant was recorded in AOG SC 3 (30.33±0.08) followed by AOG SC 1 (26.40±0.21), AOG SC 2 (25.27±0.15) and AOG SC 4 (20.47±0.14). The lowest number of leaves/plant was recorded in donor parent AOG (20.23±0.14). LSD and CV% of no. of leaves/plant were 1.3252 and 1.0495 respectively.

The mean performance of no. of leaves/plant of other six strawberry varieties and their somaclones showed significant differences as shown in Appendix 14- 19. The highest number of leaves/plant was recorded in JP-2 SC 2 (24.50 ±0.17) and lowest number of leaves/plant was recorded in donor parent JP-2 (20.27±0.39). LSD and CV% of no. of leaves/plant were 1.3090 and 1.6006 respectively.

In JP-3 and its somaclones the highest number of leaves/ plant was recorded in JP-3 SC 1 (19.60±0.20) and lowest number of leaves/plant was recorded in donor parent JP-3 (15.47±0.20). LSD and CV% of no. of leaves/plant were 1.3090 and 2.0112 respectively.

In Camarosa and its somaclones the highest number of leaves/ plant was recorded in Camarosa SC 2 (22.50±0.17) and lowest number of leaves/plant was recorded in donor parent Camarosa (18.47±0.26). LSD and CV% of no. of leaves/plant were 1.3748 and 1.8482 respectively.

In Sweet Charly and its somaclones the highest number of leaves/ plant was recorded in Sweet Charly SC 1 (17.47±0.26) and lowest number of leaves/plant was recorded in donor parent Sweet Charly (12.30±0.15). LSD and CV% of no. of leaves/plant were 0.6124 and 1.1399 respectively.

In Giant Mountain and its somaclones the highest number of leaves/ plant was recorded in Giant Mountain SC 1 (16.63±0.13) and lowest number of leaves/plant was

recorded in donor parent Giant Mountain (12.27±0.15). LSD and CV% of no. of leaves/plant were 1.0362 and 2.0278 respectively.

In Festival and its somaclones the highest number of leaves/ plant was recorded in Festival SC 2 (20.33±0.09) and lowest number of leaves/plant was recorded in donor parent Festival (13.10±0.21). LSD and CV% of no. of leaves/plant were 1.0813 and 1.7246 respectively.

3.7.1.3. *Petiole length of seven strawberry varieties and their somaclones*

The mean performances of petiole length showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 20- 26. Somaclones of seven strawberry varieties showed better performances than their respective parents.

The highest petiole length was recorded in AOG SC 3 (17.23±0.14) and the lowest petiole length was recorded in donor parent AOG (14.23±0.14). LSD and CV% of petiole length were 1.8361 and 2.2758 respectively.

The highest petiole length was recorded in JP-2 SC 2 (15.33 ±0.17) and lowest petiole length was recorded in donor parent JP-2 (13.57±0.23). LSD and CV% of petiole length were 1.3006 and 2.4299 respectively.

In JP-3 and its somaclones the highest petiole length was recorded in JP-3 SC 1 (14.23±0.15) and lowest petiole length was recorded in donor parent JP-3 (13.80±0.15). LSD and CV% of petiole length were 0.9394 and 1.8755 respectively.

In Camarosa and its somaclones the highest petiole length was recorded in Camarosa SC 2 and Camarosa SC 1 (15.27±0.15) and lowest petiole length was recorded in donor parent Camarosa (14.30±0.15). LSD and CV% of petiole length were 1.0573 and 1.9445 respectively.

In Sweet Charly and its somaclones the highest petiole length was recorded in Sweet Charly SC 2 (15.30±0.12) and lowest petiole length was recorded in donor parent Sweet Charly (14.27±0.15). LSD and CV% of petiole length were 1.0362 and 1.9360 respectively.

In Giant Mountain and its somaclones the highest petiole length was recorded in Giant Mountain SC 1 (16.63±0.13) and lowest petiole length was recorded in donor parent Giant Mountain (15.20±0.15). LSD and CV% of petiole length were 1.1279 and 1.9649 respectively.

In Festival and its somaclones the highest petiole length was recorded in Festival SC 1 and Festival SC 2 (15.20±0.15) and lowest petiole length was recorded in donor parent Festival (13.23±0.15). LSD and CV% of petiole length were 1.0573 and 1.9980 respectively.

3.7.1.4. *No. of stolons/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of stolons/plant showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 27- 33. Somaclones of seven strawberry varieties showed better performances than their respective parents.

The highest no. of stolons/plant was recorded in AOG SC 3 (16.37±0.18) and the lowest no. stolons/plant was recorded in donor parent AOG (10.07±0.43). LSD and CV% of no. stolons/plant were 2.3382 and 3.4236 respectively.

The highest no. of stolons/plant was recorded in JP-2 SC 2 (5.83 ±0.07) and lowest no. of stolons/ plant was recorded in donor parent JP-2 (4.27±0.12). LSD and CV% of no. of stolons/ plant were 0.5286 and 2.8243 respectively.

In JP-3 and its somaclones the highest no. of stolons/plant was recorded in JP-3 SC 1 (6.27±0.15) and lowest no. of stolons/plant was recorded in donor parent JP-3 (4.40±0.05). LSD and CV% of no. of stolons/plant were 0.7377 and 3.6279 respectively.

In Camarosa and its somaclones the highest no. of stolons/plant was recorded in Camarosa SC 2 (4.77±0.09) and lowest no. of stolons/plant was recorded in donor parent Camarosa (4.33±0.09). LSD and CV% of no. of stolons/plant were 0.6417 and 3.8437 respectively.

In Sweet Charly and its somaclones the highest no. of stolons/plant was recorded in Sweet Charly SC 1 (6.33±0.12) and lowest number of stolons/plant was recorded in donor parent Sweet Charly (4.60±0.06). LSD and CV% of no. of stolons/plant were 0.5558 and 2.7441 respectively.

In Giant Mountain and its somaclones the highest number of stolons/ plant was recorded in Giant Mountain SC 2 (5.67±0.09) and lowest number of stolons/plant was recorded in donor parent Giant Mountain (3.40±0.06). LSD and CV% of no. of stolons/plant were 0.4851 and 2.7460 respectively.

In Festival and its somaclones the highest number of stolons/ plant was recorded in Festival SC 1 (3.57±0.12) and lowest number of stolons/plant was recorded in donor parent Festival (2.63±0.09). LSD and CV% of no. of stolons/plant were 0.6474 and 5.0843 respectively.

3.7.1.5. *No. of nodes/stolon of seven strawberry varieties and their somaclones*

The mean performances of no. of nodes/stolon showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 34- 40. Somaclones of seven strawberry varieties also showed better performances than their respective parents.

The highest no. of nodes/stolon was recorded in AOG SC 3 (6.27±0.14) and the lowest no. of nodes/stolon was recorded in donor parent AOG (4.27±0.14). LSD and CV% of no. of nodes/stolon were 1.3902 and 5.3822 respectively.

The highest no. of nodes/stolon was recorded in JP-2 SC 2 (3.30 ±0.11) and lowest no. of nodes/stolon was recorded in donor parent JP-2 (2.43±0.09). LSD and CV% of no. of nodes/stolon were 0.2426 and 2.2388 respectively.

In JP-3 and its somaclones the highest no. of nodes/stolon was recorded in JP-3 SC 2 (4.23±0.12) and lowest no. of nodes/stolon was recorded in donor parent JP-3 (2.33±0.09). LSD and CV% of no. of nodes/stolon were 0.2101 and 1.7856 respectively.

In Camarosa and its somaclones the highest no. of nodes/stolon was recorded in Camarosa SC 2 (3.27±0.09) and lowest no. of nodes/stolon was recorded in donor parent Camarosa (2.47±0.09). LSD and CV% of no. of nodes/stolon were 0.6643 and 6.2241 respectively.

In Sweet Charly and its somaclones the highest no. of nodes/stolon was recorded in Sweet Charly SC 1 (4.13±0.09) and lowest no. of nodes/stolon was recorded in donor parent Sweet Charly (2.47±0.09). LSD and CV% of no. of nodes/stolon were 0.6967 and 5.8026 respectively.

In Giant Mountain and its somaclones the highest no. of nodes/stolon was recorded in Giant Mountain SC 2 (3.03±0.09) and lowest no. of nodes/stolon was recorded in donor parent Giant Mountain (2.33±0.07). LSD and CV% of no. of nodes/stolon were 0.1918 and 1.8898 respectively.

In Festival and its somaclones the highest no. of nodes/stolon was recorded in Festival SC 2 (3.20±0.06) and lowest no. of nodes/stolon was recorded in donor parent Festival (2.30±0.06). LSD and CV% of no. of nodes/stolon were 0.5073 and 5.0604 respectively.

3.7.1.6. *Stolon length of seven strawberry varieties and their somaclones*

The mean performances of stolon length showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 41- 47. Somaclones of seven strawberry varieties also showed better performances than their respective parents.

The highest stolon length was recorded in AOG SC 3 (163.1±0.20) and the lowest stolon length was recorded in donor parent AOG (120.4±0.35). LSD and CV% of stolon length were 55.2871 and 7.2115 respectively.

The highest stolon length was recorded in JP-2 SC 2 (155.33±0.24) and lowest stolon length was recorded in donor parent JP-2 (114.23±3.03). LSD and CV% of stolon length were 11.6129 and 2.3083 respectively.

In JP-3 and its somaclones the highest stolon length was recorded in JP-3 SC 2 (150.13±0.23) and lowest stolon length was recorded in donor parent JP-3 (100.70±0.44). LSD and CV% of stolon length were 1.8710 and 0.3851 respectively.

In Camarosa and its somaclones the highest stolon length was recorded in Camarosa SC 2 (148.20±0.12) and lowest stolon length was recorded in donor parent Camarosa (113.73±3.38). LSD and CV% of stolon length were 12.3271 and 2.5021 respectively.

In Sweet Charly and its somaclones the highest stolon length was recorded in Sweet Charly SC 1 (155.23±0.15) and lowest stolon length was recorded in donor parent Sweet Charly (109.37±5.33). LSD and CV% of stolon length were 18.8898 and 3.7726 respectively.

In Giant Mountain and its somaclones the highest stolon length was recorded in Giant Mountain SC 1 (155.67±0.12) and lowest stolon length was recorded in donor parent Giant Mountain (115.93±5.53). LSD and CV% of stolon length were 12.8639 and 2.5145 respectively.

In Festival and its somaclones the highest stolon length was recorded in Festival SC 1 (151.73±3.37) and lowest stolon length was recorded in donor parent Festival (113.03±0.32). LSD and CV% of stolon length were 12.4074 and 2.4650 respectively.

3.7.1.7. *No. of crowns/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of crowns/plant showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 48-54. Somaclones of seven strawberry varieties also showed better performances than their respective parents.

The highest no. of crowns/plant was recorded in AOG SC 3 (7.50±0.57) and the lowest no. of crowns/plant was recorded in donor parent AOG (3.20±0.58). LSD and CV% of no. of crowns/plant were 0.5272 and 1.8233 respectively.

The highest no. of crowns/plant was recorded in JP-2 SC 2 (6.47 ±0.09) and lowest no. of crowns/plant was recorded in donor parent JP-2 (3.27±0.12). LSD and CV% of no. of crowns/plant were 0.7123 and 4.1657 respectively.

In JP-3 and its somaclones the highest no. of crowns/plant was recorded in JP-3 SC 2 (5.40±0.06) and lowest no. of crowns/plant was recorded in donor parent JP-3 (3.03±0.26). LSD and CV% of no. of crowns/plant were 1.0147 and 5.5531 respectively.

In Camarosa and its somaclones the highest no. of crowns/plant was recorded in Camarosa SC 2 (6.30±0.06) and lowest no. of crowns/plant was recorded in donor parent Camarosa (2.80±0.06). LSD and CV% of no. of crowns/plant were 0.4113 and 2.2213 respectively.

In Sweet Charly and its somaclones the highest no. of crowns/plant was recorded in Sweet Charly SC 1 (6.17±0.12) and lowest no. of crowns/plant was recorded in donor parent Sweet Charly (2.50±0.06). LSD and CV% of no. of crowns/plant were 0.6752 and 3.9865 respectively.

In Giant Mountain and its somaclones the highest no. of crowns/plant was recorded in Giant Mountain SC 1 (6.20±0.06) and lowest no. of crowns/plant was recorded in donor parent Giant Mountain (3.30±0.12). LSD and CV% of no. of crowns/plant were 0.5753 and 3.1207 respectively.

In Festival and its somaclones the highest no. of crowns/plant was recorded in Festival SC 1 (6.23±0.19) and lowest no. of crowns/plant was recorded in donor parent Festival (2.80±0.06). LSD and CV% of no. of crowns/plant were 0.8489 and 4.7191 respectively.

3.7.1.8. *Canopy size (cm^2) of seven strawberry varieties and their somaclones*

The mean performances of canopy size showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 55- 61. Somaclones of seven strawberry varieties also showed better performances than their respective parents.

The highest canopy size was recorded in AOG SC 3 (594.03±0.52) and the lowest canopy size was recorded in donor parent AOG (364.23±8.14). LSD and CV% of canopy size were 31.5607 and 1.3446 respectively.

The highest canopy size was recorded in JP-2 SC 2 (474.40±0.14) and lowest canopy size was recorded in donor parent JP-2 (382.77±1.22). LSD and CV% of canopy size were 4.4029 and 0.2777 respectively.

In JP-3 and its somaclones the highest canopy size was recorded in JP-3 SC 2 (462.37±0.30) and lowest canopy size was recorded in donor parent JP-3 (383.40±3.31). LSD and CV% of canopy size were 11.6663 and 0.7417 respectively.

In Camarosa and its somaclones the highest canopy size was recorded in Camarosa SC 1 (474.37±0.30) and lowest canopy size was recorded in donor parent Camarosa (361.60±2.27). LSD and CV% of canopy size were 8.8358 and 0.6020 respectively.

In Sweet Charly and its somaclones the highest canopy size was recorded in Sweet Charly SC 1 (474.43±0.23) and lowest canopy size was recorded in donor parent Sweet Charly (337.40±3.05). LSD and CV% of canopy size were 11.6672 and 0.8109 respectively.

In Giant Mountain and its somaclones the highest canopy size was recorded in Giant Mountain SC 2 (474.53±0.15) and lowest canopy size was recorded in donor parent Giant Mountain (357.50±1.31). LSD and CV% of canopy size were 4.5604 and 0.2932 respectively.

In Festival and its somaclones the highest canopy size was recorded in Festival SC 2 (333.20±0.17) and lowest canopy size was recorded in donor parent Festival (218.83±4.41). LSD and CV% of canopy size were 16.0482 and 1.5537 respectively.

3.7.2. Fruit Yield and Yield Contributing Characters of Selected Somaclones and Comparison with their Respective Seven Strawberry Parents

Data on eight fruit yield and yield contributing characters were recorded from different selected somaclones and their donor parents. Out of eight characters three characters were recorded after 70 days, three characters were recorded after 80 days and other two characters (average fruit weight and fruit weight/plant) were recorded at harvesting. The collected data were analyzed to study variation between somaclones and their donor parents.

Table 3.37

3.7.2.1. *Days to flowering of seven strawberry varieties and their somaclones*

The mean performances of days to flowering showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 62-68. In this case somaclones of seven strawberry varieties also showed better performances than their respective parents.

In AOG somaclones showed more or less same performances. Days to flowering of somaclones were AOG SC 1 (61.67±0.33), AOG SC 2 (62.67±0.33), AOG SC 3 (60.00±0.33) and AOG SC 4 (61.67±0.33). Donor parent AOG had taken longer time to flowering (67.67±0.33) than its somaclones. LSD and CV% of days to flowering were 2.3011 and 0.7144 respectively.

In JP-2 somaclones showed more or less same performances. Days to flowering of somaclones were JP-2 SC 1 (61.33±0.33) and JP-2 SC 2 (61.67±0.33). Donor parent JP-2 had taken longer time to flowering (69.67±0.33) than its somaclones. LSD and CV% of days to flowering were 2.4255 and 1.0381 respectively.

In JP-3 somaclones showed same performances. Days to flowering of somaclones were JP-3 SC 1 and JP-3 SC 2 (61.67±0.33). Donor parent JP-3 had taken longer time to flowering (70.67±0.33) than its somaclones. LSD and CV% of days to flowering were 1.9176 and 0.8178 respectively.

In Camarosa somaclones showed more or less same performances. Days to flowering of somaclones were Camarosa SC 1 (61.67±0.33) and Camarosa SC 2 (61.00±0.58). Donor parent Camarosa had taken longer time to flowering (70.00±0.33) than its somaclones. LSD and CV% of days to flowering were 1.9176 and 0.8207 respectively.

In Sweet Charly somaclones showed same performances. Days to flowering of somaclones were Sweet Charly SC 1 and Sweet Charly SC 2 (61.67±0.33). Donor parent Sweet Charly had taken longer time to flowering (71.67±1.66) than its somaclones. LSD and CV% of days to flowering were 7.2766 and 3.0769 respectively.

In Giant Mountain somaclones showed more or less same performances. Days to flowering of somaclones were Giant Mountain SC 1 (62.00±0.33) and Giant Mountain SC 2 (61.67±0.33). Donor parent Giant Mountain had taken longer time to

flowering (70.33±0.33) than its somaclones. LSD and CV% of days to flowering were 1.4853 and 0.6313 respectively.

In Festival somaclones showed same performances. Days to flowering of somaclones were Festival SC 1 (62.00±0.58) and Festival SC 2 (61.67±0.33). Donor parent Festival had taken longer time to flowering (75.00±0.58) than its somaclones. LSD and CV% of days to flowering were 3.8351 and 1.5918 respectively.

3.7.2.2. *No. of flower clusters/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of flower clusters/plant showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 69-75.

The highest no. of flower clusters/plant was recorded in AOG SC 3 (8.67±0.33) and the lowest no. of flower clusters/plant was recorded in AOG SC 2 and donor parent AOG (6.33±0.33). LSD and CV% of no. of flower clusters/plant were 3.0440 and 8.1414 respectively.

The highest no. of flower clusters/plant was recorded in JP-2 SC 1 (7.67±0.33) and lowest no. of flower clusters/plant was recorded in donor parent JP-2 (5.67±0.33). LSD and CV% of no. of flower clusters/plant were 2.1006 and 8.6603 respectively.

In JP-3 and its somaclones the highest no. of flower clusters/plant was recorded in JP-3 SC 1 (7.67±0.30) and lowest no. of flower clusters/plant was recorded in donor parent JP-3 (6.00±0.33). LSD and CV% of no. of flower clusters/plant were 1.2128 and 4.9180 respectively.

In Camarosa and its somaclones the highest no. of flower clusters/plant was recorded in Camarosa SC 2 (6.67±0.33) and lowest no. of flower clusters/plant was recorded in donor parent Camarosa (5.33±0.33). LSD and CV% of no. of flower clusters/plant were 1.2128 and 5.6604 respectively.

In Sweet Charly and its somaclones the highest no. of flower clusters/plant was recorded in Sweet Charly SC 2 (7.00±0.58) and lowest no. of flower clusters/plant was recorded in donor parent Sweet Charly (5.33±0.33). LSD and CV% of no. of flower clusters/plant were 1.2128 and 5.3571 respectively.

In Giant Mountain and its somaclones the highest no. of flower clusters/plant was recorded in Giant Mountain SC 1 (6.67±0.33) and lowest no. of flower clusters/plant was recorded in donor parent Giant Mountain (5.67±0.33). LSD and CV% of no. of flower clusters/plant were 2.4255 and 10.7143 respectively.

In Festival and its somaclones the highest no. of flower clusters/plant was recorded in Festival SC 2 (7.00±0.58) and lowest no. of flower clusters/plant was recorded in donor parent Festival (5.33±0.33). LSD and CV% of no. of flower clusters/plant were 2.9707 and 12.8921 respectively.

3.7.2.3. *No. flowers/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of flowers/plant showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 76-82.

In AOG the highest no. of flowers/plant was recorded in AOG SC 3 (21.67±0.33) and the lowest no. of flowers/plant was recorded in donor parent AOG (15.67±0.33). LSD and CV% of no. of flowers/plant were 3.0440 and 3.2387 respectively.

In JP-2 the highest no. of flowers/plant was recorded in JP-2 SC 2 (18.67±0.33) and lowest no. of flowers/plant was recorded in donor parent JP-2 (14.33±0.33). LSD and CV% of no. of flowers/plant were 2.4255 and 3.9474 respectively.

In JP-3 and its somaclones the highest no. of flowers/plant was recorded in JP-3 SC 1 (18.67±0.33) and lowest no. of flowers/plant was recorded in donor parent JP-3 (14.33±0.33). LSD and CV% of no. of flowers/plant were 2.4255 and 3.7500 respectively.

In Camarosa and its somaclones the highest no. of flowers/plant was recorded in Camarosa SC 2 (18.67±0.33) and lowest no. of flowers/plant was recorded in donor parent Camarosa (16.33±0.33). LSD and CV% of no. of flowers/plant were 2.4255 and 3.7500 respectively.

In Sweet Charly and its somaclones the highest no. of flowers/plant was recorded in Sweet Charly SC 1 (18.33±0.33) and lowest no. of flowers/plant was recorded in

donor parent Sweet Charly (15.33±0.33). LSD and CV% of no. of flowers/plant were 2.1006 and 3.3962 respectively.

In Giant Mountain and its somaclones the highest no. of flowers/plant was recorded in Giant Mountain SC 1 (18.33±0.33) and lowest no. of flowers/plant was recorded in donor parent Giant Mountain (15.33±0.33). LSD and CV% of no. of flowers/plant were 3.2087 and 5.2219 respectively.

In Festival and its somaclones the highest no. of flowers/plant was recorded in Festival SC 1 (16.00±0.58) and lowest no. of flowers/plant was recorded in donor parent Festival (13.00±0.58). LSD and CV% of no. of flowers/plant were 3.2087 and 5.9233 respectively.

3.7.2.4. *No. of fruits/cluster of seven strawberry varieties and their somaclones*

The mean performances of no. of fruits/cluster showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 83-89.

In AOG the highest no. of fruits/cluster was recorded in AOG SC 3 (6.33±0.33) and the lowest no. of fruits/cluster was recorded in donor parent AOG (2.67±0.33). LSD and CV% of no. of fruits/cluster were 3.3871 and 15.6733 respectively.

In JP-2 the highest no. of fruits/cluster was recorded in JP-2 SC 1 (4.33±0.33) and lowest no. of fruits/cluster was recorded in donor parent JP-2 (2.33±0.33). LSD and CV% of no. of fruits/cluster were 2.4255 and 21.4286 respectively.

In JP-3 and its somaclones the highest no. of fruits/cluster was recorded in JP-3 SC 2 (6.00±0.57) and lowest no. of fruits/cluster was recorded in donor parent JP-3 (2.00±0.33). LSD and CV% of no. of fruits/cluster were 2.8442 and 21.9863 respectively.

In Camarosa and its somaclones the highest no. of fruits/cluster was recorded in Camarosa SC 2 (6.67±0.33) and lowest no. of fruits/cluster was recorded in donor parent Camarosa (2.00±0.33). LSD and CV% of no. of fruits/cluster were 1.2128 and 8.5714 respectively.

In Sweet Charly and its somaclones the highest no. of fruits/cluster was recorded in Sweet Charly SC 2 (3.00±0.33) and lowest no. of fruits/cluster was recorded in donor parent Sweet Charly (2.00±0.33). LSD and CV% of no. of fruits/cluster were 1.2128 and 13.0435 respectively. Giant Mountain showed same result.

In Festival and its somaclones the highest no. of fruits/cluster was recorded in Festival SC 2 (3.00±0.33) and lowest no. of fruits/cluster was recorded in donor parent Festival (2.00±0.33). LSD and CV% of no. of flowers/plant were 1.2128 and 13.6364 respectively.

3.7.2.5. *No. of fruits/plant of seven strawberry varieties and their somaclones*

The mean performances of no. of fruits/plant showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 90-96.

In AOG the highest no. of fruits/plant was recorded in AOG SC 3 (20.33±0.35) and the lowest no. of fruits/plant was recorded in donor parent AOG (9.67±0.33). LSD and CV% of no. of fruits/plant were 3.7576 and 4.6814 respectively.

In JP-2 the highest no. of fruits/plant was recorded in JP-2 SC 1 (14.33±0.33) and lowest no. of fruits/plant was recorded in donor parent JP-2 (6.67±0.33). LSD and CV% of no. of fruits/plant were 1.9176 and 4.5610 respectively.

In JP-3 and its somaclones the highest no. of fruits/plant was recorded in somaclones JP-3 SC 1and JP-3 SC 2 (14.33±0.33) and lowest no. of fruits/plant was recorded in donor parent JP-3 (6.67±0.33). LSD and CV% of no. of fruits/plant were 1.2128 and 2.8302 respectively.

In Camarosa and its somaclones the highest no. of fruits/plant was recorded in Camarosa SC 1 (13.67±0.33) and lowest no. of fruits/plant was recorded in donor parent Camarosa (5.33±0.33). LSD and CV% of no. of fruits/plant were 2.4255 and 6.1856 respectively.

In Sweet Charly and its somaclones the highest no. of fruits/plant was recorded in Sweet Charly SC 1 (14.33±0.33) and lowest no. of fruits/plant was recorded in donor

parent Sweet Charly (6.00±0.33). LSD and CV% of no. of fruits/plant were 1.4853 and 3.6022 respectively.

In Giant Mountain and its somaclones the highest no. of fruits/plant was recorded in Giant Mountain SC 2 (13.67±0.33) and lowest no. of fruits/plant was recorded in donor parent Giant Mountain (4.67±0.33). LSD and CV% of no. of fruits/plant were 2.4255 and 6.3158 respectively.

In Festival and its somaclones the highest no. of fruits/plant was recorded in Festival SC 2 (12.67±0.33) and lowest no. of fruits/plant was recorded in Festival SC 1 and donor parent Festival (5.67±0.33). LSD and CV% of no. of fruits/plant were 2.1006 and 5.4127 respectively.

3.7.2.6. *Days to fruit harvest of seven strawberry varieties and their somaclones*

The mean performances of days to fruit harvest showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 97-103. Here Somaclones of seven strawberry varieties also showed better performances than their respective parents.

In AOG somaclones showed more or less same performances. Days to fruit harvest of somaclones were AOG SC 1 (84.67±0.33), AOG SC 2 (84.00±0.33), AOG SC 3 (81.67±0.33) and AOG SC 4 (82.67±0.33). Donor parent AOG had taken longer time to fruit harvest (90.33±0.33) than its somaclones. LSD and CV% of days to fruit harvest were 2.8954 and 0.6646 respectively.

In JP-2 somaclones showed same performances. Days to fruit harvest of somaclones were JP-2 SC 1 and JP-2 SC 2 (84.67±0.33). Donor parent JP-2 had taken longer time to fruit harvest (90.33±0.33) than its somaclones. LSD and CV% of days to fruit harvest were 2.4255 and 0.7702 respectively.

In JP-3 somaclones showed more or less same performances. Days to fruit harvest of somaclones were JP-3 SC 1 (83.67±1.20) and JP-3 SC 2 (84.67±0.33). Donor parent JP-3 had taken longer time to fruit harvest (92.67±0.33) than its somaclones. LSD and CV% of days to fruit harvest were 3.6383 and 1.1494 respectively.

In Camarosa somaclones showed more or less same performances. Days to fruit harvest of somaclones were Camarosa SC 1 (84.33±0.33) and Camarosa SC 2 (85.00±0.33). Donor parent Camarosa had taken longer time to fruit harvest (90.33±0.33) than its somaclones. LSD and CV% of days to fruit harvest were 3.2087 and 1.0189 respectively.

In Sweet Charly somaclones showed same performances. Days to fruit harvest of somaclones were Sweet Charly SC 1 (84.67±0.33) and Sweet Charly SC 2 (84.33±0.33). Donor parent Sweet Charly had taken longer time to fruit harvest (71.67±1.66) than its somaclones. LSD and CV% of days to fruit harvest were 2.4255 and 0.7712 respectively. Giant Mountain, Festival and somaclones showed same result.

3.7.2.7. *Average fruit weight of seven strawberry varieties and their somaclones*

The mean performances of average fruit weight showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 104-110.

In AOG the highest average fruit weight was recorded in AOG SC 3 (38.00±0.11) and the lowest average fruit weight was recorded in donor parent AOG (25.30±0.33). LSD and CV% of average fruit weight were 1.0975 and 0.7031 respectively.

In JP-2 the highest average fruit weight was recorded in JP-2 SC 1 (32.17±0.73) and lowest average fruit weight was recorded in donor parent JP-2 (20.37±0.33). LSD and CV% of average fruit weight were 5.2738 and 5.8266 respectively.

In JP-3 and its somaclones the highest average fruit weight was recorded in somaclones JP-3 SC 1and JP-3 SC 2 (32.43±0.23) and lowest average fruit weight was recorded in donor parent JP-3 (22.27±0.15). LSD and CV% of average fruit weight were 1.7491 and 1.6796 respectively.

Plate 10

Plate 11

Plate 12

In Camarosa and its somaclones the highest average fruit weight was recorded in Camarosa SC 2 (32.43±0.09) and lowest average fruit weight was recorded in donor parent Camarosa (21.90±1.55). LSD and CV% of average fruit weight were 5.7960 and 5.5983 respectively.

In Sweet Charly and its somaclones the highest average fruit weight was recorded in Sweet Charly SC 1 (31.93±0.87) and lowest average fruit weight was recorded in donor parent Sweet Charly (23.10±0.23). LSD and CV% of average fruit weight were 4.0296 and 4.2381 respectively.

In Giant Mountain and its somaclones the highest average fruit weight was recorded in Giant Mountain SC 2 (32.60±0.15) and lowest average fruit weight was recorded in donor parent Giant Mountain (23.37±0.19). LSD and CV% of average fruit weight were 0.5355 and 0.5007 respectively.

In Festival and its somaclones the highest average fruit weight was recorded in Festival SC 2 (30.93±0.59) and lowest average fruit weight was recorded in donor parent Festival (21.67±0.07). LSD and CV% of average fruit weight were 4.6751 and 4.5656 respectively.

3.7.2.8. *Fruit weight/plant of seven strawberry varieties and their somaclones*

The mean performances of fruit weight/plant weight showed significant differences among the somaclones and the seven strawberry varieties as shown in Appendix 111-117.

In AOG the highest fruit weight/plant was recorded in AOG SC 3 (772.60±10.67) and the lowest fruit weight/plant was recorded in donor parent AOG (244.33±9.67). LSD and CV% of fruit weight/plant were 91.2854 and 3.6672 respectively.

In JP-2 the highest fruit weight/plant was recorded in JP-2 SC 1 (504.33±2.89) and lowest fruit weight/plant was recorded in donor parent JP-2 (144.93±0.74). LSD and CV% of fruit weight/plant were 31.1646 and 2.6024 respectively.

In JP-3 and its somaclones the highest fruit weight/plant was recorded in somaclones JP-3 SC 2 (497.03±2.57) and lowest fruit weight/plant was recorded in donor parent

JP-3 (164.57±0.43). LSD and CV% of fruit weight/plant were 14.2024 and 1.0328 respectively.

In Camarosa and its somaclones the highest fruit weight/plant was recorded in Camarosa SC 1 (438.37±1.44) and lowest fruit weight/plant was recorded in donor parent Camarosa (132.67±5.36). LSD and CV% of fruit weight/plant were 21.7791 and 1.8001 respectively.

In Sweet Charly and its somaclones the highest fruit weight/plant was recorded in Sweet Charly SC 1 (461.33±2.62) and lowest fruit weight/plant was recorded in donor parent Sweet Charly (144.00±1.53). LSD and CV% of fruit weight/plant were 10.0375 and 0.8806 respectively.

In Giant Mountain and its somaclones the highest fruit weight/plant was recorded in Giant Mountain SC 2 (463.43±1.62) and lowest fruit weight/plant was recorded in donor parent Giant Mountain (122.67±0.19). LSD and CV% of fruit weight/plant were 0.5355 and 0.5007 respectively.

In Festival and its somaclones the highest fruit weight/plant was recorded in Festival SC 2 (445.83±1.09) and lowest fruit weight/plant was recorded in donor parent Festival (138.87±5.88). LSD and CV% of fruit weight/plant were 22.4657 and 1.8270 respectively.

3.8. GENETIC PARAMETERS

The estimates of phenotypic (δ^2g), genotypic (δ^2p), error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability were calculated separately for eight morphological and eight yield contributing characters of seven strawberry varieties and their somaclones and the results were presented in Tables 3.38, 3.39, 3.40, 3.41,3.41, 3.42, 3.43 and 3.44.

3.8.1. Genotypic Variation (δ^2g)

In AOG and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 35696.880, while the lowest genotypic variatiation was recorded for no. of nodes/stolon with a value of 0.621 (Table 3.38).

In JP-2 and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 32328.683, while the lowest genotypic variatiation was recorded for no. of nodes/stolon with a value of 0.223 (Table 3.39).

In JP-3 and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 37476.381, while the lowest genotypic variatiation was recorded for no. of nodes/stolon with a value of 0.212 (Table 3.40).

In Camarosa and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 29986.014, while the lowest genotypic variatiation was recorded for no. of stolons/plant with a value of 0.041 (Table 3.41).

In Sweet Charly and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 25510.361, while the lowest genotypic variatiation was recorded for no. of fruits/cluster with a value of 0.222 (Table 3.42).

In Giant Mountain and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 35628.770, while the lowest genotypic variatiation was recorded for no. of nodes/stolon with a value of 0.155 (Table 3.43).

In Festival and its somaclones, the highest genotypic variation (δ^2g) wsa found for fruit weigth/plant with a value of 29790.107, while the lowest genotypic variatiation was recorded for no. of nodes/stolon with a value of 0.196 (Table 3.44).

3.8.2. Phenotypic Variation (δ^2p)

In AOG and its somaclones, the highest phnotypic variation (δ^2p) was found for fruit weigth/plant with a value of 314.756, while the lowest phenotypic variatiation was recorded for no. of crowns/plant with a value of 0.011 (Table 3.38).

In JP-2 and its somaclones, the highest phnotypic variation (δ^2p) was found for fruit weigth/plant with a value of 73.371, while the lowest phnotypic variation was recorded for no. of nodes/stolon with a value of 0.004 (Table 3.39).

In JP-3 and its somaclones, the highest phnotypic variation (δ^2p) was found for fruit weigth/plant with a value of 15.238, while the lowest phnotypic variation was recorded for no. of nodes/stolon with a value of 0.003 (Table 3.40).

In Camarosa and its somaclones, the highest phnotypic variation (δ^2p) was found for fruit weigth/plant with a value of 35.833, while the lowest phnotypic variation was recorded for no. of stolons/plant with a value of 0.031 (Table 3.41).

In Sweet Charly and its somaclones, the highest phnotypic variation (δ^2p) was found for stolon length with a value of 26.956, while the lowest phnotypic variation (δ^2p) was recorded for no. of stolons/plant with a value of 0.023 (Table 3.42).

In Giant Mountain and its somaclones, the highest phnotypic variation (δ^2p) was found for fruit weigth/plant with a value of 55.5219, while the lowest phnotypic variation was recorded for no. of nodes/stolon with a value of 0.003 (Table 3.43).

In Festival and its somaclones, the highest phnotypic variation (δ^2p) wsa found for fruit weigth/plant with a value of 38.128, while the lowest phnotypic variation was recorded for no. of nodes/stolon with a value of 0.019 (Table 3.44).

3.8.3. Error Variation (δ^2e)

In AOG and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 36011.636, while the lowest error variation was recorded for no. of nodes/stolon with a value of 0.694 (Table 3.38).

In JP-2 and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 32402.054, while the lowest error variation was recorded for no. of nodes/stolon with a value of 0.228 (Table 3.39).

In JP-3 and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 37491.619, while the lowest error variation was recorded for petiole length with a value of 0.279 (Table 3.40).

In Camarosa and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 30021.847, while the lowest error variation was recorded for no. of stolons/plant with a value of 0.072 (Table 3.41).

In Sweet Charly and its somaclones, the highest error variation (δ^2e) was found for stolon length with a value of 25517.972, while the lowest error variation was recorded for no. of fruits/cluster with a value of 0.333 (Table 3.42).

In Giant Mountain and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 35644.463, while the lowest error variation was recorded for no. of nodes/stolon with a value of 0.158 (Table 3.43).

In Festival and its somaclones, the highest error variation (δ^2e) was found for fruit weigth/plant with a value of 29828.234, while the lowest error variation was recorded for no. of nodes/stolon with a value of 0.216 (Table 3.44).

3.8.4. Coefficients of Variability

The estimates of genotypic (GCV), phenotypic (PCV) and error coefficient of variability of eight morphological and eight yield contributing characters of seven strawberry varieties and their somaclones were calculated and the results were presented in Tables 3.38, 3.39, 3.40, 3.41, 3.42, 3.43 and 3.44.

3.8.4.1. *Genotypic coefficient of variability (GCV)*

In AOG and its somaclones, the highest genotypic coefficient of variability was found for fruit weigth/plant with a value of 39.2260, while the lowest genotypic coefficient of variability was recorded for days to fruit harvest with a value of 4.0227 (Table 3.38).

In JP-2 and its somaclones, the highest genotypic coefficient of variability was found for fruit weigth/plant with a value of 54.6890, while the lowest genotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.8318 (Table 3.39).

In JP-3 and its somaclones, the highest genotypic coefficient of variability was found for no. of fruits/cluster with a value of 62.8894, while the lowest genotypic coefficient of variability was recorded for petiole length with a value of 3.8361 (Table 3.40).

In Camarosa and its somaclones, the highest genotypic coefficient of variability was found for no. of fruits/cluster with a value of 63.5674, while the lowest genotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.8894 (Table 3.41).

In Sweet Charly and its somaclones, the highest genotypic coefficient of variability was found for fruit weigth/plant with a value of 50.9910, while the lowest genotypic coefficient of variability was recorded for petiole length with a value of 3.9442 (Table 3.42).

In Giant Mountain and its somaclones, the highest genotypic coefficient of variability was found for fruit weigth/plant with a value of 55.5341, while the lowest genotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.9513 (Table 3.43).

In Festival and its somaclones, the highest genotypic coefficient of variability was found for fruit weigth/plant with a value of 51.0679, while the lowest genotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.8318 (Table 3.44).

3.8.4.2. *Phenotypic coefficient of variability (PCV)*

In AOG and its somaclones, the highest phenotypic coefficient of variability was found for fruit weigth/plant with a value of 39.0542, while the lowest phenotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.9674 (Table 3.38).

In JP-2 and its somaclones, the highest phenotypic coefficient of variability was found for fruit weigth/plant with a value of 54.6270, while the lowest phenotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.9674 (Table 3.39).

In JP-3 and its somaclones, the highest phenotypic coefficient of variability was found for no. of fruits/cluster with a value of 58.9210, while the lowest phenotypic coefficient of variability was recorded for petiole length with a value of 3.3463 (Table 3.40).

In Camarosa and its somaclones, the highest phenotypic coefficient of variability was found for no. of fruits/cluster with a value of 62.9869, while the lowest phenotypic coefficient of variability was recorded for petiole length with a value of 3.5618 (Table 3.41).

In Sweet Charly and its somaclones, the highest phenotypic coefficient of variability was found for fruit weigth/plant with a value of 50.9834, while the lowest phenotypic coefficient of variability was recorded for petiole length with a value of 3.4363 (Table 3.42).

In Giant Mountain and its somaclones, the highest phenotypic coefficient of variability was found for fruit weigth/plant with a value of 55.5219, while the lowest

phenotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.8753 (Table 3.43).

In Festival and its somaclones, the highest phenotypic coefficient of variability was found for no. of fruits/plant with a value of 40.7450, while the lowest phenotypic coefficient of variability was recorded for days to fruit harvest with a value of 3.7536 (Table 3.44).

3.8.4.3. *Error coefficient of variability (GCV)*

In AOG and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 34.5033, while the lowest error coefficient of variability was recorded for days to fruit harvest with a value of 0.6646 (Table 3.38).

In JP-2 and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 21.4286, while the lowest error coefficient of variability was recorded for canopy size with a value of 0.2777 (Table 3.39).

In JP-3 and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 21.9863, while the lowest error coefficient of variability was recorded for stolon length with a value of 0.3851 (Table 3.40).

In Camarosa and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 8.5714, while the lowest error coefficient of variability was recorded for canopy size with a value of 0.6020 (Table 3.41).

In Sweet Charly and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 13.0435, while the lowest error coefficient of variability was recorded for days to fruit harvest with a value of 0.7712 (Table 3.42).

In Giant Mountain and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 13.0435, while the lowest error coefficient of variability was recorded for canopy size with a value of 0.2932 (Table 3.43).

In Festival and its somaclones, the highest error coefficient of variability was found for no. of fruits/cluster with a value of 13.6364, while the lowest error coefficient of variability was recorded for days to fruit harvest with a value of 0.7702 (Table 3.44).

Table 3.38. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in AOG and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	45.603	0.187	45.790	29.3614	29.3614	29.3614
No. of leaves/plant	18.150	0.066	18.217	17.3924	17.3924	17.3924
Petiole length	1.152	0.127	1.280	7.2144	7.2144	7.2144
No. of stolons/plant	5.301	0.207	5.507	17.6803	17.6803	17.6803
No. of nodes/stolon	0.621	0.073	0.694	16.5950	16.5950	16.5950
Stolon length	267.770	115.456	383.226	13.1384	13.1384	13.1384
No. of crowns/plant	2.554	0.011	2.564	28.4920	28.4920	28.4920
Canopy size	8686.443	37.624	8724.067	20.4750	20.4750	20.4750
Days to flowering	8.567	0.200	8.767	4.7298	4.7298	4.7298
No. of flower clusters/plant	0.850	0.350	1.200	15.0749	15.0749	15.0749
No. of flowers/plant	4.683	0.350	5.033	12.2820	12.2820	12.2820
No. of fruits/cluster	1.667	0.433	2.100	34.5033	34.5033	34.5033
No. of fruits/plant	15.400	0.533	15.933	25.5876	25.5876	25.5876
Days to fruit harvest	11.283	0.317	11.600	4.0227	3.9674	0.6646
Average fruit weight	22.148	0.045	22.193	15.5273	15.5114	0.7031
Fruit weight/plant	35696.880	314.756	36011.636	39.2260	39.0542	3.6672

Table 3.39. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in JP-2 and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	20.279	0.027	20.306	21.9101	21.8957	0.7940
No. of leaves/plant	4.464	0.129	4.593	9.5348	9.3995	1.6006
petiole length	0.942	0.128	1.070	7.0315	6.5983	2.4299
No. of stolons/plant	0.633	0.021	0.654	15.7253	15.4696	2.8243
No. of nodes/stolon	0.223	0.004	0.228	16.0274	15.8703	2.2388
Stolon length	455.540	10.188	465.728	15.6068	15.4352	2.3083
No. of crowns/plant	2.631	0.038	2.669	34.7590	34.5085	4.1657
Canopy size	2255.401	1.464	2256.866	10.9007	10.8972	0.2777
Days to flowering	22.111	0.444	22.556	7.3951	7.3218	1.0381
No. of flower clusters/plant	0.889	0.333	1.222	16.5831	14.1421	8.6603
No. of flowers/plant	5.000	0.444	5.444	13.8158	13.2399	3.9474
No. of fruits/cluster	1.000	0.444	1.444	38.6309	32.1429	21.4286
No. of fruits/plant	17.944	0.278	18.222	36.9411	36.6585	4.5610
Days to fruit harvest	10.556	0.444	11.000	3.8318	3.7536	0.7702
Average fruit weight	39.897	2.101	41.998	26.0496	25.3897	5.8266
Fruit weight/plant	32328.683	73.371	32402.054	54.6890	54.6270	2.6024

Table 3.40. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in JP-3 and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	20.468	0.009	20.478	21.4919	21.4869	0.4616
No. of leaves/plant	4.607	0.129	4.737	12.1661	11.9988	2.0112
petiole length	0.212	0.067	0.279	3.8361	3.3463	1.8755
No. of stolons/plant	1.053	0.041	1.094	18.7185	18.3636	3.6279
No. of nodes/stolon	0.909	0.003	0.912	29.5393	29.4853	1.7856
Stolon length	807.926	0.264	808.190	21.2913	21.2879	0.3851
No. of crowns/plant	3.321	0.078	3.399	36.7090	36.2866	5.5531
Canopy size	1823.414	10.282	1833.696	9.9048	9.8770	0.7417
Days to flowering	23.056	0.278	23.333	7.4955	7.4508	0.8178
No. of flower clusters/plant	0.667	0.111	0.778	13.0119	12.0467	4.9180
No. of flowers/plant	5.000	0.444	5.444	13.8158	13.2399	3.9474
No. of fruits/cluster	4.389	0.611	5.000	62.8894	58.9210	21.9863
No. of fruits/plant	19.556	0.111	19.667	37.6532	37.5467	2.8302
Days to fruit harvest	24.000	1.000	25.000	5.7471	5.6310	1.1494
Average fruit weight	30.619	0.231	30.850	19.4055	19.3326	1.6796
Fruit weight/plant	37476.381	15.238	37491.619	51.2302	51.2198	1.0328

Table 3.41. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in Camarosa and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	22.021	0.131	22.152	22.7251	22.6577	1.7483
No. of leaves/plant	4.024	0.143	4.167	9.9843	9.8118	1.8482
Petiole length	0.283	0.084	0.368	4.0580	3.5618	1.9445
No. of stolons/plant	0.041	0.031	0.072	5.8564	4.4185	3.8437
No. of nodes/stolon	0.162	0.033	0.196	15.0756	13.7307	6.2241
Stolon length	352.421	11.479	363.900	14.0876	13.8636	2.5021
No. of crowns/plant	3.929	0.013	3.942	39.0164	38.9532	2.2213
Canopy size	3813.192	5.898	3819.090	15.3186	15.3068	0.6020
Days to flowering	25.056	0.278	25.333	7.8372	7.7941	0.8207
No. of flower clusters/plant	0.444	0.111	0.556	12.6570	11.3208	5.6604
No. of flowers/plant	1.444	0.444	1.889	7.7308	6.7604	3.7500
No. of fruits/cluster	6.000	0.111	6.111	63.5674	62.9869	8.5714
No. of fruits/plant	22.111	0.444	22.556	44.0654	43.6291	6.1856
Days to fruit harvest	10.556	0.778	11.333	3.8894	3.7536	1.0189
Average fruit weight	31.876	2.538	34.413	20.6156	19.8409	5.5983
Fruit weight/plant	29986.014	35.833	30021.847	52.1038	52.0726	1.8001

Table 3.42. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in Sweet Charly and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	21.035	0.165	21.200	22.4238	22.3363	1.9783
No. of leaves/plant	6.705	0.028	6.733	17.5725	17.5355	1.1399
Petiole length	0.256	0.081	0.337	3.9442	3.4363	1.9360
No. of stolons/plant	0.773	0.023	0.797	16.0340	15.7975	2.7441
No. of nodes/stolon	0.682	0.037	0.719	25.6931	25.0293	5.8026
Stolon length	601.931	26.956	628.887	18.2221	17.8273	3.7726
No. of crowns/plant	3.661	0.034	3.696	41.2922	41.0993	3.9865
Canopy size	5018.713	10.283	5028.997	17.9321	17.9137	0.8109
Days to flowering	32.000	4.000	36.000	9.2308	8.7029	3.0769
No. of flower clusters/plant	0.667	0.111	0.778	14.1737	13.1223	5.3571
No. of flowers/plant	2.222	0.333	2.556	9.4036	8.7689	3.3962
No. of fruits/cluster	0.222	0.111	0.333	22.5920	18.4463	13.0435
No. of fruits/plant	21.389	0.167	21.556	40.9658	40.8072	3.6022
Days to fruit harvest	11.222	0.444	11.667	3.9513	3.8753	0.7712
Average fruit weight	24.839	1.227	26.066	19.5361	19.0709	4.2381
Fruit weight/plant	25510.361	7.611	25517.972	50.9910	50.9834	0.8806

Table 3.43. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in Giant Mountain and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	10.977	0.153	11.130	16.4343	16.3207	1.9290
No. of leaves/plant	5.233	0.081	5.314	16.4144	16.2886	2.0278
petiole length	0.539	0.096	0.636	5.0528	4.6551	1.9649
No. of stolons/plant	1.590	0.018	1.608	26.1140	25.9693	2.7460
No. of nodes/stolon	0.155	0.003	0.158	14.2427	14.1167	1.8898
Stolon length	459.958	12.501	472.459	15.4583	15.2524	2.5145
No. of crowns/plant	2.395	0.025	2.420	30.7033	30.5443	3.1207
Canopy size	3820.810	1.571	3822.381	14.4613	14.4584	0.2932
Days to flowering	24.056	0.167	24.222	7.6107	7.5845	0.6313
No. of flower clusters/plant	0.889	0.444	1.333	17.9178	14.6298	10.3448
No. of flowers/plant	2.000	0.778	2.778	9.8684	8.3736	5.2219
No. of fruits/cluster	0.222	0.111	0.333	22.5920	18.4463	13.0435
No. of fruits/plant	25.889	0.444	26.333	48.6152	48.2032	6.3158
Days to fruit harvest	11.222	0.444	11.667	3.9513	3.8753	0.7712
Average fruit weight	27.327	0.022	27.349	17.7878	17.7808	0.5007
Fruit weight/plant	35628.770	15.693	35644.463	55.5341	55.5219	1.1653

Table 3.44. Genotypic (δ^2g), phenotypic (δ^2p) and error (δ^2e) components of variation and genotypic (GCV), phenotypic (PCV) and error (ECV) coefficient of variability of eight morphological and eight yield contributing characters in Festival and its somaclones.

Characters	δ^2g	δ^2p	δ^2e	GCV	PCV	ECV
Plant height	25.104	0.152	25.256	23.7799	23.7084	1.8428
No. of leaves/plant	13.852	0.088	13.940	21.6652	21.5964	1.7246
petiole length	1.261	0.084	1.346	7.9754	7.7211	1.9980
No. of stolons/plant	0.687	0.032	0.719	24.2249	23.6854	5.0843
No. of nodes/stolon	0.196	0.019	0.216	16.8489	16.0710	5.0604
Stolon length	477.151	11.629	488.780	15.9807	15.7894	2.4650
No. of crowns/plant	3.478	0.054	3.532	38.0108	37.7167	4.7191
Canopy size	3449.364	19.456	3468.820	20.7464	20.6881	1.5537
Days to flowering	57.444	1.111	58.556	11.5553	11.4451	1.5918
No. of flower clusters/plant	0.556	0.667	1.222	17.4559	11.7688	12.8921
No. of flowers/plant	2.444	0.778	3.222	12.0563	10.5009	5.9233
No. of fruits/cluster	0.222	0.111	0.333	23.6189	19.2847	13.6364
No. of fruits/plant	18.889	0.333	19.222	41.1030	40.7450	5.4127
Days to fruit harvest	10.556	0.444	11.000	3.8318	3.7536	0.7702
Average fruit weight	31.123	1.651	32.774	20.3411	19.8221	4.5656
Fruit weight/plant	29790.107	38.128	29828.234	51.1006	51.0679	1.8270

Part B: Evaluation of Variants to Different Biotic and Abiotic Stress Condition

Plants do not grow in optimum conditions during their life cycle, but suffer many adverse situations that cause different types of stress, and prevent them from reaching maximum development. In addition, the physiological optimum for any one species differs from what is known as the ecological optimum and therefore in each particular case, the plant has to adapt to the environmental conditions prevailing in its habitat. Stress is considered as a change in any environmental factor that has an impact on the plant by affecting its biochemical and physiological response to such changes and may on occasions lead to damage or injury. In general, the stressful situations cause a series of physiological changes in the plant to offset these situations that are intended to maintain the plant's vital functions. Strawberry (*Fragaria* x *ananassa* Duch.) plants are most susceptible to disease causing organisms when stressed. Stress results from planting in clay or high salt soil, incorrect planting depth, too much or too little water, too much shade, winter drying and frost heaving.

Biotic stress is stress that occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. It is a major focus of agricultural research, due to the vast economic losses caused by biotic stress to cash crops. The relationship between biotic stress and plant yield affects economic decisions as well as practical development. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment (Hamish 1998). The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way (George 1993). Abiotic stress is essentially unavoidable. Abiotic stress affects animals, but plants are especially dependent on environmental factors, so it is particularly constraining. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide (Chawla 2002).

Strawberry is high value fruit mainly grown in temperate and sub-temperate region. There are many strawberry genotypes grown in tropical and sub-tropical environment but fruits of these genotypes are mostly unpalatable. Elite and tasty strawberry

cultivars of temperate and sub-tropical origin can be grown by importing plantlets every year and planting them in the beginning of the winter season preferably in October-November. Recent media reports showed that imported plants grow well producing flowers and fruits up to March in Bangladesh. During hot summer and humid rainy season almost all of the imported plants are perished due to different diseases. Strawberry cultivation in tropical climate is vulnerable due to different biotic and abiotic factors. It has been observed that strawberry cultivation in Bangladesh is highly affected with different disease and environmental factors. Among the different diseases, verticillium wilt and crown rots are found to be very prominent. Hot and dry weather (above 38°C) and soil pH are major abiotic factors that affect strawberry cultivation. These problems should be overcome for sustainable strawberry cultivation in Bangladesh. Different biotic and abiotic factors that affect strawberry plants are investigated in following heads.

In the present study seven strawberry varieties and their somaclones were evaluated for studying different biotic and abiotic stresses that affect strawberry cultivation. The effect of different biotic and abiotic factors on strawberry cultivation and causing yield loss are described below.

3.1. BIOTIC FACTORS

3.1.1. Phytophthora Crown Rot

Phytophthora cactorum (Lebert & Cohn) J. Schort produces leather rot on fruits and crown rot and wilt of plants. Leather rot symptoms on green fruit are characterized by dark brown diseased areas or natural green outlined with a brown margin, and the whole berry eventually becomes brown, with a rough texture and is leathery in appearance. Infection of ripe fruit may cause a little change in color or can cause discoloration, making the fruit dull. The mature fruit will become tough and leathery, and under high moisture conditions, a white moldy growth appears on them. Infected fruits have an unpleasant odor and a bitter in taste, otherwise no other symptoms are obvious. The leather fruit rot incidence increased when wetness duration increased, and infection increased up to the optimum temperatures (21ºC) and then declined. *P. cactorum* causes a crown disease. Young leaves turn bluish green and wilt suddenly and then turn brown and the whole plant dies within a few days. Internal crown tissue

shows more or less extensive necrosis. The initial crowns are brown with disintegrated vascular tissue (Figure 12. A). Crown symptoms appear first on the upper part. In internal tissues, the first symptom of infection is a water soaked appearance, light brown in color. Later, the crown becomes intensely and homogeneously brown and this is typically characteristic of crown rot infection by *Phytophthora* species depends on the distribution of infected plants or infested soils. High temperatures and prolonged wetness favor crown rot.

3.1.2. Verticillium Wilt

Verticillium wilt, caused by the soil-borne fungus *Verticillium albo-atrum*, can be a major factor limiting production. The first symptoms of *Verticillium* wilt in new strawberry plantings often appear about the time runners begin to form. When a plant is severely infected, the probability of it surviving to produce a crop is greatly reduced. On infected strawberry plants, the outer and older leaves drop, wilt, turn dry, and become reddish-yellow or dark brown at the margins and between veins (Figure 12. B). Few new leaves develop and those that do tend to be stunted and may wilt and curl up along the mid vein. Severely infected plants may appear stunted and flattened with small yellowish leaves. Brownish-to-bluish black streaks or blotches may appear on the runners or petioles. Inner leaves are stunted but tend to remain green and turgid until the plants die.

3.1.3. Leaf Scorch

Leaf scorch is caused by the fungus *Diplocarpon earliana*. The leaf scorch fungus can infect leaves, petioles, runners, fruit stalks and caps of strawberry plants. Leaf scorch symptoms are very similar to the early stages of leaf spot. Round to angular or irregular dark-purple spots up to 1/4 inch in diameter are scattered over the upper leaf surface (Figure 12 D). As spots enlarge, they resemble small drops of tar. The centers of the spots remain dark purple. This distinguishes the disease from leaf spot where the center turns white. If many infections occur on the same leaf, the entire leaf becomes reddish or light purple. Severely infected leaves dry up and appear scorched. Similar, but elongated, spots may appear on other affected plant parts. Lesions may girdle fruit stalks causing flowers and young fruit to die. Infections on green berries

are rare, appearing as red to brown discolorations or a flecking on the fruit surface. The leaf scorch fungus can infect strawberry leaves at all stages of development.

3.1.4. Leaf Spot

Leaf spot is caused by the fungus *Mycosphaerella fragariae*. Also referred to as Ramularia leaf spot, "rust," bird's eye spot, "gray spotness," and white spot. The fungus overwinters in purple spots on infected plants. These spots on the upper leaf surface produce spores. This disease first appears on leaves as purplish spots that resemble leaf scorch. Later these spots enlarge to a diameter of 0.13 to 0.25 in (3 to 6 mm). The centers become grey or white with reddish to purplish borders. Leaf petioles or stalks, stolons, runners and, to some extent, calyxes (caps or hulls) may be attacked (Figure 12. C). Plants and leaves are mostly susceptible early in the growing season and in late summer, especially where growth is very succulent. The leaf spot fungus may also attack the fruit and cause black seed. Mowing the strawberry bed afer the plants have fnished fruiting will remove old infected leaves and reduce infectious material.

3.1.5. Phomopsis Leaf Blight

Leaf blight is caused by the fungus *Phomopsis obscurans*. Leaf blight is found most commonly on plants after harvest. The disease is distinctively different from both leaf spot and leaf scorch. The enlarging leaf spots of this disease are round to elliptical or angular and a quarter of an inch to an inch in diameter (Figure 12. E). Spots are initially reddish-purple. Later, they develop a darker brown or reddish brown center surrounded by a light brown area with a purple border. Similar spots may sometimes develop on the fruit caps. Usually, only one to six lesions develop on a leaflet. Often the infected area becomes V-shaped with the widest part of the "V" at the leaf margin. Older leaves become blighted and may die in large numbers. The same fungus can cause an enlarging, soft, pale pink rot at the stem end of the fruit.

3.1.6. Botrytis Fruit Rot (Gray mold)

One of the most serious and common fruit rot diseases of strawberry is gray mold. Gray mold is caused by the fungus *Botrytis cinerea*. Under favorable environmental conditions for disease development, serious losses can occur. The gray mold fungus

can infect petals, flower stalks (pedicels), fruit caps and fruit. The disease is most severe during prolonged rainy and cloudy periods during bloom and harvest. Abundant gray brown, fluffy, fungal growth on infected tissue is responsible for the disease's name "gray mold". Young blossoms are very susceptible to infection. One to several blossoms in a cluster may show blasting (browning and drying) that may spread down the pedicel. Fruit infections usually appear as soft, light brown, rapidly enlarging areas on the fruit (Figure 12 F). If it remains on the plant, the berry usually dries up, "mummifies", and becomes covered with a gray, dusty powder. Berries resting on soil or touching another decayed berry or a dead leaf in dense foliage are most commonly affected.

Disease incidence (%) of seven strawberry varieties and their somaclones are presented in Table 3.45. Verticillium wilt, phytophthora crown rot, leaf scotch, leaf spot, leaf blight and botrytis fruit rot disease were found in the strawberry field. Among the six strawberry diseases, disease incidences (%) of verticillium wilt and phytophthora crown rot is high (60%) in seven donor parent’s viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival but in somaclones the incidence was 15%.

In other diseases viz. leaf scotch, leaf spot, leaf blight and botrytis fruit rot, disease incidence (%) were high (45-50%) in donor plants but in their somaclones it was low (10%). Most of the plants were severally affected with these diseases during the summer mounths and were perished. There were no plants found resistant to fungal diseases.

Table 3.45. Disease incidence (%) of seven strawberry cultivars and their somaclones. Data were recorded after 90–120 days after plantation in the field.

Cultivers/ Somaclones	Disease incidence (%)					
	Verticillium wilt	Phytophthora crown rot	Leaf scotch	Leaf spot	Leaf blight	Botrytis fruit rot
AOG	60	60	50	50	50	45
AOG SC 1	15	10	10	10	10	10
AOG SC 2	15	15	10	10	10	10
AOG SC 3	10	10	00	00	00	00
AOG SC 4	15	15	10	10	10	10
JP-2	60	60	50	50	50	50
JP-2 SC 1	15	15	10	10	10	10
JP-2 SC 2	15	15	10	10	10	10
JP-3	60	60	50	50	50	50
JP-3 SC 1	15	15	10	10	10	10
JP-3 SC 2	15	15	10	10	10	10
Camarosa	60	60	50	50	50	50
Camarosa SC 1	15	15	10	10	10	10
Camarosa SC 2	15	15	10	10	10	10
Sweet Charly	60	60	50	50	50	50
Sweet Charly SC 1	15	15	10	10	10	10
Sweet Charly SC 2	15	15	10	10	10	10
Giant Mountain	60	60	50	50	50	50
Giant Mountain SC 1	15	15	10	10	10	10
Giant Mountain SC 2	15	15	10	10	10	10
Festival	60	60	50	50	50	50
Festival SC 1	15	15	10	10	10	10
Festival SC 2	15	15	10	10	10	10

Plate 13

Figure 13. Phytophthora crown rot disease of strawberry (A), strawberry plant dying from Verticillium wilt (B), strawberry leaf spot symptoms on leaflet (C), leaf scotch on strawberry (D), Pomopsis leaf blight on strawberry (E) and Botrytis fruit rot on mature strawberry fruit (F).

3.2. ABIOTIC FACTORS

Hot and dry weather and soil pH are major abiotic factors that affect strawberry cultivation in Bangladesh. During the summer mounth April-May temperature become high (above 38°C) and the plants failed to perpetuate in the field.

In terms of summer overcoming capacity, majority of plants were found heat sensitive in donor plants. In their somaclones, 75- 80% plants showed moderate summer overcoming capacity and only 15-20% plants showed high summerovercomming capacity. Among the somaclones AOG SC 3 showed better performance than other somaclones and donor parents in terms of summer overcoming capacity.

Table 3.46. **Summer overcoming potentiality of seven strawberry varieties and their somaclones. Data were recorded 120 days after plantation in the field. There were ten plants in each variety.**

Varieties/Somaclones	Summer overcoming potentiality (%)		
	No	Moderate	High
AOG	95	5	--
AOG SC 1	10	75	15
AOG SC 2	10	75	15
AOG SC 3	--	80	20
AOG SC 4	10	75	15
JP-2	95	5	--
JP-2 SC 1	10	75	15
JP-2 SC 2	10	75	15
JP-3	95	5	--
JP-3 SC 1	10	75	15
JP-3 SC 2	10	75	15
Camarosa	95	5	--
Camarosa SC 1	10	75	15
Camarosa SC 2	10	75	15
Sweet Charly	95	5	--
Sweet Charly SC 1	10	75	15
Sweet Charly SC 2	10	75	15
Giant Mountain	95	5	--
Giant Mountain SC 1	10	75	15
Giant Mountain SC 2	10	75	15
Festival	95	5	--
Festival SC 1	10	75	15
Festival SC 2	10	75	15

Table 3.5. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. AOG. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	20	+	All are cream in colour	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	90	+++		S	-
		Mature leaf	90	+++		S	-
		Node	70	++		S	-
	2.5	Young leaf	55	++		LC	-
		Mature leaf	60	++		LC	-
		Node	45	+		LC	-
NAA	1.0	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	S	-
		Node	20	+	LCr	LC	-
	2.0	Young leaf	90	+++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	80	++	LCr	S	-
	2.5	Young leaf	60	++	Cr	LC	-
		Mature leaf	70	++	Cr	LC	-
		Node	50	+	Cr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.6. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. JP-2. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	15	+	All are cream	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	83	+++	W	S	-
		Mature leaf	81	+++	W	S	-
		Node	70	++	CrB	S	-
	2.5	Young leaf	70	++	All are cream	LC	-
		Mature leaf	60	++		LC	-
		Node	60	++		LC	-
NAA	1.0	Young leaf	25	+	All are cream	LC	-
		Mature leaf	30	+		S	-
		Node	15	+		LC	-
	2.0	Young leaf	82	+++	L Cr	S	-
		Mature leaf	86	+++	L Cr	S	-
		Node	80	++	L Cr	S	-
	2.5	Young leaf	60	++	All are cream	LC	-
		Mature leaf	70	++		LC	-
		Node	50	+		LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.7. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. JP-3. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	20	+	All are cream	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	84	+++	W	S	-
		Mature leaf	81	+++	W	S	-
		Node	60	++	Cr B	S	-
	2.5	Young leaf	70	++	All are cream	LC	-
		Mature leaf	60	++		LC	-
		Node	60	++		LC	-
NAA	1.0	Young leaf	25	+	LCr	LC	-
		Mature leaf	30	+	LCr	LC	-
		Node	15	+	LCr	LC	-
	2.0	Young leaf	85	+++	LCr	S	-
		Mature leaf	86	+++	LCr	S	-
		Node	70	++	LCr	S	-
	2.5	Young leaf	60	++	All are cream	S	-
		Mature leaf	70	++		S	-
		Node	55	++		S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.8. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. Camarosa. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	20	+	All are cream	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	85	+++	W	S	-
		Mature leaf	85	+++	W	S	-
		Node	65	++	CrB	S	-
	2.5	Young leaf	70	++	All are cream	LC	-
		Mature leaf	60	++		LC	-
		Node	60	++		LC	-
NAA	1.0	Young leaf	30	+	LCr	LC	-
		Mature leaf	35	+	LCr	LC	-
		Node	20	+	LCr	LC	-
	2.0	Young leaf	90	+++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	70	++	LCr	S	-
	2.5	Young leaf	60	++	All are cream	S	-
		Mature leaf	70	++		S	-
		Node	50	+		S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.9. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. Sweet Charly. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	15	++	All are cream	LC	-
		Mature leaf	30	++		S	-
		Node	10	++		LC	-
	2.0	Young leaf	85	+++	W	S	-
		Mature leaf	85	+++	W	S	-
		Node	70	++	CrB	S	-
	2.5	Young leaf	60	++	All are cream	LC	-
		Mature leaf	70	++		LC	-
		Node	50	++		LC	-
NAA	1.0	Young leaf	30	+	All are cream	LC	-
		Mature leaf	40	++		S	-
		Node	20	++		LC	-
	2.0	Young leaf	90	+++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	80	++	LCr	S	-
	2.5	Young leaf	60	++	All are cream	LC	-
		Mature leaf	70	++		LC	-
		Node	50	+		LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.10. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. Giant Mountain. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	20	+	All are cream in colour	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	82	+++		S	-
		Mature leaf	82	+++		S	-
		Node	60	++		S	-
	2.5	Young leaf	55	++		LC	-
		Mature leaf	60	++		LC	-
		Node	40	+		LC	-
NAA	1.0	Young leaf	30	+	LCr	LC	-
		Mature leaf	35	+	LCr	S	-
		Node	20	+	LCr	LC	-
	2.0	Young leaf	90	+++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	70	++	LCr	S	-
	2.5	Young leaf	70	++	Cr	LC	-
		Mature leaf	75	++	Cr	LC	-
		Node	70	++	Cr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.11. Effect of different concentrations of 2,4-D and NAA alone in MS medium on callus formation from different parts of strawberry cv. Festival. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D	1.0	Young leaf	20	+	All are cream	LC	-
		Mature leaf	30	+		S	-
		Node	10	+		LC	-
	2.0	Young leaf	82	+++	W	S	-
		Mature leaf	83	+++	W	S	-
		Node	70	++	CrB	S	-
	2.5	Young leaf	70	++	All are cream	LC	-
		Mature leaf	60	++		LC	-
		Node	60	++		LC	-
NAA	1.0	Young leaf	30	+	All are cream	LC	-
		Mature leaf	35	+		S	-
		Node	20	+		LC	-
	2.0	Young leaf	90	+++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	70	++	LCr	S	-
	2.5	Young leaf	60	++	All are cream	LC	-
		Mature leaf	65	++		LC	-
		Node	50	+		LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, W= White, CrB= Creamy Brown, LCr = Light Creamy.

Table 3.12. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. AOG. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	10	+	All are LCr	C	-
		Mature leaf	30	+		C	-
		Node	10	+		C	-
	0.5+1.0	Young leaf	20	+		LC	-
		Mature leaf	40	+		LC	-
		Node	40	+		LC	-
	1.0+0.5	Young leaf	40	+		L	-
		Mature leaf	55	++		L	-
		Node	40	+		L	-
	1.0+1.0	Young leaf	60	++		S	-
		Mature leaf	60	++		S	-
		Node	50	+		S	-
	0.5+1.5	Young leaf	90	+++		S	2
		Mature leaf	90	+++		S	3
		Node	80	++		S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.13. Effect of different concentrations of NAA +BA in MS medium on callus formation from different parts of strawberry cv. JP-2. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	00	+	LCr	C	-
		Mature leaf	10	+	LCr	C	-
		Node	10	+	LCr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	30	+	Cr	LC	-
		Node	20	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	LCr	LC	-
		Mature leaf	55	++	LCr	LC	-
		Node	40	+	LCr	LC	-
	1.0+1.0	Young leaf	40	+	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	50	+	LCr	LC	-
	0.5+1.5	Young leaf	70	++	LCr	S	-
		Mature leaf	90	+++	LCr	S	-
		Node	80	++	LCr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.14. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. JP-3. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	10	+	Cr	C	-
		Mature leaf	30	+	Cr	C	-
		Node	10	+	Cr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	30	+	Cr	LC	-
		Node	20	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	Cr	L	-
		Mature leaf	55	++	Cr	L	-
		Node	40	+	Cr	L	-
	1.0+1.0	Young leaf	40	+	Cr	L	-
		Mature leaf	50	+	Cr	L	-
		Node	50	+	Cr	L	-
	0.5+1.5	Young leaf	70	++	Cr	S	2
		Mature leaf	90	+++	Cr	S	2
		Node	80	++	Cr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, L = Loose, Cr = Creamy.

Table 3.15. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. Camarosa. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	10	+	LCr	C	-
		Mature leaf	20	+	LCr	C	-
		Node	10	+	LCr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	30	+	Cr	LC	-
		Node	10	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	LC	-
		Node	40	+	LCr	LC	-
	1.0+1.0	Young leaf	40	+	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	50	+	LCr	LC	-
	0.5+1.5	Young leaf	70	++	LCr	S	-
		Mature leaf	90	+++	LCr	S	2
		Node	70	++	LCr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, L = Loose, LCr= Light Creamy, Cr = Creamy.

Table 3.16. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. Sweet Charly. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	10	+	LCr	C	-
		Mature leaf	20	+	LCr	C	-
		Node	10	+	LCr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	20	+	Cr	LC	-
		Node	10	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	LC	-
		Node	30	+	LCr	LC	-
	1.0+1.0	Young leaf	40	+	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	40	+	LCr	LC	-
	0.5+1.5	Young leaf	70	++	LCr	S	1
		Mature leaf	90	+++	LCr	S	2
		Node	70	++	LCr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.17. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. Gaint Mountain. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	10	+	LCr	C	-
		Mature leaf	30	+	LCr	C	-
		Node	10	+	LCr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	30	+	Cr	LC	-
		Node	10	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	LC	-
		Node	40	+	LCr	LC	-
	1.0+1.0	Young leaf	40	+	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	50	+	LCr	LC	-
	0.5+1.5	Young leaf	70	++	LCr	S	-
		Mature leaf	90	+++	LCr	S	2
		Node	70	++	LCr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, L Cr= Light Creamy, Cr = Creamy.

Table 3.18. Effect of different concentrations of NAA+BA in MS medium on callus formation from different parts of strawberry cv. Festival. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
NAA+BA	0.5+0.5	Young leaf	00	+	LCr	C	-
		Mature leaf	20	+	LCr	C	-
		Node	10	+	LCr	C	-
	0.5+1.0	Young leaf	10	+	Cr	LC	-
		Mature leaf	20	+	Cr	LC	-
		Node	10	+	Cr	LC	-
	1.0+0.5	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	LC	-
		Node	30	+	LCr	LC	-
	1.0+1.0	Young leaf	30	+	LCr	LC	-
		Mature leaf	40	+	LCr	LC	-
		Node	30	+	LCr	LC	-
	0.5+1.5	Young leaf	70	++	LCr	S	1
		Mature leaf	90	+++	LCr	S	2
		Node	70	++	LCr	S	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, LCr= Light Creamy, Cr = Creamy.

Table 3.19. Effect of different concentrations of 2,4-D+BA in MS medium on callus formation from different parts of strawberry cv. AOG. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	80	++	Cr	LC	-
		Mature leaf	90	+++	Cr	LC	-
		Node	80	++	Cr	LC	-
	3.0+1.5	Young leaf	70	++	Cr	LC	-
		Mature leaf	70	++	Cr	LC	-
		Node	60	++	Cr	LC	-
	4.0+1.0	Young leaf	90	+++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	80	++	LCr	LC	-
	4.0+1.5	Young leaf	80	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	70	++	LCr	LC	-
		Node	50	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.20. Effect of different concentrations of 2,4-D+BA in MS medium on callus formation from different parts of strawberry cv. JP-2. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	90	+++	Cr	LC	-
		Mature leaf	90	+++	Cr	LC	-
		Node	70	++	Cr	LC	-
	3.0+1.5	Young leaf	70	++	Cr	LC	-
		Mature leaf	80	++	Cr	LC	-
		Node	70	++	Cr	LC	-
	4.0+1.0	Young leaf	90	+++	Cr	LC	-
		Mature leaf	80	++	Cr	LC	-
		Node	70	++	Cr	LC	-
	4.0+1.5	Young leaf	80	++	Cr	LC	-
		Mature leaf	80	++	Cr	LC	-
		Node	70	++	Cr	LC	-
	5.0+1.0	Young leaf	60	++	Cr	LC	-
		Mature leaf	50	+	Cr	LC	-
		Node	50	+	Cr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.21. Effect of different concentrations of 2,4-D +BA in MS medium on callus formation from different parts of strawberry cv. JP-3. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	90	+++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	3.0+1.5	Young leaf	70	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	80	++	LCr	LC	-
	4.0+1.5	Young leaf	60	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	40	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.22. Effect of different concentrations of 2,4-D+BA in MS medium on callus formation from different parts of strawberry cv. Camarosa. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	70	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	3.0+1.5	Young leaf	70	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	80	++	LCr	LC	-
	4.0+1.5	Young leaf	60	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	35	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.23. Effect of different concentrations of 2,4-D+BA in MS medium on callus formation from different parts of strawberry cv. Sweet Charly. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	70	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	3.0+1.5	Young leaf	70	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.5	Young leaf	60	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	35	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.24. Effect of different concentrations of 2,4-D +BA in MS medium on callus formation from different parts of strawberry cv. Giant Mountain. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	3.0+1.5	Young leaf	70	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	60	++	LCr	LC	-
	4.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.5	Young leaf	60	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	35	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.25. Effect of different concentrations of 2,4-D+BA in MS medium on callus formation from different parts of strawberry cv. Festival. Data were recorded after four weeks incubation in dark. There were 20 explants in each treatment.

Growth regulators (mg/l)	Concentrations	Explants	Callus induction (%)	Degree of callus induction	Callus colour	Callus nature	Adventitious shoot formation
2,4-D+BA	3.0+1.0	Young leaf	70	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	70	++	LCr	LC	-
	3.0+1.5	Young leaf	70	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	4.0+1.0	Young leaf	80	++	LCr	LC	-
		Mature leaf	90	+++	LCr	LC	-
		Node	80	++	LCr	LC	-
	4.0+1.5	Young leaf	60	++	LCr	LC	-
		Mature leaf	80	++	LCr	LC	-
		Node	70	++	LCr	LC	-
	5.0+1.0	Young leaf	60	++	LCr	LC	-
		Mature leaf	50	+	LCr	LC	-
		Node	35	+	LCr	LC	-

- = No response, + = little callusing (0-50%), ++ = Moderate callusing (51-80%), +++ = Highly callusing (81-100%), LC = Loosely Compact, C = Compact, S = Soft, Cr = Creamy, LCr = Light Creamy.

Table 3.34. Effect of rooting media formulation on root induction of *in vitro* regenerated shoots of strawberry. Data were recorded after 4 weeks of culture.

Treatment (mg/l)			Shoots induced root development (%)							No. of roots/callus (Mean±SE)						
			AOG	JP-2	JP-3	Camarosa	Sweet Charly	Giant Mountain	Festival	AOG	JP-2	JP-3	Camarosa	Sweet Charly	Giant Mountain	Festival
Full strength	NAA	0.1	70	65	66	70	65	69	67	18.2±0.23	17.6±0.25	18.4±0.21	18.0±0.23	17.5±0.25	18.5±0.25	18.5±0.21
		0.5	90	86	85	88	85	85	88	17.5±0.25	16.5±0.35	16.5±0.33	17.0±0.25	16.5±0.35	16.5±0.33	16.5±0.33
		1.0	95	90	90	90	88	90	90	17.5±0.35	15.5±0.35	14.0±0.35	17.3±0.35	15.5±0.35	14.0±0.35	14.5±0.35
		2.0	91	90	90	90	90	90	90	15.4±0.21	15.4±0.25	16.4±0.25	14.2±0.21	15.4±0.25	16.4±0.25	14.4±0.25
	IBA	0.1	60	60	58	60	60	58	60	12.4±0.42	12.0±0.41	11.0±0.45	12.5±0.41	12.0±0.41	11.0±0.45	12.1±0.42
		0.5	88	87	87	88	80	80	80	13.6±0.32	12.5±0.30	12.5±0.31	13.6±0.32	12.5±0.30	12.5±0.31	12.4±0.30
		1.0	90	90	90	90	90	90	90	17.9±0.12	15.0±0.32	15.0±0.32	17.9±0.12	15.0±0.32	15.0±0.32	15.6±0.35
		2.0	88	85	84	83	83	85	83	12.3±0.21	12.5±0.24	11.5±0.84	12.3±0.21	12.5±0.24	11.5±0.84	11.5±0.25
Half strength	NAA	0.1	65	60	65	65	66	60	65	9.2±0.25	8.9±0.24	10.9±0.84	9.2±0.25	8.9±0.24	10.9±0.84	9.8±0.84
		0.5	65	60	60	60	63	63	60	15.4±0.76	15.2±0.76	13.2±0.76	15.4±0.76	15.2±0.76	13.2±0.76	15.5±0.76
		1.0	85	80	80	85	85	85	80	12.2±0.80	12.4±0.81	11.4±0.81	12.2±0.80	12.4±0.81	11.4±0.81	12.1±0.81
		2.0	55	55	56	57	56	55	56	11.4±0.34	10.5±0.35	10.5±0.35	11.4±0.34	10.5±0.35	10.5±0.35	10.0±0.25
	IBA	0.1	45	45	43	50	50	55	44	9.8±0.09	9.8±0.09	9.8±0.09	9.8±0.09	9.8±0.09	9.8±0.09	9.8±0.09
		0.5	60	57	58	58	60	57	58	11.6±0.47	11.6±0.50	12.6±0.50	11.6±0.47	11.6±0.50	12.6±0.50	12.5±0.50
		1.0	75	75	70	77	77	75	72	12.9±0.77	13.9±0.75	13.9±0.75	12.9±0.77	12.9±0.75	13.9±0.75	11.0±0.75
		2.0	60	60	59	60	58	60	60	10.5±0.53	11.5±0.55	12.5±0.55	10.5±0.53	12.5±0.55	12.5±0.55	12.5±0.55
Without growth regulators	½ MSo		96	95	95	96	95	92	92	18.5±0.33	19.3±0.45	17.5±0.53	19.6±0.70	18.5±0.55	19.3±0.45	18.9±0.25
	MSo		100	100	100	100	100	100	100	28.5±0.58	25.7±0.62	24.5±0.52	25.2±0.30	25.5±0.45	25.7±0.62	25.0±0.55

Table 3.36. Morphological characters of selected somaclones and comparison with their respective seven strawberry parents.

Varieties/ somaclones	Plant height (cm) (Mean±SE)	No. of leaves/plant (Mean±SE)	Petiole length (cm) (Mean±SE)	No. of stolons/ Plant (Mean±SE)	No. of nodes/ Stolon (Mean±SE)	Stolon length (cm) (Mean±SE)	No. of crowns/ Plant (Mean±SE)	Canopy size (cm^2) (Mean±SE)
AOG	16.40±0.21	20.23± 0.14	14.23±0.14	10.07±0.43	4.27±0.14	120.4±0.35	3.20±0.058	364.23±8.14
AOG SC 1	21.47±0.58	26.40±0.21	16.07±0.26	14.23±0.14	4.90±0.21	161.5±13.8	6.50±0.057	474.40±0.26
AOG SC 2	28.00±0.29	25.27±0.15	15.40±0.20	12.43±0.23	4.40±0.15	155.0±0.40	5.40±0.057	374.37±0.30
AOG SC 3	31.97±0.32	30.33±0.08	17.23±0.14	16.37±0.18	6.27±0.14	163.1±0.20	7.50±0.057	594.03±0.52
AOG SC 4	17.40±0.21	20.47±0.14	15.47±0.14	13.27±0.14	5.27±0.14	145.0±0.11	5.50±0.057	473.87±0.63
LSD value (at 5% level)	**2.2250**	**1.3252**	**1.8361**	**2.3382**	**1.3902**	**55.2871**	**0.5272**	**31.5607**
CV%	**1.8763**	**1.0495**	**2.2758**	**3.4236**	**5.3822**	**7.2115**	**1.8233**	**1.3446**
JP-2	15.47±0.20	20.27±0.39	13.57±0.23	4.27±0.12	2.43±0.09	114.23±3.03	3.27±0.14	382.77±1.22
JP- 2 SC 1	24.00±0.29	22.67±0.12	15.23±0.14	5.33± 0.12	3.20±0.10	145.27±0.14	4.37±0.07	450.27±0.15
JP- 2 SC 2	22.23±0.21	24.50±0.17	15.33±0.17	5.83±0.067	3.30±0.11	155.33±0.24	6.47±0.09	474.40±0.14
LSD value (at 5% level)	**0.5941**	**1.3090**	**1.3006**	**0.5286**	**0.2426**	**11.6129**	**0.7123**	**4.4029**
CV%	**0.7940**	**1.6006**	**2.4299**	**2.8243**	**2.2388**	**2.3083**	**4.1657**	**0.2777**
JP-3	16.13±0.18	15.47±0.20	13.27±0.15	4.40±0.05	2.33±0.09	100.70±0.44	3.03±0.26	383.40±3.31
JP-3 SC 1	25.03±0.26	19.60±0.20	14.23±0.15	6.27±0.15	3.13±0.09	149.73±0.39	5.40±0.06	451.23±0.16
JP-3 SC 2	22.00±0.23	18.60±0.21	13.80±0.15	6.10±0.10	4.23±0.12	150.13±0.23	6.63±0.09	462.37±0.30
LSD value (at 5% level)	**0.3536**	**1.3090**	**0.9394**	**0.7377**	**0.2101**	**1.8710**	**1.0147**	**11.6663**
CV%	**0.4616**	**2.0112**	**1.8755**	**3.6279**	**1.7856**	**0.3851**	**5.5531**	**0.7417**
Camarosa	15.43±0.23	18.47±0.26	14.30±0.15	4.33±0.09	2.47±0.09	113.73±3.38	2.80±0.06	361.60±2.27
Camarosa SC 1	22.27 ±0.15	20.37±0.09	15.27±0.15	4.67±0.12	3.27±0.09	144.30±0.15	6.17±0.09	474.37±0.30
Camarosa SC 2	24.43 ±0.12	22.50±0.17	15.27±0.15	4.77±0.09	3.07±0.12	148.20±0.12	6.30±0.06	374.30±0.36
LSD value (at 5% level)	**1.3174**	**1.3748**	**1.0573**	**0.6417**	**0.6643**	**12.3271**	**0.4113**	**8.8358**
CV%	**1.7483**	**1.8482**	**1.9445**	**3.8437**	**6.2241**	**2.5021**	**2.2213**	**0.6020**
Sweet Charly	15.33 ±0.17	12.30±0.15	14.27 ±0.15	4.60 ±0.06	2.47±0.09	109.37 ±5.33	2.50 ±0.06	337.40±3.05
Sweet Charly SC 1	22.23±0.15	17.47±0.26	14.57±0.18	6.33±0.12	4.13±0.09	155.23±0.15	6.17±0.12	474.43±0.23
Sweet Charly SC 2	24.03±0.29	14.53±0.15	15.30±0.12	5.77±0.09	3.30±0.12	148.27±0.15	5.30±0.15	374.57±0.12
LSD value (at 5% level)	**1.4779**	**0.6124**	**1.0362**	**0.5558**	**0.6967**	**18.8898**	**0.6752**	**11.6672**
CV%	**1.9783**	**1.1399**	**1.9360**	**2.7441**	**5.8026**	**3.7726**	**3.9865**	**0.8109**
Giant Mountain	16.47±0.15	12.27±0.15	15.20±0.15	3.40 ±0.06	2.33 ±0.07	115.93 ±3.53	3.30 ±0.12	357.50 ±1.31
Giant Mountain SC 1	22.30±0.15	16.63 ±0.13	16.63±0.13	5.50±0.06	3.00±0.06	155.67±0.12	6.20±0.06	450.53±0.20
Giant Mountain SC 2	22.13±0.32	13.23±0.15	15.50±0.29	5.67±0.09	3.03±0.09	150.23±0.12	5.70±0.06	474.53±0.15
LSD value (at 5% level)	**1.4247**	**1.0362**	**1.1279**	**0.4851**	**0.1918**	**12.8639**	**0.5753**	**4.5604**
CV%	**1.9294**	**2.0278**	**1.9649**	**2.7460**	**1.8898**	**2.5145**	**3.1207**	**0.2932**
Festival	15.57±0.23	13.10 ±0.21	13.23 ±0.15	2.63 ±0.09	2.30 ±0.06	113.03 ±0.32	2.80 ±0.06	218.83±4.41
Festival SC 1	25.30±0.41	18.27±0.15	15.20±0.15	3.57±0.12	2.77±0.09	151.73±3.37	6.23±0.19	299.63±0.19
Festival SC 2	22.53 ±0.14	20.33±0.09	15.20±0.15	4.30±0.06	3.20±0.06	150.27±0.12	5.80±0.06	333.20±0.17
LSD value (at 5% level)	**1.4169**	**1.0813**	**1.0573**	**0.6474**	**0.5073**	**12.4074**	**0.8489**	**16.0482**
CV%	**1.8428**	**1.7246**	**1.9980**	**5.0843**	**5.0604**	**2.4650**	**4.7191**	**1.5537**

Table 3.37. Fruit yield and yield contributing characters of selected somaclones and comparison with their respective seven strawberry parents.

Varieties/ somaclones	Days to Flowering (Mean±SE)	No. of flower clusters/plant (Mean±SE)	No. of flowers/ Plant (Mean±SE)	No. of fruits/ Cluster (Mean±SE)	No. of fruits/plant (Mean±SE)	Days to fruit Harvest (Mean±SE)	Average fruit wt. (g) (Mean±SE)	Fruit wt./plant (Mean±SE)
AOG	67.67±0.33	6.33±0.33	15.67±0.33	2.67±0.33	9.67±0.33	90.33±0.33	25.30±0.15	244.33±9.67
AOG SC 1	61.67±0.33	7.67±0.33	17.67±0.33	4.00±0.33	15.33±0.33	84.67±0.33	30.03±0.14	460.43±8.28
AOG SC 2	62.00±0.33	6.33±0.33	17.67±0.33	4.33±0.57	17.67±0.57	84.00±0.33	28.23±0.14	498.80±10.04
AOG SC 3	60.00±0.33	8.67±0.33	21.67±0.33	6.33±0.33	20.33±0.35	81.67±0.33	38.00±0.11	772.60±10.67
AOG SC 4	61.67±0.33	7.33±0.33	18.67±0.33	3.67±0.33	15.00±0.58	82.67±0.33	30.13±0.23	442.73±7.99
LSD value (at 5% level)	**2.3011**	**3.0440**	**3.0440**	**3.3871**	**3.7576**	**2.8954**	**1.0975**	**91.2854**
CV%	**0.7144**	**8.1414**	**3.2387**	**15.6733**	**4.6814**	**0.6646**	**0.7031**	**3.6672**
JP-2	69.67±0.33	5.67±0.33	14.33±0.33	2.33±0.33	6.67±0.33	90.33±0.33	20.37±0.09	144.93±0.74
JP- 2 SC 1	61.33±0.33	7.67±0.33	17.67±0.33	4.33±0.33	14.33±0.33	84.67±0.33	32.17±0.73	504.33±2.89
JP- 2 SC 2	61.67±0.33	6.67±0.33	18.67±0.33	2.67±0.33	13.67±0.33	84.67±0.33	22.10±1.70	338.17±9.27
LSD value (at 5% level)	**2.4255**	**2.1006**	**2.4255**	**2.4255**	**1.9176**	**2.4255**	**5.2738**	**31.1646**
CV%	**1.0381**	**8.6603**	**3.9474**	**21.4286**	**4.5610**	**0.7702**	**5.8266**	**2.6024**
JP-3	70.00±0.33	6.00±0.33	14.33±0.33	2.00±0.33	6.67±0.33	92.67±0.33	22.27±0.15	154.57±0.43
JP-3 SC 1	61.67±0.33	7.67±0.33	17.67±0.33	2.67±0.33	14.33±0.33	83.67±1.20	31.17±0.65	482.27±3.05
JP-3 SC 2	61.67±0.33	6.67±0.33	18.67±0.33	6.00±0.57	14.33±0.33	84.67±0.33	32.43±0.23	497.03±2.57
LSD value (at 5% level)	**1.9176**	**1.2128**	**2.4255**	**2.8442**	**1.2128**	**3.6383**	**1.7491**	**14.2024**
CV%	**0.8178**	**4.9180**	**3.9474**	**21.9863**	**2.8302**	**1.1494**	**1.6796**	**1.0328**
Camarosa	70.00±0.33	5.33±0.33	16.33 ±0.33	2.00±0.33	5.33±0.33	90.33±0.33	21.90 ±1.55	132.67 ±5.36
Camarosa SC 1	61.67±0.33	5.67±0.33	18.33±0.33	3.00±0.33	13.67 ±0.33	84.33±0.67	31.03±0.20	438.37±1.44
Camarosa SC 2	61.00±0.58	6.67±0.33	18.67±0.33	6.67±0.33	13.33±0.33	85.00±0.33	32.43±0.09	426.60±2.01
LSD value (at 5% level)	**1.9176**	**1.2128**	**2.4255**	**1.2128**	**2.4255**	**3.2087**	**5.7960**	**21.7791**
CV%	**0.8207**	**5.6604**	**3.7500**	**8.5714**	**6.1856**	**1.0189**	**5.5983**	**1.8001**
Sweet Charly	71.67 ±1.66	5.33 ±0.33	15.33±0.33	2.00 ±0.33	6.00 ±0.33	90.33±0.33	23.10±0.23	144.00±1.53
Sweet Charly SC 1	61.67±0.33	6.33±0.33	18.33±0.33	2.67±0.33	14.33±0.33	84.67±0.33	31.93±0.87	461.33±2.62
Sweet Charly SC 2	61.67±0.33	7.00±0.58	17.33±0.33	3.00±0.33	13.67±0.33	84.33±0.33	23.37±0.44	334.50±0.58
LSD value (at 5% level)	**7.2766**	**1.2128**	**2.1006**	**1.2128**	**1.4853**	**2.4255**	**4.0296**	**10.0375**
CV%	**3.0769**	**5.3571**	**3.3962**	**13.0435**	**3.6022**	**0.7712**	**4.2381**	**0.8806**
Giant Mountain	70.33 ±0.33	5.67 ±0.33	15.33 ±0.33	2.00 ±0.33	4.67±0.33	90.33 ±0.33	23.37±0.19	122.67±3.71
Giant Mountain SC 1	62.00±0.33	6.67±0.33	18.33±0.33	2.67±0.33	13.33±0.33	84.33±0.33	32.23±0.12	433.80±0.31
Giant Mountain SC 2	61.67±0.33	6.33±0.33	17.00±0.58	3.00±0.33	13.67±0.33	84.67±0.33	32.60±0.15	463.43 ±1.62
LSD value (at 5% level)	**1.4853**	**2.4255**	**3.2087**	**1.2128**	**2.4255**	**2.4255**	**0.5355**	**14.4131**
CV%	**0.6313**	**10.7143**	**5.2219**	**13.0435**	**6.3158**	**0.7712**	**0.5007**	**1.1653**
Festival	75.00±0.58	5.33±0.33	13.00 ±0.58	2.00 ±0.33	5.67±0.33	90.33 ±0.33	21.67 ±0.07	138.87±5.88
Festival SC 1	62.00±0.58	6.67±0.33	16.00±0.58	2.33±0.33	5.67±0.33	84.67±0.33	30.93±0.59	445.83±1.09
Festival SC 2	61.67±0.33	7.00±0.58	15.67±0.33	3.00±0.33	12.67±0.33	84.67±0.33	31.83±0.87	429.23±0.80
LSD value (at 5% level)	**3.8351**	**2.9707**	**3.2087**	**1.2128**	**2.1006**	**2.4255**	**4.6751**	**22.4657**
CV%	**1.5918**	**12.8921**	**5.9233**	**13.6364**	**5.4127**	**0.7702**	**4.5656**	**1.8270**

Plate 1

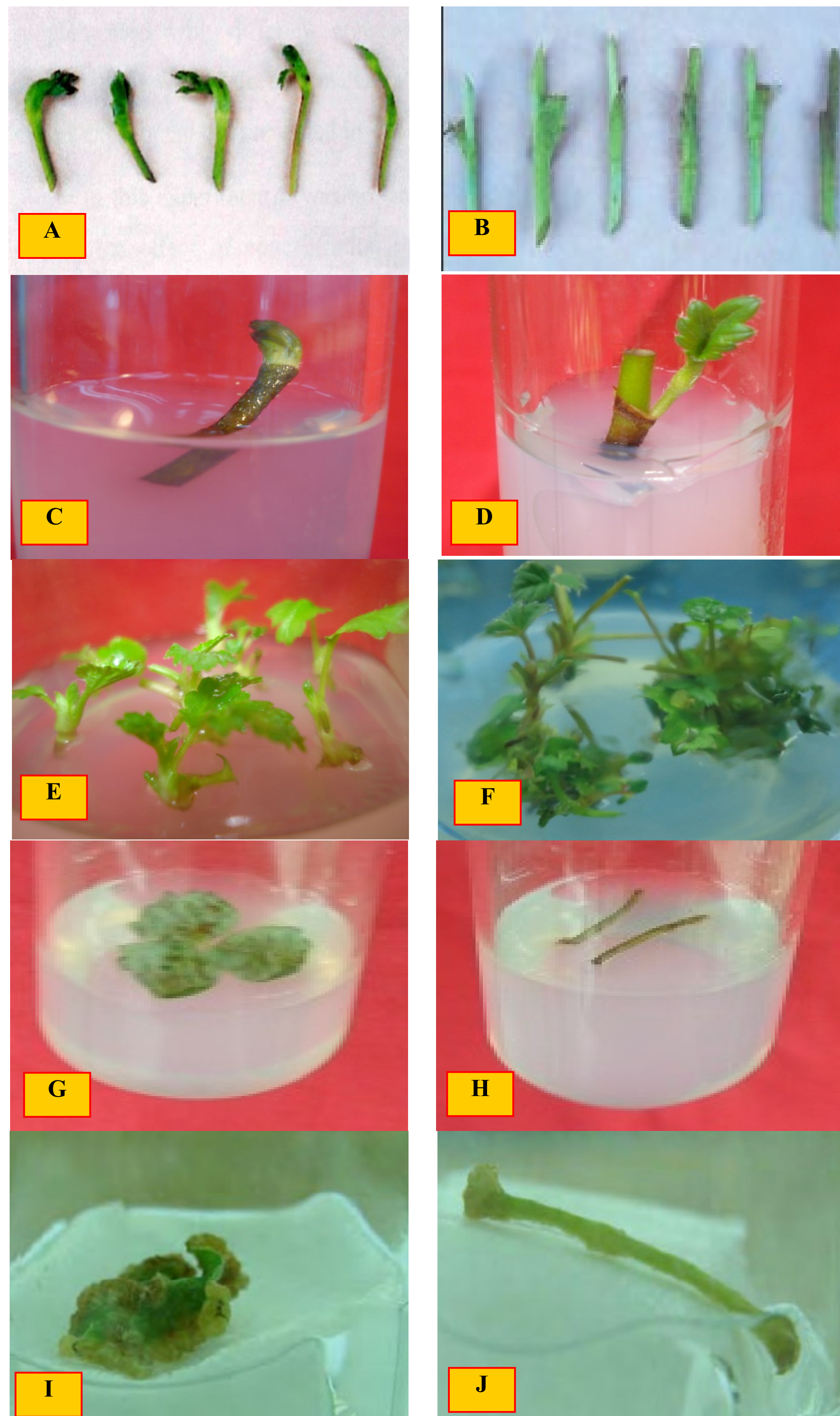


Figure 1. Runner tip (A) and nodal segment (B) explants for initial culture establishment of strawberry for sufficient source of leaf and node for callus induction and shoot regeneration. Primary establishment of runner tip (C) and nodal segment (D) in MS medium supplemented with 1.0 mg/l BA after one week of culture. Shoot multiplication on MS + 1.5 mg/l BA + 0.5 mg/l KIN after 15 days (E), after 25 days (F) of culture. *In vitro* callus induction of strawberry from leaf (G) and node (H) explants. Initiation of callus from *in vitro* grown leaf (I) and node (J) in MS supplemented with 1.5 mg/l BA + 0.5 mg/l NAA, 20 days after culture.

Plate 2

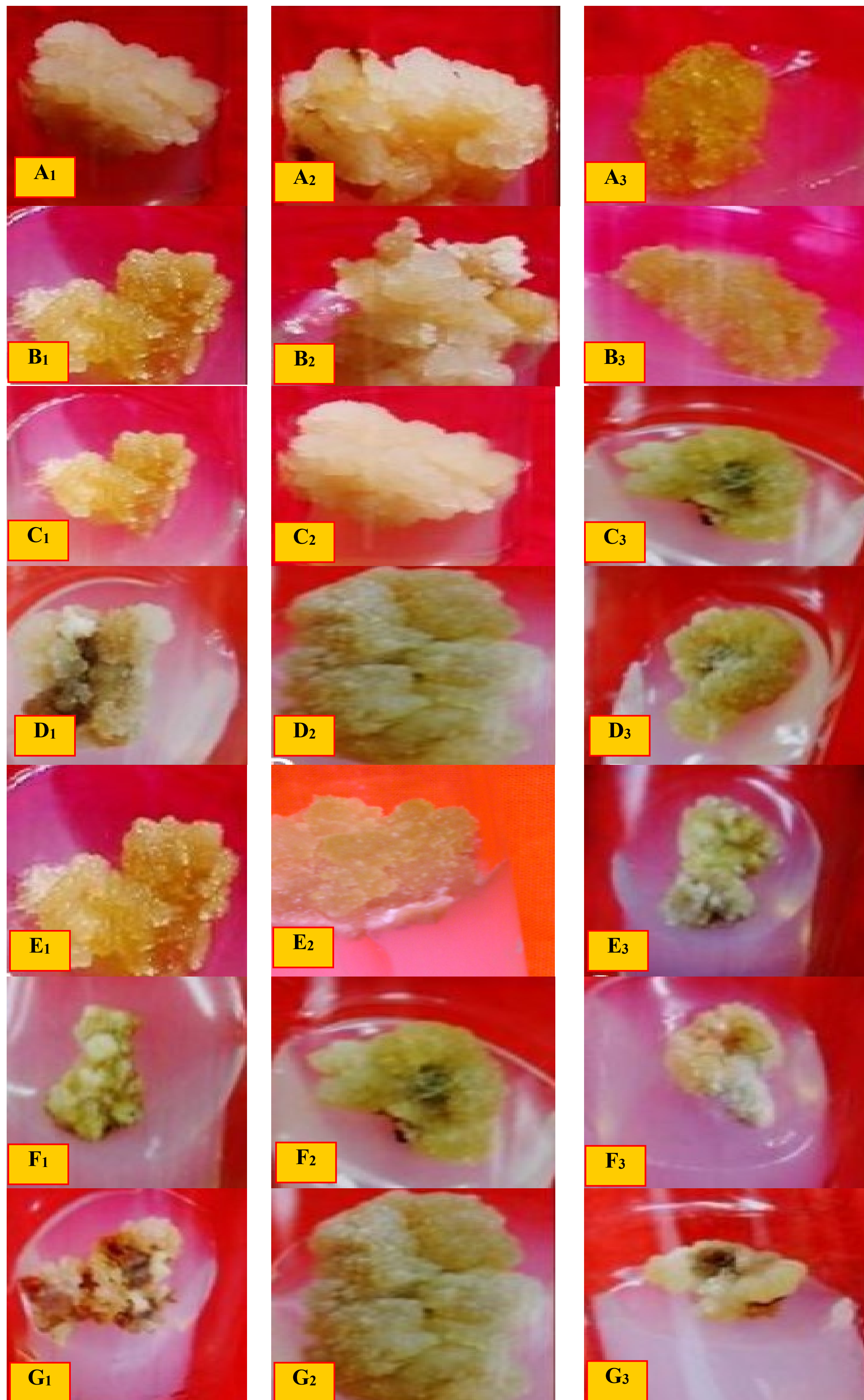


Figure 2. Effect of 2,4-D (2.0 mg/l) on callus induction in strawberry. A $_{1-3}$ = Callus development gradually from young leaf, mature leaf and node in AOG, B $_{1-3}$ = callus development gradually from young leaf, mature leaf and node in JP-2, C$_{1-3}$ = callus development gradually from young leaf, mature leaf and node in JP-3, D$_{1-3}$ = callus development gradually from young leaf, mature leaf and node in Camarosa, E $_{1-3}$ = callus development gradually from young leaf, mature leaf and node in Sweet Charly, F $_{1-3}$ = callus development gradually from young leaf, mature leaf and node in Giant Mountain and G $_{1-3}$ = callus development gradually from young leaf, mature leaf and node in Festival.

Plate 3

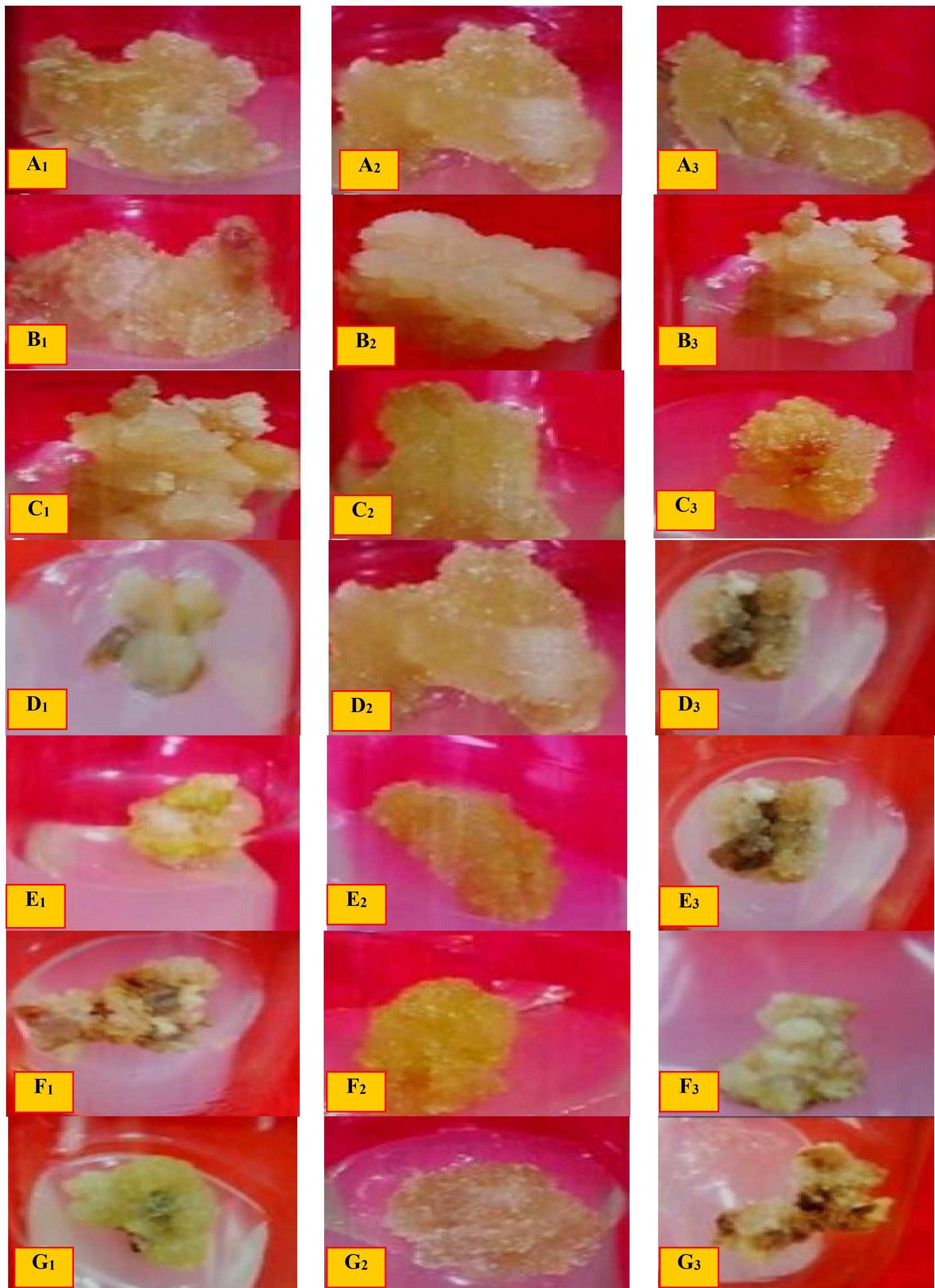


Figure 3. Effect of NAA (2.0 mg/l) on callus induction in strawberry. A $_{1\text{-}3}$ = Callus development gradually from young leaf, mature leaf and node in AOG, B $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-2, C $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-3, D $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Camarosa, E $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Sweet Charly, F $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Giant Mountain and G $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Festival.

Plate 4

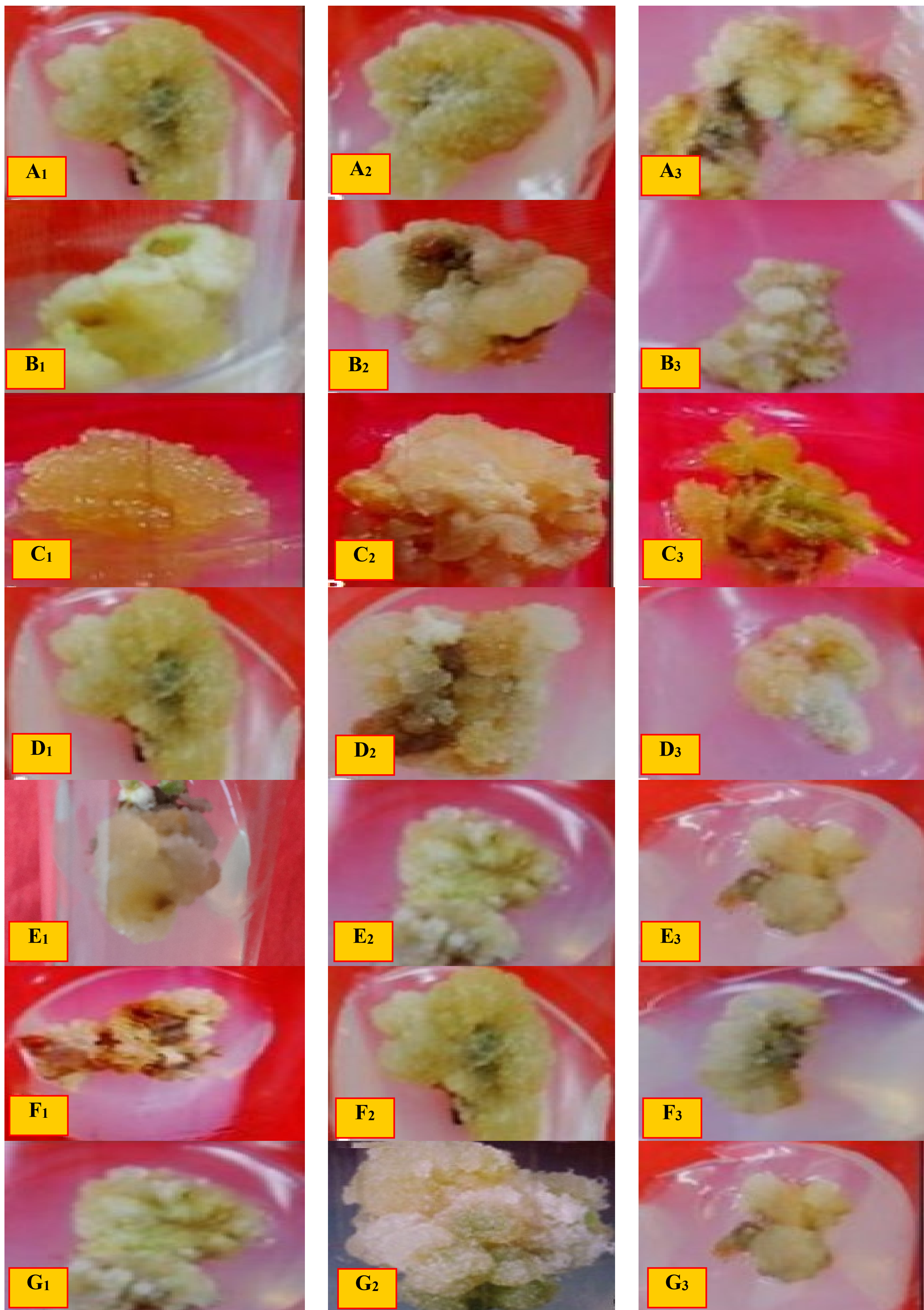


Figure 4. Effect of NAA+BA (0.5 mg/l+1.5 mg/l) on callus induction in strawberry. A $_{1\text{-}3}$ = Callus development gradually from young leaf, mature leaf and node in AOG, B $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-2, C $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-3, D $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Camarosa, E $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Sweet Charly, F $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Giant Mountain and G $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Festival.

Plate 5

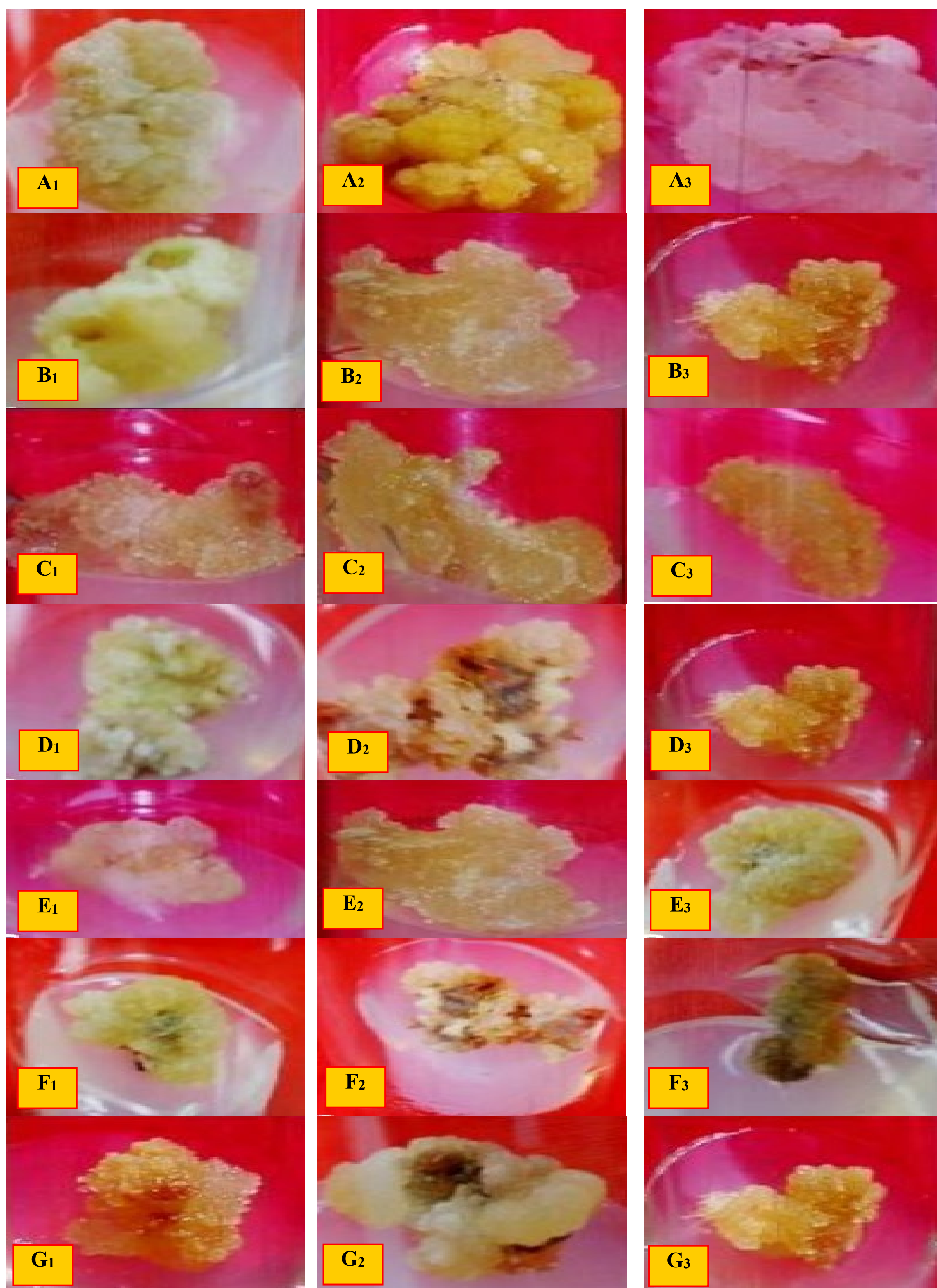


Figure 5. Effect of 2,4-D +BA (1.0 mg/l+0.5 mg/l) on callus induction in strawberry. A $_{1\text{-}3}$ = Callus development gradually from young leaf, mature leaf and node in AOG, B $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-2, C $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in JP-3, D $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Camarosa, E $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Sweet Charly, F $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Giant Mountain and G $_{1\text{-}3}$ = callus development gradually from young leaf, mature leaf and node in Festival.

Plate 6

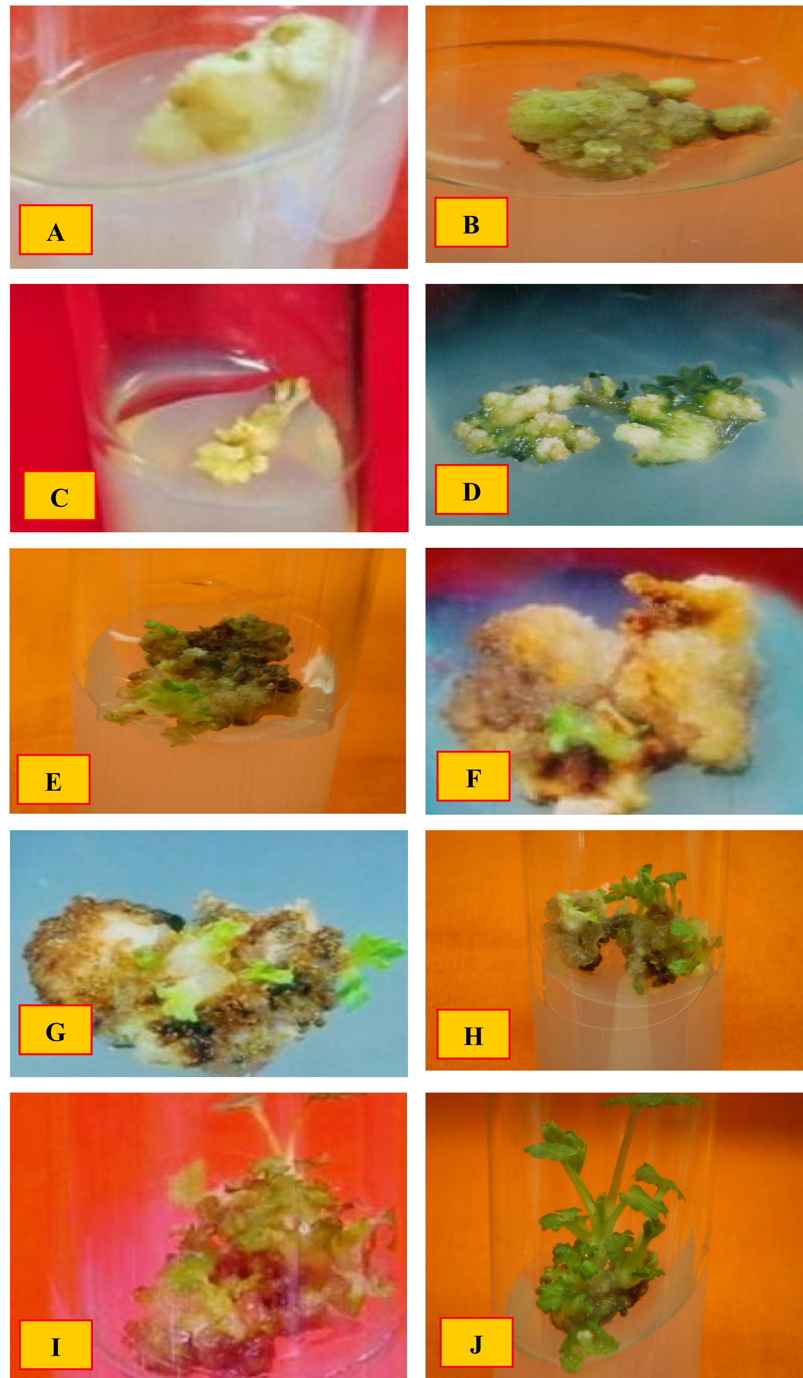


Figure 6. Shoot regeneration in MS medium with different concentrations of BA+NAA.

Shoot regeneration in MS medium with 1.5 mg/l BA + 0.5 mg/l NAA from mature leaf derived calli (B, D, F, H and J).

Shoot regeneration in MS medium with 1.5 mg/l BA + 0.5 mg/l NAA from young leaf derived calli (A, C, E, G and I).

Plate 7

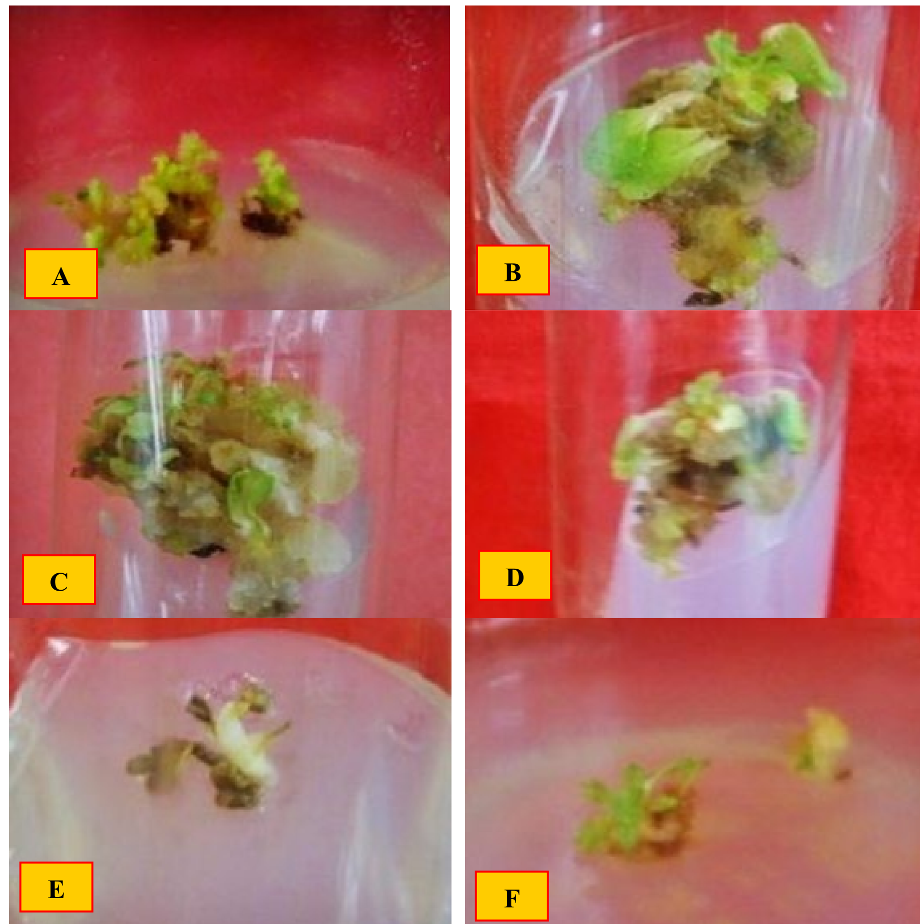


Figure 7. Shoot regeneration in different concentrations of BA.

Shoot regeneration in MS medium with 0.5 mg/l BA from young leaf which developed in 0.5 mg/l NAA + 1.5 mg/l BA (A, C, E).

Shoot regeneration in MS medium with 0.5 mg/l BA from mature leaf which developed in 0.5 mg/l NAA + 1.5 mg/l BA (B, D, F).

Plate 8

Figure 8. Photographs showing adventitious root formation from micro-cuttings in MS + 0.5 mg/l IBA (A), MS + 0.5 mg/l NAA (B) ½ MS_0 (C), MS_0 (D) after 15 days and after 30 days (E, F). Root number in MS_0 (G), in ½ MS_0 (H), MS + 0.5 mg/l IBA (I), MS + 0.5 mg/l NAA (J).

Plate 9

Figure 9. Photographs showing different stages of acclimatization and field establishment of rooted plantlets in open bottle in room temperature (A), in sand (B), in thump pots (C), in field (D), in field with stolons (E) and in field with flowers (F).

Plate 10

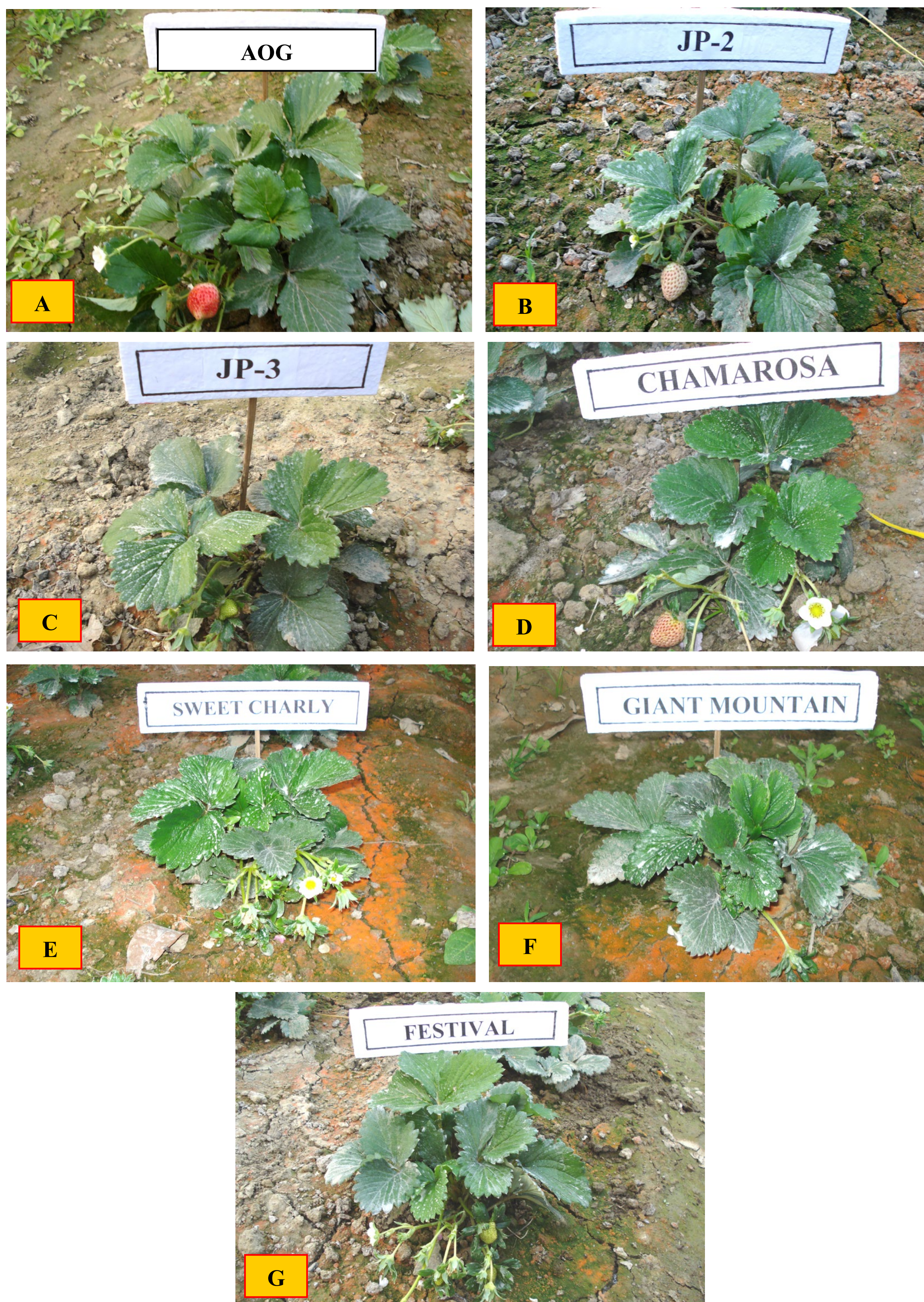


Figure 10. Seven strawberry donor parents. AOG (A), JP-2 (B), JP-3 (C), Camarosa (D), Sweet Charly (E), Giant Mountain (F) and Festival (G).

Plate 11

Figure 11. Different plant types showing somaclonal variation (A-H).

Plate 12

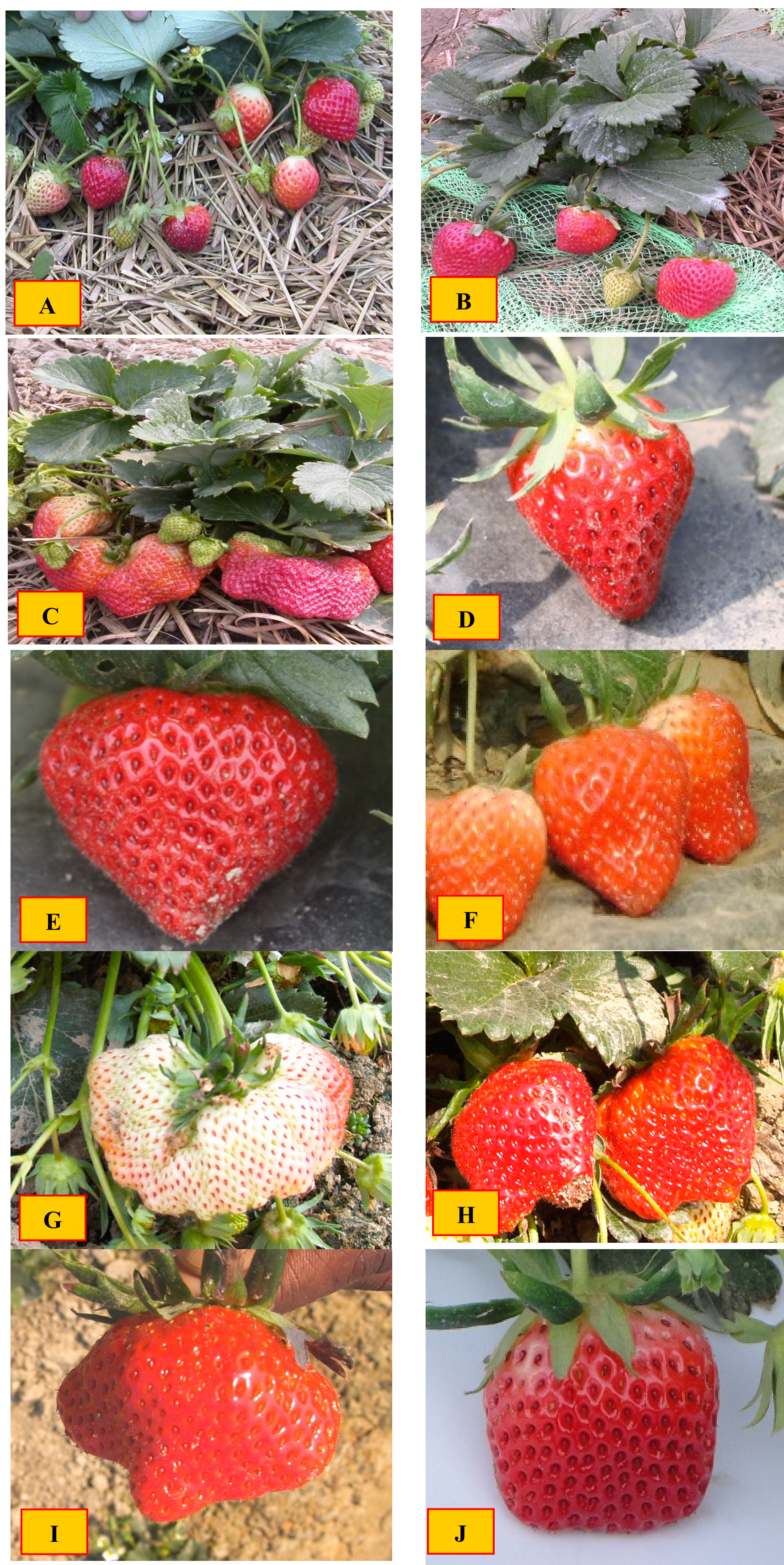


Figure 12. Different fruit shapes showing somaclonal variation (A-J).

IV. DISCUSSION

Strawberry (*Fragaria* x *ananassa* Duch.) is a widely adapted crop that is grown in geographically diverse areas ranging from the low altitude tropics to subtropics to high altitude continental areas. The strawberry cultivated on over 200,000 ha worldwide and world production in 2000 was estimated to be over three million Mt (FAO STAT Database, 2001). Although strawberry has been cultivated in some of the neighboring countries like India, Nepal, Thailand etc, but commercial cultivation of strawberry in Bangladesh is still by at large. Bangladesh is a tropical country with subtropical weather. Distinct winter season with pleasant and mild weather condition (November to March) Bangladesh should be favorable for strawberry cultivation. However, strawberry cultivation has not been flourishing in Bangladesh because there is no suitable cultivar adaptive to local agro climatic condition. Attempts have been made by the researchers of BARI and by personal initiative to grow strawberry from imported cultivar but failed in most of cases. Moreover, the scope of improvement of strawberry through conventional breeding is also very limited, since there is no indigenous germplasm here. Novel *in vitro* techniques have the potential to aid conventional breeding programs. Somaclonal variation is considered to be a useful source of variation and has been demonstrated to be feasible in many crop species. Strawberry cultivation in tropical climate is vulnerable due to different biotic and abiotic factors. It has been observed that strawberry cultivation in Bangladesh is highly affected with different disease and environmental factors. Among the different diseases, *Verticillium* wilt and crown rots are found to be very prominent. Hot and dry weather (above 38°C) and soil pH are the major abiotic factors that affect strawberry cultivation. These problems should be overcome for sustainable strawberry cultivation in Bangladesh. A study has taken up at Plant Breeding and Gene Engineering Laboratory, University of Rajshahi, Bangladesh, for the development of new strawberry cultivars adaptive to the local agro-climate through the exploitation of novel in vitro techniques. The present study was undertaken as a part of this endeavor for callus induction, maintenance and regeneration of seven imported strawberry varieties to study somaclonal variation for both qualitative and quantitative characters

and selection of suitable somaclonal variants and evaluation of their performances under biotic and abiotic stress condition. As a prerequisite to induce somaclonal variation, methods and techniques for optimizing the regeneration rate of callus tissues derived from leaf and node explants were evaluated.

In vitro techniques like production of doubled haploids through anther culture, wide hybridization through protoplast fusion and *in vitro* selection have the potential to overcome such barriers as difficulties in crossing, undesirable linkages in subsequent generations, enormous space and time requirements of conventional breeding programs. One of the applications of *in vitro* techniques is the exploitation of genetic changes occurring in plants regenerated from callus cultures for producing useful somaclonal variants (Larkin and Scowcroft 1981). The mutant cells with genetic and cytogenetic alterations need to undergo differentiation to produce plantlets to have these changes represented in the plants. It is thought that, at the morphogenic stage, a severe selection pressure is exerted against the cells with major cytogenetic, change as a result of which most of the major changes are not represented in the regenerated plants, the somaclones. Thus the situations in plant improvement program that require induced mutagenesis to create additional variation are the same conditions under which somaclonal variability finds its application. The advantage however is the high frequency mostly of gene mutations in somaclones and the experimental opportunities available for selection of cells with altered biochemical features. The occurrence of useful somaclonal variation has been demonstrated in many crop species like wheat (Hasim *et al.* 1990), rice (Abe *et al.* 1989) and maize (Lananyi *et al*. 1990). There are also instances where somaclonal variation has produced agriculturally useful changes like, increased fruit solids and resistance to *Fusarium* race 2 in tomato, high yield and proteins in wheat. In sorghum, somaclonal variation for leaf morphology and growth habit was reported for the first time by Gamborg *et al*. (1977). A sorghum germplasm line, GAC, tolerant to 50% aluminum saturation in acid soil stressed environments was developed by using tissue culture procedure (Duncan *et al.* 1991). The prerequisite for applying these techniques for plant improvement depends on the availability of reliable methods for induction of morphogenic callus cultures, their maintenance and subsequent plant regeneration in the target species. The present studies used seven popular strawberry varieties imported from Japan and America for

callus induction, regeneration and field establishment of callus derived plants and study the induction of somaclonal variations in SC_0 generations.

In this technique collection of explants from the mother plant does not hamper the growth and development of mother plant. Although micropropagtion of plants is presently more expensive, it offers significant advantages in terms of maintenance and mass propagation of selected genotypes for collecting leaf and node for callus induction and regeneration. Moreover, this method may overcome the difficulties related to seasonal changes, age and genetic background of the mother plants and ensure the rapidly of multiplication (Nemeth 1986).

The present study was conducted to determine the appropriate growth regulator concentration and combination to establish a mass production system of friable callus and its regeneration ability using different vegetative organs from selected strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival to study somaclonal variation. These experiments on strawberry were conducted with a view to (i) *in vitro* shoot proliferation from the runner tip and nodal explants for rapid multiplication for collection of leaves and nodes for callus induction and shoot regeneration, (ii) standardization of suitable culture media composition for rapid callus induction from different parts of strawberry, (iii) plant regeneration from induced callus, (iv) optimization of root induction from regenerated shoots, (v) acclimatization and field evaluation of somaclonal variants, (vi) selection of suitable somaclonal variants and evaluation of their performances under biotic and abiotic stress condition. To fulfill these objectives established tissue culture techniques were used. The results obtained from different experiments of the present investigation have been discussed in the following paragraphs with an endeavour to provide with the logical explanation for the work undertaken.

At the initial stage of the present study bacteria and fungi were observed as most common contaminants of the cultured explants. These microbes are very common in our environment and are coming in contact with the culture media easily. Because the explants collected from field grown plants having many microbes that cherish the nutrient media, which are used in tissue culture techniques and they find conditions

optimal there in and grow much faster than the cultured tissue. Consequently, death of the cultured tissue due to contamination of fungi and bacteria was noticed, a very common case found in other instances (Anon 1998). For this purpose one potent and well known surface sterilizing agent was employed in the present investigation.

In the present investigation, the runner tip (RT) and nodal segment (NS) from the field grown plants of seven strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival were treated with $HgCl_2$ for raising aseptic culture. In doing so, the collected explants from the field grown plants were washed thoroughly under running tap water and then washed with savlon water for 10-12 minute. Then the plant materials (runner tips and nodal segments) were treated with 0.1% $HgCl_2$ for different duration of time viz. 4, 6, 7 and 8 min to surmount the contamination difficulty and to increase viability rate of the explant types used for direct regeneration systems. It was found that treatment of the explants in different durations in 0.1% $HgCl_2$ and treatment durations were adjusted according to the resistance of explants to the sterilant solution as suggested by earlier authors (Druart and Gruselle 1986).

The explants of strawberry were very sensitive to $HgCl_2$ treatment. The results show that 85% runner tips and 80% nodal segments were found contamination free when these two types of explants were treated with 0.1% $HgCl_2$ for 6 min. On the other hand 15% runner tips and 25% nodal segments were found contamination free when the explants were treated with 0.1% $HgCl_2$ for 4 min. In this experiment, 100% contamination free explants were found when the explants of the selected seven strawberry varieties were treated with 0.1% $HgCl_2$ for 8 min. However, incase of 7 and 8 min duration the explants died due to tissue killing. The most of the cultured explants showed fungal and bacterial contamination within 3-8 days of inoculation. There are also many others reports on using mercuric chloride ($HgCl_2$) for surface sterilization of explants (Nekrosova 1964, Bennette and McComb 1982, Roy *et al.* 1987 etc.). Although mercuric chloride ($HgCl_2$) is used as one of the effective surface sterilant but its residual inhibitory effect is also greater than the other sterilizing agents commonly used in plant tissue culture (Bhojwani and Rajdan 1983, Torres 1988, Razdan 1993). There have been many reports on using calcium hypochlorite [$Ca(OCl)_2$] (Bini and

Belling 1973, Monin 1974, Quoirin *et al.* 1977 etc.) or sodium hypochlorite (NaOCl) (Hammerschalag 1982, Jones *et al.* 1977) for surface sterilization of explants. So, choice of the surface sterilizing agent depends upon the sensitivity of the explants as well as researchers preference to the chemical. Considering the sensitivity of the two types of explants of the selected seven strawberry varieties (AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival), treatment with 0.1% $HgCl_2$ for 6 min duration was found to be most suitable for runner tip and nodal explants.

In vitro methods of plant propagation include shoot culture with proliferation of axillary bud or adventitious shoots and callus culture with organogenesis or embryogenesis. The direct regeneration is essential to obtain plantlets with uniform characteristics that are to make the regenerated plantlets resemble to mother plants in terms of growth characteristics and habits. Though all plant cells are theoretically totipotent (Huberlandt 1902), attempts with many tissue to get whole plants lead to failure due to lack of proper techniques and insufficient knowledge about nutrient media and other physical and chemical conditions which are essential for proper growth of cells, tissue and organs of the plant concern (Johri 1982). Among the most popular tissue culture media, the MS medium (Murashige and Skoog 1962) was formulated with comparatively higher concentrations of nutrient salts and most plant species grow-well on the MS media (Bhojwani and Rajdan 1983, Razdan 1993, Reinert and Bajaj 1977). Although most plants exhibit a range of responses to MS medium the herbaceous and semi woody species respond better than the woody ones (Bhojwani and Rajdan 1983). The technology of plant multiplication by *in vitro* process from runner tip and nodal explants are simple, efficient and also economical (Choudury and Prakash 1992).

For rapid propagation development and multiplication of shoot was necessary. Many workers used different media supplemented with various combinations and concentrations of vitamins and hormones for runner tip and node culture of strawberry. In the present investigation, different concentrations of cytokinin alone or combination of cytokinin or gibberellic acid or both of cytokinin were used to see the response of runner tips and nodes explants obtained from field grown plants and from *in vitro* grown plantlets. Various media viz. MS (Murashige and Skoog 1962), MMS_1

(MS with ½ strength of the macro-nutrients and full strength of the micro-nutrients and vitamins) and MMS_2 (MS with ½ strength of both the macro-nutrients and micro-nutrients) were commonly used for *in vitro* technique. The herbaceous and semi woody species showed better response to MS medium (Bhojwani and Rajdan 1983).

In this experiment, the results regarding shoot development of seven strawberry varieties viz. AOG, JP-2 JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival were recorded after 4 weeks of culture on MS medium supplemented with BA, KIN and GA_3 alone at different concentration for primary culture establishment of runner tips and nodal segments. Through this experiment it was realized that BA was more effective growth regulator and the most preferred concentration of BA was 1.0 mg/l for the primary culture establishment for the selected seven strawberry varieties. The highest percentage of explant responded 90% from runner tip and 80% from nodal segment are noted in medium with 1.0 mg/l BA concentration. Generally, shoot formation was noticed after 12-14 days of culture. However in 1.0 mg/l BA concentration, shoot formation was observed after 7-10 days of culture. Similar results were obtained by Khan *et al.* (1994). Superiority of BA over other cytokinins in producing *in vitro* shoots has also been established in other plants like grape (Barlass and Skene 1978) and *Momordica charantia* (Islam *et al.* 1994), banana (Cronaur and Krikorian 1984; Vessey and Rivera 1981).

For regenerating axillary shoots from *in vitro* grown shoots BA+KIN showed the best shooting performance. In this experiment excised shoots from *in vitro* grown primary cultures were cultured on MS medium supplemented with different concentrations of BA viz. 0.5, 1.5 and 2 mg/l in combination with KIN viz. 0.1 and 0.5 mg/l. Among the various combinations of BA and KIN, best result was observed on medium supplemented with 1.5 mg/l BA + 0.5 mg/l KIN in seven strawberry varieties. On this growth regulator combination shoots derived from both type of explants showed 100% multiple shoot proliferation in AOG within 8-10 days of culture. The highest number of shoots/culture produced from both type of explants were 12 and shoot among which 10 were useable. The maximum length of the shoots was 3.2 cm and it was observed in 1.5 mg/l BA + 0.5 mg/l KIN combination. This result is in consistent with the findings of Cononer and Litz (1978) as well as Teixetra and Da Silva (1990).

There are also reports (Wilna De Winer 1988; Kabir *et al.* 1993) that GA_3 with BA or NAA formulation stimulated both the proliferation and elongation of shoots *in vitro*.

In the present study, leaf explants and node from in vitro grown plants were cultured on to MS medium supplemented with different concentrations and combinations of plant growth regulators (2,4-D, NAA and BA), and the cultures were incubated in dark for callus induction. The strawberry leaf explants produced callus in most of the culture media formulations. However, the effect of different PGR formulations on the degree and types of callus formation were different. Among the different PGR formulations MS + 0.5 mg/l NAA with 1.5 mg/l BA was found to be the most effective media formulation in terms of % of explants induced to develop callus and the degree of callus development. Auxin alone (NAA and 2, 4-D) at 2.0 mg/l was also found very effective for callus development from leaf explants.

Leaf tissue has been studied and shown to have the greatest regeneration capacity of strawberry plant tissues (Jones *et al*. 1988; Liu and Sanford 1988; Nehra and Stushnoff 1989; Nehra *et al*. 1990; Jelenkovic *et al*. 1991; Popescu *et al*. 1997 and Passey *et al*. 2003). Callus production is also more prolific from the leaf tissue. In addition, leaf derived callus produces more shoots than node and root derived callus (Popescu *et al*. 1997). Moreover, most researchers use young tissue culture stock as the source of plant material for regeneration and transformation studies. The *in vitro* stock is usually very young. The runner tips, stipules, leaf disks etc. are brought into tissue culture for the purpose of the experiment and cultured to the point of growth necessary for use in the study or in some cases researchers acquired *in vitro* cultures from outside sources for study (Barcelo *et al*. 1998; Liu and Sanford 1988; James *et al*. 1990 and Alsheikh *et al*. 2002). No matter which part of the plant is used for regeneration, research reports indicate actively dividing cells are more readily undergo morphogenic dedifferentiation to callus tissue formation and subsequent re-differentiation to plant regeneration. If cell division has ceased in the leaf tissue being used morphogenic responses to *in vitro* culture also become slower (James *et al*. 1990).

Previous studies have shown that PGR concentrations and selections are vital to strawberry callus induction and regeneration. Various formulations of BA, IBA, 2,4-D,

CH, and KNO_3 have all been reported used in callus induction and plant regeneration studies in strawberry (Liu and Sanford 1988; Nehra *et al*. 1990 and Goffreda *et al*. 1995). Liu and Sanford (1988) reported using casein hydrolysate (CH) and potassium nitrate on leaf explants of 'Allstar' strawberry. Both stimulated the production of callus and shoot and reportedly had an additive effect. Best callus and 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. In the present investigation auxin in combination with cytokinin (NAA+ BA) was found the most effective for callus induction, which is concomitant with most of the previous report. Kartha *et al*. (1980) studied the effects of light intensity and media on greenhouse grown and *in vitro* grown cultures of 'Redcoat'. Early young leaf and mature leaf formed callus and shoots when BA and 2, 4-D were used at different concentrations. Severe browning occurred when fully expanded leaves of greenhouse grown 'Redcoat' were used. BA caused browning and NAA and that it occurred even when young leaves were used. They suggested that the browning would not have occurred in the presence of activated charcoal added to the medium. However, activated charcoal will inhibit callus formation. They also saw a relationship between PGR concentrations and explants polarity when tissue from the upper part of the leaf regenerated at a higher frequency with higher concentrations of PGRs than those on the basal end exposed to lower concentrations of PGRs.

Protocols in previous reports indicate that a pretreatment in darkness is vital for callus induction and plantlet regeneration (Nehra and Stushnoff 1989; Popescu *et al.* 1997). One possible explanation is the photo inactivation of the phytohormone IAA (Nehra and Stushnoff 1989). One study examined the effects of a dark period from one to four weeks with no significant differences found. However, all dark period groups had improved regeneration over the groups with no dark period (Nehra and Stushnoff 1989). In present study indirect shoot regeneration was obtained by sub culturing the calli onto regeneration medium and incubating the culture in light. To do so the calli proliferated in dark were sub cultured either onto corresponding callus initiation media or onto regeneration media consisted of MS medium supplemented with different concentrations of BA alone or in combination with NAA (considered as

regeneration media) and the cultures were incubated in light for 8 weeks. It was observed that PGR formulations present in all but except root callus induction and regeneration media showed profound effect on shoot induction. The calli induced in auxin (2, 4-D and NAA) supplemented MS callus inducing media were hardly induced to regenerate shoot. These calli failed to regenerate or even perpetuate when the regeneration media contained only BA. On the other hand, only a few calli derived from auxin fortified callusing media were induced to develop shoots when the regeneration contained higher proportion of BA (0.5–1.5 mg/l) and lower proportion of NAA (0.1–0.5 mg/l). Whereas, the calli proliferated in NAA – BA supplemented callusing media showed very high regeneration potential. The calli proliferated in NAA – BA fortified callusing media showed the highest response to indirect regeneration when regeneration medium contained 1.5 mg/l BA and 0.5 mg/l NAA. It is evident from the present study that PGR formulations in callus induction as well as regeneration media are critical factors for successful indirect regeneration of strawberry through callus.

Indirect regeneration from leaf derived callus of strawberry has also reported previously. Nehra and Stushnoff (1989) reported successful regeneration using greenhouse grown stock plants with surface sterilized leaves excised and cut into strips avoiding the midrib and placed adaxial side down onto the media. Nehra and Stushnoff (1989) discovered that leaf disks from young plants had a higher regeneration rate than older plants. A study of the regeneration and transformation of the cultivar ‘Rapella’ showed higher regeneration rates when the youngest leaves were used (James *et al.* 1990). Passey *et al*. (2003) studied seven commercial cultivars of strawberry using leaf disks, petioles, roots and stipules as explant material. They started out by establishing and growing runner tips *in vitro* and sub culturing them every three weeks until enough material was produced to begin the experiments. The leaf disks had the highest regeneration rates (14) for all cultivars with greater than 90% of explants producing shoots (Passey *et al*. 2003). Graham *et al*. (1995) used stem tissue for their regeneration and transformation studies of ‘Melody’, ‘Rhapsody’ and ‘Symphony’.

Whitely (2004) reported successful indirect regeneration of popular strawberry cv. ‘Chandler’ from leaf explant. She cultured *in vitro* derived leaf explants onto modified MS with PGR incubated in dark for 4 weeks and then transferred under light after sub culturing onto fresh regeneration medium. She reported dark treatment of initial culture accentuates indirect regeneration from strawberry leaf, which is concomitant to the results of the present study. Other regeneration methods tested include use of embryos. Wang *et al*. (1984) used embryos excised from strawberry achenes. The tiny strawberry embryos were excised from surface sterile achenes under a microscope and placed on hormone modified regeneration media.

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 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. Shoot regeneration was observed at 16 weeks post callus initiation until 24 weeks. When calli cultures were placed on media containing BA and NAA incubated in a light intensity of 62.5 μ E. $m^{-2}s^{-1}$, a regeneration rate of 50% was attained (Nehra *et al*. 1990). The kind of plant hormone and the amount used is as varied as the protocols for regeneration of strawberry. Nehra and Stushnoff (1989) were successful with IAA and BA, while six years later; Finstad (1995) touted the success of 2, 4-D and BA. The difference being, Nehra was studying ‘Redcoat’ and Finstad studied ‘Totem’ and ‘Hood’ (Finstad and Martin 1995). Jelenkovic *et al*. (1991) studying different cultivars than Nehra or Finstad (1995) studied, tested hypocotyls, runners, petioles and lamina. Only young, fully expanded leaves were used in the lamina study. They determined in preliminary tests that BA and 2, 4-D were the most effective phytohormones to use. However, regeneration was noticeably lower in inoculated explants than in controls possibly due to the explants dying from the toxic effects of the bacteria. The researchers expressed frustration over the inconsistency of response of the same explant material exposed to the same conditions.

The most important aspect of regeneration is the choice of species and cultivar (James *et al*. 1990). Barcelo *et al*. (1998) used ‘Chandler’ in transformation and regeneration

studies. Leaf disks from greenhouse grown plants as well as in vitro grown plants were used. BA and IBA were tested as growth regulators in the media. Also, dark period of 1 - 4 weeks was evaluated. They found no significant differences in the darkness tests. However, the group receiving no dark period had significantly lower regeneration rates. They also did not see any difference in greenhouse verses *in vitro* source plant material. Owen and Miller (1996) compared auxins, cytokinins and carbohydrates for their effect on indirect regeneration of strawberry. Glucose was the carbohydrate source, which produced, shoots for all three cultivars used. 'Chandler' performed best when sucrose, IAA, and BA were used. It responded with 65% forming callus and 19% forming shoots. BA was also used by Lopez-Aranda *et al*. (1994). They studied 'Chandler' to see the effects of mineral salts, BA levels and the number of times an explant was sub cultured on the regeneration capacity. The best quality regeneration with 'Chandler' was achieved with a formulation of N30K and 1.48 μM BA.

Regenerated shoots needed root induction to grow into plantlets and to establish them into soil. To induce root individual shoots proliferated in regeneration media from the calli developed in different callusing media, were excised and cultured in MS and half MS media with or without different PGR formulations (IBA and NAA). The micro shoots of strawberry inoculated in MS and ½ MS media without plant growth regulators (MS_0 and ½ MS_0) developed roots without developing any callus at their base. Cent percent cultured shoots induced to develop roots when cultured in MS_0 rooting medium within 7-12 days of inoculation. Whereas, 96% shoots were induced root development in ½ MS_0 rooting medium. Addition of auxin in rooting media accentuated rooting but also microcuttings developed callus at their base that hamper their field establishment. Similar results on the rooting and subsequent field establishment were also reported by Boxus (1974), Owen and Miller (1996) and Jimenez-Bermudez and Redondo-Nevado (2002).

Tissue culture generates a wide range of genetic variation in plant species, which can be incorporated in plant breeding programmes. By *in vitro* selection, mutants with useful agronomic traits, e.g. salt or drought tolerance or disease resistance, can be isolated in a short duration. Regenerated plants from calli, cells and protoplasts in

different crops including strawberry (Heinz *et al*. 1977; Larkin and Scowroft 1983; Ramos Leal and Maribona 1991; Kaushal *et al*. 2004) have been shown to exhibit great variability in agronomic traits. This genetic alternation termed somaclonal variation (Larkin and Scowroft 1981), which is being exploited to shorten the time needed to produce new breeding lines with desirable traits. The first commercial sugarcane cv. obtained by biotechnological methods was a somaclonal one improved for sugar content (Maribona *et al*. 1989).

The somaclonal variation may be attributed to either (i) pre-existing variation in the somatic cells of the explant (genetic) or (ii) variation generation during tissue culture (epigenetic). Often both factors may contribute. Factors such as explant source, time of culture, number of sub-cultures, phytohormons, genotype, media composition, the level of ploidy and genetic mosaicism are capable for inducing *in vitro* variability (Silvarolla 1992).

In the present study, somaclonal variation in the form of different morphological and yield contributing characters were observed among *in vitro* callus derived plants. It was observed that regenerated plants grown in the field were not identical to their parent plants. Wide ranges of variations for different morphological and yield contributing characters were noticed among the indirectly regenerated plant populations. Among the different quantitative parameters, plant height, no. of leaves/plant, stolon length, canopy size, days to flowering, average fruit weight (g), fruit weight/plant (g) showed wide range of variations.

Strawberry plants spread vegetatively using runners and this enables them to be easily transplanted and propagated as clones. However, many of the plants lost their capacity to develop runner. Strawberry is perennial herb. The plant body is comprised of a compressed stem known as crown from which leaves, runners, leaves, roots, axillary crown and inflorescence arise. A strawberry plant normally develops a numbers of crowns during their growth. However, many of the plants as observed in the present study did not develop axillary crowns. In addition to these, many of the plants also failed to flowering. Moreover, similar sorts of variations were also recoded among the plants for other characters such as: fruit shape colour, attractiveness and test,

summer overcoming potential and the degree of disease tolerance. Plant off-types, i.e. non true-to-type and genetically not identical to the mother plant, may simply be the result from a change in the genetic make-up of the resulting plants.

It has been recognized that all plants regenerated from tissue culture are not exact replicas of a parental form and exhibit great variability in agronomic traits (Heinz *et al*. 1977; Larkin and Scowcroft 1983; Ramos Leal and Maribona 1991). Heritable genetic variation found in plants regenerated from any type of *in vitro* culture is termed somaclonal variation (Larkin and Scowcroft 1981). In most cases, *in vitro* differentiation is a major cause of genetic variation (Swartz 1991). According to De Klerk (1990), only random variations found in regenerated plants that are transmitted to the progeny through meiosis and are not reversible can be called as somaclonal variation. Such variation in callus regenerated plants has been documented in many plant species for a wide array of characters (Larkin and Scowcroft 1981; Reisch 1983; Vasil 1986; Bajaj 1990).

In the present study the occurrence of novel variations in some the plants were also noticed. These plants were exhibited some traits such as: plant height, no. of leaves/plant, petiole length, no. of stolons/plant, no. of nodes/stolon, stolon length, no. of crowns/plant, canopy size, days to flowering, no. of flower clusters/plant, no. of flowers/plant, no. of fruits/cluster, no. of fruits/plant, days to fruit harvest, average fruit weight, fruit weight/plant, seemed to be superior to mother plants.

Somaclonal variation has been successful in identification of new varieties in sugarcane, sorghum, tomato, wheat, celery, flax and Pelargonium (Skirvin and Janick 1976; Compton and Veilleux 1991; Sears *et al*. 1992; Duncan *et al*. 1995; Karp 1995). Variation in morphological characters among callus regenerated plants were observed in rice for grain size, tiller number, leaf number, maturity (Sun and Zheng 1990), panicle number, seed weight, mature plant height and culm height (Lal and Lal 1990) and in potato for maturation time, shape, size, number and colour of tubers, leaf shape and size and yield (Karp 1990; Lal and Lal 1990). Sood *et al*. (2006) demonstrated that tissue culture derived sugarcane var. CoJ 64 plants attained better height, millable cane height, a greater number of live buds, increased cane yield and sugar recovery % as compared to conventionally propagated sugarcane plants under parallel agronomic

practices in the field. They also reported that high tillering is resulted in thinner canes because thickness of the canes is directly proportional to the number of tillers per clump and is also related to the cytokinin effect. Introduction of *in vitro* culture also allowed production of pathogen free plants and widened the genetic variability of existing banana cultivars, as a result of somaclonal variation (Daniells and Smith 1992, Reuveni *et al*. 1996, Walther *et al*. 1997, Khayat *et al*. 2004). At the same time due to the variation that occurs during the *in vitro* propagation, plants of the same cultivar selected in different locations often differ in same characteristics.

The next step of the present investigation was to study genetic diversity in seven strawberries and their somaclones. Genetic information of the inheritance of the quantitative characters is necessary for the preparation of effective meaningful breeding program in any crop for its improvement. Although reports on the inheritance of quantitative characters of strawberry have been reported by different investigators, more information on this aspect will be helpful for strawberry breeding program in Bangladesh.

In the present study seven strawberry genotypes and their somaclones were evaluated for sixteen characters (eight morphological and eight yield contributing characters). Collected data were analyzed in order to estimate mean with standard error, analysis of variance (ANOVA), least significant difference (LSD) and coefficient of variability.

In the analysis of variance the main item genotype was highly significant for all characters at 5% level of significance. These results indicate that genotypes were significant and genotypically different from each other and justify their inclusion in the present investigation as materials. The replication items were non significant for all characters. Haque (1997) and Hoque (1997) obtained similar results in chickpea.

In the analysis of components of variation it was found that phenotypic variation was greater than genotypic variation. This result was conformity with the findings of Samad (1991), Deb (1994) and Biswas *et al.* (2008). But here error variance is greater than genotypic and phenotypic variance. High value of error variance than genotypic and phenotypic variance indicated that environmental influences were not negligible for the expression of these traits.

In the present study the highest genotypic and phenotypic variation was observed for fruit weight/plant. As yield (fruit weight/plant) is the ultimate goal, the positive association of these characters will help for selecting best genotype. Large genotypic value is always helpful to effective selection. Similar result has also been reported by Mondal (2003) and Aysunnahar (2012).

Phenotypic coefficient of variability (PCV) was greater than genotypical coefficient of variability (GCV). This result is in agreement with the findings of Samad (1991) and Deb (1994).

These results suggested that the greater variability for these traits among the variance was due to environmental causes which are less affected by genetic cause. To create new genetic variability indirect plant regeneration could be open an opportunity for selection of elite clones.

In the second part of this research, field evaluation of the seven strawberry varieties and their somaclones was conducted under biotic and abiotic stress condition. Verticillium wilt, phytophthora crown rot, leaf scotch, leaf spot, leaf blight and botrytis fruit rot disease were found in the strawberry field. Among the six strawberry diseases, disease incidences (%) of verticillium wilt and phytophthora crown rot was high (60%) in seven donor parent's viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival but in somaclones it was 15%. In other diseases viz. leaf scotch, leaf spot, leaf blight and botrytis fruit rot, disease incidence (%) was high (45-50%) in donor plants but in their somaclones it was low (10%). Most of the plants were severally affected with these diseases during the summer mounths and were perished. There were no plants found resistance to fungal diseases. Hot and dry weather and soil pH are major abiotic factors that affect strawberry cultivation. During the summer month April-May temperature becomes high (above 38°C) and the plants do not perpetuate in the field. In terms of summer overcoming capacity, majority of plants were found heat sensitive in donor plants. In their somaclones, 75-80% plants showed moderate summer overcoming capacity and 15-20% plants showed high summer overcomming capacity. Somaclone AOG SC 3 showed better performance than other somaclones and donor parents in terms of summer overcoming capacity. These somaclones can be acceptable commercially if the good characters exhibited are transmitted through generations or could be used in future breeding programme for the improvement of strawberry varieties in Bangladesh.

V. SUMMARY

The main goal of this study was to select and characterize several somaclonal lines in strawberry populations derived from callus cultured regenerated plants which might be useful as a potential source for the development of new strawberry cultivar adaptive to the local agro-climate through the exploitation of novel *in vitro* techniques. The present study was conducted to standardize a suitable protocol of *in vitro* plant regeneration potentiality of seven strawberry varieties viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival and evaluate their field performance under biotic and abiotic stress condition. As a prerequisite to induce somaclonal variation, methods and techniques for optimizing the condition for callus induction and subsequent plant regeneration from the callus tissue were evaluated. Occurrence of somaclonal variation was evaluated by planting the regenerants in the field.

Young leaves, mature leaves and nodes of seven strawberry varieties were used as explants. The nodes and leaf segments were collected from the *in vitro* runner tip derived shoots and cultured in MS medium supplemented with different concentrations of 2, 4-D, or NAA alone or in combination with BA and the cultures were incubated in dark for four weeks for callus induction. The highest degree of percentage of callus was observed in some formulations such as 2.0 mg/l 2, 4-D, 2.0 mg/l NAA and 0.5 mg/l NAA + 1.5 mg/l BA. The medium with 1.5 mg/l BA + 0.5 mg/l NAA showed the best response to indirect shoot regeneration. Mature leaves were favorable for callus induction and shoot regeneration more than young leaves and nodes. After shoot proliferation, the mini regenerated shoots were separated aseptically and cultured in MS medium and ½ MS medium without growth regulators and with different concentrations of NAA, IBA alone. Cent percent cultured shoots induced to develop roots when cultured in MS0 medium. After sufficient root formation, the *in vitro* regenerated plantlets were acclimatized gradually and then successfully acclimatized plantlets were transplanted to the field.

In the present study the occurrence of novel variations in some the plants were noticed. These plants exhibited higher plant height, no. of leaves/plant, petiole length,

no. of stolons/plant, no. of nodes/stolon, stolon length, no. of crowns/plant, canopy size, days to flowering, no. of flower clusters/plant, no. of flowers/plant, no. of fruits/cluster, no. of fruits/plant, days to fruit harvest, average fruit weight, fruit weight/plant than their mother plants.

In the next step of the present investigation was to study genetic diversity in seven strawberries and their somaclones. In the present study seven strawberry genotypes and their somaclones were evaluated for sixteen characters (eight morphological and eight yield contributing characters). Collected data were analyzed in order to estimate mean with standard error, analysis of variance (ANOVA), least significant difference (LSD) and coefficient of variability. In the analysis of variance the main item genotype was highly significant for all characters. In the analysis of components of variation it was found that phenotypic variation was greater than genotypic variation. In the present study the highest genotypic and phenotypic variation was observed for fruit weight/plant. Genetic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were calculated.

In the second part of this research, field evaluation of the seven strawberry varieties and their somaclones was conducted under biotic and abiotic stress condition. Verticillium wilt, phytophthora crown rot, leaf scotch, leaf spot, leaf blight and botrytis fruit rot disease were found in the strawberry field. Among the six strawberry diseases, disease incidences (%) of verticillium wilt and phytophthora crown rot was high (60%) in seven donor parents viz. AOG, JP-2, JP-3, Camarosa, Sweet Charly, Giant Mountain and Festival but in somaclones it was 15%. Most of the plants were severally affected with these diseases during the summer months and were perished. There were no plants found resistant to fungal diseases. In terms of summer overcoming capacity, majority of plants were found heat sensitive in donor plants. In their somaclones, 75- 80% plants showed moderate summer overcoming capacity and 15-20% plants showed high summer overcomming capacity. Somaclone AOG SC 3 showed better performance than other somaclones and donor parents in terms of summer overcoming capacity. These somaclones can be acceptable commercially if the good characters exhibited are transmitted through generations or could be used in future breeding programme for the improvement of strawberry varieties in Bangladesh.

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APPENDICES

APPENDIX 1

COMPOSITION OF MS (MURASHIGE AND SKOOG 1962) BASAL MEDIUM

Components	Concentrations (mg/l)
Macro-nutrients	
KNO_3	1900
NH_4 NO_3	1650
$CaCl_2.2H_2o$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micro-Nutrients	
KI	0.83
H_3BO_4	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$Na_2EDTA.$ $2H_2O$	37.3
$FeSO_4.$ $7H_2O$	27.8
Vitamins and organics	
myo-Inositol	100.0
Nicotinic acid	0.50
Pyridoxine-HCI	0.50
Thaimine-HCI	0.10
Glycine	2.0
Sucrose	30g

pH was adjusted to 5.8 before autoclaving

APPENDIX 2

MODIFIED MUASHIGE AND SKOOG (1/2 MS) MEDIUM

Components	Concentrations (mg/l)
Macro-nutrients	
KNO_3	950.00
NH_4 NO_3	825.00
KH_2PO_4	85.00
$CaCl_2.2H_2o$	220.00
$MgSO_4.7H_2O$	185.00
Micro-Nutrients	
$FeSO_4$. $7H_2O$	13.90
Na_2EDTA	18.65
$MnSO_4.4H_2O$	11.15
H_3BO_4	3.10
$ZnSO_4.7H_2O$	4.30
KI	0.83
$Na_2MoO_4.2H_2O$	1.125
$CuSO_4.5H_2O$	0.0125
$CoCl_2.6H_2O$	0.0125
Vitamins and organics	
Glycine	2.0
Nicotinic acid	0.50
Pyridoxine-HCI	0.50
Thaimine-HCI	0.10
Inositol	100.0
Sucrose	30g
pH was adjusted to 5.7/5.8 before autoclaving	

APPENDIX 3

MODIFIED MUASHIGE AND SKOOG (MMS_1) MEDIUM

Components	Concentrations (mg/l)
Macro-nutrients	
KNO_3	950.00
NH_4 NO_3	825.00
KH_2PO_4	85.00
$CaCl_2.2H_2o$	220.00
$MgSO_4.7H_2O$	185.00
Micro-Nutrients	
$FeSO_4$. $7H_2O$	27.80
Na_2EDTA	37.30
$MnSO_4.4H_2O$	22.30
H_3BO_4	6.20
$ZnSO_4.7H_2O$	8.60
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Vitamins and organics	
Glycine	2.00
Nicotinic acid	0.50
Pyridoxine-HCI	0.50
Thaimine-HCI	0.10
Inositol	100.00
Sucrose	30,000.00
pH was adjusted to 5.7/5.8 before autoclaving	

APPENDIX 4

MODIFIED MUASHIGE AND SKOOG (MMS$_2$) MEDIUM

Components	Concentrations (mg/l)
Macro-nutrients	
KNO_3	1900.00
NH_4 NO_3	1650.00
KH_2PO_4	170.00
$CaCl_2.2H_2o$	440.00
$MgSO_4.7H_2O$	370.00
Micro-Nutrients	
$FeSO_4$. $7H_2O$	13.90
Na_2EDTA	18.65
$MnSO_4.4H_2O$	11.15
H_3BO_4	3.10
$ZnSO_4.7H_2O$	4.30
KI	0.415
$Na_2MoO_4.2H_2O$	0.125
$CuSO_4.5H_2O$	0.0125
$CoCl_2.6H_2O$	0.0125
Vitamins and organics	
Glycine	2.00
Nicotinic acid	0.05
Pyridoxine-HCI	0.05
Thaimine-HCI	0.10
Inositol	100.00
Sucrose	30,000.00
pH was adjusted to 5.7/5.8 before autoclaving	

APPENDIX 5

COMPOSITION OF HOAGLAND SOLUTION

Composition	Stock solution (g)	Use
1. $MgSO_4$. $7H_2O$	246 g/l	1.0 ml/l
2. $Ca(NO_3)_2$. $4H_2O$	236 g/l	2.3 ml/l
3. KH_2PO_4	136 g/l	0.5 m/l
4. KNO_3	101 g/l	2.5 ml/l
5. Micronotrients	Micronutrient solution (see below)	0.5 ml/l
6. Fe.EDTA	Fe-EDTA solution (added last, see blew)	10 ml/l

Adjust the pH to 5.8 with NaOH or HCL

Preparation of micronutrient solution

Addition	Stock solution
1. H_3BO_3	2.86 g/l
2. $MnCl_2$. $4H_2O$	1.82 g/l
3. $ZnSO_4$. $7H_2O$	0.22 g/l
4. Na_2MoO_4. $2H_2O$	0.09 g/l
5. $CuSO_4$. $5H_2O$	0.09 g/l

Preparation of Fe-EDTA solution

Addition	Stock solution
1. $FeCl_3.6H_2O$	0.968 g/l
2. EDTA	3.8 g/l

APPENDIX 6. ANOVA for plant height of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	2.14	1.06866	0.007801 (NS)
Var.	4	547.98	136.996	732.5989*
Error	8	1.50	0.187	
Total	14	551.62	39.4012	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 7. ANOVA for plant height of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.93	0.463333	0.007613 (NS)
Var.	2	121.73	60.86333	2282.375*
Error	4	0.11	0.026667	
Total	8	122.76	15.345	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 8. ANOVA for plant height of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.90	0.447778	0.007291 (NS)
Var.	2	122.83	61.41444	6502.706*
Error	4	0.04	0.009444	
Total	8	123.76	15.47028	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 9. ANOVA for plant height of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.02	0.007778	0.0001127 (NS)
Var.	2	132.39	66.19444	504.8729*
Error	4	0.52	0.131111	
Total	8	132.93	16.61611	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 10. ANOVA for plant height of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.14	0.07	0.001106 (NS)
Var.	2	126.54	63.27	383.4545*
Error	4	0.66	0.165	
Total	8	127.34	15.9175	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 11. ANOVA for plant height of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.26	0.13	0.003929 (NS)
Var.	2	66.17	33.08333	215.7609*
Error	4	0.61	0.153333	
Total	8	122.76	15.345	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 12. ANOVA for plant height of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.89	0.443333	0.005875 (NS)
Var.	2	150.93	75.46333	497.5604*
Error	4	0.61	0.151667	
Total	8	152.42	19.0525	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 13. ANOVA for no. of leaves/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.16	0.078	0.001431 (NS)
Var.	4	218.07	54.51733	821.8693*
Error	8	0.53	0.066333	
Total	14	218.76	15.62543	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 14. ANOVA for no. of leaves/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.68	0.337778	0.024982 (NS)
Var.	2	27.04	13.52111	104.4549*
Error	4	0.52	0.129444	
Total	8	28.24	3.529444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 15. ANOVA for no. of leaves/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.23	0.114444	0.008203 (NS)
Var.	2	27.90	13.95111	107.7768*
Error	4	0.52	0.129444	
Total	8	28.65	3.581111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 16. ANOVA for no. of leaves/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.06	0.031111	0.002547 (NS)
Var.	2	24.43	12.21444	85.54864*
Error	4	0.57	0.142778	
Total	8	25.06	3.132778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 17. ANOVA for no. of leaves/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.56	0.28	0.0139 (NS)
Var.	2	40.29	20.14333	710.9412*
Error	4	0.11	0.028333	
Total	8	40.96	5.12	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 18. ANOVA for no. of leaves/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.04	0.017778	0.001127 (NS)
Var.	2	31.56	15.78111	194.5616*
Error	4	0.32	0.081111	
Total	8	31.92	3.990278	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 19. ANOVA for no. of leaves/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.08	0.04	0.000961 (NS)
Var.	2	83.29	41.64333	471.434*
Error	4	0.35	0.088333	
Total	8	83.72	10.465	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 20. ANOVA for petiole length of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.03	0.014	0.003906 (NS)
Var.	4	14.34	3.584333	28.14921*
Error	8	1.02	0.127333	
Total	14	15.38	1.098857	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 21. ANOVA for petiole length of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.11	0.054444	0.018428 (NS)
Var.	2	5.91	2.954444	23.12174*
Error	4	0.51	0.127778	
Total	8	6.53	0.816111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 22. ANOVA for petiole length of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.13	0.063333	0.090047 (NS)
Var.	2	1.41	0.703333	10.55*
Error	4	0.27	0.066667	
Total	8	1.80	0.225	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 23. ANOVA for petiole length of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.06	0.027778	0.029727 (NS)
Var.	2	1.87	0.934444	11.06579*
Error	4	0.34	0.084444	
Total	8	2.26	0.282778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 24. ANOVA for petiole length of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.07	0.034444	0.040629 (NS)
Var.	2	1.70	0.847778	10.45205*
Error	4	0.32	0.081111	
Total	8	2.09	0.261111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 25. ANOVA for petiole length of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.36	0.181111	0.105638 (NS)
Var.	2	3.43	1.714444	17.83815*
Error	4	0.38	0.096111	
Total	8	4.18	0.521944	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 26. ANOVA for petiole length of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.07	0.034444	0.008905 (NS)
Var.	2	7.74	3.867778	45.80263*
Error	4	0.34	0.084444	
Total	8	8.14	1.017778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 27. ANOVA for no. of stolons/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.26	0.130667	0.008111 (NS)
Var.	4	64.44	16.109	78.00969*
Error	8	1.65	0.2065	
Total	14	66.35	4.739238	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 28. ANOVA for no. of stolons/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.12	0.057778	0.030075 (NS)
Var.	2	3.84	1.921111	91*
Error	4	0.08	0.021111	
Total	8	4.04	0.505278	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 29. ANOVA for no. of stolons/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.04	0.021111	0.006595 (NS)
Var.	2	6.40	3.201111	77.86486*
Error	4	0.16	0.041111	
Total	8	6.61	0.826111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 30. ANOVA for no. of stolons/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.06	0.027778	0.179856 (NS)
Var.	2	0.31	0.154444	4.964286*
Error	4	0.12	0.031111	
Total	8	0.49	0.061111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 31. ANOVA for no. of stolons/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.06	0.03	0.012802(NS)
Var.	2	4.69	2.343333	100.4286*
Error	4	0.09	0.023333	
Total	8	4.84	0.605	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 32. ANOVA for no. of stolons/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.02	0.007778	0.001625 (NS)
Var.	2	9.58	4.787778	269.3125*
Error	4	0.07	0.017778	
Total	8	9.66	1.207778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 33. ANOVA for no. of stolons/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.03	0.013333	0.006369 (NS)
Var.	2	4.19	2.093333	66.10526*
Error	4	0.13	0.031667	
Total	8	4.34	0.5425	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 34. ANOVA for no. of nodes/stolon of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.20	0.098	0.05062 (NS)
Var.	4	7.74	1.936	26.52055*
Error	8	0.58	0.073	
Total	14	8.52	0.608857	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 35. ANOVA for no. of nodes/stolon of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.17	0.084444	0.125206 (NS)
Var.	2	1.35	0.674444	151.75*
Error	4	0.02	0.004444	
Total	8	1.54	0.191944	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 36. ANOVA for no. of nodes/stolon of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.17	0.083333	0.030525 (NS)
Var.	2	5.46	2.73	819*
Error	4	0.01	0.003333	
Total	8	5.64	0.705	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 37. ANOVA for no. of nodes/stolon of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.05	0.023333	0.044872 (NS)
Var.	2	1.04	0.52	15.6*
Error	4	0.13	0.033333	
Total	8	1.22	0.1525	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 38. ANOVA for no. of nodes/stolon of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.03	0.013333	0.0064 (NS)
Var.	2	4.17	2.083333	56.81818*
Error	4	0.15	0.036667	
Total	8	4.34	0.5425	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 39. ANOVA for no. of nodes/stolon of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.08	0.041111	0.087886 (NS)
Var.	2	0.94	0.467778	168.4*
Error	4	0.01	0.002778	
Total	8	1.03	0.128611	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 40. ANOVA for no. of nodes/stolon of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.01	0.004444	0.007313 (NS)
Var.	2	1.22	0.607778	31.25714*
Error	4	0.08	0.019444	
Total	8	1.30	0.162778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 41. ANOVA for stolon length of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	230.19	115.094	0.12527 (NS)
Var.	4	3675.06	918.765	7.957672*
Error	8	923.65	115.4565	
Total	14	4828.90	344.9214	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 42. ANOVA for stolon length of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	14.95	7.474444	0.005429 (NS)
Var.	2	2753.62	1376.808	135.1431*
Error	4	40.75	10.18778	
Total	8	2809.32	351.1644	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 43. ANOVA for stolon length of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.34	0.667778	0.000275 (NS)
Var.	2	4848.08	2424.041	9166.542*
Error	4	1.06	0.264444	
Total	8	4850.48	606.3094	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 44. ANOVA for stolon length of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	23.03	11.51444	0.010774 (NS)
Var.	2	2137.48	1068.741	93.10042*
Error	4	45.92	11.47944	
Total	8	2206.43	275.8036	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 45. ANOVA for stolon length of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	62.04	31.01778	0.016924 (NS)
Var.	2	3665.50	1832.748	67.99007*
Error	4	107.82	26.95611	
Total	8	3835.36	479.4194	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 46. ANOVA for stolon length of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	25.08	12.53778	0.009005 (NS)
Var.	2	2784.75	1392.374	111.3801*
Error	4	50.00	12.50111	
Total	8	2859.83	357.4786	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 47. ANOVA for stolon length of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	22.20	11.10111	0.007693 (NS)
Var.	2	2886.16	1443.081	124.0886*
Error	4	46.52	11.62944	
Total	8	2954.88	369.3603	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 48. ANOVA for no. of crowns/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.02	0.008	0.001043 (NS)
Var.	4	30.68	7.671	730.5714*
Error	8	0.08	0.0105	
Total	14	30.78	2.198857	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 49. ANOVA for no. of crowns/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.05	0.023333	0.002942 (NS)
Var.	2	15.86	7.93	206.8696*
Error	4	0.15	0.038333	
Total	8	16.06	2.0075	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 50. ANOVA for no. of crowns/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.16	0.081111	0.008078 (NS)
Var.	2	20.08	10.04111	129.1*
Error	4	0.31	0.077778	
Total	8	20.56	2.569444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 51. ANOVA for no. of crowns/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.04	0.017778	0.001506 (NS)
Var.	2	23.60	11.80111	923.5652*
Error	4	0.05	0.012778	
Total	8	23.69	2.961111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 52. ANOVA for no. of crowns/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.11	0.054444	0.004942 (NS)
Var.	2	22.04	11.01778	319.871*
Error	4	0.14	0.034444	
Total	8	22.28	2.785278	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 53. ANOVA for no. of crowns/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.02	0.01	0.001387 (NS)
Var.	2	14.42	7.21	288.4*
Error	4	0.10	0.025	
Total	8	14.54	1.8175	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 54. ANOVA for no. of crowns/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.03	0.014444	0.001377 (NS)
Var.	2	20.98	10.48778	192.6327*
Error	4	0.22	0.054444	
Total	8	21.22	2.652778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 55. ANOVA for canopy size of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	101.20	50.598	0.001939 (NS)
Var.	4	104387.82	26096.95	693.6283*
Error	8	300.99	37.62383	
Total	14	104790.00	7485	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 56. ANOVA for canopy size of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	3.62	1.807778	0.000267 (NS)
Var.	2	13535.34	6767.668	4621.321*
Error	4	5.86	1.464444	
Total	8	13544.81	1693.101	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 57. ANOVA for canopy size of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	25.25	12.62333	0.002303 (NS)
Var.	2	10961.05	5480.523	533.0384*
Error	4	41.13	10.28167	
Total	8	11027.42	1378.427	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 58. ANOVA for canopy size of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	8.70	4.347778	0.00038 (NS)
Var.	2	22890.95	11445.47	1940.642*
Error	4	23.59	5.897778	
Total	8	22923.24	2865.404	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 59. ANOVA for canopy size of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	15.14	7.57	0.000502 (NS)
Var.	2	30132.85	15066.42	1465.13*
Error	4	41.13	10.28333	
Total	8	30189.12	3773.64	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 60. ANOVA for canopy size of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	4.41	2.204444	0.000192 (NS)
Var.	2	22928.00	11464	7296.748*
Error	4	6.28	1.571111	
Total	8	22938.70	2867.337	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 61. ANOVA for canopy size of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	39.23	19.61444	0.001892 (NS)
Var.	2	20735.10	10367.55	532.8685*
Error	4	77.82	19.45611	
Total	8	20852.15	2606.519	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 62. ANOVA for days to flowering of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.40	0.2	0.007722 (NS)
Var.	4	103.60	25.9	129.5*
Error	8	1.60	0.2	
Total	14	105.60	7.542857	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 63. ANOVA for days to flowering of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.001664 (NS)
Var.	2	133.56	66.77778	150.25*
Error	4	1.78	0.444444	
Total	8	135.56	16.94444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 64. ANOVA for days to flowering of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.0016 (NS)
Var.	2	138.89	69.44444	250*
Error	4	1.11	0.277778	
Total	8	140.22	17.52778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 65. ANOVA for days to flowering of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.56	0.777778	0.010309 (NS)
Var.	2	150.89	75.44444	271.6*
Error	4	1.11	0.277778	
Total	8	153.56	19.19444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 66. ANOVA for days to flowering of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	2.00	1	0.01 (NS)
Var.	2	200.00	100	25*
Error	4	16.00	4	
Total	8	218.00	27.25	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 67. ANOVA for days to flowering of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.004608 (NS)
Var.	2	144.67	72.33333	434*
Error	4	0.67	0.166667	
Total	8	146.00	18.25	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 68. ANOVA for days to flowering of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.000641 (NS)
Var.	2	346.89	173.4444	156.1*
Error	4	4.44	1.111111	
Total	8	351.56	43.94444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 69. ANOVA for no. of flower clusters/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.53	0.266667	0.091954 (NS)
Var.	4	11.60	2.9	8.285714*
Error	8	2.80	0.35	
Total	14	14.93	1.066667	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 70. ANOVA for no. of flower clusters/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.111111 (NS)
Var.	2	6.00	3	9*
Error	4	1.33	0.333333	
Total	8	8.00	1	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 71. ANOVA for no. of flower clusters/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.89	0.444444	0.210526 (NS)
Var.	2	4.22	2.111111	19*
Error	4	0.44	0.111111	
Total	8	5.56	0.694444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 72. ANOVA for no. of flower clusters/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.56	0.777778	0.538462 (NS)
Var.	2	2.89	1.444444	13*
Error	4	0.44	0.111111	
Total	8	4.89	0.611111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 73. ANOVA for no. of flower clusters/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	2.89	1.444444	0.684211 (NS)
Var.	2	4.22	2.111111	19*
Error	4	0.44	0.111111	
Total	8	7.56	0.944444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 74. ANOVA for no. of flower clusters/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.035714 (NS)
Var.	2	6.22	3.111111	7*
Error	4	1.78	0.444444	
Total	8	8.22	1.027778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 75. ANOVA for no. of flower clusters/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.142857 (NS)
Var.	2	4.67	2.333333	3.5*
Error	4	2.67	0.666667	
Total	8	8.00	1	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 76. ANOVA for no. of flowers/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.53	0.266667	0.018519 (NS)
Var.	4	57.60	14.4	41.14286*
Error	8	2.80	0.35	
Total	14	60.93	4.352381	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 77. ANOVA for no. of flowers/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.007194 (NS)
Var.	2	30.89	15.44444	34.75*
Error	4	1.78	0.444444	
Total	8	32.89	4.111111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 78. ANOVA for no. of flowers/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.007194 (NS)
Var.	2	30.89	15.44444	34.75*
Error	4	1.78	0.444444	
Total	8	32.89	4.111111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 79. ANOVA for no. of flowers/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.023256 (NS)
Var.	2	9.56	4.777778	10.75*
Error	4	1.78	0.444444	
Total	8	11.56	1.444444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 80. ANOVA for no. of flowers/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.047619 (NS)
Var.	2	14.00	7	21*
Error	4	1.33	0.333333	
Total	8	16.00	2	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 81. ANOVA for no. of flowers/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.016393 (NS)
Var.	2	13.56	6.777778	8.714286*
Error	4	3.11	0.777778	
Total	8	16.89	2.111111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 82. ANOVA for no. of flowers/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.56	0.777778	0.09589 (NS)
Var.	2	16.22	8.111111	10.42857*
Error	4	3.11	0.777778	
Total	8	20.89	2.611111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 83. ANOVA for no. of fruits/cluster of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.20	0.6	0.110429 (NS)
Var.	4	21.73	5.433333	12.53846*
Error	8	3.47	0.433333	
Total	14	26.40	1.885714	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 84. ANOVA for no. of fruits/cluster of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.032258 (NS)
Var.	2	6.89	3.444444	7.75*
Error	4	1.78	0.444444	
Total	8	8.89	1.111111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 85. ANOVA for no. of fruits/cluster of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.008065 (NS)
Var.	2	27.56	13.77778	22.54545*
Error	4	2.44	0.611111	
Total	8	30.22	3.777778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 86. ANOVA for no. of fruits/cluster of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.006135 (NS)
Var.	2	36.22	18.11111	163*
Error	4	0.44	0.111111	
Total	8	36.89	4.611111	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 87. ANOVA for no. of fruits/cluster of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.142857 (NS)
Var.	2	1.56	0.777778	7*
Error	4	0.44	0.111111	
Total	8	2.22	0.277778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 88. ANOVA for no. of fruits/cluster of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.142857 (NS)
Var.	2	1.56	0.777778	7*
Error	4	0.44	0.111111	
Total	8	2.22	0.277778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 89. ANOVA for no. of fruits/cluster of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.142857 (NS)
Var.	2	1.56	0.777778	7*
Error	4	0.44	0.111111	
Total	8	2.22	0.277778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 90. ANOVA for no. of fruits/plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.40	0.2	0.00428 (NS)
Var.	4	186.93	46.73333	87.625*
Error	8	4.27	0.533333	
Total	14	191.60	13.68571	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 91. ANOVA for no. of fruits/plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.89	0.444444	0.008214 (NS)
Var.	2	108.22	54.11111	194.8*
Error	4	1.11	0.277778	
Total	8	110.22	13.77778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 92. ANOVA for no. of fruits/plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.56	0.777778	0.013233 (NS)
Var.	2	117.56	58.77778	529*
Error	4	0.44	0.111111	
Total	8	119.56	14.94444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 93. ANOVA for no. of fruits/plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.001664 (NS)
Var.	2	133.56	66.77778	150.25*
Error	4	1.78	0.444444	
Total	8	135.56	16.94444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 94. ANOVA for no. of fruits/plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.005181 (NS)
Var.	2	128.67	64.33333	386*
Error	4	0.67	0.166667	
Total	8	130.00	16.25	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 95. ANOVA for no. of fruits/plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.001422 (NS)
Var.	2	156.22	78.11111	175.75*
Error	4	1.78	0.444444	
Total	8	158.22	19.77778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 96. ANOVA for no. of fruits/plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.67	0.333333	0.005848 (NS)
Var.	2	114.00	57	171*
Error	4	1.33	0.333333	
Total	8	116.00	14.5	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 97. ANOVA for days to fruit harvest of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.13	0.066667	0.001951 (NS)
Var.	4	136.67	34.16667	107.8947*
Error	8	2.53	0.316667	
Total	14	139.33	9.952381	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 98. ANOVA for days to fruit harvest of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.00346 (NS)
Var.	2	64.22	32.11111	72.25*
Error	4	1.78	0.444444	
Total	8	66.22	8.277778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 99. ANOVA for days to fruit harvest of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	6.00	3	0.041096 (NS)
Var.	2	146.00	73	73*
Error	4	4.00	1	
Total	8	156.00	19.5	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 100. ANOVA for days to fruit harvest of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.003425 (NS)
Var.	2	64.89	32.44444	41.71429*
Error	4	3.11	0.777778	
Total	8	68.22	8.527778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 101. ANOVA for days to fruit harvest of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.003257 (NS)
Var.	2	68.22	34.11111	76.75*
Error	4	1.78	0.444444	
Total	8	70.22	8.777778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 102. ANOVA for days to fruit harvest of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.003257 (NS)
Var.	2	68.22	34.11111	76.75*
Error	4	1.78	0.444444	
Total	8	70.22	8.777778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 103. ANOVA for days to fruit harvest of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.22	0.111111	0.00346 (NS)
Var.	2	64.22	32.11111	72.25*
Error	4	1.78	0.444444	
Total	8	66.22	8.277778	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 104. ANOVA for average fruit weight of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.44	0.218	0.003279 (NS)
Var.	4	265.96	66.489	1461.297*
Error	8	0.36	0.0455	
Total	14	266.76	19.054	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 105. ANOVA for average fruit weight of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	12.17	6.084444	0.049958 (NS)
Var.	2	243.58	121.7911	57.9651*
Error	4	8.40	2.101111	
Total	8	264.16	33.01944	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 106. ANOVA for average fruit weight of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	2.06	1.027778	0.011161 (NS)
Var.	2	184.18	92.08778	398.4567*
Error	4	0.92	0.231111	
Total	8	187.16	23.39444	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 107. ANOVA for average fruit weight of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	4.60	2.301111	0.023441 (NS)
Var.	2	196.33	98.16444	38.68126*
Error	4	10.15	2.537778	
Total	8	211.08	26.38528	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 108. ANOVA for average fruit weight of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	1.15	0.573333	0.007569 (NS)
Var.	2	151.49	75.74333	61.74728*
Error	4	4.91	1.226667	
Total	8	157.54	19.6925	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 109. ANOVA for average fruit weight of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.35	0.173333	0.002114 (NS)
Var.	2	164.01	82.00333	3784.769*
Error	4	0.09	0.021667	
Total	8	164.44	20.555	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 110. ANOVA for average fruit weight of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	0.10	0.047778	0.000503 (NS)
Var.	2	190.04	95.02111	57.5498*
Error	4	6.60	1.651111	
Total	8	196.74	24.59278	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 111. ANOVA for fruit wt./plant of variety AOG and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	124.57	62.286	0.00058 (NS)
Var.	4	429621.58	107405.4	341.2338*
Error	8	2518.05	314.756	
Total	14	432264.20	30876.01	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 112. ANOVA for fruit wt./plant of variety JP-2 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	276.18	138.0878	0.001423 (NS)
Var.	2	194118.84	97059.42	1322.856*
Error	4	293.48	73.37111	
Total	8	194688.50	24336.06	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 113. ANOVA for fruit wt./plant of variety JP-3 and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	35.67	17.83444	0.000159 (NS)
Var.	2	224888.76	112444.4	7379.316*
Error	4	60.95	15.23778	
Total	8	224985.38	28123.17	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 114. ANOVA for fruit wt./plant of variety Camarosa and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	66.00	33.00111	0.000367 (NS)
Var.	2	179987.75	89993.87	2511.496*
Error	4	143.33	35.83278	
Total	8	180197.08	22524.64	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 115. ANOVA for fruit wt./plant of variety Sweet Charly and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	26.72	13.36111	0.000175 (NS)
Var.	2	153077.39	76538.69	10056.18*
Error	4	30.44	7.611111	
Total	8	153134.56	19141.82	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 116. ANOVA for fruit wt./plant of variety Giant Mountain and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	36.14	18.07	0.000169 (NS)
Var.	2	213804.01	106902	6811.937*
Error	4	62.77	15.69333	
Total	8	213902.92	26737.87	

* = Significant at 5% level of probability and NS = Non significant

APPENDIX 117. ANOVA for fruit wt./plant of variety Festival and their somaclones.

Source of variation	Df	SS	MS	*F*
Rep.	2	65.63	32.81444	0.000367 (NS)
Var.	2	178816.90	89408.45	2344.969*
Error	4	152.51	38.12778	
Total	8	179035.04	22379.38	

* = Significant at 5% level of probability and NS = Non significant