STUDIES ON *IN VITRO* PROPAGATION OF PROMISING GRAPE (*Vitis vinifera* L.) ROOTSTOCKS

Thesis

Submitted to the Punjab Agricultural University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
In
FRUIT SCIENCE
(Minor Subject: Biotechnology)

By

Avtar Singh
(L-2012-A-66-M)

Department of Fruit Science
College of Agriculture
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LUDHIANA-141004

2014

CERTIFICATE I

This is to certify that the thesis entitled, **"Studies on *in vitro* Propagation of Promising Grape (*Vitis vinifera* L.) Rootstocks"** submitted for the degree of **M.Sc.** in the subject of **Fruit Science** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Avtar Singh (L-2012-A-66-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. M.I.S. Gill)
Senior Horticulturist - cum- Head
Deptt. of Fruit Science
Punjab Agricultural University
Ludhiana

CERTIFICATE II

This is to certify that the thesis entitled, **"Studies on *in vitro* Propagation of Promising Grape (*Vitis vinifera* L.) Rootstocks"** submitted by **Avtar Singh (L-2012-A-66-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of **M.Sc.** in the subject of **Fruit Science** (Minor Subject: **Biotechnology**) has been approved by the Student's Advisory Committee along with the Head of Department after an oral examination of the same.

____

(Dr. M.I.S. Gill)
Major Advisor

____

(Dr. J.S. Randhawa)
Head (Retired)
Deptt. of Fruit Science
Punjab Agricultural University
Ludhiana-141004.

____

(Dr. M.I.S. Gill)
Head of the Department

____

(Dr. Gursharan Singh)
Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

First of all, I offer my humble thanks with folded hands and bowed head to ***"AKAL PURKH"*** *the* ***ALMIGHTY*** *by whose kindness I have been able to clear another chapter of my life.*

I am highly grateful to my major advisor, ***Dr. M.I.S. Gill,*** *Senior Horticulturist – cum – Head, Department of Fruit Science, Punjab Agricultural University, Ludhiana for his motivation, ingenious guidance, encouragement and enlightened supervision from time to time throughout the course of my research. He has been generous in lending his intellectual idea, inculcating in me the spirit of an independent research worker and training my mind to think systematically and logically. His friendly attitude of guidance will always be acknowledged.*

I pay my sincere thanks to all the members of my advisory committee, namely ***Dr. N.K. Arora*** *(Assistant Horticulturist) Department of Fruit Science,* ***Dr. Gurupkar Singh*** *(Assistant Horticulturist) School of Agricultural Biotechnology and* ***Dr. H.S. Rattanpal*** *(Dean PG's Nominee) Department of Fruit Science for their keen interest and helpful suggestions during the course of studies.*

I duly acknowledge the help rendered by ***Dr M. I. S. Gill****, Head, Department of Fruit Science, Punjab Agricultural University, Ludhiana, for providing guidance and necessary research facilities. My sincere thanks are also to the faculty of the Department of Fruit Science for their encouragement and cooperation.*

I find no lexis complementary to acknowledge my loving father ***Sh. Darshan Singh*** *and mother* ***Smt. Shinder Kaur,*** *my pillars of support, source of strength and inspiration, for their love, blessings, enduring patience and care that brought me here up to. I dedicate this golden chapter of my life to my parents.*

I am also thankful to the farm, laboratory and other staff of the department of Vegetable Science for their cooperation in the conduct of these studies.

I proudly and profoundly appreciate the support of my friends ***Onkar, Sarvpriya, Dalvinder, Sukhsagar, Simran, Harman, Jaspreet*** *and* ***Sukhpreet*** *for being ever prepared to accomplish myriad of tasks I asked for and rallying around me in the hour of need.*

I feel proud to be a part of ***PAU****, Ludhiana where I learnt a lot and spent some unforgettable moments of my life.*

Needless to say, errors and omissions if any are all mine.

Place…………………….

Date……………………..

(Avtar Singh)

Title of the Thesis	**:**	Studies on *in vitro* propagation of promising grape (*Vitis Vinifera* L.) rootstocks
Name of the Student and Admission No.	**:**	Avtar Singh (L-2012-A-66-M)
Major Subject	**:**	Fruit Science
Minor Subject	**:**	Biotechnology
Name and Designation of Major Advisor	**:**	Dr. M.I.S. Gill Senior Horticulturist-cum-Head
Degree to be Awarded	**:**	M. Sc.
Year of award of degree	**:**	2014
Total pages in thesis	**:**	42 + VITA
Name of the University	**:**	Punjab Agricultural University, Ludhiana – 141 004, Punjab, India

ABSTRACT

The present investigation entitled "Studies on *in vitro* propagation of promising grape (*Vitis Vinifera* L.) rootstocks" was carried out in the Tissue Culture Laboratory of the Department of Fruit Science, Punjab Agricultural University, Ludhiana during 2013-2014. The MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l) shows maximum establishment 45.54 % and 40.80 % from nodal segments and shoot tips respectively. Rootstock R 110 and 1613 C rootstock exhibited the maximum nodal segment and shoot tip establishment 47.37 % and 35.42 % respectively. The maximum shoot regeneration (65.67 %) was recorded in MS medium supplemented with BAP (1.0 mg/l) and Kinetin (1.0 mg/l). Degrasset rootstock gave the maximum (52.89 %) shoot regeneration. Maximum number of shoots (3.63) per explant were produced at BAP (1.5 mg/l). Maximum shoot length (3.13 cm) was recorded on MS supplemented with BAP (0.5 mg/l) and Kinetin (0.5 mg/l) medium. The maximum shoot length (1.73 cm) was recorded in 1613 C rootstock. Maximum rooting was (64.95 %) recorded with MS media fortified with IBA (0.1 mg/l) and NAA (0.1 mg/l). Maximum rooting (55.64 %) was recorded in Degrasset rootstock. Maximum number of roots (13.75) was recorded in R 110 rootstock on IBA (0.01 mg/l) and NAA (0.01 mg/l). Maximum length of roots (5.66 cm) was recorded in rootstock Degrasset cultured on MS medium supplemented with IBA (0.1 mg/l). The 2:1:1 ratio of peat perlite and vermiculite and Degrasset rootstock is the best rootstock for plantlet establishment under *ex vitro* conditions.

Keywords: MS Medium, BAP, Kinetin, NAA, IBA, Rootstock.

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Signature of Major Advisor	**Signature of the Student**

ਖੋਜ ਗ੍ਰੰਥ ਦਾ ਸਿਰਲੇਖ	:	ਅੰਗੂਰ (*ਵਿਟਿਸ ਵਿਨੀਫੇਰਾ* ਐਲ.) ਦੇ ਸੰਭਾਵਤ ਜੜ੍ਹ ਮੁੱਢਾਂ ਦੀ ਇੰਨ ਵਿਟਰੋ ਵੰਸ਼ ਵਾਧੇ ਦਾ ਮੁਲਾਂਕਣ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਅਵਤਾਰ ਸਿੰਘ (ਐੱਲ-2012-ਏ-66-ਐੱਮ)
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਫ਼ਲ ਵਿਗਿਆਨ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਬਾਇਓਤਕਨੋਲੋਜੀ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁੱਦਾ	:	ਡਾ. ਐੱਮ.ਆਈ.ਐੱਸ. ਗਿੱਲ ਸੀਨੀਅਰ ਹੋਰਟੀਕਲਚਰਿਸਟ-ਕਮ-ਮੁੱਖੀ
ਡਿਗਰੀ	:	ਐਮ.ਐਸ.ਸੀ.
ਡਿਗਰੀ ਨਾਲ ਸਨਮਾਨਿਤ ਕਰਨ ਦਾ ਸਾਲ	:	2014
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	42 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ – 141004, ਪੰਜਾਬ, ਭਾਰਤ ।

ਸਾਰ

ਮੌਜੂਦਾ ਅਧਿਐਨ "ਅੰਗੂਰ (*ਵਿਟਿਸ ਵਿਨੀਫੇਰਾ* ਐਲ.) ਦੇ ਸੰਭਾਵਤ ਜੜ੍ਹ ਮੁੱਢਾਂ ਦੀ ਇੰਨ ਵਿਟਰੋ ਵੰਸ਼ ਵਾਧੇ ਦਾ ਮੁਲਾਂਕਣ" ਸਿਰਲੇਖ ਅਧੀਨ ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ ਲੁਧਿਆਣਾ ਦੇ ਫ਼ਲ ਵਿਗਿਆਨ ਵਿਭਾਗ ਦੀ ਟੀਸ਼ੂ ਕਲਚਰ ਪ੍ਰਯੋਗਸ਼ਾਲਾ ਵਿਖੇ ਸੰਨ 2013-14 ਦੌਰਾਨ ਕੀਤਾ ਗਿਆ। ਬੀ.ਏ.ਪੀ. (1.0 ਮਿ.ਗਾ./ਲਿ.) + ਕਾਇਨੇਟਿਨ (1.0 ਮਿ.ਗ੍ਰਾ./ਲਿ.) ਵਾਲੇ ਐਮ.ਐਸ. ਮੀਡੀਅਮ ਉਪਰ ਪੌਦੇ ਦੀਆਂ ਗੰਢਾਂ ਦੇ ਭਾਗ ਅਤੇ ਸ਼ਾਖਾਵਾਂ ਦੇ ਸਿਰੇ ਸਭ ਤੋਂ ਵਧੇਰੇ ਕ੍ਰਮਵਾਰ 45.54% ਅਤ 40.80% ਪਾਏ ਗਏ। ਜੜ੍ਹ ਮੁੱਢ ਆਰ 110 ਅਤੇ 1613 ਸੀ ਵਿੱਚ ਪੌਦੇ ਦੀਆਂ ਗੰਢਾਂ ਦੇ ਭਾਗ ਅਤੇ ਸ਼ਾਖਾਵਾਂ ਦੇ ਸਿਰਿਆਂ ਦੀ ਸਥਾਪਨਾ ਸਭ ਤੋਂ ਵਧੇਰੇ ਪਾਈ ਗਈ ਜੋ ਕਿ ਕ੍ਰਮਵਾਰ 47.37% ਅਤੇ 35.42% ਸੀ। ਬੀ.ਏ.ਪੀ. (1.0 ਮਿ.ਗਾ./ਲਿ.) + ਕਾਇਨੇਟਿਨ (1.0 ਮਿ.ਗ੍ਰਾ./ਲਿ.) ਵਾਲੇ ਐਮ.ਐਸ. ਮੀਡੀਅਮ ਵਿੱਚ ਸਭ ਤੋਂ ਵਧੇਰੇ (65.67%) ਸ਼ਾਖਾਵਾਂ ਉਤਪੰਨ ਹੋਈਆਂ। ਜੜ੍ਹ ਮੁੱਢ ਡੀ-ਗ੍ਰਾਸੈਟ ਵਿੱਚ ਸਭ ਤੋਂ ਵਧੇਰੇ (52.89%) ਸ਼ਾਖਾਵਾਂ ਉਤਪੰਨ ਹੋਈਆਂ। ਬੀ.ਏ.ਪੀ. (1.5 ਮਿ.ਗਾ./ਲਿ.) ਮੀਡੀਅਮ ਉਪਰ ਪ੍ਰਤੀ ਪੌਦਾ ਸ਼ਾਖਾਵਾਂ ਦੀ ਸੰਖਿਆ ਸਭ ਤੋਂ ਵਧੇਰੇ (3.63) ਪਾਈ ਗਈ। ਬੀ.ਏ.ਪੀ. (0.5 ਮਿ.ਗਾ./ਲਿ.) + ਕਾਇਨੇਟਿਨ (0.5 ਮਿ.ਗ੍ਰਾ./ਲਿ.) ਵਾਲੇ ਐਮ.ਐਸ. ਮੀਡੀਅਮ ਉਪਰ ਸ਼ਾਖਾਵਾਂ ਦੀ ਲੰਬਾਈ ਸਭ ਤੋਂ ਵਧੇਰੇ (3.13 ਸੈ.ਮੀ.) ਸੀ। ਸਭ ਤੋਂ ਵਧੇਰੇ ਸ਼ਾਖਾਵਾਂ ਦੀ ਲੰਬਾਈ (1.73 ਸੈ.ਮੀ.) ਜੜ੍ਹ ਮੁੱਢ 1613 ਸੀ ਵਿੱਚ ਵੇਖਣ ਨੂੰ ਮਿਲੀ। ਆਈ.ਬੀ.ਏ. (0.1 ਮਿ.ਗਾ./ਲਿ.) + ਐਨ.ਏ.ਏ. (0.1 ਮਿ.ਗ੍ਰਾ./ਲਿ.) ਵਾਲੇ ਐਮ.ਐਸ. ਮੀਡੀਅਮ ਨਾਲ ਸਭ ਤੋਂ ਵਧੇਰੇ (64.95%) ਜੜ੍ਹਾਂ ਵਿਕਸਿਤ ਹੋਈਆਂ। ਜੜ੍ਹ ਮੁੱਢ ਡੀ-ਗ੍ਰਾਸੈਟ ਵਿੱਚ ਸਭ ਤੋਂ ਵਧੇਰੇ ਜੜ੍ਹਾਂ (55.64%) ਦਰਜ ਕੀਤੀਆਂ ਗਈਆਂ। ਆਈ.ਬੀ.ਏ. (0.1 ਮਿ.ਗਾ./ਲਿ.) + ਐਨ.ਏ.ਏ. (0.1 ਮਿ.ਗ੍ਰਾ./ਲਿ.) ਮੀਡੀਅਮ ਉਪਰ ਜੜ੍ਹ ਮੁੱਢ ਆਰ 110 ਵਿੱਚ ਜੜ੍ਹਾਂ ਦੀ ਸੰਖਿਆ ਸਭ ਤੋਂ ਵਧੇਰੇ (13.75) ਪਾਈ ਗਈ। ਆਈ.ਬੀ.ਏ. (0.1 ਮਿ.ਗਾ./ਲਿ.) ਵਾਲੇ ਐਮ.ਐਸ. ਮੀਡੀਅਮ ਉਪਰ ਕਲਚਰ ਕੀਤੇ ਗਏ ਜੜ੍ਹ ਮੁੱਢ ਡੀ-ਗ੍ਰਾਸੈਟ ਵਿੱਚ ਜੜ੍ਹਾਂ ਦੀ ਲੰਬਾਈ ਸਭ ਤੋਂ ਵਧੇਰੇ (5.66 ਸੈ.ਮੀ.) ਪਾਈ ਗਈ। ਐਕਸ. ਵਿਟਰੋ ਹਾਲਾਤਾਂ ਅਧੀਨ ਸਥਾਪਨ ਲਈ ਪੀਟ ਪਰਲਾਇਟ ਅਤੇ ਵਰਮੀਕੁਲਾਈਟ ਦਾ 2:1:1 ਅਨੁਪਾਤ ਅਤੇ ਡੀ ਗ੍ਰਸੈਟ ਜੜ੍ਹ ਮੁੱਢ ਸਭ ਤੋਂ ਵਧੀਆ ਪਾਇਆ ਗਿਆ।

ਮੁੱਖ ਸ਼ਬਦ: ਐਮ.ਐਸ. ਮੀਡੀਅਮ, ਬੀ.ਏ.ਪੀ., ਕਾਇਨੇਟਿਨ, ਐਨ.ਏ.ਏ., ਆਈ.ਬੀ.ਏ., ਜੜ੍ਹ ਮੁੱਢ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

CHAPTER –I

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important fruit crop cultivated worldwide and is considered most refreshing and nourishing fruit of the world. It is a temperate fruit crop but over the years has adapted well in hot tropical and sub-tropical climate regions of world. Grape was introduced in India in 1300 AD by invaders from Iran and Afghanistan. At the present, India is among the top countries in the world in grape productivity. The major producers of grapes in the world are China, Italy, USA, Spain, France, Turkey and Argentina. India has occupied ninth rank with 3.31 percent share in the world grape production (Anonymous 2014). It occupies an area of 1.1 lakh ha (1.7 % of total fruit area) with an annual production of 12.3 lakh MT (Anonymous 2014). In India, major grape growing states are Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and North-Western region covering Punjab, Haryana, Delhi, Uttar Pradesh, Rajasthan and Madhya Pradesh. Maharashtra is a largest producer of the grapes in the country.

At present, Punjab occupies 441 ha area under grapes with an annual production of 12.5 thousand MT (Anonymous 2014). In India, Punjab has highest per hectare productivity i.e 28.4 tonnes of grapes which is around three times than productivity of other states. The grapes can be successfully grown in arid- irrigated zone of Punjab. Amongst the various grape varieties recommended for cultivation in the state 'Perlette' occupies more than 95 percent area. Almost all the commercial plantations of grapes in the state are on own roots. The quality of fruit produced of a commercial variety in Punjab is comparatively inferior from the commercial varieties grown in rest of the major grape growing regions in the country. Climate plays an important role in production of quality fruits. Apart from unfavourable climate particularly pre-monsoon rains under North Indian conditions, the production of quality grapes is further limited due to their susceptibility to fungal diseases such as anthracnose and powdery mildew. Diversity in terms of varieties is very limited in Punjab due to its climatic conditions. A very short period is available for ripening in the sub-tropical climate of Punjab. Moreover early rains during ripening play a major havoc in vineyards. Area under grapes is declining primarily due to monoculture of Perlette vis-à-vis poor quality of grapes and low economic returns to farmers. The panic harvesting of unripe fruits due to pre-monsoon rains further add to the marketing problems. Keeping in view the decline of area under grape cultivation in Punjab and with a view to diversify and to uphold the economic stability of grape growers, a new grape hybrid H-516 (Catawba x Beauty Seedless) has been introduced from ARI Pune. Its performance is promising under agro-climatic conditions of Punjab (Gill and Arora 2009). It has been released under the name Punjab MASC Purple and recommended by PAU especially for juice and red wine production. In the recent past biotic and abiotic stresses like disease, drought and salinity have restricted grape production and productivity. Troncoso *et al* (1999) reported that the use of resistant rootstocks against disease and drought are of great importance in major viticultural countries of the world. In India, rootstocks have been introduced from several countries and are being used for commercial grape cultivation (Upadhyay *et al* 2007). Degrasset is a promising rootstock for viticulture and have been screened for high tolerance to drought (Patil *et al* 2003). The grafted plants had higher salt tolerance as well as high graft compatibility with Thomson Seedless grapes (Deshmukh *et al* 2008).

In vitro propagation is now a known mean to propagate selected genotypes on large scale. Depending on the plant species and culture conditions, tissue culture may enable the mass production of genetically homogeneous populations from elite (e.g., high-yielding or disease resistant) individuals. *In vitro* multiplication is the most appropriate method for producing large number of quality plantlets for vineyard establishment (Skiada *et al* 2009) in considerably lesser time. Micropropagation of grapevines is well established for wide range grape species (Gray and Klein 1987, Gray and Benton 1991). Micropropagation of selected *Vitis* genotypes can be carried out, among others, by the culture of intact or fragmented shoot apical meristems, axillary-bud microcuttings or through adventitious bud formation (Heloir *et al* 1997; Barlass and Skene, 1980; Gray and Fischer 1985; Monette 1988). Efficient protocols have been reported for muscadine or other than *V. vinifera* grapes (Thies and Graves 1992; Qiu *et al* 2004), while studies with cultivars of *V. vinifera* L. have met with less success (Chee and Pool 1983; Zatiko and Molnar 1985; Mhatre *et al* 2000). The degree of competence is highly dependent on the particular genotype, as various *Vitis* species, cultivars or hybrids respond differently to certain culture conditions (Chee and Pool 1983; Monette, 1988, Qiu *et al* 2004). Despite years of investigation, the application of tissue culture techniques in the grape-growing industry is still limited. The major obstacles for the widespread utilization of this technology is relatively high cost compared with the conventional methods (Chee *et al* 1984, Monette 1988) and the possibility of genetic discrepancy in micropropagated plants, often called as somaclonal variation. Somaclonal variation is highly undesirable in applications aiming to reproduce selected elite genotypes, e.g., ancient and rare clones, pathogen-resistant or drought salinity-tolerant genotypes and it is most likely caused by high levels of plant growth regulators (PGRs), mainly cytokinins, usually applied to promote shoot multiplication and thus increase the yield (Gray *et al* 2005). Unfortunately, there is huge variation among various genotypes and as such there is no routine tissue culture regeneration and transformation system in grapes (Botha 2001). Moreover, there are few reports on micropropagation of potential rootstocks having utility under north Indian conditions. Due to onset of several biotic and abiotic stresses in Punjab and their adverse effect on commercial grape production, it is most timely to use appropriate rootstocks for commercial grape cultivation. Thus to have more number of plants in less time, it is pertinent to standardize protocol for *in vitro* propagation of rootstocks.

Thus the present study was under taken with the following objectives:

1. To standardize a protocol for *in vitro* shoot regeneration in promising grape rootstocks.
2. To optimize conditions for efficient rooting of *in vitro* shoots and plantlet establishment.

CHAPTER- II

REVIEW OF LITERATURE

In vitro culture is a useful tool for rapid and disease free propagation of plants. After the concept of totipotency given by Haberlandt in 1902 and *in vitro* chemical regulation of plant growth by Miller and Skoog in 1957, a considerable success has been achieved in the field of plant tissue culture. The biotic stresses up to some extent can be overcome through conventional breeding approaches viz., inter and intraspecific hybridization but the genetic improvement of grape through conventional breeding is severely limted by several factors such as long prebearing age, polyploidy and the highly heterozygous nature of existing cultivars (Gray and Mezredith 1992, Nakano *et al* 1994). In such difficult conditions, one the promising germplasm line is identified large scale propagation is the prime objective. Through, grape can be propagated vegetatively, the *in vitro* propagation can be used to rapidly produce large number of disease-free clones, and is economically feasible if the demands for a species or cultivar are enough to justify cost. The selection of suitable explant is a crucial step for efficient initiation of micropropagation of grapevine as well as other woody species (Grenan 1992). Benzyl Adenine (BA) is the most effective cytokinin among other cytokinins for inducing shoot development in *Vitis* (Lee and Wetzstein 1990; Heloir *et al* 1997).

Morel (1944) was the first who established callus, in grapevine. Plant tissue culture forms the back bone of plant biotechnology i.e micropropagation, induction of somaclonal variation, somatic hybridization, cryopreservation, genetic transformation etc. Plant cell and tissue culture has contributed significantly to crop improvement and has potential for the future. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cell, tissues and organs under sterile and controlled environmental conditions. Tissue culture techniques are now being widely applied for improvement of horticulture and plantation crops. This technology is being exploited mainly for large scale production or micropropagation of elite planting material with desirable characteristics. This technology has been commercialized globally and has contributed significantly towards the enhanced and mass production of high quality true-to-type planting material. The use of tissue culture for fruit and nut species has increased substantially since the early 1970's with various degree of success, but till now there is no reliable regeneration procedure that is applicable to all major rootstocks.

The literature relevant to *in vitro* propagation/ micropropagation of grape rootstocks is reviewed under the following headings.

2.1 Shoot regeneration / proliferation

2.1.1 Genotype

Genotype is one of the most critical factors for the *in vitro* proliferation response. Chee *et al* (1984) studied the shoot production potential of 21 different genotypes. The greatest number of shoots (48) per explant was obtained in cultivar Cabernet Sauvignon while, least was obtained in cultivar Seyval. While studying plantlet regeneration by organogenesis, Clog *et al* (1990) found that 'Kober 5BB' gave the best results with 38.6 per cent embryogenic calli. Whereas, 'Pinot Noir' and 'Chardonnay' did not regenerate. The shoot regeneration response in *V. rotundifolia* proved to be cultivar dependent. The best response was observed with 'Carlos' followed by 'Tarheel', 'Regale' and 'Noble' (Sudarsonu and Goldy 1991). Out of three grape cultivars (Ribier, Thompson Seedless and Black Seedless) evaluated for shoot regeneration potential, 'Thompson Seedless' proved to be the most responsive i.e. 2,8,08,990 shoots/explants/year followed by 26,494 in 'Ribier' and only 1,213 shoots/explants/year in 'Black Seedless' (Claudia *et al* 1993). According to Harst *et al* (1995) 'Seyval Blanc' showed a high frequency of embryogenic explant (75 %), while this frequency decreased to 15 % for 'Chancellor' and to 25 % for other species e.g. *V. thumbergi*. Mozsar and Vicizian (1996) noted the genotype effect for somatic embryogenesis response in 8 grape cultivars. Of the tested cultivars, 'Chasselas' was superior in somatic embryos induction (5.5 %), while the efficiency of somatic embryos induction in other cultivars was quite low (less than 1%). EI-Din *et al* (1997) evaluated the *in vitro* propagation of Muscadine grape cvs. Triumph, Fry, Regale, Golden and Nespitt. The best survival of explant was recorded for 'Regale', while the percentage shooting ranged from 20 to 80 per cent and differed with the cultivar.

EI and Kamal (1998) found that cv. Flame Seedless was superior in regeneration to Thompson Seedless and King Ruby. Baydar (2000) found that adventitious shoot regeneration from leaf explants was highest (34 %) in 'Kalecik Karasi' while, it was the lowest in cultivar '41 BMG', among various genotypes tested. Zlenko *et al* (2005) found that cv. 'Bianca' produced shoots on hormone free medium, while in cv. 'Podarok Magaracha' the shoots were produced on medium with BA. Similarly Alizadeha *et al* (2010) evaluated performance of *in vitro* multiplication of four rootstocks namely Dogridge, SO_4, H-144 and 3309 C. The genotype 3309 C showed highest culture establishment (54.5 %) followed by

Dogridge (45.02 %) and SO_4 (40.12 %). The genotype H-144 was found to give poor response in this regard and only 38.31 % culture establishment was achieved.

2.1.2 Explant, its source, type and sterilization

Explant source, its maturity, type, season of collection all affect the success or failure of micropropagation protocol. Shoot tips from current season's growth have been widely used as explants for *in vitro* propagation of grapes. Besides shoot tip which is just one per shoot, nodal buds have also been tried by various workers with good success.

The *in vitro* regeneration from explants depends on a number of factors. These include the selection of organs that serve as tissue source, size of the explant, original position of the explants in the plant, age of the plant and overall quality of the plant from which the explants are to be obtained (Murashige 1974). Explant source directly influence the *in vitro* response for shoot regeneration. Shoot tips from green house (Harris and Stevenson 1982) and field grown vines (Chee and Pool 1982) have been successfully used for micropropagation. Fanizza *et al* (1984) found that shoot tips from field grown vines had a much lower per cent survival than those from green house or shade house grown vines. Yu and Meredith (1986) noted that explants taken from shoots grown in full sun had a lower percentage of survival than those from shoots grown in complete shade. Novak and Juvova (1980) reported that shoot development was increasingly successful in buds taken from nodes at increasing distance from the apex, while Yu and Meredith (1986) obtained a higher explant survival in axillary shoot tips than in terminal shoot tips. Gray and Benton (1991) observed that meristems taken from 10 cm long shoots had less contamination (3 %) and higher survival rate (94 %) than those from shorter or longer shoots of 9 muscadine grape cultivars tested. Pandeliev *et al* (1990) cultured the microcuttings as explants from different bud positions along the shoot length. The regenerative ability was weakest in cuttings of nodes 1 to 4 (from the apex) and best in cuttings from sector 5 to 8. Explant (axillary buds) originating from the 10 basal nodes of a shoot with atleast 25 nodes gave better shoot proliferation than explant originating from the 10 distal nodes (Sudarsono and Goldy 1991). Stamp and Meredith (1988) while studying somatic embryogenesis in several grape genotypes found that leaves less than 5mm long and translucent pale green anthers and higher embryogenic potential as compared to large sized leaves mature anthers. Adventitious bud regeneration potential was higher in first top leaf as compared to other leaves of cv. Red Globe (Li *et al* 2002). Thomas (1997) used shoot tips and single node (leaf bearing or leafless) explants for clonal propagation of grape cv. Arka Neelmani. Shoot tip explants gave the highest (83.4 %) proportion of hardenable plantlets followed by leaf-bearing nodes (45 %) and leafless nodes (25 %) but, Singh *et al* (2002) reported nodal segments as an ideal explants as compared to shoot tips for *in vitro* culture establishment of 7 grape genotypes, due to the less polyphenol content of the former. Baydar (2000) found that adventitious shoot regeneration was higher from petiole explants as compared to lamina explants among the several grape cultivars and rootstock tested. Shoot tips proved to be superior over axillary buds for getting grapevine leaf roll associated virus-S (GLRaV-3) free plants in cv. Napoleon (Valero *et al* 2003). Galzy (1969) described shoot apex culture of *Vitis rupestris*. Fragmented shoot apex has been used for clonal propagation of cv. Cabernet Sauvignon (Barlass and Skene 1980). Similarly, shoot tips have been used to culture *Vitis* hybrid 'Remaily Seedless' (Chee and Pool 1982, 1983, 1985), *V.bellandieri* x *V. riparia* (Clone-Craciunel) *V. vinifera* spp. *sativa* (Novak and Juvova 1982), *V. vinifera* cv. Chen in Blanc (Goussard 1981), *Vitis vinifera* cvs. Thompson Seedless, Ribier and Black Seedless (Claudia *et al* 1993), *V. vinifera* cv. Arka Neelmani (Thomas 1997). The extreme tip of the shoot is always virus and disease free. The potential of this zone has been utilized in getting virus free plants and for propagation in cv. Cabernet Sauvignon (Duran-Vila *et al* 1988), *Vitis* spp. cvs. Perlette and Italia (Gok *et al* 1997), *V.vinifera* cv. Napoleon (Valero *et al* 2003). Pool and Powell (1975) used small microcutting for mass multiplication of 'Concord' grapes. Axillary buds have been used to culture *V.vinifera* cv. Sylvaner Riesling (Jona and Webb 1978), *V. rotundifolia cvs.* Carlos, Noble, Regale and Tarheel (Sudarsono and Goldy 1991). Similarly, nodal segments have been used as starting material in *V. rotundifolia* cv. Summit (Lee and Wetzstein 1990), *V vinifera* cv. Arka Neelrnani (Thomas 1997), rootstock Jales and Campinas (Biasi *et al* 1997), *V vinifera cvs*. Thompson Seedless, Sonaka and Tas-e-Ganesh (Minal *et al* 2000), *V.vinifera* cv. Napoleon (Ibanez *et al* 2003), cv. Tash-e-Ganesh (Nalwade and Shitole 2004). Keeping in view, the high efficiency of leaves for somatic embryogenesis and adventitious shoot regeneration, these have also been used as explant. Leaves were cultured on NN medium supplemented with growth regulators for somatic embryos induction in *V vinifera* cv. Cabernet Sauvignon and *Vitis rupestris* 'St. George' (Stamp and Meredith 1988). Whole leaf has been used as explants for adventitious shoots regeneration in 'Kyoho' grape (Kwon *et al* 2000), *V. vinifera cvs*. Heiwang and Nioriai (Han *et al* 2000), *V. vinifera* cv. Red Globe (Li *et al* 2002). Baydar (2000) used petiole and lamina explants for adventitious shoot production in 5 grape cultivars and 2 rootstocks. Swelling within 4 weeks of culture followed by root formation occurred when fully opened leaves of cv. Perlette were cultured on MS media fortified with BAP (1.0 mg/l) and NAA (0.5 mg/l) (Kumar *et al* 2005). Claudia *et al* (1993) evaluated three grape cultivars (Black Seedless, Ribier, and Thompson Seedless) for shoot regeneration potential. Thompson Seedless proved to be the most responsive cultivar followed by 'Ribier' and 'Black Seedless'. In Thompson Seedless, 2,808,990 shoots per explant per year with an average number of shoots was obtained in 3 sub-cultures.

2.1.3 Season / month of explant collection

Murashige (1974) reported that beside other factors, the season of explant collection also influence the *in vitro* regeneration success of the explants. The regeneration ability in grapevine microcutting is influenced by the month in which it is cultured and highest regenerative ability is found in explants at active growth stage i.e. June and July (Pandeliev *et al* 1990). The shoot regeneration potential among cultivars Thompson Seedless, Ribier and Black Seedless varied with the date of culture. The highest shoot proliferation rate for three cultivars was obtained, when these were cultured in end of September, mid of October and mid of November; respectively (Claudia *et al* 1993). Singh *et al* (2002) correlated the polyphenol content with explants survival in 7 grape genotypes. The higher explant survival was achieved, when explants were cultured in mid of April, due to lesser polyphenol content in vines during this period of year. Akbas *et al* (2004) studied the proliferation efficiency of lateral buds in grape cv. Perle de Csaba during different periods of the year (June to May) to determine the best time for proliferation. Maximum shoot bud proliferation (80 %) was achieved when explants were cultured in December while least proliferation (15 %) was achieved in June cultured explants. Baydar *et al* (2006) examined changes in endogenous hormones and phenolic contents in shoot tips of two grape cultivars (‘Cauvus’ and ‘Hafizali’) collected during different months. The survival and subsequent growth of explants was affected by phenolics and endogenous hormones to various extents. In both the grape cultivars, the shoot tips collected in the month of May exhibited highest survival rate and growth during culture period.

Sterilization of explant

Plant parts carry a wide range of contaminants and hence obtaining sterile plant material is very difficult. The woody plants are grown in soil for many years under ambient conditions and they are routinely infected with microorganisms both internally and externally which are often difficult to control *in vitro* (Skirvin 1983). Therefore, explants need surface sterilization before culturing. This is done by using various surface sterilizing agents. The kind, concentration and duration of disinfection treatment depend upon the degree of contamination and the hardiness of explant. Many sterilizing agents such as calcium hypochlorite, chlorine water, bleaching water, mercuric chloride, hydrogen peroxide etc have been used. Keeping the shoot tips and nodal segments in running water for an hour prior to a single surface sterilization has been found effective. This treatment also caused leaching of water soluble phenols and other growth inhibitors (Jones *et al* 1978).

2.1.4 Nutrient medium

The concept of growing plants from individual cells was suggested by Haberlandt (1902), who tried to grow leaf cell cultures in simple mineral solutions. Various modification in nutrient media has been reported by Tukey (1933), White (1943), Nitsch (1951), Heller (1953), Murashige and Skoog (1962), Gamborg *et al* (1976), Favre (1977), and Lloyd and McCown (1980). There are differences in micronutrient compositions among MS, B5 and WPM media which might cause differences in shoot proliferation rate of different species (Lu

2005). Using Driver and Kuniyuki Walnut media for the establishing proliferating cultures gave better results than Murashige and Skoog (MS) media in case of all used rootstocks. (Krizan *et al* 2012). Virtually all media contain inorganic salts and sucrose as a carbon source. Concentration of these ingredients in basal medium depends on the type of plant being cultured and the stage of culture development (Murashige 1974). The pH of the nutrient media is also a critical factor; most media have pH values between 5.5 and 6.2. For establishment of shoot tip culture and subsequent shoot multiplication, the MS medium supplemented with 3% sucrose and solidified 0.8% agar has been commonly used (Novak and Juvova 1983). Torregrosa and Bouquet (1995) worked on micropropagation of *Vitis* x *Muscadinia* hybrid by microcutting or axillary budding using different culture media. (MS/2, CP/4 and HLR). Li *et al* (2002) studied the effect of different culture media (MS, B5, and NN) supplemented with different concentrations of BA and IBA on adventitious bud regeneration on leaf and petiole explants of cv. Red Globe. NN medium supplemented with 2.0-2.5 mg BA/litre + 2.0 mg/l.

IBA proved superior over other media in bud regeneration. Park *et al* (2001) found that Nitsch medium with 1mg/l BA was optimal for caulogenesis and the type of shoot dependent upon the pH of the medium with vigorous multiple shoot development occurring at pH 6.0 and single shoot at pH 5.0. On contrary Nookaraju *et al* (2008) found that single node segments of Crimson Seedless cultured on 6 different basal media i.e Murashige and Skoog (MS), Erikason (ER), Gamborg (B5), Nitsch and Nitsch (NN), WPM, and C2D showed different percentage of shoot initiation and morphogenic responses. The maximum shoot initiation (90.0 %) was observed in MS medium. Chee and Pool (1982) used a medium for shoot tip culture of cv. Rougeon (Seibel 5898) which contained macronutrients, micronutrients and glycine according to MS (1962) medium. Vitamins were those of Galzy (1972) medium but modified by deletion of calcium pantothenate and biotin. By modifying, the MS salt formulation Chee *et al* (1984) increased the rate of shoot multiplication by 40% to 350% depending upon the grapevine variety. The modifications consisted of substituting calcium nitrate for calcium chloride, eliminating potassium iodide and decreasing manganese sulphate concentration from 100 to 5 µM. Chee and Pool (1985) proposed that the addition of vitamins in the medium should be adjusted according to need of cultivar and culture of concern. They found that thiamine and inositol were necessary, while nicotinic acid and pyridoxine were beneficial for shoot multiplication. Shoot tips of *Vitis* hybrid 'Remaily Seedless' were cultured on improved MS media lacking chloride and potassium iodide but low in manganese sulphate, showed improved shoot multiplication. It was suggested that KI is non essential for higher plants as this inhibits IAA transport (Chee and Pool 1988). Zlenko *et al* (1995) used shoot length, leaf quality, number, length and quality of roots as a criteria for the optimization of medium in rootstock (Kober SBB) and 3 wine cultivars (Podarok, Zhemchug Magaracha and Sverkhrannii bessernyannyi Magaracha). The medium elements, 20 mg/l NH_4NO_3, 922 mg/l KNO_3, 597 mg/l $MgSO_4.7H_2O$, 122 mg/l KH_2PO_4, 331 mg/l $CaCl_2$, ½ MS Ferrum chelate, ½ MS micro elements, 20 mg/l myo- inositol, 0.5 mg/l nicotinic acid , 0.2 mg/l pyridoxine, 0.1 mg/l thiamine, 0.1 mg/l IAA, 10 g/l sucrose and 7 g/l agar. Dimitrova (2000) used a mathematical approach to optimize the nutrient medium for *in vitro* cultivation of cv. Boglar (*V vinifera* L. ssp. *sativa*). The media consisted of macrocomponent concentration 0.4 of MS, microcomponent concentration 1.2 of MS and 0.98 mg/l IAA supplemented to the organic substances of the medium. Chee and Pool (1988) studied the effect of sucrose concentration in the medium on shoot vigour and quality of *Vitis* hybrid 'Remaily Seedless'. Shoot vigour and condition was good when the medium contained 1 % sucrose. The further increase in sucrose concentration deteriorated the quality of shoots. Peixoto and Pasqual (1995) observed the influence of medium pH and agar concentration on shoot proliferation of the rootstock RP-101-14 (*Vitis spp*). Analysis after 60 days showed that culture at pH 3.7 or 4.7 with a concentration of 3.5 agar per litre resulted in the most vigorous shoot growth. Kwon *et al* (2000) studied the effect of various gelling agents *viz*., agar, agarose and phytgel for shoot regeneration in 'Kyoho' grape. Agar when used as gelling agent showed higher shoot regeneration than agarose and phytagel. In grape rootstock 1613 C, with an objective to achieve *in vitro* cloning shoot regeneration has been standardized (Krishan *et al* 2008). The maximum shoot regeneration in nodal segments was obtained on MS medium supplemented with BAP (1 mg/l) + Kin(1 mg/l), while from shoot tips, it was obtained on MS medium supplemented with BAP (1 mg/l) + Kin(0.5 mg/l). Kumar *et al* (2005) found the callusing of nodal segments on MS basal media. Best response (85.6%) for the culture initiation was obtained when MS medium fortified with BAP (4.0 mg/l) and NAA (0.2 mg/l) was used . Kalatejari *et al* (2007) reported MS medium supplemented with 0.05 mg/l of BA as best rooting medium for grape cv 'Bidane Sefid' and 'Shahroodi'. Singh *et al* (2004) multiplied 'Pusa Urvashi' and 'Pusa Navrang' from nodal segments collected from field-grown vines on Murashige and Skoog medium supplemented with 2.0 or 4.0 mg/l BAP + 0.2 mg/l NAA. The sprouted shoots were successfully proliferated and rooted on half-strength MS medium supplemented with 2.0 mg/l IBA + 200 mg/l activated charcoal. Chang and Reisch (1989) also obtained normal plants from leaf (15 %) and petiole (70 %) derived from *in vitro* grown shoots of 'Catawba' (*Vitis vinifera* X *Vitis labrusca*) and regeneration was reported from basipetal end. Mukherjee *et al* (2010) while optimizing standardized protocol for *in vitro* multiplication of grape rootstock Degrasset (*Vitis champinii* Planch.) studied four medium compositions, MS, MS with ½ nitrates (MS-1), B5 and WPM, were tested for shoot growth from nodal explants and MS-1 medium produced significantly higher rate of shoot proliferation. MS-1 medium supplemented with 2.0 mg/l BAP was found to be optimum for culture establishment. The first subculturing on the same medium resulted in the production of 4-6, mostly short, hyperhydrated shoots per explant. For subsequent subcultures, a reduced concentration of BAP (1.0 mg/l) was used to prevent hyperhydricity, and that resulted in distinct individual shoot elongation.

The basal MS medium contains a relatively high level of nitrogen in the form ammonium and nitrate ions in addition to high concentration of calcium than other media. The most commonly included vitamins are thiamine, nicotinic acid and pyridoxine. Thiamine is critical and is usually provided in the range of 0.1 to 0.4 mg/l. Inositol is not essential; nevertheless, it has been clearly beneficial and has been used at the rate of 100 mg/l (Murashige 1974).

A number of carbohydrates have been used in tissue culture, but the sucrose has been the most popular and versatile. It is generally incorporated at a concentration of 2-3 per cent. Carbohydrates serve two principle functions (a) they provide an energy source to the tissues, and (b) they maintain an osmotic balance with the medium (Skirvin 1983). Carbohydrates effect the growth and the frequencies of shoot developed and shoot proliferation of woody Rosaceous species (Marino *et al* 1993).

One of the most common organic complexes used in tissue culture is agar. The MS medium includes 10.0 g/l (1%) agar, but many researchers have reduced agar concentration to 8.0 g/l or even less. The osmotic potential of high agar concentration and the concomitant reduction in nutrient and organic matter availability to the tissue is the common reason for reducing the agar concentration (Skirvin 1983).

2.1.5 Growth regulators

A balance between endogenous and exogenous growth regulators controls the initiation and development of shoots, roots, plantlet and callus. Skoog and Miller (1957) reported for the first time that the ratio of auxin to cytokinin determines the type and extent of organogenesis in tissue cultures. In general, cytokinins favour meristem proliferation, auxins induce callus formation and rooting and gibberlins induce stem elongation (Kumar and Kumar 1998).

Cytokinins are required for the proper development of shoots. Skene And Kerrridge (1967) demonstrated a relationship between the cytokinin content of grape xylem sap and the vigour of shoot growth. Similarly, Pool and Powell (1975) found that cutting without roots must be provided with cytokinins to sustain the development of shoots. In general, BA/BAP is the most effective cytokinin for stimulating axillary shoot proliferation followed by in decreasing order Kinetin and 2-iso-pentenyl adenine (2-ip) (Hu and Wang 1983). While studying, the effects of various cytokinins on ‘Concord’ *in vitro* shoot cultures, Pool and Powell (1975) reported that BAP at 1mg/l or above induced better development as compared to Zeatin riboside and 2-ip. Shoot regeneration in axillary bud culture of cultivar Sylvaner Riesling was obtained in medium containing 10^{-5} M benzyl adenine (BA) while shoot proliferation was achieved at 2 x 10^{-5} M BA, however high BA retarded shoot elongation (Jona and Webb 1978). Similarly, Barlass and Skene (1980) in cultivar Cabernet Sauvignon, Lee and Wetzstein (1990) in muscadine grape cv. Summit had reported that adventitious shoot regeneration was achieved when in 10^{-5} M BAP was added to the medium. The cytokinins, BAP and Zeatin riboside resulted in the optimum shoot proliferation of *V. vinifera* cv. Chenin Blanc when both of these were added at a concentration of 2 mg/l (Goussard 1981). Shoot production in the French hybrid 'Baco' grown on Murashige's minimal organic medium (MMO) supplemented with 80 mg/l adenine sulphate, 170 mg/l sodium phosphatell (Monobasic) and 3-4 mg/l BAP was increased 7 fold by starting on solid media and then reculturing on liquid medium (Harris and Stevenson 1982). Novak and Juvova (1983) studied the morphogenic effect of Kinetin, 2-ip and BAP on shoot tip growth in 8 grapevine clones. BAP (5, 10 or 20 µM) strongly stimulated shoot tip growth and axillary bud and shoot development, while Kinetin at 5-20 µM and 2-ip at 10-20 µM produced shoots. Kinetin at lower concentration exhibited root formation and 2-ip at lower concentration formed callus at the base. BAP (0.5-5 µM) in combination with TDZ (5-20 µM) resulted in more number of shoots/shoot apex in cultivar Blanc du Bois (Gray and Klein 1989). Thidiazuron (TDZ) at concentration of 2.3-4.5 µM were effective for *in vitro* explant establishment and shoot proliferation in muscadine grapes. However, addition of BAP or Kinetin at 1.0-5.0 µM in combination with TDZ increased the number of shoot production (Sudarsono and Goldy 1991). BA encouraged the best morphogenic response and at concentrations of 6.67 and 8.9 µM resulted in the average 8.7 axillary buds and 2.5 shoots per explant (Ibanez *et al* 2003). Addition of BAP at highest concentration i.e. 2.5 mgl^{-1} gave the best meristem differentiation in 3 cultivars of grapes (Gomes *et al* 2004). Chee (1980) found that addition of NAA (5 x 10^{-7} M) along with BAP (5 x 10^{-6} M) in the medium were effective for establishment of shoot cultures in grape cv. Rougeon. Reisch (1986) observed that BAP at 5-10 µM resulted in shoot tip proliferation of all the genotypes tested. Addition of auxin, picloram or adenine sulphate to the BAP supplemented medium resulted in decreased shoot production. Dimitrova (1995) found that addition of NAA at concentrations above 0.05 mg/l inhibited regeneration and induced callus at the base of cultured microcuttings in grape cvs. Cabernet Sauvignon, Chardonnay and rootstock 'Rupestris du Lot' However, Singh *et al* (2004) noted that addition of NAA (0.2 mg/l) along with BAP (4 mg/l) improved explant establishment in cultivar Pusa Urvashi. The maximum number of shoots in grape cv. Calmeria were obtained on MS medium containing 1.0 mg/l BA, 0.05 mg/l IAA and 1.0 mg/l PP 333 (Jiang 2000). Lu *et al* (2004) noted that one half strength MS medium supplemented with 1.0 mg/l BA + 0.1 mgl IAA was most suitable for axillary bud formation in wine grape cv. NW 196. Addition of auxins along with BA/BAP improves the adventitious shoot formation in leaves of several grape cultivars while in others it was achieved on media supplemented with BAP only. The frequency was higher with 2 mg/l BAP than with 1 or 4 mg/l BAP with or without auxins (Stamp *et al* 1990). Half MS medium supplemented with BA (2 mg/l) and IBA (0.02 mg/l) induced the highest shoot regeneration frequency in 'Kyoho' grape (Kwon *et al* 2000). Han *et al* (2000) reported that addition of IBA (0.02 mg/l) along with BAP (1-2 mg/l) in NN medium resulted in induction of 86.7 and 93 % adventitious buds in grape cvs. Heiwang and Thompson Seedless, respectively. Addition of BAP (2 mgl^{-1}) to MS or NN medium resulted in adventitious buds induction in several grape genotypes (Bayder 2000). Heloir *et al* (1997) reported that maintenance of cultures under higher BA concentration (8.9 µM) resulted in hyperhydricity in cultivar Pinot Noir. Similar results were also reported by Lee and Wetzstein (1990) in muscadine grape cv. Summit. Goussard (1982) repeatedly subcultured shoots obtained from apics of grapevine on nutrient media containing different concentration of 6-benzylaminopurine (BAP) and Zeatin Riboside (ZR) and obtained optimum shoot proliferation with combination of these cytokinins each at 2mg/l. Mukherjee *et al* (2010) while optimizing standardized protocol for *in vitro* multiplication of grape rootstock Degrasset (*Vitis champinii* Planch.) evaluated three plant growth regulators, BAP, ZEA and TDZ for further shoot proliferation and BAP at 1.0 mg/l added to MS-1 medium displayed the highest proliferation rate (4.75 new explants per explants inoculated, in 6 weeks). For *in vitro* rooting of shoots, IAA at 0.2 mg/l was found to be optimum to produce highest response (84 %) and an average number of 2.03 roots per shoot whereas use of IBA or NAA resulted in rooting with high frequency of callus formation.

2.1.6 *Ex vitro* and *in vitro* physical conditions

Alleweldt and Radler (1962) reported that a photoperiodic pretreatment of the mother plant affected subsequent growth activity in a genotype dependent manner. Chee and Pool (1982) found that 10 hour photo period was optimum for establishment of 'Rougeon' (Seibel 5898) culture. After surveying a range of grapevines genotypes, Chee and Pool (1983) found that shoot production was better or equal with short (10 hr) days than it was with long (16 hr) days. They also reported that at 1:1 combination of gro lux and cool white fluorescent or cool white light alone was satisfactory for shoot production. Chee *et al* (1984) recommended an irradiance of 1900 $\mu W/cm^2$ at explant level with a temperature of 27ºC during the period of illumination and 23ºC in the dark. A 16h photoperiod has been commonly used by others with either a constant temperature of about 24ºC (Harris and Stevenson 1982, Monette 1983, Fanizza *et al* 1984, Morini *et al* 1985) or a temperature alternating between 27ºC during the light period and 24ºC in the dark (Goussard 1981). Burr and Kurtz (1988) reported that maintaining shoot tip cultures of grape in 10 hr light and 14 hr dark cycle at 26ºC to 29ºC was optimum for plantlet regeneration. The continous 60 days *in vitro* exposure of cultured nodal segments and shoot tips of grapevine to 7 Gy gamma irradiation resulted in increased shoot length of cultivars Helwani and Cabernet Franc out of various doses tested (0, 2, 5, 7 Gy)

(Charbaji and Nabulsi 1999). Kwon *et al* (2000) while working on 'Kyoho' grape showed that dark incubation of cultures for initial 3-4 weeks improved the shoot regeneration. Cui *et al* (2001) found that *in vitro* CO_2 enrichment at relatively high photosynthetic photon flux (PPF) improved the vigour and growth of cultured plantlets of cultivar Cabernet Sauvignon and it also enhanced their survival rate after acclimatization and transplanting on vermiculite medium.

CHAPTER-III

MATERIALS AND METHODS

Present research entitled, 'Studies on *in vitro* propagation of promising grape (*Vitis vinifera.* L) rootstocks' was carried out in the Tissue Culture Laboratory of the Department of Fruit Science in collaboration with School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during 2013-2014. Description of the materials and methods used during the investigation are explained under following headings:

3.1 Experimental plant material

3.2 Culture medium

- 3.2.1 Preparation of nutrient medium
- 3.2.2 Preparation of growth regulator solutions
  - 3.2.2.1 Preparation of stock solution of NAA
  - 3.2.2.2 Preparation of stock solution of BAP
  - 3.2.2.3 Preparation of stock solution of Kinetin
  - 3.2.2.4 Preparation of stock solution of IBA
- 3.2.3 Sterilization of media
- 3.2.4 Glassware and plasticware
- 3.2.5 Laboratory sanitation

3.3 Preparation of explants

- 3.3.1 Surface sterilization

3.4 Inoculation and incubation of cultures

3.5 Observations

3.1 Experimental plant material

Grape rootstocks i.e Degrasset, R 110, 1613 C, 1616 C, were selected for *in vitro* propagation studies. The explants were collected from vines grown on Y-trellis system in the Department of Fruit Science, PAU. The explants viz., shoot tips and nodal segments from mature field grown plants were obtained during periodic (5 days) intervals from April-June 2014.

3.2 Culture medium

Murashige and Skoog medium (MS, 1962) was used as basal culture medium (Table 3.1) for explant culture, establishment and shoot regeneration.

3.2.1 Preparation of nutrient medium

The stock solutions (1, 2, 3, 4 and 5) of MS media were prepared by dissolving the required amount of salts in measured quantity of distilled water as mentioned in Table 3.1. The stock solution number 4 was stored in dark brown glass bottle to avoid photo-oxidation. All the stock solutions were stored at 4±1ºC in the refrigerator.

The basal MS medium was prepared by mixing the required quantity of each of the five stock solutions in 500 ml of distilled water by continous stirring. Myo-inositol (100 mg/l) and sucrose (3 %) were added to this solution. The basal medium was modified by adding measured quantity of different growth regulators.

Table 3.1 Composition of Murashige and Skoog (1962) medium.

Stock solution no.	Strength	Constituent salts	Quantity used	Use (ml/l)	Actual amount in culture medium (mgl^{-1})
Per 2 Litre					
1.	X20	Ammonium nitrate (NH_4NO_3)	66.0g	50	1650
		Potassium nitrate (KNO_3)	76.0g		1900
		Potassium dihydrogen orthophosphate (KH_2PO_4)	6.800g		170
		Boric acid (H_3BO_3)	0.248g		6.2
		Manganese sulphate ($MnSO_4.7H_2O$)	0.892g		22.3
		Zinc sulphate ($ZnSO_4.7H_2O$)	0.033g		0.825
		Potassium Iodide (KI)	0.033g		0.825
	a)	Copper sulphate ($CuSO_{4.}5H_2O$)	1.0 ml		0.025
	b)	Sodium molybdate (Na_2MoO_4)	1.0 ml		0.250
	c)	Cobalt chloride ($CoCl_{2.}6H_2O$)	1.0 ml		0.025
Per ½ Litre					
2.	X50	Calcium chloride ($CaCl_2.2H_2O$)	11.0g	20	440
3.	X50	Magnesium sulphate ($MgSO_4.7H_2O$)	9.250	20	370
Per 1 Litre					
4.	X100	Ferrous sulphate ($FeSO_4.7H_2O$)	2.780g	10	27.8
		Ethylene diamine tetra acetic acid and disodium salt (Na_2EDTA)	3.728g		37.28
Per 1 litre					
5.	X100	Thiamine HCl	0.010	10	0.1
		Nicotinic acid	0.050g		0.5
		Pyridoxine HCl	0.050g		0.5
		Glycine	0.200g		2.0
		Myo-inositol			100
		Sucrose			30,000

a) 100mg $CuSO_4.5H_2O$ was dissolved in 100ml distilled water

b) 1g $Na_2MoO_4.2H_2O$ was dissolved in 100ml distilled water

c) 100mg $CoCl_{2.}6H_2O$ was dissolved in 100ml distilled water

Note:

1) 1ml of concentrated HCl was added in stock solution No. 1 to avoid precipitation.

2) Stock solution No.4 was heated slightly to avoid precipitation.

*Myo-inositol @ 100 mg and sucrose @ 30 gm were added in solid form while preparing the final medium.

The final volume was adjusted to 1 litre by adding more distilled water. The pH of the medium was adjusted to 5.8 with pH meter (SAB 5000, LAB India. Mumbai) using 1 N HCl or 1 N NaOH before adding agar.

Bacto agar (Qualigenes) at the rate of 7.5 gml^{-1} was used as solidifying agent in the medium. After adding agar, the medium was stirred regularly to avoid formation of agar clumps till it started boiling. Then, it was allowed to cool for few minutes at room temperature and 30 ml of the media was dispensed into each jar (400 ml, Screw cap bottles) and 15 ml of media was poured into each of the culture tube (25 mm x 150 mm,Borosil). After pouring the media, the culture tubes were plugged with non-absorbent cotton wrapped in muslin cloth and jars were capped with polypropylene caps.

3.2.2 Preparation of growth regulator solutions

Depending upon the nature of experiment, different concentration of the growth regulators were added to the basal medium. The stock solution of different growth regulators were prepared by dissolving them in few drops of required solvent as mentioned in Table 3.2. The freshly prepared growth regulator solutions were used each time.

Table 3.2 Preparation of growth regulators stock solution.

Sr. No.	Growth Regulator	Solvent
1.	6-Benzyl amino purine (BAP)	1 N HCl / 1 N NaOH
2.	6-Furfuryl amino purine (Kinetin)	1 N HCl / 1 N NaOH
3.	α-Naphthalene acetic acid (NAA)	Absolute alcohol
4.	Indole butyric acid (IBA)	Absolute alcohol

3.2.2.1 Preparation of stock solution of NAA

10 mg of NAA (Hi Media) taken in a dry test tube was dissolved in 1-2 ml of absolute alcohol and the final volume was scaled upto 10 ml by adding Milli Q water slowly with gentle shaking thus making a stock concentration of 1 mg/ ml.

3.2.2.2 Preparation of stock solution of BAP

10 mg of BAP (Hi Media) was taken in a dry test tube and dissolved in few drops of 1 N HCl, shaken well till it dissolved and then final volume was scaled upto 10 ml by adding Milli Q water. The concentration of stock solution of BAP, thus prepared was 1 mg/ ml.

3.2.2.3 Preparation of stock solution of Kinetin

10 mg of Kinetin (Hi Media) was taken in a dry test tube and dissolved in few drops of 1 N NaOH, shaken well till it dissolved and then final volume was scaled upto 10 ml by adding Milli Q water. The concentration of stock solution of Kinetin, thus prepared was 1 mg/ ml.

3.2.2.4 Preparation of stock solution of IBA

10 mg of IBA (Hi Media) was taken in a dry test tube and dissolved in 1-2 ml of

absolute alcohol and the final volume was scaled upto 10 ml by adding Milli Q water slowly with gentle shaking thus making a stock concentration of 1 mg/ ml.

3.2.3 Sterilization of media

The media was sterilized by autoclaving at 121ºC temperature and 15 p.s.i. pressure for 30-35 minutes. All the components of medium were added before autoclaving, except antibiotics. The antibiotics were added into the medium after autoclaving, when the medium was lukewarm under aseptic conditions (Laminar air flow cabinet). The antibiotics were sterilized by using 0.22 µM (Milipore) filter papers. Then the media was poured in the pre-autoclaved culture tubes and jars.

3.2.4 Glassware and plasticware

Various types of glassware/plasticware were used, which included 'Borosil' test tubes (25 x 150 mm), 'HNG' glass jars (400 ml), 'Erlenmmeyer' conical flasks (150 ml, 250 ml), measuring cylinders, beakers, glass petridishes, 'Tarsons' disposable petridishes (90 mm), pipettes (5 ml, 10 ml), micropipettes and microtips (1-1000 µl), disposable syringes, glass vials, 'Milipore' filter units (0.22 µm) and 'American National Can' parafilm. All glassware was washed with Teepol™ and under running tap water, and dried / autoclaved before reuse.

3.2.5 Laboratory sanitation

The inoculation and incubation rooms were daily cleaned. All inoculations were done under aseptic conditions in the Laminar Air Flow which was sterilized with methanol and then with UV light for 30 minutes. The inoculations were done over a Bunsen burner. All equipments (forcep and scalpel) were made red hot over the flame and cooled in methanol before use. The hands were properly sterilized with methanol. The culture vessels were wiped with methanol before keeping them on laminar hood and their rims were slightly flamed before and after each inoculation. The Laminar Air Flow Cabinet was fumigated with formaldehyde (30 %) after every 3 weeks.

3.3 Preparation of explants

The explants used were nodal segments and shoot tips from the juvenile branches of grapevine plantation (Y-trellis) at Fruit Research Farm, PAU. Twenty cm long apical portion was taken from the current season's growth from the four rootstocks Degrasset, R 110, 1613 C, 1616 C. These shoots were brought to the laboratory and washed thoroughly under running tap water. Then the explants were prepared into appropriate size *viz.* nodal segment (2-3 cm.) and shoot tips (1 cm.)

After preparation, the explants were washed with diluted Teepol for 1-3 minutes. Nodal segments were prepared from the middle of the shoot leaving the apical and basal nodes. The effect of explants and season/month of explants collection on the culture establishment and shoot regeneration was studied.

3.3.1 Surface sterilization

The explants were given a pre-treatment of bavistin (0.01 % to 0.05 %) depending upon the nature of the explant. The shoot tips were given a pre treatment of 0.01 % bavistin for 1-2 minutes, while, the nodal segments were given a pre-treatment of 0.05 % bavistin for 15 minutes.

The explants (nodal segments) were sterilized under aseptic condition (laminar air flow cabinet) with 0.1 per cent mercuric chloride. The treated explants were then rinsed thrice with sterile distilled water before inoculation on establishment media. The surface sterilization time was standardized, so as to achieve minimal contamination and maximum establishment.

3.4 Inoculation and incubation of cultures

The explants were cultured under aseptic conditions in laminar air flow cabinet (Klenzaids Contamination Controls Pvt Ltd. Valsad). The floor and walls of the cabinet were thoroughly cleaned with cotton dipped in methanol (spirit) just after switching on the laminar air flow cabinet. The floor was again cleaned with methanol just before the inoculation.

All the instruments such as surgical scalpel handle (Size no. 4), disposal surgical blade (Size no. 24), scissors, forceps (20 cm, stainless steel) etc. were also sterilized with cotton dipped in methanol and sterilized (red hot) by flaming for repeated use. All these instruments were also autoclaved after each use along with culture medium to avoid contamination.

Surface sterilized explants (nodal segments and shoot tips) were transferred from bottle to sterile petri dishes (90 mm, 120 mm, Borosil) with the aid of sterilized forceps. A sterile filter paper was placed in the petri dish to absorb excess water adhering to the explants. These explants were inoculated in test tubes/jars containing culture medium (MS medium fortified with growth regulators). Each test tube contained single explant in case of nodal segment or shoot tips, while, in case of jars two shoot tips were inoculated per jar.

The brown ends of nodal segments and shoot tips were removed before inoculation to remove toxic effects of mercuric chloride. The explants were inoculated with the help of sterilized forceps. After inoculation, the culture vessels were incubated at 25±2° C temperature in 16 hours continous fluorescent light (2000 lux) followed by dark period of 8 hours. The incubation period and photoperiod were similar in all experiments.

3.5 Observations

During the study on *in vitro* propagation of grape rootstocks, the following observations were recorded in all the four rootstocks:

- Explant establishment (%)
- Days taken for shoot induction
- Explants showing browning / phenol exudation (%)

- Shoot regeneration (%)
- Number of shoots per explant
- Average shoot length (cm)
- Percent root induction
- Number of roots/ shoot
- Root length (average)
- Survival percentage of plantlets under *ex vitro* conditions.

CHAPTER-IV

RESULTS AND DISCUSSION

The results of present investigation entitled, 'Studies on *In vitro* propagation of promising grape (*Vitis Vinifera* L.) rootstocks' is presented and discussed in this chapter under the following heads:

4.1 Effect of culturing time on explant establishment.

4.2 Effect of growth regulators on establishment of nodal segments.

4.3 Effect of growth regulators on establishment of shoot tips.

4.4 Effect of growth regulators on days taken for shoot induction.

4.5 Effect of growth regulators on nodal segments showing browning / phenol exudation.

4.6 Effect of growth regulators on per cent shoot regeneration.

4.6.1 Effect of growth regulators on number of shoots per explant.

4.6.2 Effect of growth regulators on average length of shoots.

4.7 Effect of growth regulators on per cent rooting.

4.7.1 Effect of growth regulators on number of roots per explant.

4.7.2 Effect of growth regulators on average length of roots.

4. 1 Effect of culturing time on explant establishment:

The data pertaining to explants establishment is presented in Table 4.1. It was observed that mean maximum explants establishment (43.51 %) was noted in April which was significantly better than May, June and July. Among rootstocks, the mean maximum establishment (48.06 %) was noted in R 110, which was however at par (43.41 %) with 1613 C rootstock. All the rootstocks showed similar trend for month wise establishment. Among different months, the minimum explants establishment/survival was attacked in the month of July. This gives clear indication that the young explants are less prove to contamination and thus have higher survival as compared to old explants used for culturing.

Table 4.1 Effect of culturing time on percent explant survival in different grape rootstocks.

Month of rootstock culturing	Explant Survival (%)				Mean
	Degrasset	R 110	1613 C	1616 C	
April	41.07 (39.81)*	57.92 (49.54)*	57.14(49.09)*	33.92(35.59)*	47.51(43.51) *
May	36.65 (37.23) *	51.78 (46.00)*	53.57(47.03)*	28.57(32.28)*	42.64(40.63)*
June	22.65 (28.37) *	44.64 (41.90)*	42.85(40.86)*	26.78(31.14)*	34.23(35.57)*
July	12.24 (20.45)*	39.28 (38.79)*	35.71(36.67)*	17.85(24.96)*	26.27(30.22) *
Mean	28.15 (31.47)*	48.40 (44.06) *	47.31(43.41)*	26.78 (30.99)*	
CD (0.05)	Rootstock (A)= 1.42, Month(B)= 1.42, A×B= 2.84				

*Figures in paranthesis are Arc Sine transformed means.

The maximum culture establishment percentage in April might be due to less contamination as compared to June and July, because in rainy season the spread of bacterial and fungal infection is generally very high. Furthermore, Singh *et al* (2002) correlated the polyphenol content with explant survival in seven grape genotypes. The higher explant survival was achieved, when explants were cultured in mid of April, due to lesser polyphenol content in vines during this period of year. These results are in accordance to the findings of Singh *et al* (2002) for cultivar Pusa Urvashi, Perlette, Centennial Seedless and Pusa Seedless.

4.2 Effect of growth regulators on establishment of nodal segments:

The nodal segments were established on MS media supplemented with varying levels of BAP and Kinetin. Data in Table 4.2 shows the effect of medium composition supplemented with various combinations of BAP and Kinetin on percent explant establishment of all genotypes *viz.,* rootstocks Degrasset, R 110, 1613 C, 1616 C. Highest explant establishment of 47.37 % was obtained in R 110 rootstock (Fig 2) followed by 1613 C (Fig 3) and 1616 C (Fig 4). As far as growth regulator combination is concerned, BAP (1.0 mg/l) and Kinetin (1.0 mg/l) proved the best combination for explant establishment (45.54 %), followed by BAP (0.5 mg/l) and Kinetin (0.5 mg/l) (44.20 %). These two treatments were significantly different from rest of the treatment combination. Lowest explant establishment (32.34 %) was obtained on MS medium supplemented with BAP (2.0 mg/l) and Kinetin (1.0 mg/l).

The increase in the BAP level more than 1 mg/l on MS medium resulted in decreased explant establishment in nodal segments.

Table 4.2: Effect of growth regulators on establishment of nodal segments in different grape rootstocks.

Growth regulators (mg/l)	Explant Establishment (%)				Mean (%)
	Degrasset	R 110	1613 C	1616 C	
BAP (0.5)	27.26 (31.32)*	50.63(45.34)*	33.56(35.38)*	33.86 (35.53)*	36.32 (36.89)*
BAP (0.5) + Kin (0.5)	28.33(32.06)*	42.10 (40.43)*	63.88 (53.04)*	60.71 (51.26) *	48.75 (44.20)*
BAP (1.0)	29.02 (32.52)*	65.21 (53.86)*	44.23 (41.65) *	46.66 (43.06)*	46.28 (42.77)*
BAP (1.0) + Kin (0.5)	30.85 (33.70) *	45.23 (42.23)*	53.33 (46.89)*	42.85 (40.86) *	43.06 (40.92) *
BAP (1.0) + Kin (1.0)	39.53 (38.92)*	63.63 (52.96)*	43.75 (41.20)*	57.14 (49.08)*	50.93 (45.54)*
BAP (1.5) + Kin (0.5)	27.13 (31.34) *	50.0 (44.98)*	56.09 (48.48) *	47.14 (43.32)*	45.09 (42.03) *
BAP (2.0)	42.40 (40.60) *	61.11 (51.41) *	48.33 (44.01) *	41.66 (40.16)*	48.37 (44.05) *
BAP(2.0) + Kin (0.5)	31.25 (33.93)*	42.85 (40.86)*	46.15 (42.76)*	22.91 (28.57) *	35.78 (36.53)*
BAP(2.0) + Kin (1.0)	20.58 (26.88) *	53.33 (46.89)*	26.66 (31.02)*	17.64 (24.55)*	29.55 (32.34)*
BAP(2.0) + Kin (2.0)	13.84 (21.54) *	66.66 (54.74)*	34.28 (35.79)*	23.40 (28.86)*	34.54 (35.23)*
Mean	29.02 (32.28) *	54.07 (47.37)*	44.99 (42.02) *	39.39 (38.53) *	
CD (0.05)	Rootstock (A) = 1.72, Media (B)= 2.72 , A×B= 5.45				

*Figures in parenthesis are Arc Sine transformed means.

Fig 1: Establishment of Degrasset from nodal segments on MS medium fortified with BAP (2.0 mg/l).

Fig 2: Establishment of R 110 from nodal segments on MS medium fortified with BAP (2.0 mg/l) + Kinetin (2.0 mg/l).

Fig 3: Establishment of 1613 C from nodal segments on MS medium fortified with BAP (0.5 mg/l) + Kinetin (0.5 mg/l).

Fig 4: Establishment of 1616 C from nodal segments on MS medium fortified with BAP (0.5 mg/l) + Kinetin (0.5 mg/l).

4. 3: Effect of growth regulators on establishment of shoot tips:

The shoot tips taken from all the four rootstocks were established on MS media supplemented with varying levels of BAP and Kinetin.

Data in Table 4.3 shows the effect of medium composition supplemented with various combinations of BAP and Kinetin on percent explant establishment of all genotypes *viz.,* rootstocks Degrasset , R 110, 1613 C, 1616 C. Highest explant establishment of 35.42 percent was obtained in 1613 C, rootstock (Fig 7) followed by 1616 C (Fig 8) and R 110 (Fig 6). As far as growth regulator combination is concerned, BAP (1.0 mg/l) and Kinetin (1.0 mg/l) proved to be the best combination for explant establishment (40.80 %). Lowest explant establishment (31.15 %) was obtained on MS medium supplemented with BAP (2.0 mg/l) and Kinetin (1.0 mg/l).

Among the rootstocks the maximum shoot tip establishment in R 110 rootstock (Fig 6) was recorded on MS media supplement with BAP (1.0 mg/l) and Kinetin (1.0 mg/l) and minimum (22.9 %) was recorded in Degrasset rootstock when cultured on MS medium supplemented with BAP (1.5 mg/l) and Kinetin (0.5 mg/l).

Table 4.3: Effect of growth regulators on establishment of shoot tips in different grape rootstocks

Growth regulators (mg/l) / Rootstock	Explant(Shoot tips) Establishment (%)				Mean (%)
	Degrasset	R 110	1613 C	1616 C	
BAP (0.5)	21.05 (27.14)*	26.08 (30.62)*	28.94 (32.41)*	35.00 (36.24)*	27.77 (31.60)*
BAP (0.5) + Kin (0.5)	31.25 (33.95) *	32.14 (33.71) *	39.27 (38.73)*	42.85 (40.86) *	36.08 (36.81)*
BAP (1.0)	26.31 (30.81)*	31.42 (34.04)*	29.72 (33.00) *	26.66 (31.03)*	28.52 (32.22)*
BAP (1.0) + Kin (0.5)	18.18 (24.97)*	37.03 (37.34) *	31.57 (34.08)*	36.36 (37.22)*	30.87 (33.40)*
BAP (1.0) + Kin (1.0)	40.74 (39.62) *	52.94 (46.66)*	34.61 (35.99)*	42.93 (40.91)*	42.80 (40.80)*
BAP (1.5) + Kin (0.5)	15.38 (22.90) *	33.33 (35.23)*	38.46 (38.14)*	32.43 (34.53)*	29.90 (32.70)*
BAP (2.0)	31.57 (34.15)*	34.28 (35.79)*	36.36 (37.05)*	42.10 (40.42)*	36.06 (36.85)*
BAP (2.0) + Kin (0.5)	23.07 (28.66)*	27.27 (31.45) *	32.00 (34.36)*	31.03 (33.83)*	28.34 (32.08)*
BAP (2.0) + Kin (1.0)	30.43 (33.40)*	23.52 (28.83)*	26.08 (34.32)*	22.22 (28.06)*	27.04 (31.15)*
BAP (2.0) + Kin (2.0)	28.57 (32.22)*	28.00 (31.83) *	35.48 (36.15) *	18.18 (25.20)*	27.55 (31.35)*
Mean	26.65 (30.78)*	32.48 (34.55) *	33.84 (35.42)*	33.00 (34.83)*	
CD (0.05)	Rootstock (A)= 1.97, Media (B) = 3.12, A×B=6.25				

*Figures in parenthesis are Arc Sine transformed means.

4.4 Effect of growth regulators on days taken for shoot induction:

Data in Table 4.4 shows the effect of medium composition supplemented with various combinations of BAP and Kinetin on days taken for percent shoot induction of all genotypes *viz.,* rootstocks Degrasset, R 110, 1613 C, 1616 C. Shoot induction occurs early in rootstock Degrasset followed by R 110 and 1613 C. As far as growth regulator combination is concerned, BAP (1.0 mg/l) and Kinetin (0.5 mg/l) proved the best combination for shoot induction, as it was earliest to induce shoot induction (13.17 days) followed by BAP (0.5 mg/l). These two treatments were significantly different from rest of the treatment combinations. Maximum number of days were required for shoot induction obtained on MS medium supplemented with BAP (2.0 mg/l) and Kinetin (0.5 mg/l).

The earliest shoot induction (12 days) was recorded in rootstock R 110 and 1613 C on BAP (0.5 mg/l) and Kinetin (0.5 mg/l). The shoot induction was delayed in 1613 C and 1616 C rootstock when MS medium was supplemented with BAP (2.0 mg/l) and Kinetin (2.0 mg/l).

Table 4.4 Effect of growth regulator concentration and combination on days taken for shoot induction in different rootstocks.

Growth regulators (mg/l) /Rootstock	Days taken for shoot induction				Mean (d)
	Degrasset	R 110	1613 C	1616 C	
BAP (0.5)	13	12	12	13	13.25
BAP (0.5) + Kin (0.5)	14	12	12	13	13.75
BAP (1.0)	14	15	14	14	13.91
BAP (1.0) + Kin (0.5)	13	14	14	13	13.17
BAP (1.0) + Kin (1.0)	15	14	16	16	15.33
BAP (1.5) + Kin (0.5)	18	16	15	17	17.92
BAP (2.0)	18	20	19	18	21.08
BAP (2.0) + Kin (0.5)	22	20	23	22	22.91
BAP (2.0) + Kin (1.0)	20	23	22	22	20.83
BAP (2.0) + Kin (2.0)	21	22	23	23	22.41
Mean	17.03	17.27	17.37	18.16	
CD (0.05)	Rootstock (A)= 0.60, Media (B)= 0.95, A×B=1.90				

4.5 Effect of growth regulators on nodal segments showing browning/phenol exudation:

Data in Table 4.5 shows the effect of different growth regulator concentrations and combinations on induction of browning in nodal segments of all the four rootstocks. Data clearly revealed that rootstock and growth regulator combination had significant effect on browning during establishment stage of explants.

The effect of growth regulators on browning in different rootstocks is presented in Table 4.5. The data reveals that both rootstocks and growth regulator concentration and combinations had significant effect on browning and explant establishment. The mean minimum (0.13 %) browning was recorded on MS medium fortified with BAP (1.0 mg/l) and mean maximum (0.17 %) browning was recorded with BAP (1.5 mg/l) and Kinetin (0.5

Fig 5: Establishment of Degrasset from shoot tips on MS medium fortified with BAP (2.0 mg/l).

Fig 6: Establishment of R 110 on MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l).

Fig 7: Establishment of 1613 C on MS medium fortified with BAP (0.5 mg/l) + Kinetin (0.5 mg/l).

Fig 8: Establishment of 1616 C on MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l).

mg/l). The maximum browning was recorded in R 110 and 1616 C rootstock which was statistically at par with Degrasset and 1613 C rootstock. The browning percentage does not differ significantly with in the media combination.

Table 4.5 Effect of growth regulators on nodal segments showing percent browning / phenol exudation (%).

Growth regulator combination (mg/l) / Rootstocks	Explants showing browning / phenol exudation (%)				Mean(%)
	Degrasset	R 110	1613 C	1616 C	
BAP(0.5)	0.18	0.14	0.16	0.19	0.14
BAP (0.5) + Kin (0.5)	0.10	0.18	0.14	0.17	0.16
BAP (1.0)	0.15	0.13	0.10	0.15	0.13
BAP (1.0) + Kin (0.5)	0.12	0.13	0.18	0.18	0.15
BAP (1.0) + Kin (1.0)	0.17	0.12	0.15	0.19	0.16
BAP (1.5) + Kin (0.5)	0.13	0.18	0.19	0.16	0.17
BAP (2.0)	0.15	0.11	0.10	0.18	0.14
BAP(2.0) + Kin (0.5)	0.12	0.18	0.16	0.17	0.16
BAP(2.0) + Kin (1.0)	0.15	0.10	0.17	0.17	0.14
BAP(2.0) + Kin (2.0)	0.13	0.18	0.15	0.13	0.15
Mean	0.14	0.16	0.14	0.16	
CD (0.05)	**Rootstock (A)= 0.33, Media (B) = 0.52, A×B= 0.10**				

4.6 Effect of growth regulators on per cent shoot regeneration:

Shoots from established explants were cultured on different media supplemented with varying doses of growth regulators. Initially, the shoot growth was very slow in all media at all levels of BAP. However, after two subcultures on respective media, two to three axillary shoots arose from the base of cultured shoots.

Perusal of data in Table 4.6 clearly revealed that proliferated culture percentage was highest in rootstock Degrasset (52.89 %), which is significantly higher than 49.84 % in R 110 and 45.62 % in 1616 C. Proliferated culture percentage increased significantly with increase in concentration of BAP. There was significant interaction between rootstock and medium composition in determining the percent proliferated culture during proliferation stage. As far as growth regulator combination is concerned, BAP (1.0 mg/l) and Kinetin (1.0 mg/l) proved to be the best combination for highest proliferated cultures, followed by BAP (1.0 mg/l). MS medium containing BAP (1.0 mg/l) and Kinetin (1.0 mg/l) resulted in significantly highest proliferated cultures (65.67 %).

Cytokinin had significant effect on shoot regeneration in different rootstocks. Data pertaining to shoot regeneration in different rootstocks when cultured on MS media with varying concentration of BAP and Kinetin is presented in Table 4.6. The mean maximum (65.67 %) shoot regeneration was recorded in MS medium supplemented with BAP (1 mg/l) and kin (1 mg/l) which was significantly better than all other media combination. Mean minimum (32.82 %) shoot regeneration was recorded in MS medium supplemented with BAP

10 mg / l. Out of four rootstocks, Degrasset gave the maximum (52.89 %) shoot regeneration , which was at par with R 110 but significantly better than 1613 C and 1616 C rootstock .

Best shoot regeneration (Fig 9) was recorded in DeGrasset (77.00 %) and R 110 (72.57 %) rootstock (Fig 10) when cultured on MS medium supplemented under BAP (1.0 mg/l) and kin (1.0 mg/l). Similarly 1613 C (61.58 %) and 1616 C (65.04 %) gave maximum shoot regeneration on MS medium supplemented under BAP (0.5 mg/l) and kin (0.5 mg/l) as shown in Fig 11 and Fig 12.

Table 4.6: Effect of growth regulators on per cent shoot regeneration in different grape Rootstocks

Growth regulators (mg/l) / Rootstock	**Shoot regeneration (%)**				**Mean (%)**
	Degrasset	**R 110**	**1613 C**	**1616 C**	
BAP (0.5) + Kin (0.5)	85.00(67.72)*	72.00 (58.05)*	77.27 (61.58)*	82.14 (65.04)*	79.10 (63.10)*
BAP(1.0)	92.80 (74.63)*	81.25 (64.34)*	66.66 (54.70)*	78.57 (62.43)*	79.82 (64.02)*
BAP(1.5)	83.33 (65.96)*	77.27 (61.51)*	70.37 (57.03)*	74.28 (59.53)*	76.31 (61.01)*
BAP(1.0) + Kin (0.5)	75.00 (60.12)*	73.33 (58.93)*	68.75 (55.99)*	73.33 (58.89)*	72.60 (58.48)*
BAP(1.5) + Kin (0.5)	72.22 (58.18)*	88.46 (70.80)*	55.0 (47.86)*	60.52 (57.06)*	69.05 (56.98)*
BAP(1.0) + Kin (1.0)	94.73 (77.00)*	90.90 (72.57)*	61.53 (51.65)*	77.14 (61.48)*	81.07 (65.67)*
BAP (2.0)	83.33 (66.39)*	85.18 (67.47)*	68.75 (56.00)*	81.25 (64.39)*	79.62 (63.56)*
BAP (2.0) + Kin (0.5)	85.71 (67.86)*	80.64 (63.91)*	67.85 (55.45)*	65.00 (53.71)*	74.80 (60.23)*
BAP (2.0) + Kin (1.0)	71.42 (57.78)*	75.00 (60.05)*	58.33 (49.77)*	42.85 (40.87)*	62.00 (52.12)*
BAP (2.0) + Kin (2.0)	67.64 (55.34)*	68.75 (56.05)*	27.27 (31.45)*	40.74 (39.63)*	51.10 (45.62)*
BAP (4.0)	76.66 (61.09)*	47.05 (43.28)*	23.07 (28.69)*	35.71 (36.66)*	45.62 (42.63)*
BAP (4.0) + Kin(0.5)	57.14 (49.08)*	45.55 (42.42)*	30.76 (33.66)*	27.27 (28.09)*	38.93 (38.31)*
BAP (4.0) + Kin(2.0)	38.46 (38.30)*	28.57 (32.29)*	38.88 (38.48)*	33.33 (35.22)*	34.81 (36.07)*
BAP (6.0)	44.00 (41.53)*	34.61 (36.01)*	31.25 (33.91)*	37.50 (37.73)*	36.84 (37.30)*
BAP (6.0) + Kin(0.5)	36.36 (37.06)*	38.46 (38.30)*	36.84 (37.35)*	36.36 (37.06)*	37.00 (37.44)*
BAP (6.0) + Kin(2.0)	45.45 (42.37)*	42.10 (40.43)*	38.09 (38.09)*	42.85 (40.86)*	42.12 (40.44)*
BAP (8.0)	38.09 (38.09)*	53.84 (47.18)*	44.82 (42.00)*	42.30 (40.54)*	44.76 (41.95)*
BAP (8.0) + Kin(0.5)	46.15 (42.78)*	48.14 (43.91)*	15.38(23.04)*	30.76 (33.60)*	35.10 (35.83)*
BAP (8.0) + Kin(2.0)	40.74 (39.64)*	42.30 (40.54)*	21.05 (27.26)*	31.57 (34.16)*	33.91 (35.40)*
BAP (10.0)	39.39 (38.84)*	22.22 (28.07)*	17.64 (24.81)*	40.62 (39.55)*	29.96 (32.82)*
BAP (10.0) + Kin(0.5)	45.83 (42.58)*	36.00 (36.79)*	14.28 (22.18)*	48.27 (44.00)*	36.09 (36.39)*
BAP (10.0) + Kin(2.0)	43.75 (41.36)*	30.43 (33.46)*	18.18 (25.19)*	40.00 (39.21)*	33.09 (34.80)*
Mean	61.97 (52.89)*	57.36 (49.84) *	43.28 (40.73)*	50.79(45.62) *	
C.D(0.05)	**Rootstock (A)= 0.90, Media (B) = 2.13, A×B= 4.26**				

*Figures in parenthesis are Arc Sine transformed means.

Fig 9: Proliferation of Degrasset on MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l).

Fig 10: Proliferation of R 110 on MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l).

Fig 11: Proliferation of 1613 C on MS medium fortified with BAP (0.5 mg/l) + Kinetin (0.5 mg/l).

Fig 12: Proliferation of 1616 C on MS medium fortified with BAP (0.5 mg/l) + Kinetin (0.5 mg/l).

4.6.1 Effect of growth regulators on number of shoots per explant:

Similarly number of shoots per explant showed positive relation with BAP concentration upto a level, after which further increase in BAP concentration inhibited shoot proliferation (Table 4.7). Maximum number of shoots per explant (3.63) was recorded in MS medium suplemented with BAP (1.5 mg/l) which was significantly higher than 3.49 at BAP (0.5 mg/l) and Kin (0.5 mg/l). The minimal number of shoots per explants (1.71) were obtained in MS media supplemented with BAP (10.0 mg/l) and Kin (2.0 mg/l).

Furthermore, the highest rate of proliferation (8-10 shoots per cutting) were obtained with a high level of BAP (2.0 mg/l), but the internodes were short; fewer (4-6) but longer shoots were obtained with less BAP (0.5 mg/l) (Silvestroni 1981).

Similarly Lee *et al* (1990) observed that with higher BAP level (20, 40 µM); shoots were unexpanded and exhibited higher mortalities. However, shoot morphology was somewhat distorted at (BAP) 5-10 µM than at 2.5-3.75 µM (Hicks and Dorey 1988).

The increase in cytokinin levels beyond optimum limit resulted in vitrification. Moreover, maintenance of cultures under higher cytokinin concentration resulted in hyperhydricity of cultures (Lee and Wetzstein 1990, Heloir *et al* 1997).

Table 4.7: Effect of growth regulators on number of shoots /explant in different grape rootstocks

Growth regulators (mg/l) Rootstock	Number of shoots/explant				Mean (Number of shoots)
	Degrasset	R 110	1613 C	1616 C	
BAP(0.5) + Kin (0.5)	4.16	2.33	3.60	3.90	3.49
BAP (1.0)	2.50	2.60	2.75	1.80	2.41
BAP (1.5)	6.40	2.20	2.55	3.40	3.63
BAP (1.0) + Kin (0.5)	3.33	2.50	2.80	2.83	2.87
BAP (1.5) + Kin (0.5)	3.00	2.80	2.33	2.50	2.65
BAP (1.0) + Kin (1.0)	3.14	3.33	2.84	1.33	2.66
BAP (2.0)	2.28	3.00	2.20	2.25	2.43
BAP (2.0) + Kin (0.5)	3.25	1.57	1.16	2.40	2.09
BAP (2.0) + Kin(1.0)	3.75	2.75	1.11	2.20	2.45
BAP (2.0) + Kin (2.0)	3.33	1.75	1.73	2.80	2.40
BAP (4.0)	7.33	1.50	2.83	2.12	3.44
BAP (4.0) + Kin (0.5)	2.40	2.40	2.16	2.40	2.34
BAP (4.0) + Kin (2.0)	1.80	2.60	2.50	2.16	2.26
BAP (6.0)	2.20	1.66	2.83	2.60	2.32
BAP (6.0) + Kin (0.5)	2.00	2.00	2.85	2.50	2.33
BAP (6.0) + Kin (2.0)	2.60	1.75	2.83	2.33	2.38
BAP (8.0)	2.33	2.50	2.14	2.37	2.33
BAP (8.0) + Kin (0.5)	2.50	2.28	2.00	2.71	2.37
BAP (8.0) + Kin (2.0)	2.75	2.40	1.83	2.50	2.37
BAP (10.0)	1.75	1.83	2.33	2.66	2.14
BAP (10.0) + Kin (0.5)	2.60	1.80	1.80	1.57	1.94
BAP (10.0) + Kin (2.0)	2.00	1.60	1.60	1.66	1.71
Mean	3.06	2.23	2.30	2.40	
C.D (0.05)	Rootstock (A) = 0.13, Media (B) = 0.32, A×B = 0 .64				

Among different rootstocks, Degrasset had maximum (3.06) response to number of shoots per explant followed by 1616 C (2.40) and minimum (2.23) obtained in R 110 rootstock.

4.6.2 Effect of growth regulators on average length of shoots:

The effect of growth regulators on average shoot length is presented in Table 4.8. Average length of shoots also varied with type of media and change in growth regulator and its concentrations. There was decrease in average length of shoots with an increase in level of BAP. It was observed that mean maximum (3.13 cm) shoot length was recorded on MS medium supplemented with BAP (0.5 mg/l)and Kinetin (0.5) mg/l which was significantly better than all other media combination . The mean minimum (0.66 cm) shoot length was recorded in MS medium supplemented with BAP (10.0) and Kin (2.0 mg/l). . The media combination and rootstock has significant effect on length of shoots. The length of shoots in different rootstocks does not vary significantly. The mean maximum shoot length (1.73 cm) was recorded in 1613 C rootstock, followed by Degrasset (1.71 cm).

Table 4.8: Effect of growth regulators on average shoot length in different grape rootstocks.

Growth regulators (mg/l) / Rootstock	Average length of shoots (cm)				Mean
	Degrasset	R 110	1613 C	1616 C	
BAP (0.5) + Kin (0.5)	2.77	2.10	3.82	3.83	3.13
BAP (1.0)	4.06	2.75	2.45	2.30	2.89
BAP (1.5)	1.77	2.47	2.72	1.71	2.16
BAP (1.0) + Kin (0.5)	1.70	2.52	2.40	2.58	2.30
BAP (1.5) + Kin (0.5)	1.96	2.51	1.82	2.04	2.08
BAP (1.0) + Kin 1.0)	2.91	1.59	2.52	2.20	2.30
BAP (2.0)	3.55	1.82	2.30	2.46	2.53
BAP (2.0) + Kin (0.5)	2.80	2.82	2.45	2.22	2.57
BAP (2.0) + Kin (1.0)	1.95	2.95	2.27	1.76	2.23
BAP (2.0) + Kin (2.0)	2.10	1.67	1.98	1.55	1.82
BAP (4.0)	1.75	2.08	2.47	1.72	1.98
BAP (4.0) + Kin(0.5)	2.20	1.78	1.75	1.62	1.83
BAP (4.0) + Kin(2.0)	1.20	1.06	1.47	1.56	1.32
BAP (6.0)	0.90	0.96	0.87	0.92	0.91
BAP (6.0) + Kin (0.5)	0.82	0.80	1.04	1.30	0.99
BAP (6.0) + Kin (2.0)	0.90	0.94	0.98	0.88	0.92
BAP (8.0)	0.54	0.92	1.00	1.06	0.87
BAP (8.0) + Kin (0.5)	0.94	0.76	0.92	1.07	0.92
BAP (8.0) + Kin (2.0)	0.96	1.08	0.72	0.75	0.87
BAP (10.0)	0.70	0.92	0.76	0.95	0.83
BAP (10.0) + Kin (0.5)	0.72	0.77	0.72	0.68	0.72
BAP (10.0) + Kin (2.0)	0.48	0.80	0.75	0.62	0.66
Mean	1.71	1.63	1.73	1.62	
CD (0.05)		Rootstock (A)=0.61, Media(B)= 0.14, A×B= 0.28			

4.7 Effect of growth regulators on percent rooting:

The Table 4.9 revealed the effect of growth regulators on percent rooting. On an average of 2-3 cm shoot was used to induce rooting in all the rootstocks. It was noted that mean maximum percent (64.95) rooting was recorded with IBA (0.1 mg/l) and NAA (0.1 mg/l), which was at par with IBA (0.1 mg/l). Similarly maximum percent (55.64) rooting was recorded in Degrasset rootstock which was significantly better than all other rootstocks. In R 110 rootstock (55.07) percent rooting was recorded followed by 1616 C and 1613 C. The best rooting (82.03) percent was recorded in Degrasset rootstock on MS medium supplemented with IBA (0.1 mg/l) as shown in Fig 15.

Table 4.9: Effect of growth regulators on per cent rooting in different rootstocks

Growth regulators (mg/l) / Rootstock	Per cent rooting				Mean
	Degrasset	R 110	1613 C	1616 C	
IBA (0.01)	73.91 (59.26)*	71.94 (58.01)*	61.74 (51.77)*	64.66 (53.51)*	68.06 (55.64)*
IBA (0.01) + NAA (0.01)	66.66 (54.73)*	76.92 (61.26)*	63.63 (52.89)*	77.77 (61.88)*	71.24 (57.69)*
IBA (0.05)	83.33 (65.37)*	75.00 (59.98)*	70.00 (56.77)*	71.42 (57.67)*	74.77 (59.94)*
IBA (0.05) + NAA (0.05)	75.43 (60.33)*	78.44 (62.33)*	68.76 (56.03)*	61.13 (51.43)*	70.94 (57.53)*
IBA (0.1)	97.8 (82.03)*	80.00 (63.42)*	70.58 (57.13)*	68.75 (56.00)*	79.28 (64.65)*
IBA (0.1) + NAA (0.1)	89.47 (71.09)*	88.23 (69.97)*	78.57 (62.43)*	69.23 (56.29)*	81.37 (64.95)*
IBA (0.2) + NAA (0.2)	76.47 (60.96)*	64.28 (53.30)*	63.63 (52.89)*	87.50 (69.47)*	72.97 (59.15)*
IBA (0.3) + NAA (0.3)	63.63 (52.89)*	62.50 (52.22)*	58.33 (49.77)*	66.66 (54.71)*	62.78 (52.40)*
IBA (0.25)	73.33 (58.90)*	85.71 (67.79)*	82.60 (65.32)*	81.25 (64.42)*	80.72 (64.11)*
IBA (0.5)	62.85 (52.43)*	72.22 (58.27)*	66.66 (54.71)*	76.92 (61.27)*	69.66 (56.67)*
NAA (0.5)	57.14 (49.08)*	64.70 (53.52)*	68.42 (55.79)*	73.68 (59.11)*	65.98 (54.38)*
IBA (0.5) + NAA (0.5)	61.53 (51.65)*	68.42 (55.79)*	61.53 (51.64)*	64.28 (53.28)*	63.94 (53.09)*
Control	0.00 (0.00)*	0.00 (0.00)*	0.00 (0.00)*	0.00 (0.00)*	0.00 (0.00)*
Mean	67.76 (55.64)*	68.33 (55.07)*	62.65 (51.32)*	66.40 (53.77)*	
CD (0.05)	**Rootstock (A)= 0.85, Media (B) = 1.54, A×B = 3.08**				

*Figures in parenthesis are Arc Sine transformed means

Likewise, Lu *et al* (2004) obtained 98 % rooting, when proliferated shoots of 'NW 196' were cultured on half strength medium MS medium supplemented with IBA 1mg/l + activated charcoal 0.2 mg/l.

4.7.1 Effect of growth regulators on number of roots per explants:

The effect of growth regulators on number of roots per explant is discussed in Table 4.10. Significantly maximum (10.15) number of roots were induced in MS medium fortified with IBA (0.01 mg/l) and NAA (0.01 mg/l). Similarly rootstock R 110 and 1616 C gave maximum (6.28) roots per explants followed by Degrasset (5.99) and 1613 C (5.26). The genotype and media combination has significant effect on number of roots per explant. Maximum number of roots (13.75) were recorded in R 110 rootstock on IBA (0.01 mg/l) and NAA (0.01 mg/l) followed by 1616 C rootstock (10.66) on IBA (0.5 mg/l).

Table 4.10: Effect of growth regulators on number of roots/explant in different rootstocks.

Growth regulators (mg/l) / Rootstock	Number of roots/explant				Mean
	Degrasset	R 110	1613 C	1616 C	
IBA (0.01)	5.59	8.75	6.11	9.14	7.39
IBA (0.01) + NAA (0.01)	7.40	13.75	9.80	9.66	10.15
IBA (0.05)	5.66	6.85	6.60	7.83	6.73
IBA (0.05) + NAA (0.05)	6.77	4.33	4.25	5.01	5.09
IBA (0.1)	4.50	3.28	2.87	3.33	3.49
IBA (0.1) + NAA (0.1)	5.60	5.50	6.16	6.25	5.88
IBA (0.2) + NAA (0.2)	7.66	3.60	3.20	2.50	4.24
IBA (0.3) + NAA (0.3)	3.50	3.40	3.50	7.66	4.51
IBA (0.25)	6.80	7.16	5.90	6.50	6.59
IBA (0.5)	9.80	8.80	6.40	10.66	8.91
NAA (0.5)	7.83	7.66	7.40	6.85	7.43
IBA (0.5) + NAA (0.5)	6.85	8.66	6.25	6.33	7.02
Control	0.00	0.00	0.00	0.00	0.00
Mean	5.99	6.28	5.26	6.28	
CD (0.05)	Rootstock (A)= 0.25, Media (B) = 0.46, A×B= 0.92				

4.7.2. Effect of growth regulators on average length of roots.

The Table 4.11 depicts the effect of growth regulators on average length of roots of different grape rootstockss. It as observed that average length of roots was maximum (3.60) on MS medium supplemented with IBA (0.1 mg/l) which was significant better than all other treatments. Similarly mean maximum length of roots (2.17) was recorded in rootstock Degrasset and it was at par with all other rootstocks. The maximum average length of roots (5.66) was recorded in Degrasset rootstock when cultured on MS medium supplemented with IBA (0.1 mg/l) followed by the same rootstock on IBA (0.01 mg/l). The media combination and the genotypes had significant effect on average length of roots.

Fig 13: Rooting of 1613 C on MS medium fortified with IBA (0.25 mg/l)

Fig 14: Rooting of R 110 on MS medium fortified with IBA (0.1 mg/l) + NAA (0.1 mg/l)

Fig 15: Rooting of Degrasset on MS medium fortified with IBA (0.1 mg/l).

Fig 16: Rooting of 1616 C on MS medium fortified with IBA (0.2 mg/l) + NAA (0.2 mg/l).

Fig 17: Hardening of in vitro plantlets on cotton moist with distilled water.

Fig 18: Transfer of plantlets in potting mixture combination of peat: perlite: vermiculite (2:1:1)

Fig 19: Survival of plants in potting mixture combination of peat: perlite: vermiculite (2:1:1) under *ex vitro* conditions

Table 4.11: Effect of growth regulators on average root length in different grape rootstocks.

Growth regulators (mg/l) / Rootstock	Average length of roots (cm)				Mean
	Degrasset	R 110	1613 C	1616 C	
IBA (0.01)	4.33	2.34	2.66	2.23	2.89
IBA (0.01) + NAA (0.01)	1.92	1.46	2.85	2.76	2.24
IBA (0.05)	2.34	2.12	2.97	2.40	2.45
IBA (0.05) + NAA (0.05)	4.12	1.87	2.12	3.10	2.80
IBA (0.1)	5.66	2.75	2.88	3.14	3.60
IBA (0.1) + NAA (0.1)	1.12	2.22	2.87	1.03	1.81
IBA (0.2) + NAA (0.2)	1.38	1.17	2.80	0.80	1.53
IBA (0.3) + NAA (0.3)	1.62	1.22	1.20	1.24	1.32
IBA (0.25)	2.75	2.10	1.60	3.08	2.38
IBA (0.5)	0.82	4.93	0.92	1.10	1.94
NAA (0.5)	0.97	0.93	0.70	1.22	0.94
IBA (0.5) + NAA (0.5)	1.20	1.02	0.66	1.42	1.07
Control	0.00	0.00	0.00	0.00	0.00
Mean	2.17	1.85	1.86	1.80	
CD (0.05)	Rootstock (A)= 0.88, Media (B) = 0.15, A×B= 0.31				

Plantlet acclimatization

The shoot cultures, each with two or more strong roots, after 3 weeks on rooting medium were washed thoroughly and transferred to wide mouthed bottles containing quarter-strength liquid MS medium (without sucrose) and absorbant cotton as a support for hardening and maintained under culture room conditions (Fig 17). After 2 weeks, the plantlets were transferred to small plastic pots containing mixture of peat, perlite and vermiculite with different combinations, watered and transferred to net house in shade (Fig 18). The potted plants were covered with transparent polythene bags to maintain high humidity. After 10 days, the leaves started to emerge and therefore to reduce excessive humidity the polythene bags were removed. Finally in about 4 weeks time the plants grew in length and became hardy and therefore the established plants were further transferred to large pots and rate of establishment was recorded (Fig 19). The combination of peat perlite and vermiculite in the form of 2:1:1 is the best combination. Among rootstocks, Degrasset rootstock is the best rootstock for plantlet establishment under *ex vitro* conditions.

CHAPTER-V

SUMMARY

Grape (*Vitis vinifera* L.) is an important fruit crop cultivated worldwide and is considered most refreshing and nourishing fruit of the world. The major producers of grapes in world are China, Italy, USA, Spain, France, Turkey and Argentina. India has occupied ninth rank with 3.31 percent share in world grape production. At present, Punjab occupies 441 ha area under grapes with an annual production of 12.5 thousand MT. Almost all the commercial plantations of grapes in the state are on own roots. The quality of Perlette grapes produced in Punjab are comparatively inferior from commercial grapes produced in rest of the major grape growing regions in the country. Apart from unfavourable climate under North Indian conditions, the production of quality grapes is further limited due to their susceptibility to fungal diseases such as anthracnose and powdery mildew. Further more, the abiotic factors such as salinity and drought are likely to limit/expansion of grapes in potential areas. To promote grape cultivarion in such areas, promising varieties should be promoted on rootstocks in near future. *In vitro* multiplication is the most appropriate method for producing large number of quality plantlets for vineyard establishment in considerably lesser time.

Despite years of investigation, the application of tissue culture techniques in the grape-growing industry is still limited. Among the major obstacles for the widespread utilization of this technology is relatively high cost compared with the conventional methods and the possibility of genetic discrepancy in micropropagated plants. Unfortunately, there is huge variation among various genotypes and as such there is no routine tissue culture regeneration and transformation system in grapes. Moreover, there are few reports on micropropagation of potential rootstocks having utility under north Indian conditions. Keeping in view the decline of area under grape cultivation in Punjab and with a view to diversify and to uphold the economic stability of grape growers, the present study was under taken to standardize a protocol for *in vitro* shoot regeneration in promising grape rootstocks and to optimize conditions for efficient rooting of *in vitro* shoots and plantlet establishment.

In vitro culture is a useful tool for rapid and disease free propagation of plants. Genotype is one of the most critical factors for the *in vitro* proliferation response. Explant source, its maturity, type, season of collection all affect the success or failure of micropropagation protocol. In rootstocks April to October have been widely used as the choice months for taking explants for *in vitro* propagation of grapes. The *in vitro* regeneration from explants depends on a number of factors. These include the selection of organs that serve as tissue source, size of the explant, original position of the explants in the plant, age of the plant and overall quality of the plant from which the explants are to be obtained. The regeneration ability in grapevine microcutting is influenced by the month in which it is cultured and highest regenerative ability is found in explants at active growth stage i.e. June and July. A balance between endogenous and exogenous growth regulators controls the initiation and development of shoots, roots, plantlet and callus.

The present investigation "Studies on *in vitro* propagation of promising grape (*Vitis vinifera*.L) rootstocks" was carried out to standardize a protocol for *in vitro* shoot regeneration in promising grape rootstocks and to optimize conditions for efficient rooting of *in vitro* shoots using basal medium supplemented with different growth regultors and plantlet establishment. The explants *viz* shoot tips and nodal segments cultured in month of April, exhibited the highest establishment i.e 43.51 % which may be due to presence of lesser polyphenolic content in the vines in the month of April. Rootstock R 110 exhibited the mean maximum establishment i.e 44.06 %. The MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l) induced maximum 45.54 % from nodal segments. Rootstock R 110 exhibited the mean maximum nodal segment establishment i.e 47.37 %.The MS medium fortified with BAP (1.0 mg/l) + Kinetin (1.0 mg/l) induced maximum of 40.80 % establishment from shoot tips. 1613 C rootstock exhibited the mean maximum shoot tip establishment i.e 35.42 %.

The mean minimum (0.13 %) browning was recorded in MS medium fortified with BAP (1.0 mg/l) and mean maximum (0.17 %) was recorded with BAP (1.5 mg/l) and Kinetin (0.5 mg/l). The maximum browning was recorded in R 110 rootstock which was statistically at par with Degrasset and 1613 C rootstock. The browning percentage does not differ significantly within the media combination. BAP (1.0 mg/l) and Kinetin (0.5 mg/l) proved to be the best combination for shoot induction followed by BAP (0.5 mg/l). More number of days were required for shoot induction obtained on MS medium supplemented with BAP (2.0 mg/l) and kinetin (0.5 mg/l). The earliest shoot induction (12 days) was recorded in rootstock R 110 and 1613 C on BAP (0.5 mg/l) or BAP (0.5 mg/l) and Kinetin (0.5 mg/l). The shoot induction was delayed in 1613 C and 1616 C rootstock, when MS medium was supplemented with BAP (2.0 mg/l) and Kinetin (2.0 mg/l).

The mean maximum (65.67 %) shoot regeneration was recorded in MS medium supplemented with BAP (1.0 mg/l) and Kin (1.0 mg/l) which was significantly better than all other media combination. Mean minimum (32.82 %) shoot regeneration was recorded in MS medium fortified with BAP (10.0 mg/l.) Degrasset gave the maximum (52.89 %) shoot regeneration, which was at par with R 110 but significant better than 1613 C and 1616 C rootstock. Best shoot regeneration was recorded in Degrasset and R 110 rootstock when cultured on MS medium supplemented with BAP (1.0 mg/l) and Kin (1.0 mg/l). Similarly 1613 C and 1616 C gave maximum shoot regeneration on MS_1 medium supplemented with BAP (0.5 mg/l) and Kinetin (0.5 mg/l) . Maximum number of shoots per explant (3.63) were produced at BAP (1.5 mg/l), which was significantly higher than 3.49 at BAP (0.5 mg/l) and

Kinetin (0.5 mg/l). Mean maximum (3.13 cm) shoot length was recorded on MS supplemented with BAP (0.5 mg/l) and Kinetin (0.5 mg/l) medium which was significantly better than all other media combination. The mean minimum (0.66 cm) shoot length was recorded in MS medium fortified with BAP (10.0 mg/l) and Kinetin (2.0 mg/l). The media combination and rootstock had significant effect on length of shoots. The mean maximum shoot length (1.73 cm) was recorded in 1613 C rootstock followed by Degrasset (1.71 cm).

Mean maximum (64.95 %) rooting was recorded with IBA (0.1 mg/l) and NAA (0.1 mg/l) which was at par with MS medium fortified with IBA (0.1 mg/l) and MS medium fortified with IBA (0.25 mg/l). Among rootstocks maximum (55.64 %) rooting was recorded in Degrasset rootstock which was significantly better than all other rootstocks. In R 110 rootstock (55.07 %) rooting was recorded followed by 1616 C and 1613 C. The best rooting (82.03 %) was recorded in Degrasset rootstock on MS medium supplemented with IBA (0.1 mg/l). Maximum (10.15) roots were induced in MS medium fortified with IBA (0.01 mg/l) and NAA (0.01 mg/l). Rootstock R 110 and 1616 C gave maximum (6.28) roots followed by Degrasset (5.99) and 1613 C (5.36). Maximum number of roots (13.75) was recorded in R 110 rootstock on IBA (0.01 mg/l) and NAA (0.01 mg/l) followed by 1616 C rootstock (10.66) on IBA (0.5 mg/l). Average length of roots was maximum (3.6 cm) was obtained on MS medium supplemented with IBA (0.1 mg/l) which was significant better than all other treatments. Mean maximum length of roots was recorded in rootstock Degrasset and it was at par with all other rootstocks. The maximum average length of roots was recorded in Degrasset rootstock when cultured on MS medium supplemented with IBA (0.1 mg/l). The shoot cultures, each with two or more strong roots, after 3 weeks on rooting medium were washed thoroughly and transferred to wide mouthed bottles containing quarter-strength liquid MS medium (without sucrose) and absorbant cotton as a support for hardening and maintained under culture room conditions. After 2 weeks, the plantlets were transferred to small plastic pots containing mixture of peat, perlite and vermiculite with different combinations. The combination of peat perlite and vermiculite in the form of 2:1:1 is the best combination. Among rootstocks, Degrasset rootstock is the best rootstock for plantlet establishment under *ex vitro* conditions.

Thus, *in vitro* shoot and root regeneration procedure reported in this study is rapid, efficient and reproducible thus can be used for large scale production and is expected to contribute to the future genetic improvement of this important multipurpose fruit crop.

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VITA

Name of the student	:	Avtar Singh
Father's name	:	Sh. Darshan Singh
Mother's name	:	Smt. Shinder Kaur
Nationality	:	Indian
Date of birth	:	03.10.1984
Permanent home address	:	Village & Post Office Nathana Teh & Distt. Bathinda (Pb.)

EDUCATIONAL QUALIFICATIOINS

Bachelor degree	:	B. Sc. Agriculture (Hons.)
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2010
OCPA/% marks	:	63.3%
Master's degree	:	M. Sc. Fruit Science
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2014
OCPA	:	7.03/10.00
Title of Master's Thesis	:	Studies on *in vitro* propagation of promising grape (*Vitis Vinifera* L.) rootstocks