In vitro Propagation Studies in Quince (*Cydonia oblonga* Mill.)

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(2010-A-857-M)

Division of Fruit Science

Faculty of Postgraduate Studies

Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Thesis

Submitted to

The Faculty of Postgraduate Studies
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in partial fulfilment of requirement for the award of the degree of

Master of Science in Horticulture
(Fruit Science)

2012


Dedicated
to my beloved Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Fruit Science, Shalimar Campus, Srinagar
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CERTIFICATE – I

This is to certify that the thesis entitled "***In vitro* Propagation Studies in Quince (*Cydonia oblonga* Mill.)**" submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Horticulture (Fruit Science),** to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,** is a record of bonafide research work carried out by **Ms. Yusra Ali Basu (Regd. No. 2010-A-857-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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CERTIFICATE – II

We, the members of the Advisory committee of **Ms. Yusra Ali Basu (Regd. No. 2010-A-857-M)**, a candidate for the degree of **Master of Science in Horticulture (Fruit Science),** have gone through the manuscript of the thesis entitled, **"*In vitro* Propagation Studies in Quince (*Cydonia oblonga* Mill.)"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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CERTIFICATE – III

This is to certify that the thesis entitled, ***"In vitro* Propagation Studies in Quince (*Cydonia oblonga* Mill.)"** submitted by **Ms. Yusra Ali Basu (Regd. No. 2010-A-857-M)** to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Horticulture (Fruit Science)** was examined and approved by the Advisory Committee and external examiner on ……..

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ABSTRACT

The present investigation entitled "*in vitro* Propagation Studies in Quince (*Cydonia oblonga* Mill.)" was conducted in order to develop appropriate techniques of stock plant manipulations for obtaining explants suitable for *in vitro* culture, and to standardize media formulation and plant growth regulator regime for various stages of *in vitro* propagation of quince. The studies were conducted on explants derived from forced and unforced stock plants. Satisfactory culture asepsis (55.99% aseptic culture) was achieved in forced explants by subjecting them to the treatment of 0.1 per cent Mercuric chloride for 10 minutes followed by 70 per cent ethyl alcohol for 10 seconds. Murashige and Skoog's full strength medium with 0.50 mg /l BAP and 0.01 mg/l IBA gave the best response in terms of establishment of cultures. Murashige and Skoog's full strength basal media supplemented with BAP and IBA at different concentrations were tried for culture proliferation of quince and it was found that BAP at 0.50 mg/l and IBA at

0.01 mg/l gave the highest per cent proliferated cultures (72.49 %). However, the lowest percentage of proliferated cultures (41.66%) were observed in WPM medium supplemented with BAP 0.50mg/l and IBA 0.01mg/l. The elongated shoots were then transferred to MS medium supplemented with different concentrations of IBA and it was found that highest rooting percentage was achieved when the IBA was used at 1.25 mg/l. The present studies culminated in standardization of protocol for *in vitro* propagation of quince.

Key words : Aseptic culture, *in vitro* propagation, *in vitro* rooting, Proliferation, Quince, Tissue culture

Signature of Student

Dated : _____

Signature of Major Advisor

Dated: _____

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"Success is the journey not the destination in synthesis"
"Nothing worth is ever achieved without deep thought and hard work"
"Give me a point of support, I will rotate the entire world" This point of support is almighty Allah who showered his best of zeal, enthusiasm, dedication, perseverance, vigour and wit with a thick positive outcome. So it is the time to bow down my head in his praise and laud "He who wishes to fulfil his mission in the world must be a man of one idea that is of one great overmastering purpose, overshadowing all his aims and guiding and controlling his entire life"

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Yusra Ali Basu

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Chapter – 1

INTRODUCTION

Quince (*Cydonia oblonga* Mill.) belongs to the family Rosaceae and sub family Pomoideae. It is the sole member of the genus Cydonia and is supposed to be the 'golden apple of hesperides'. Quince is the relative of apples and pears as they all belong to the pome fruit family. Latin name for quince is cydonia, it originated in cydon where it has been grown from time immemorial. Quince is a good source of vitamin A, fibre, iron, but due to the astringency from their high tannin content, quince fruits are not eaten fresh. They contain high level of pectin, due to which they are popular for use in jams, marmalades, preserves etc. The far famed cotignac preserve of the French and Italians is also made from this fruit. However India is the leading exporter of *quince compote, quince sulc, quince sudzuk moravac* (a kind of pasta) and a lot of foreign currency is earned from these delicacies. The marketability and consumer acceptability of quince rum is more in abroad as compared to other fruits. It is rich in potassium, phosphorus, sodium, magnesium and provides energy upto 60 K/cal.

Quince contain large amount of hydroxycinnamic derivatives which are mainly composed of 3 and 5-caffeoylguinic acid and polymeric procyanidins. Major producing areas of world are Turkey, USA, New York, Europe, New Zealand, Brazil and J&K (Chadha, 2001). Turkey ranks first in the world quince production by producing the quarter of the total world's production. The total area under quince cultivation is about 3.96 m ha. In India, Quince is mostly grown in J&K and some parts of H.P. The principal cultivars grown in other parts of the world are Orange, Champion, Vandeman, Portugal, Smyrna and Pineapple (Ahmad *et al*., 2004).

Tissue culture is used widely in plant science, although it has a number of commercial applications. Infact, tissue culture is a very important tool not for the agriculture and forestry but also for the biotechnology. Consequently, it has

become the world's fastest growing and most rapidly changing technology. It has contributed tremendously to crop improvement and has a great potential for future. Plant tissue culture is used as a blanket term for protoplast, cell, tissue and organ culture under aseptic conditions. Infact, an important contribution or technique made through this is the revelation of the unique capacity of plant cells called cellular totipotency. Tissue culture has acquired many practical applications. One of the best established uses is as an efficient, safe and often economical method of plant propagation. Tissue culture techniques are used extensively for genetic modification of crop plants. Such manipulations are especially important for the improvement of tree fruit species due to long juvenility and high heterozygosity which often complicate conventional breeding. Infact some of the physiological processes such as nutrient uptake and utilization can be more easily studied using cultured plantlets and tissues. Meristem culture to raise virus free plant from those infected with virus is being widely practiced.

Further more, micropropagation is becoming increasingly important for a number of areas in horticulture which includes mass propagation of root stocks, scion cultivars and elite planting material. Apart from virus elimination, *in vitro* techniques offer exciting opportunities in quince improvement by way of using it as a tool for developing genotypes resistant to adverse biotic and abiotic factors, production of haploids, germplasm exchange and preservation. Among the various applied techniques of biotechnology, tissue culture and genetic engineering have opened new avenues for rapid and mass clonal propagation of superior genotypes all the year round, independent of seasonal limitations. Plant tissue culture has become a valuable tool for producing true to type, uniform, disease free plants, with distinctive characteristics of increased vigour, besides this *in vitro* techniques offer tremendous opportunities for improvement in fruit crops through genetic engineering so that novel and useful genotypes are available to cope with environmental stress through disease and pest resistance, salt and herbicide tolerance etc. (Pandey, 1993).

Tissue culture of higher plants has rapidly evolved as a dynamic and important field of endeavour during the last two decades. Tissue culture studies of quince has received much less attention as compared to the other pome fruits. Although some work has been reported on quince in abroad.

It would be valuable to propagate fruit trees by tissue culture techniques because the propagation rate is much higher than with the traditional methods. Quince is used as a root stock for pear. Pear is usually propagated by grafting on to quince (Brian and Duron, 1971). Quince as a root stock produce dwarf trees, which are suitable for high density planting system. The usual method of propagation of quince is by rooting of cuttings or layering which is very cumbersome and time consuming. Hence for rapid techniques of propagation, micropropagation is important. The *in vitro* technique is considered superior to conventional methods of propagation because of quick multiplication of plants in a relatively shorter period irrespective of season. Growing quince as alternative sort of fruit in relation to other fruits that are in current crises, due to over production opens new possibilities. Quince require less initial investment than other fruits. The production costs are also much lower than costs with apple and pear. However great advantage of quince in relation to other fruits is that, quinces are put in packaging which can be used repeatedly and ultimately it helps in the final financial results.

The fruit industry profile of J&K has long been dominated by apple. There is a need to diversify fruit industry which calls for widening of cultivars of different types of fruits including quince. This is possible only when the industry has an access to suitable varietal complex. For diversification the Division of Fruit Science under the survey and selection programme of quince has identified some elite selections of quince. Among them SKAU-016 is one of the best on the basis of its physical and chemical characteristics. To release the selection as a variety as well as to popularize the fruit crop among the farmers, there is a need to propagate the promising quince selection on a large scale which is possible only

through micropropagation. The traditional methods of propagation are proving inadequate to cope with the increasing demand of the planting material of desired genotypes. Micropropagation is the only alternative technique for increasing the supply of quality planting material. Keeping in view the above facts the present studies are proposed with the two-fold objectives outlined as follows :

1) To develop appropriate techniques of stock plant manipulation for obtaining explant suitable for *in vitro* culture.

2) To standardize media formulation and plant growth regulator regime for various stages of *in vitro* propagation of quince.

Chapter – 2

REVIEW OF LITERATURE

Many woody fruit trees (rootstock and scion cultivars) have been successfully propagated *In vitro* through shoot tips, buds, organogenesis and embryogenesis (Evans *et al*., 1981; Skirvin, 1981) during the last few years. Al-Maarri *et al.* (1986) studied the *in vitro* propagation of quince (*Cydonia oblonga* Mill.) and described the methods for the *in vitro* propagation of province quince for pear root stock making possible the production of a large number of plants from micro cuttings. The best rate of shoot multiplication was obtained with 2 mg/l 6-benzylaminopurine.However the best treatment which resulted in maximum percentage and quality of roots was with 0.1 mg/l naphthalene acetic acid. Baviera *et al.* (1988) studied the commercial *in vitro* micropropagation of pear cv.. conference. Though the traditional propagation technique of pear trees by grafting on quince, seedlings or clonal selection of *Pyrus communis* is not satisfactory because of the lack of compatibility with some other rootstocks, and also due to the sensitivity of the grafted plants to pear decline. However growing pear trees with their own roots is the another possibility in order to overcome this problem.

Vitagliano *et al.* (1991) worked on the effect of NaCl on quince(*Cydonia oblonga* Mill.) tissue culture and studied the response of NaCl at different stages of *in vitro* tissue culture of quince root stock and found that quince clones exhibit different sensitivities to salt concentration. NaCl negatively affect explant survival, halves shoot chlorophyll content and reduced cell growth. Sanjuan *et al*. (1991a) worked on plant regeneration from cultured leaves of *Cydonia oblonga* Mill. and found that the adventitious shoots of cv. East Malling Quince A were obtained from leaves cultured on MS-N6 medium containing TDZ, BAP and NAA. The frequency of regeneration was highest i.e. 78 per cent of the cultured leaves with 3.2 shoots per regenerating leaf at 32 μM TDZ plus 0.3 μM NAA. The regenerated shoots were rooted on the same medium containing 5 μM

NAA. Sanjuan *et al.* (1991b) studied the *in vitro* manipulations of pyrus species and quince (*Cydonia oblonga* Mill.). They regenerated adventitious shoots from leaf discs of *P. amygdaliformis* Mill. and *Cydonia oblonga* on medium containing Thidiazuron (TDZ) and alpha-naphthaleneacetic acid (NAA). The optimal combination was 3 μM TDZ and 1 μM NAA for *P. amygdaliformis* and 32 μM TDZ and 0.3 μM NAA for *Cydonia oblonga*. Micropropagated shoots, plantlets and roots of the two species differed dramatically in the ability to reduce Fe^{3+}. Roots of *P. amygdaliformis*, a species with high tolerance to low Fe availability reduced Fe^{3+} at the rate 12 times higher than *Cydonia oblonga* which is sensitive to low Fe.

Morini and Sciutli (1991) studied the *in vitro* propagation of quince clonal root stocks. They cultured shoot tip explants on MS medium and observed that maximum shoot proliferation was recorded with the growth regulator regime of 0.8 mg/l BA, 0.08 mg/l IBA and 0.1 mg/l GA_3. 13-14 shoots per explant were produced after three weeks of culture.

Gulsen and Dumanoglu (1991) conducted the research on the effect of sucrose, agar and pH on shoot multiplication rate and examined the quality in quince A root stock. Shoots were cultured on MS medium supplemented with 3 mg/l BA, 0.1 mg/l IAA, 0.1 mg/l GA_3. They observed the best shoot proliferation and shoot development with 30 g sucrose and 5g agar/l at pH 5.5. The average rooting percentage ranged from 56-73 per cent being highest with NAA at 0.7 mg/l as compared to IBA.

Wang (1991) studied the shoot multiplication of pear in double phase medium culture where in shoot number and length were significantly increased on double phase medium as compared to solidified medium. Different combinations of medium in liquid and solid phase strongly influenced shoot multiplication with the optimum results obtained in QL liquid and solid medium. Adding liquid medium to one month old cultures or transplanting the cultures to a fresh solid medium produced the similar effects on shoot multiplication.

Klerk *et al.* (1992) worked on the factors affecting the adventitious root formation in micro cuttings of Malus. They studied the rooting of *in vitro* shoots of apple cultivars Jork, Nicolai, Elstar and Golden delicious and *Cydonia oblonga* (Quince) cv. Leskovacz, under various conditions. They observed that when the application of IBA was delayed, the percentage of rooted shoots and the number of plantlets decreased significantly.

Vinterhalter and Neskovic (1992) studied the factors which affect the *In vitro* propagation of quince and found that the best multiplication and elongation medium was MS supplemented with 4.5 µM BA and 0.1 µM IBA. Only 35 per cent rooting was observed when shoots were cultured in MS medium containing 10 µM IBA for four days followed by their transfer to half strength MS medium devoid of any growth regulators. They also observed that by using the double phase medium i.e. by adding 5 to 10 ml of liquid medium over the agar surface, the average multiplication rate was 8.5 in a four week period with 66.3 per cent of shoots being longer than 10 mm.

Adventitious shoot regeneration from leaf explants of quince was developed on the medium containing 1.5 µM TDZ and 2.5 µM NAA and 85 per cent of the leaf discs regenerated shoots with the average of 8 shoots per leaf disc. Leaves excised from 4-6week old cultures gave a higher per cent shoot regeneration. Regeneration percentage was significantly reduced when sucrose concentration in the medium was less than 3 per cent (Baker and Bhatia, 1993).

Chartier-Hollis (1993) studied the induction and maintenance of caulogenesis from undifferentiated callus of quince *(Cydonia oblonga* Mill.). Stem callus from axenic shoots on multiplication medium was transferred to MSPI medium [MS salts, 85 mM sucrose,10.6 µM alpha-Naphthaleneacetic acid, 2.25 µM 6-Benzyleadenine(BA) pH 5.81] and cultured at 25^0 C with a 16h photo period, 35 $\mu mol.m^{-2}. S^{-1}$. Caulogenesis initially observed on this medium could not be sustained and a medium containing GA_3 in addition to BA and IBA was required for continued adventitious shoot production. Elongated shoots were

rooted *in vitro* and plantlets were transferred to glass house where they completed acclimatization.

Hummer (1993) studied the genetic resources of pyrus and related genera at the Corvallis repository where in about 82% of the clones have tested negative for the significant pear viruses. Infected clones are subjected to virus elimination through thermotherapy, meristem culture, micrografting and about 10% of the clones are preserved as *In vitro* cultures, stored at 4 ^{o}C.

Dumanoglu and Gulsen (1994) worked on *in vitro* rooting of Quince A [*Cydonia oblonga* Mill]. They conducted two experiments of auxin treatments on *In vitro* rooting of micropropagated shoots of East Malling Quince A. In the first experiment IBA, IAA, NAA at 0.0, 0.1, 0.5 and 1.0 mg/l concentrations were added to solidified MS (1962) basal medium. NAA was found to be most effective auxin with 1.0 mg/l giving the highest rooting rate (26.7%). In the second experiment, they compared the addition of NAA at concentrations of 0.0, 0.5, 1.0 and 1.5 mg/l in the medium with the rapid immersion of microcuttings for 5 seconds and 1 minute. However in both the experiments microcuttings were cultured in MS medium containing full and half macro elements for 30 days. It was observed that the rooting was best in MS medium with half strength macro elements after a 5 second (90.9% rooting) or 1 minute (83.3% rooting).

Denis (1995) studied the micropropagation of 3 Pyrus rootstocks, in which *Pyrus calleryana* Oregon pear rootstock (OPR) 157, *P. betulifolia* Bunge OPR 260 and *P. communis* L. Old home x Farmingdale 230(OHX F 230) were used OPR 260 and OHX F 230 shoots cultured on cheng medium with IBA proliferated better than those on NAA.NAA and IBA concentrations at >0.5 μM inhibited shoot multiplication. Overall, the best micropropagation medium for OPR 260 and OH x F 230 was cheng medium with 8 μM BA and 0.5 μM IBA. Shoot multiplication of OPR 157 was best on 8 μM BA and NAA 0.5 μM or no auxin than on IBA.

Suzuki *et al.* (1997) conducted a research on shoot formation and plant regeneration of vegetative pear buds cryopreserved at – 150^{o}C. Vegetative winter buds of pear were successfully cryopreserved at – 150^{o}C after pre-freezing to -30 ^{o}C. Meristems were excised from the buds and cultured on WPM medium containing 1.0 mg/l 6 BAP,25 g/l sucrose and 0.8%(w/v) agar. Partial dehydration at 25^{o}C prior to pre-freezing at -30^{o}C improved the subsequent shoot formation rate. However pre-freezing with a daily decrease at 5^{o}C increments to -30 ^{o}C followed by slowly thawing in air at 0^{o}C was effective for producing the highest rate of shoot formation.

Fisichella *et al.* (2000a) studied the effect of auxin-cytokinin ratio and of auxin and gibberelins inhibitors on *in vitro* rooting and development of quince BA 29 shoots [*Cydonia oblonga* Mill]. Microcuttings of BA 29 quince clone were rooted on MS medium suppemented with different NAA concentrations, several auxin-cytokinin ratio and auxin (TIBA)or gibberelin (ancymidol) inhibitors.No difference was detected in rooting frequency among NAA concentration while 1.0 mg/l NAA induced the highest root number for rooted shoots, but decreased root length. Moreover, cytokinin stimulated growth of shoots.

Fisichella *et al.* (2000b) studied regeneration of somatic embryos and roots from quince leaves cultured in media with different macro-element composition. They studied the influence of genotype and macronutrient combination on somatic embryo and root regeneration. Clone BA 29 showed the highest embryogenic properties and MS medium appeared to be the most favourable for somatic embryo formation. However, root regeneration was highest on WPM medium.

Japanese quince (*Chaenomeles japonica*) does not proliferate or root easily *in vitro* proliferation and rooting of three genotypes(I, II and III) of Japanese quince were tested in seven modified MS media and six rooting treatments in order to optimize the micropropagation method. The proliferation media were modified by varying the concentration of either N6-benzyladenine (BA 0.5-2.0 mg/l) or by adding indole-3-butyric acid (IBA 0.1 mg/l). However pretreated

shoots (68%) suggested that the absence of BA improved *Pyrus communis* rooting (Kauppinen *et al.,* 2001).

Previati *et al.* (2002) studied the development of protocols for *In vitro* rooting of advanced selections of root stocks. They optimized the *In vitro* rooting of several genotypes under selection, A74, C91, D41, D46, E82, E105, E110. Results indicate that the rooting percentage significantly differed among genotypes with highest rates for D41 and lowest values for A74. Rooting was in general higher with MS than with QL medium.

Freire *et al.* (2002) studied the improved culture media for the *in vitro* establishment of pear from nodal cuttings in which the experiments were carried out aiming at an efficient *in vitro* establishment from uninodal cuttings. However the explants obtained from the shoots of three adult "Rocha" trees in the field and from a Williams tree. Revealed that an increase in BAP concentration resulted in the higher number of shoots per explants.

Bouvier *et al.* (2002) studied the first double haploid plants of pear (*Pyrus communis).* They investigated the procedures for the production of haploid plants from pear. Oryzalin which was an efficient mitotic agent for chromosome doubling of haploid shoots *in vitro* was tried on *in vitro* on haploid pear. Clones from three cultivars were tested and successfully doubled.

Muleo *et al.* (2002) studied different responses induced by bicarbonate and Fe deficiency on micro shoots of quince and pear. They investigated the effects of bicarbonate induced chlorosis and Fe deficiency using shoots of quince clone MA and pear cultivar Conference in *in vitro* culture. The ability of "conference" to reduce the pH value of media was higher than that of clone "MA". The difference of H^+ extrusion between the two genotypes was less evident under all treatments, although "conference" maintained a higher degree of acidification.

Bell and Reed (2002) conducted a research on *in vitro* tissue culture of pear:advances in techniques for micropropagation and germplasm preservation.

They studied the micropropagation techniques in which the solid media gelled with agar,sometimes in combination with gellan gum have been used, but two phase liquid overlay or intermittent liquid immersion techniques have greatly increased shoot proliferation.

Onofrio and Morini (2003) evaluated the capacity of quince leaves to regenerate somatic embryos and shoots and/or roots simultaneously. Leaves from *In vitro* cultures were tested for two days in liquid medium containing 2.5 mg/l, 2 4-D mg/l followed by their culturing for different time durations with different growth regulator combinations. Proportions among the various types of regenerating leaves varied according to both the duration of NAA plus kinetin treatment and the presence or absence of growth regulators in the transfer medium.

Bianchi *et al*. (2003) studied the effect of the explant type and time of immersion in sodium hypochlorite on *in vitro* establishment of Quince and observed that when MS medium supplemented with 5.0 µM BA, 0.6 µM GA_3 and 0.5 µM IBA was used for the explant inoculation, the meristems showed the lowest percentage of contamination i.e., 36 per cent and the highest percentage of explant survival i.e., 79.2 and 85.1 per cent with 10 and 20 minutes of immersion in $NaOCl_2$ respectively.

Kauppinen *et al.* (2003) studied the propagation of Japanese quince (*Chaenomeles japonica)* plants. They propagated the Japanese quince based on soft wood cuttings, for this purpose 20cm long top cuttings with the basal portion of the cuttings soaked in 30 mg/l IBA for 18 hours were used. However micropropagation was shown to be very efficient method for propagation of Japanese quince, with an average proliferation rate of 3.4 and a rooting percentage of over 90%.

Staniene and Stanys (2004) while working on plant regeneration from leaves of *Cydonia oblonga* cultivars and studied the microplant regeneration of

cultivars K.11,K.16,K.19 of *Cydonia oblonga* in which leaves were isolated from microshoots and grown on MS medium supplemented with inositol, vitamins, naphthaleneacetic acid, sucrose and various amounts of TDZ. It was determined that by modifying the TDZ concentration in nutrient medium it was possible to induce morphogenesis in a sufficient frequency for transformation. The highest output of regeneration was obtained by using *Cydonia oblonga* cultivar K.11.

Erig and Schuch (2004a) worked to determine the ideal explant orientation as well as the sucrose and agar concentration in the culture medium for the *in vitro* multiplication of quince. They used MS medium with 1.25 salt strength added with the myo-inositol 10mg /l, BAP 13.3 μM, IAA 0.6 μM and GA_3 0.3 μM and found that maximum proliferation of quince shoots was observed in the above medium supplemented with 30g sucrose/l solidified with 5g agar/l and the culturing of the explants in the horizontal orientation.

Erig and Schuch (2004b) studied the *In vitro* rooting of quince and found that both the auxin type and concentration had an effect on *in vitro* rooting of quince. The microcuttings were cultured for 7 days in half strength MS medium containing three types of auxins (IBA, NAA and IAA) at five concentrations (i.e., 0, 5, 10, 15, and 20 μM) and then transferred to hormone free medium. All the auxins had the same effect on rooting per cent and rooting was maximum at 10 μM. However, NAA resulted in maximum mean root length but callus formation was highest with IBA.

Erig *et al*. (2004) conducted an experiment to determine the optimum salts and sucrose concentration of the culture medium that favour *in vitro* rooting of scions from quince cultivars MC and Adams and the substrate that results in the highest scion survival under acclimatization. In the first experiment, the salt concentration (50, 75 and 100%) of the original concentration and the sucrose concentration (0, 15, 30 and 45 g/l) were tested. The *in vitro* rooting of MC and Adams was favoured with the reduction of the salts of MS medium to 75 per cent of its original concentration and with 15 g sucrose /l in the culture medium.

Plantmax and Plantmax + vermiculite exhibited the highest survival of scions under acclimatized conditions.

Onofrio and Morini (2005) studied the development of adventitious shoots from *In vitro* grown *Cydonia oblonga* leaves as influenced by the different cytokinins and time durations. TDZ (4.5 μM) plus BA (4.5 μM) treatment induced marked shoot production which gradually increased with increased duration of TDZ treatment. Kinetin (4.5 μM) appeared to be effective in shoot induction but not in shoot development. The presence of TDZ and a long treatment duration appeared to be very important in inducing caulogenesis.

Erig and Schuch (2005) studied the *In vitro* regeneration of adventitious shoots and roots of quince. They found that the *In vitro* regeneration of adventitious shoots was favoured with the use of leaves as explant and with the addition of 4.5 μM TDZ to the culture medium. However, the same explant when cultured in medium supplemented with 2 μM of NAA in the absence of TDZ, resulted in root regeneration.

Cosac and Frasin (2005) studied the differentiation stages as part of *In vitro* multipilication for quince. They developed a regeneration protocol for three quince cultivars; Moldovenesti, Aurii and Aromate and two rootstocks, TIP A and BN 70. For initiating the culture the terminal and lateral meristems were used. They were put on medium in vegetative phase. The tested mediums were MS, Lepoivre, Fossard, and WPM. However the highest results were obtained on MS medium (77.77%) supplemented with 0,1 mg/l BAP and mg/l AG_3 for the "BN 70"and "TIP A" rootstock. The most favourable differentiation medium was Fossard (58.33%).

Pavlidis *et al.* (2006) studied the effect of strength of solid growth media(WPM,MS), porous substrates enriched with liquid ¼ WPM and presence of auxins for various periods as well as the inoculation of media with cultures of auxin overproducing mutants of Ectomycorrhiza Hebeloma Cylindrosporum on

rooting clones of quince (EMA and BA29) microshoots. It was observed that the rooting percentage of EMA noticed in porous substrate reached 100% irrespective of the auxin source(1mg/l IBA, 0.1mg/lNAA,0.1mg/l IAA) while that of BA29 reached only 42% with 0.1 mg/l IAA. However the survival of rooted plantlets in vermiculite during acclimatization was very high (93.3%) for EMA and (70%) for BA29.

Cosac and Frasin (2007) studied the influence of culture medium on quince explant callus. They used four mediums i.e MS , Fossard, Lepoivre, WPM along with dextrose (40g/l). agar(10g/l), IBA(0.1mg/l), AG3 (1mg/l), NaFe EDTA (3,2ml/l).The favourable medium to develop callus was Lepoivre (23,33%).

Cosac *et al.* (2008) studied the *In vitro* propagation of pear cultivars. They worked out on the regeneration protocol of 10 pear cultivars; Cure, Argessis, Euras, Carpica, Republica, Daciana, Getica, Eevina and Conference. The highest multipilication rate was obtained on Lepoivre medium, suplemented with 0.1mg/l BAP. However the highest rooting was obtained with 2.5 mg/l IBA and 2.5mg/l NAA with decreased mineral concentration of 50%.

Quiterio *et al.* (2008) studied the *In vitro* establishment and multipilication of pear rootstock. They studied the micropropagation of the selected pear rootstocks Provence, BA29, SYDO, CTS212, EMA, EMC, ADAMS332 and OHF 333and Microcuttings of CTS 212,SYDO and BA29were used to evaluate the best results of *In vitro* rooting and their survival after acclimatization. Treatments consisted of two auxin types (IBA and NAA) three concentrations (0 Mm, 5 μM and10 μM) in different ionic composition (mM) of macronutrients formulations; supplemented with (mg/l) Thiamine HCL 0.8, Nicotinic acid 1, Pyridoxine HCL 1, Myoinositol 100, Glycine 10, Arginine 200, and Phloroglucinol 80. However root stocks were renewed and were established successfully in Quoirin medium.

Anirudh and Kanwar (2008) studied the effects of cultural conditions, auxin and phloroglucinol on *in vitro* rhizogenesis in wild pear. Highest mean root

percentage (28.78%) was obtained in solid medium as compared to liquid medium (6.80%) irrespective of the growth regulator concentrations used. The rooting response was better with low concentrations (0.125 and 0.25 mg /l) of NAA than IBA. However at higher growth regulator concentrations (0.5-2.0mg/l)though the rooting response was poor, yet significantly higher rooting was observed with IBA as compared to NAA.

Giorgota *et al*. (2009) established the technology for *in vitro* propagation of "BN70" and 'A type of quince rootstock and of "Aurii" Quince cultivar. They studied the effect of culture media and time for collecting explants on the establishment of plant material *in vitro* and found that successful rooting with good multiplication rate was obtained on half MS with IBA (1 mg/l), GA_3 (0.1 mg/l), NaFeEDTA 3.2 mg/l and sucrose (20 g/l).

Chapter – 3

MATERIALS AND METHODS

3.1 General

Present investigations were carried out at the Biotechnology Laboratory of the Division of Fruit Science, SKUAST-K during the year 2010-2012. The salient features of the experiments carried out in the present studies are summarized in Table-3.1. The investigations involved study of different types of stock plant treatments, media and hormonal regime of media formulation for *in vitro* culture of SKAU-016 promising selection of quince (*Cydonia oblonga* Mill).

3.2 Plant Material

3.2.1 Stock Plants

The mature and bearing quince tree of SKAU-016 growing at the farmer's field and also the one year old plants growing at the main orchard in the Shalimar campus of Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir were used as stock plants in the present study. The genotype used in the present study was SKAU-016.

3.2.2 Chemicals

The major and minor elements required for preparation of media were obtained from Hi-media chemicals in India. The amino acids, vitamins & plant growth regulators were obtained from sigma chemicals. St. lavis, U.S.A.

3.2.3 Glasswares

The Glassware used for the experiment were procured from borosil. Glasswares were soaked in potassium dichromate Nitric acid solution for six hours followed by thorough washing in a jet of tap water so as to completely remove all traces of dichromate solution. They were then soaked in detergent solution (Teepol 0.1%) overnight and were thoroughly washed in tap water and rinsed twice with double distilled water. The glass ware was then dried in hot air oven at 100^0C for 24 hours and was stored until use.

Table-3.1 : Salient features of the experiments

S. No.	Name of Experiment	Factors and size of factorial experiment[a]	No. of replications	Media[b]	Parameters[c]	Design of Experiment[d]
1.	Standardization of sterilization procedure	Sterilants (5) Explant origin (2) (5 x 2)	3	MS	1, 2, 3	CRD
2.	Media standardization for establishment of quince selection SKAU-016	Media (2) BAP (3) IBA (1) (2 x 3 x 1)	3	MS, WPM	4	CRD
3.	Media standardization for proliferation of quince selection SKAU-016	Media (2) BAP (3) IBA (1) (2 x 3 x 1)	3	MS, WPM	5, 6, 7	CRD
4.	Media standardization for rooting of quince selection SKAU-016	Media (2) IBA (4) (2 x 4)	3	MS, WPM	8, 9, 10	CRD

a = Figures in the parentheses refer to the number of levels of each factor; b = MS, Murashige and Skoog's (1962) media, WPM, Lloyd and McCown media; c = 1, Aseptic cultures (%): 2, Explant survival (%): 3, Necrotic cultures (%): 4, Establishment cultures (%): 5, Proliferated cultures (%); 6, No. of shoots per explant; 7, Maximum shoot length (cm); 8, No. of rooted explants; 9, No. of roots per explant; 10, Maximum root length (cm); d = CRD, completely randomized block design

3.2.4 Culture Media

3.2.4.1 Selection of culture Media

Two different types of the media i.e. Murashige and Skoog's (1962) and Lloyd and McCown (1980) were used in the present investigation.

3.2.4.2 Composition of media

The composition of the different media used for the present investigation and details for preparation of stock solutions are furnished in Table-3.2 and 3.3. The stock solutions of the various chemicals were first prepared and required aliquots from the same were pippetted out and mixed together for preparing the media. The stock solutions were stored in corning reagent bottles at $4^{o}C$.

For preparation of the stock solution of iron, required quantities of $FeSo_4/H_2O$ & Na_2EDTA were dissolved separately in 50 ml each of double distilled water. Na_2EDTA solution was boiled and then added to $FeSo_4/H_2O$ gently & heated again till completely dissolved. The mixture was cooled and then volume was made up to 200 ml.

3.2.4.3 Adjuvants to the basal medium

Two different types of the medias viz., Murashige and Skoog (1962) and Lloyd and McCown (1980), were supplemented with vitamins, aminoacids and growth regulators. Stock solution of organics (vitamins and aminoacids) was prepared in double distilled water. Stock solution of Benzyl amino purine (BAP) was prepared by dissolving the known quantity in few drops of 0.1N NaOH and the stock solutions of naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) were prepared by dissolving in 5 ml of 0.5 N NaOH and then volume was made up with double distilled water.

Table-3.2 : Composition and preparation of stock solutions for Murashige & skoog (1962) medium

Stock solution designation	Ingredients	Wt. of ingredient (mg)	Volume of water used (ml)	Volume of stock solution taken for making 1 lt of medium (ml)	Final conc. of the ingredient in the medium (mg l^{-1})
I.	**Macronutrients**				
A.	$NH_4 NO_3$	16500	500	50	1650
B.	KNO_3	19000	500	50	1900
	$MgSO_4 7H_2O$	3700			370
	$KH_2 PO_4$	1700			170
C.	$CaCl_2 2H_2O$	4400	500	50	440
II.	**Micronutrients**				
D.	$H_3 BO_3$	620	500	50	6.20
	KI	83			0.83
	$Na_2 MoO_4 2H_2O$	25			0.25
	$CoCl_2 6 H_2O$	2.5			0.025
E.	$CuSO_4$	2.5	500	50	0.025
	$ZnSO_4$	860			8.6
	$MnSO_4$	2230			22.30
F.	Na_2 EDTA $2H_2O$	373	200	20	37.3
	$FeSO_4 7H_2O$	278			27.8
G.	Glycine	40	200	20	2.0
	Nicotinic acid	10			0.5
	Thiamine HCL	2			0.1
	Pyridoxine HCL	10			0.5
	Myo-inositol	2000			100
H.	Sucrose	30000			30000
	Agar				8000

Table-3.3 : Composition and preparation of stock solutions for Lloyd and McCown (1980) medium

Stock solution designation	Ingredients	Weight of ingredient (mg)	Volume of water used (ml)	Volume of stock solution taken for making 1 lt. of medium (ml)	Final concentration of the ingredient the medium (mg l^{-1})
I.	**Macronutrients**				
A.	NH_4NO_3	4000	500	50	400
	$Ca(NO_3)_2$	5560			556
B.	K_2SO_4	9900			990
	$MgSO_4\ 7H_2O$	3700	500	50	370
	KH_2PO_4	1700			170
C.	$CaCl_2\ 2H_2O$	960	500	50	96
II.	**Micronutrients**				
D.	H_3BO_3	6.200	500	50	6.20
	$Na_2M_oO_4\ 2H_2O$	0.250			0.25
E.	$CuSO_4\ 5H_2O$	0.0250			0.025
	$ZnSO_4\ 7H_2O$	8.60	500	50	8.6
	$MnSO_4\ 4H_2O$	22.30			22.3
F.	Na_2 EDTA $2H_2O$	37.30	200	20	37.3
	$FeSO_4\ 7H_2O$	27.80			27.8
G.	Glycine	2.000			2.0
	Nicotinic acid	0.50	200	20	0.5
	Pyridoxine HCl	0.50			0.5
	Thiamine HCI	0.10			0.1

3.2.4.4 Preparation of culture media

The required quantities of sucrose was dissolved in double distilled water and to this, stock solutions of macronutrients, micronutrients, vitamins and growth substances were added as per treatment required. The pH of the medium was adjusted to 5.7 with 0.1 N HCl or 0.1N NaOH prior to the addition of agar. Agar (7g/l) was dissolved in boiling distilled water and added to medium and then volume was made up with double distilled water. The medium was boiled and the hot medium was then immediately dispensed in the test tubes or conical flasks which were plugged tightly with non absorbent cotton plugs and covered with aluminum foil. The media was autoclaved at 15 psi (121^{o}C for 15 minutes (Dodds and Roberts, 1982).

3.2.4.5 Transfer area & aseptic manipulations

All the aseptic manipulations like surface sterilization, preparation and inoculation of explants and subsequent sub culturing were carried out under hood of clean laminar air flow chamber. The working table of the laminar air flow chamber was first surface sterilized with absolute alcohol, then by ulta violet light for 30 minutes. The forceps and scalpel used for the inoculation as well as the petri dishes were first steam sterilized in an autoclave at 121^{0}C for 20 minutes and later flame sterilized before each inoculation. The hands were cleaned and wiped with alcohol before working. Further, the standard general procedure for sterile techniques suggested by Street (1977) were followed.

3.2.4.6 Incubation Chamber

The cultures were incubated at 24±1^{o}C in a air conditioned room with a 16 hour photoperiod (3500 lux).

3.3 Methods

3.3.1 Collection and preparation of explants

Shoot tips of 2-3 cm in length were excised from quince trees of SKAU-016 selection growing at the farmers field & the main orchard in the campus of

Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar. As far as possible fresh actively growing shoots were used for explant isolation. Shoot tips were collected in flasks containing tap water, brought to the laboratory & washed thoroughly in running water.

3.3.2 Surface sterilization and inoculation

The explants were surface sterilized with different sterilants and their combinations for varying time duration.

1. In the first experiment, the explants were surface sterilized with 10% sodium hypochlorite (NaOCl) for 10 minutes.
2. In the second case, the explants were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 10 minutes.
3. In the third case, the explants were surface sterilized with 70% ethyl alcohol (C_2H_5OH) for 10 seconds.
4. In the forth experiment, the explants were surface sterilized first with 10% sodium hypochlorite (NaOCl)solution for 10 minutes followed by treatment of 70% ethyl alcohol (C_2H_5OH) for 10 seconds.
5. In the fifth experiment, the explants were first treated with 0.1% mercuric chloride for 10 minutes followed by 70% ethyl alcohol for 10 seconds.

In all the above cases, the explants after surface sterilization process were washed thoroughly with double distilled sterile water (5-6 times) under aseptic conditions in the laminar airflow chamber. The explants were then placed on the medium in such a manner that conformed to the original polarity & exposed above the surface of the medium.

3.4 Experiments

Salient features of the experiments carried out in the present investigation are presented in Table-3.1.

3.4.1 *In vitro* culture of explants

Studies on *in vitro* culture of SKAU-016 selection of quince were undertaken to standardize the sterilization procedure and define the media and growth regulator regime for establishment of explants, shoot multiplication, and *in vitro* rooting.

3.4.1.1 Standardization of sterilization procedure

The forced & unforced explants of SKAU-016 selection of quince were subjected to different sterilization treatments. The details of the combination of different concentrations of sterilants with varying time duration are presented in Table 3.4.

Table-3.4 : Concentration of various sterliants with time duration used for surface sterilization of explants

S. No.	Name of sterilant	Concentration (%)	Time
1.	Sodium hypochlorite	10	10 minutes
2.	Mercuric chloride	0.1	10 minutes
3.	Ethyl alcohol	70	10 Sec.
4.	10% Sodium hypochlorite for 10 min followed by 70% ethyl alcohol for 10 sec.		
5.	0.1% Mercuric chloride for 10 min followed by 70% ethyl alcohol for 10 sec.		

Observations with regard to per cent aseptic cultures (cultures free of infection), per cent explant survival (explants which were alive) and per cent necrotic cultures were made within 4±1 weeks of inoculation. Each treatment combination was assigned 10 explants with one explant per test tube and replicated 3 times.

3.4.1.2 Media standardization for culture initiation/establishment

The unforced explants (E_1) taken from the field and forced ones(E_2) developed under controlled conditions in the growth chamber from the cuttings of SKAU-016 selection of quince were excised for establishment studies. The studies were conducted separately for this selection under independent experiments. The explants were cultured in two different types of media viz., Murashige and Skoog's (1962) (M1) and Lloyd and McCown (1980) (M2) supplemented with different concentrations of growth regulators. The details of the combinations of different concentrations of growth regulators are presented in Table-3.5. The composition of various designated media are given in Table 3.2 and 3.3.

Table-3.5 : Concentration of **BAP and IBA used in culture media for explant establishment studies**

Factor	No. of levels of concentration	Concentration (mg)		
Benzyl aminopurine	3	0.25	0.50	0.75
Indole-3-butyric acid	1	0.01		

Observations with regard to per cent established cultures was made within 4±1 week of inoculation. Each treatment combination was assigned 20 plants replicated 3 times.

3.4.1.3 Stock plant treatments

The *in vitro* behavior of explants shall be investigated by subjecting the stock plants to two different stock plant treatments which shall yield.

3.4.1.3.1 Unforced explants (control)

These explants were obtained from mature field grown standing trees of quince of SKAU-016 selection. Fresh actively growing shoot tips were excised from this genotype.

3.4.1.3.2 Forced plants

For obtaining forced explants, pruned wood was collected from the mature standing stock plants of SKAU-016 selection of quince in open field from the inception of dormancy during Nov-Dec. For forcing (Plate-3) dormant cuttings (terminal or subterminal) of 15-20 cm length (10-15mm diameter) were collected from mature quince trees. The cuttings were treated with 0.2 per cent Captan and stored in a cool store at 4±3^{o}C in polythene bags until use. After withdrawal from the cold store, the basal ends of the cuttings were re-cut by about 1 cm and placed in glass jars containing distilled water. The amount of distilled water in the Jar shall be sufficient to cover 5 cm of the basal portion of cuttings. The cuttings were incubated in a growth chamber. The incubation condition were same as those used for maintenance of *In vitro* cultures. The water in the glass jar was changed every 5 days using fresh distilled water. The no. of buds on the prepared cuttings shall range from 5-8. Sprouting of buds took place after incubation in the growth chamber. Shoots, thus put forth by the sprouted buds of the cuttings served as an explant source from the forced stock plants.Shoot tips from these cuttings were excised and cultured on establishment media. Forcing was done as per the method described by Dalal *et al*. (1992), Dalal *et al.* (2000).

3.4.1.4 Media standardization for culture proliferation

The established cultures/explants of SKAU-016 selection were sub cultured on proliferation medium within 4±1 weeks of culture initiation. The Murashige and Skoog's (1962) medium supplemented with 6-benzyl aminopurine (BAP) at three concentrations 0.25, 0.5 and 0.75 mg l^{-1} and IBA at one concentration i.e 0.01 mg l^{-1} was tried for the stimulation of axillary buds. Shoot production at different concentrations of BAP & IBA was recorded within 5±1weeks of culture initiation.

Observations on per cent proliferated cultures, No. of shoots per explant and maximum shoot length was recorded after 5±1 weeks in the proliferation

medium. Each treatment combination was assigned 20 cultures (1 culture per flask) which were replicated 3 times.

3.4.1.5 Media standardization for rooting

Shootlets from the proliferating cultures of SKAU-016 selection were aseptically separated and supplemented with different concentrations of Indole-3-butyric acid i.e. 0.75, 1.0, 1.25 and 1.50 mg l^{-1}. Each treatment comprised of 10 explants with one explant per flask. Observations on number of rooted explants (per cent rooted shoots) number of roots per explants and maximum root length were recorded 4±1weeks after inoculation in rooting media. For rooting 10mm microcuttings were utilized.

3.5 Statistical analysis

The data generated from the various experiments were subjected to statistical analysis in completely randomized design. To satisfying model assumptions of explants, percentages were subjected to angular/square root transformations as suggested by Steel and Torrie (1981).

Chapter – 4

EXPERIMENTAL FINDINGS

The results of the present investigation are being presented under appropriate headings with the help of tables. The details of the experimental findings are presented as follows :

4.1 Standardization of sterilization regime

In order to standardize the sterilization regime for culture asepsis and survival of the explants, two types of primary explants viz, forced and unforced, derived from the donor stock plants of SKAU-016 selection of quince were subjected to five different sterilization regimes using MS as the basal medium. Effects of various sterilization regimes and forcing on culture asepsis (Table 4.1.1; Plate-1) and survival (Table 4.1.2; Plate-2) was highly significant. Highest mean percentage of aseptic cultures to the tune of 64.99 per cent was obtained under the S_5 sterilization regime (0.1% $HgCl_2$ for 10 minutes followed by 70% ethyl alcohol for 10 seconds) followed by 56.66 per cent under S_4 sterilization regime (10% sodium hypochlorite for 10 minutes followed by 70% ethyl alcohol for 10 seconds), whereas the lowest mean percentage of aseptic cultures to the tune of 37.49 per cent was obtained by treating the explants with S_3 sterilization regime (70% ethyl alcohol for 10 seconds). As regards the main effects of forcing, maximum mean percentage of aseptic cultures of 55.99 per cent was observed when the forced explants were utilized as against 43.33 per cent when the plants were obtained from field grown donor stock plants.

The main effects due to different sterilization regimes and forcing on survival of explants was statistically significant. In terms of the main effects of sterilants, maximum mean survival of explants (62.49%) was obtained under S_2 sterilization regime where mercuric chloride was applied at 0.1 per cent for 10 minutes followed by S_1 sterilization regime (54.99%) where sodium hypochlorite at a concentration of 10% was used for 10 minutes, whereas the lowest mean survival

Table-4.1.1 : Influence of various sterilants on culture asepsis (per cent aseptic cultures) in quince

Explant origin	Sterilants					
	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**Mean**
Unforced Explant (E_1)	33.33	39.99	28.33	54.99	59.99	43.33
Forced Explant (E_2)	51.66	53.33	46.66	58.66	69.99	55.99
Mean	42.49	46.66	37.49	56.66	64.99	

CD ($p \leq 0.05$)

Sterilants = 1.26

Explant = 2.00

Sterilants x Explants = 2.83

S_1 = 10% sodium hypochlorite for 10 minutes

S_2 = Mercuric chloride (0.1%) for 10 minutes

S3 = 70% ethyl alcohol for 10 seconds

S_4 = 10% sodium hypochlorite for 10 minutes followed by 70% ethyl alcohol for 10 seconds

S_5 = Mercuric chloride (0.1%) for 10 minutes followed by 70% ethyl alcohol for 10 seconds

Table-4.1.2 : Influence of various sterilants on explant survival (%) in quince

Explant origin	Sterilants					
	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**Mean**
Unforced Explant (E_1)	46.66	48.33	43.33	36.66	34.99	41.99
Forced Explant (E_2)	63.33	76.66	54.99	44.99	43.33	56.66
Mean	54.99	62.49	49.16	40.83	39.16	

CD ($p \leq 0.05$)

Sterilants = 1.60

Explant = 2.53

Sterilants x Explants = 3.59

S_1 = 10% sodium hypochlorite for 10 minutes

S_2 = Mercuric chloride (0.1%) for 10 minutes

S_3 = 70% ethyl alcohol for 10 seconds

S_4 = 10% sodium hypochlorite for 10 minutes followed by 70% ethyl alcohol for 10 seconds

S_5 = Mercuric chloride (0.1%) for 10 minutes followed by 70% ethyl alcohol for 10 seconds

Plate-1 : **Aseptic cultures of quince selection SKAU-016 under S_5 treatment**

a) Forced explants **b) Unforced explants**

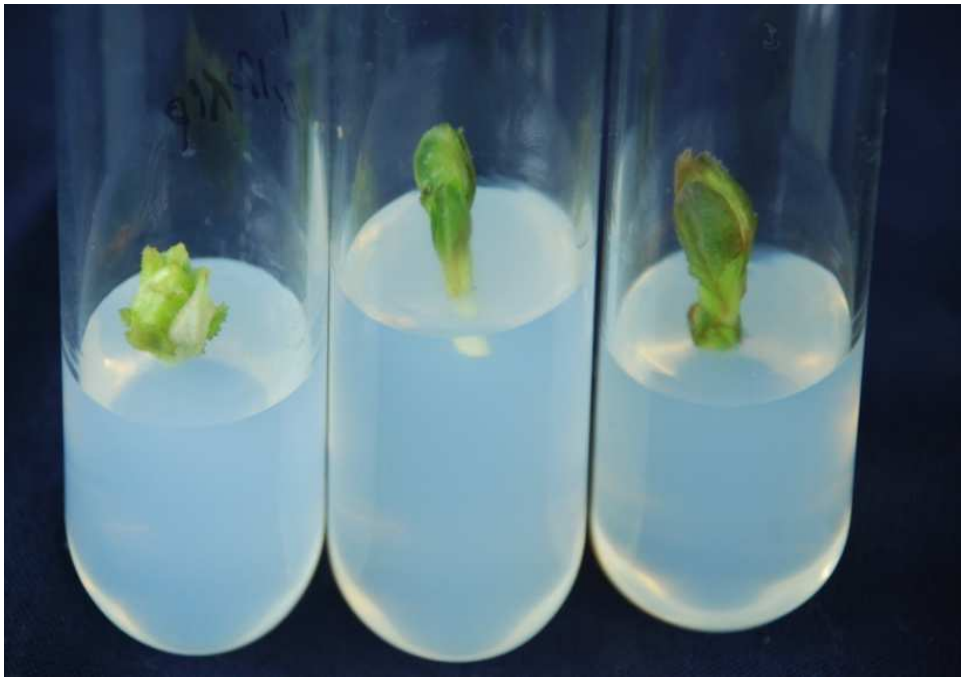
(a)

Plate – 1

(b)

Plate-2 : **Survival cultures of quince selection SKAU-016 under S_2 treatment**

a) Forced explants **b) Unforced explants**



(a)

Plate – 2

(b)

Plate-3 : **Various stages of forcing of dormant quince cuttings in the growth chamber**

(a) Dormant cuttings (0 day)

(b) Vegetative bud break (5 day)

(c) Advanced bud break (15 day)

(a)

(b)

Plate – 3

(c)

of 39.16 per cent was obtained under S_5 sterilization regime in which mercuric chloride was used at 0.1% for 10 minutes followed by treating the explants with 70 per cent ethyl alcohol for 10 seconds. Highest mean survival of 56.66 per cent was obtained in case of forced explants as against 41.99 per cent when unforced explants were utilized.

Effect of various sterilization regimes and forcing on necrotic cultures (Table 4.1.3) was highly significant. Highest mean percentage of necrotic cultures to the tune of 56.66 per cent was obtained under S_5. Sterilization regime (0.1% $HgCl_2$ for 10 minutes followed by 70% ethyl alcohol for 10 seconds) followed by 49.99 per cent under S_4 sterilization regime (10% sodium hypochlorite for 10 minutes followed by 70% ethyl alcohol for 10 seconds) where as the lowest mean percentage of necrotic cultures to the tune of 37.49 per cent was obtained by treating the explants with S_2 sterilization regime (0.1% $HgCl_2$ for 10 minutes). As regards, the main effects of forcing, maximum mean percentage of necrotic cultures of 57.99 per cent was observed when the forced explants were utilized as against 34.33 per cent when the explants were obtained from field grown donor stock plants.

4.2. Standardization of medium for culture initiation of quince SKAU-016

The main effects due to media, different hormonal concentrations and forcing on establishment cultures were statistically significant. Maximum mean establishment percentage of 50.54% was achieved when the explants were cultured in MS medium (M_1) under forced conditions followed by 47.21 per cent under unforced conditions. However the lowest mean establishment percentage of 37.76 per cent was achieved when the explants were cultured in Lloyd and McCown (M_2) medium under unforced conditions (Table 4.2.1).

Regarding the main effects of growth regulators, over all establishment percentage was increased when cytokinin and auxin were used in combination. However when cytokinin (BAP) was used alone, percentage of establishment was declined.

Table-4.1.3 : Influence of various sterilants on per cent necrotic cultures of quince

Explant origin	Sterilants					
	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**Mean**
Unforced Explant (E_1)	29.99	28.33	33.33	36.66	43.33	34.33
Forced Explant (E_2)	51.66	46.66	58.33	63.33	69.99	57.99
Mean	40.83	37.49	45.83	49.99	56.66	

CD ($p \leq 0.05$)

Sterilants = 1.26

Explant = 2.00

Sterilants x Explants = 2.83

S_1 = 10% sodium hypochlorite for 10 minutes

S_2 = Mercuric chloride (0.1%) for 10 minutes

S_3 = 70% ethyl alcohol for 10 seconds

S_4 = 10% sodium hypochlorite for 10 minutes followed by 70% ethyl alcohol for 10 seconds

S_5 = Mercuric chloride (0.1%) for 10 minutes followed by 70% ethyl alcohol for 10 seconds

Table-4.2.1 : Influence of media, explant source and growth regulator regime on per cent establishment of initiating cultures in quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
BAP(0.25)	23.33 (4.91)	26.60 (5.23)	13.30 (3.73)	20.00 (4.48)	36.66
BAP (0.50)	66.66 (8.22)	70.00 (8.41)	53.33 (7.36)	60.00 (7.79)	62.49
BAP (0.75)	23.33 (4.91)	26.66 (5.23)	16.66 (4.16)	20.00 (4.58)	21.66
BAP (0.25) +IBA (0.01)	40.00 (6.37)	43.33 (6.64)	30.00 (5.51)	33.33 (5.84)	36.66
BAP (0.50) +IBA (0.01)	83.33 (9.17)	86.66 (9.35)	76.66 (8.80)	80.00 (8.98)	81.66
BAP (0.75)+ IBA (0.01)	46.66 (6.89)	50.00 (7.11)	36.66 (6.12)	40.00 (6.37)	43.33
Mean	47.21	50.54	37.76	42.22	

Media mean (M_1)	=	48.87
Media mean (M_2)	=	39.99
Explant mean (E_1)	=	39.43
Explant mean (E_2)	=	45.55
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0. 49
Media	=	0.28
Explant	=	0.28
GR x Media	=	NS
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

Considering the effects of various treatment combinations of cytokinins and auxins, the treatment combination of BAP at 0.50 mg l^{-1} and IBA 0.01 mg l^{-1} resulted in highest establishment percentage of 81.66, followed by 62.49 per cent when the cytokinin (BAP) was used alone at a dosage level of 0.50 mg l^{-1} where as the lowest percentage of establishment 21.66 was obtained when the BAP concentration was increased from 0.50 mg l^{-1} to 0.75 mg l^{-1} without any addition of auxin. The interaction effect was non significant.

4.3 Standardization of medium for culture proliferation of quince cv SKAU-016

Established cultures were transferred to fresh medium supplemented with various concentrations of BAP and IBA. This transfer of cultures was done within 4 week of culture initiation for shoot proliferation. The results on per cent proliferation (Plate-4), number of shoots per explant , maximum shoot length were recorded after 5±1 weeks of transfer to the proliferation medium and are presented in Tables 4.3.1, 4.3.2 and 4.3.3 respectively.

Effect of various concentrations of growth regulators on proliferation per cent of established explants of quince cv. SKAU -016 was highly significant. Considering the trend of main effects of growth regulators, over all proliferation percentage was increased when cytokinin and auxin were used in combination. However when cytokinin (BAP) was used alone, percentage of proliferation was declined. Considering the effects of various treatment combinations of cytokinins and auxins, the treatment combination of BAP at 0.50 mg l^{-1} and IBA 0.01 mg l^{-1} resulted in highest proliferation percentage of 72.49 followed by 50.83 per cent when the cytokinin (BAP) was used alone at the dosage level of 0.50 mg l^{-1}, where as the lowest percentage of proliferation 21.66 % was obtained when the BAP concentration was lowest i.e. 0.25 mg l^{-1} without any addition of auxin (Table 4.3.1). As regards the main effects of media highest proliferation rate was observed in MS medium. Maximum proliferation rate of 45.55% was recorded in forced explants as compared to 39.43% in unforced explants. Interaction effect was found non significant.

Table 4.3.1 : Influence of growth regulator regime, explant source & media on proliferation percentage of initiating cultures in quince

Media	M_1		M_2		
Explant / G.R	E_1	E_2	E_1	E_2	Mean
BAP(0.25)	20.00 (4.48)	30.00 (5.51)	16.66 (4.16)	20.00 (4.48)	21.66
BAP (0.50)	50.00 (7.11)	56.66 (7.58)	43.33 (6.64)	53.33 (7.36)	50.83
BAP (0.75)	33.33 (5.84)	36.66 (5.84)	26.66 (4.91)	30.00 (4.91)	31.66
BAP (0.25) +IBA (0.01)	36.66 (6.12)	40.00 (6.37)	26.66 (5.23)	30.00 (5.51)	33.33
BAP (0.50) +IBA (0.01)	73.33 (8.61)	83.33 (9.17)	63.33 (8.01)	70.00 (7.94)	72.49
BAP (0.75)+ IBA (0.01)	43.30 (6.64)	50.00 (7.11)	40.00 (6.37)	46.66 (6.89)	44.99
Mean	42.77	49.44	36.10	41.66	

Media mean (M_1)	=	46.10
Media mean (M_2)	=	38.88
Explant mean (E_1)	=	39.43
Explant mean (E_2)	=	45.55
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0.572
Media	=	0.330
Explant	=	0.330
GR x Media	=	NS
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

Plate-4 : **Proliferating cultures of quince selection SKAU-016 in MS medium supplemented with BAP 0.50 mg/l and IBA 0.01 mg/l**

Plate – 4

Main effect of cytokinin and auxin concentrations on number of shoots per explant (Table 4.3.2) was found to be statistically significant. Maximum mean number of shoots per explant to the tune of 5.75 shoots per explant were obtained when BAP was used at 0.50 mg l^{-1} plus IBA 0.01mg l^{-1} followed by 5.42 shoots per explants when MS was supplemented with BAP 0.50 mg l^{-1} and auxin 0.01 mg l^{-1}. Whereas the lowest number of 4.79 shoots were obtained when MS medium was supplemented with BAP 0.25 mg l^{-1} and IBA 0.01mg l^{-1}. As regards the main effect of medium, it was observed that the highest mean number of shoots per explant to the tune of 5.43 shoots per explant were obtained when MS medium was used under forced conditions followed by 5.29 shoots per explants under unforced conditions. However the lowest number of shoots 5.09 were obtained when Lloyd and McCown medium was used under unforced conditions and 5.21 shoots per explants were obtained under forced conditions. The interaction effect between growth regulators and media was also found to be non significant.

Trends of the main effects of growth regulator, media & forcing on elongation of the shoots reveal that maximum shoot elongation (Table 4.3.3) to the tune of 5.49 cm was observed when BAP 0.50 mg l^{-1} plus IBA 0.01 mg l^{-1} was used followed by 4.52 cm when BAP was used at 0.50 mg l^{-1}, where as the smallest shoots of 1.38 cm were observed when BAP was used at 0.75 mg l^{-1}. As regards the main effect of media it was observed the at MS medium recorded maximum shoot length of 3.53 cm as compared to Lloyd and Mc Cown medium which recorded shoot length of 3.03 cm. Forcing also has a significant effect on shoot length. Maximum shoot length of 3.42cm was achieved in forced explants as compared to unforced ones (3.14 cm).

Table 4.3.2 : Influence of growth regulators, explants source and media on number of shoots explant^{-1} of proliferated cultures of quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
BAP (0.25)	5.03	5.10	5.03	5.13	5.07
BAP (0.50)	5.53	5.69	5.16	5.30	5.42
BAP (0.75)	5.40	5.50	5.20	5.30	5.35
BAP (0.25) +IBA (0.01)	4.90	5.00	4.56	4.70	4.79
BAP (0.50) +IBA (0.01)	5.80	5.90	5.60	5.69	5.75
BAP (0.75)+ IBA (0.01)	5.10	5.40	5.06	5.16	5.18
Mean	5.29	5.43	5.09	5.21	

Media mean (M_1)	=	5.36
Media mean (M_2)	=	5.15
Explant mean (E_1)	=	5.19
Explant mean (E_2)	=	5.32
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0.0648
Media	=	0.0374
Explant	=	0.0374
GR x Media	=	0.0917
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

Table 4.3.3 : Influence of growth regulator regime, explant source & media on maximum shoot length (cm) of proliferated cultures of quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
BAP (0.25)	2.20	2.50	1.60	1.90	2.05
BAP (0.50)	4.60	4.90	4.20	4.40	4.52
BAP (0.75)	1.40	1.70	1.10	1.30	1.38
BAP (0.25) +IBA (0.01)	3.43	3.83	3.10	3.40	3.44
BAP (0.50) +IBA (0.01)	5.56	5.83	5.10	5.40	5.49
BAP (0.75) + IBA (0.01)	3.10	3.40	2.30	2.60	2.88
Mean	3.38	3.69	2.90	3.16	

Media mean (M_1)	=	3.53
Media mean (M_2)	=	3.03
Explant mean (E_1)	=	3.14
Explant mean (E_2)	=	3.42
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0.10
Media	=	0.05
Explant	=	0.05
GR x Media	=	0.144
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

4.4 *In vitro* rooting

Shootlets from the proliferating cultures were aseptically excised and transferred to the rooting media supplemented with various concentrations of Indole 3 butyric acid (IBA). The shoots were first cultured in IBA supplemented MS & WPM medium for root induction followed by their transfer to the hormone free media for root elongation. The data from Table 4.4.1 revealed that the maximum mean percentage (81.66%) of rooted shoots (Plate-6) was observed when IBA was used at 1.25 mg l^{-1} followed by 68.33 % when IBA was used at 1.0 mg l^{-1} where as the lowest percentage of 45.00 was observed when IBA was used at 0.75 mg l^{-1}. As regards the main effect of media it was revealed that maximum mean percentage to the tune of 64.16 per cent was observed when shoots of SKAU-016 were cultured in MS media compared to 59.16 per cent in Lloyd and McCown media.

The main effects due to the media, forcing and different concentrations of Indole-3-butyric acid on number of roots per explants was statistically significant. The data from Table 4.4.2 revealed on the mean number of roots per explant (Plate-7) revealed that maximum mean number of roots (5.0 roots/explant) were obtained when IBA was used at 1.25 mg l^{-1} followed by 3.54 roots/explant at 1.0 mg l^{-1} IBA, whereas only 2.26 roots/explant were obtained when IBA was used at 0.75 mg l^{-1}. Regarding the main effect of medium it was observed that SKAU-016 under MS medium recorded 3.52 roots/explant as compared to 3.11 roots/explant incase of SKAU-016 under Llyod and Mc Cown medium.

The data on the mean length (cm) of roots (maximum root length) [Plate-8] was recorded after 20 days of transfer of root initiated shoots to the hormone free media. The data from Table 4.4.3 revealed that maximum mean length of 6.15 cm was observed when the shoots were cultured in MS medium supplemented with IBA at 1.25 mg l^{-1} followed by their transfer to hormone free media, where as the smallest roots of 2.42 cm length were obtained when IBA was used at 0.75 mg l^{-1}. Trends of the main effect of media reveal that maximum mean length (cm) of roots (4.59 cm) was observed in case of SKAU-016 when cultured in MS medium as compared to Lloyd and Mc Cown media (4.02 cm).

Table 4.4.1 : Influence of growth regulator regime, forcing & media on per cent rooting of quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
IBA (0.75)	43.30	50.00	40.00	46.66	45.00
IBA (1.00)	70.00	73.33	63.33	66.66	68.33
IBA (1.25)	83.33	86.66	76.66	80.00	81.66
IBA (1.50)	50.00	56.66	46.66	53.33	51.66
Mean	61.66	66.66	56.66	61.66	

Media mean (M_1)	=	64.16
Media mean (M_2)	=	59.16
Explant mean (E_1)	=	59.16
Explant mean (E_2)	=	64.16
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	6.12
Media	=	4.32
Explant	=	4.30
GR x Media	=	NS
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

Table 4.4.2 : Influence of growth regulator regime, explant source & media on number of roots per explant of quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
IBA (0.75)	2.10	2.40	2.20	2.30	2.26
IBA (1.00)	3.46	4.10	3.10	3.50	3.54
IBA (1.25)	5.10	5.40	4.60	4.90	5.00
IBA (1.50)	2.50	2.80	2.00	2.30	2.40
Mean	3.37	3.67	2.97	3.25	

Media mean (M_1)	=	3.52
Media mean (M_2)	=	3.11
Explant mean (E_1)	=	3.17
Explant mean (E_2)	=	3.46
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0.13
Media	=	0.09
Explant	=	0.09
GR x Media	=	0.19
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	NS

*Values given in the parenthesis are square root transformed values

Table 4.4.3 : Influence of growth regulator regime, explant source & media on maximum root length (cm) of quince

Media	M_1		M_2		
G.R \ Explant	E_1	E_2	E_1	E_2	Mean
IBA (0.75)	2.60	2.80	2.00	2.30	2.42
IBA (1.00)	5.10	5.60	4.60	4.90	5.05
IBA (1.25)	6.19	6.63	5.80	6.00	6.15
IBA (1.50)	3.80	4.10	3.10	3.50	3.62
Mean	4.42	4.77	3.87	4.17	

Media mean (M_1)	=	4.59
Media mean (M_2)	=	4.02
Explant mean (E_1)	=	4.14
Explant mean (E_2)	=	4.47
CD ($p \leq 0.05$)		
Growth regulator (GR)	=	0.06
Media	=	0.04
Explant	=	0.04
GR x Media	=	0.19
GR x Explant	=	NS
Media x Explant	=	NS
GR x Media x Explant	=	0.124

*Values given in the parenthesis are square root transformed values

Plate-5 : **Development of a complete rooted plantlet of selection SKAU-016 in MS medium supplemented with IBA 1.25 mg/l**

Plate – 5

Plate-6 : **Number of roots per explant of quince selection SKAU-016 in MS medium supplemented with IBA 1.25mg/l**

Plate – 6

Plate-7 : Maximum root length of quince selection SKAU-016 in MS medium

Plate – 7

Chapter – 5

DISCUSSION

Inspite of many encouraging research reports in many crops, tissue culture has not been even competitive with nursery practices (Murashige, 1990). The usual method of propagation of quince is cuttings or layering. This technique is very cumbersome, time consuming and slow. Hence for rapid multiplication of desired clones of quince, micro-propagation is an important alternative.

Application of tissue culture technology has developed rapidly in recent years, but if schemes entailing use of tissue culture are to be result-oriented, they must be efficient. Therefore, attention must be given to all details of procedure requisite to successful *in vitro* culture, especially when mature trees of woody species are to be brought within its ambit. The details of the published micro-propagation methods are not always precise and exact protocols details are often deliberately omitted where commercial ventures are involved. The present investigation is an initiating step for multiplication of the quince through micro-propagation and to overcome the increasing demand of the planting material of desired genotypes.

Quince being limited in distribution, very little work on its micropropagation has been carried out in India and abroad. The present investigation is an attempt to develop a micropropagation protocol for clonal propagation and production of quince plantlets irrespective of time and season on commercial scale.

5.1 Sterilization procedures

The present studies on sterilization of explants with different sterilants/sterilant combinations revealed that maximum aseptic cultures were obtained when explants were treated with 0.1 per cent $HgCl_2$ for 10 minutes + 70 per cent ethyl alcohol for 10 seconds.Combined sterilization treatments have been found effective in increasing culture asepsis (Modgil *et al.*,1994). Explant source

had a significant effect on culture asepsis. Unforced explants resulted in less per cent aseptic cultures than the forced ones. This variation in response may be due to relatively lesser inoculum load of the forced explants developed from the cuttings incubated in the growth chamber, however the unforced explants taken from stock plants grown in the open field conditions are exposed to the attack of different micro-organisms. As far as the survival of cultures are concerned, the treatment (S_2) of 0.1 per cent $HgCl_2$ for 10 minutes gave maximum survival percentage of the cultures both in case of forced and unforced explants than the S_5 treatment. Though S_5 treatment resulted in maximum per cent aseptic cultures but the survival percentage was less because this treatment resulted in the necrosis and injury of the explants where S_2 treatment resulted in less percentage of aseptic cultures but the survival rate was higher as most of workers have found that a single sterilant is more effective than the combinations in increasing explant survival.. These results are in close conformity with those of Dalal *et al*. (1992) in grape, Modgil *et al*. (1994) in apple, Hammerschlag (1980) in peach, Norton and Norton (1986) in prunus species, and Muna *et al.* (1999) in cherry.

5.2 Culture establishment

The first step of initating *in vitro* culture is to successfully adapt the plant tissue or explants to heterotrophic mode of nutrition. This has been termed as establishment stage (Murashige, 1974). The culture establishment medium is useful for conditioning the explants and for stimulating its initial growth. *in vitro* studies of quince has been carried out by a number of workers using different type of media viz, MS full strength, Llyod Mc Cown (WPM).

In the present investigation MS and WPM media were tried during the culture establishment stage. Effect of medium on culture establishment was found significant. Murashige and Skoog's full strength medium resulted in maximum explant establishment than WPM medium in SKAU-016 selection of quince. Superiority of using cytokinins in combination with auxins for culture establishment was evident from the results, BAP + IBA (0.50+0.01mg l^{-1})

resulting in maximum establishing cultures. Growth regulator concentration higher or lower than this resulted in significant reduction in culture establishment. Requirement of growth hormones varies considerably with the nature of tissue because of endogenous hormonal levels (Bhojwani, 1990).

The variations in response of explants of SKAU-016 selection to different types of media supplemented with different growth regulators in terms of establishment may be due to the reason that these media contain different concentration of nutrients which may or may not suit to explants during initial stages. Requirement of growth hormones is also specific for different tissues due to the endogenous levels of hormones in different tissues.These observations are in accordance with Morini and Sciutli (1991), who found that shoot tips of quince cultivar established best on MS medium supplemented with 0.08 mg l^{-1} IBA, 0.8 mg l^{-1} BA, and 0.1 mg l^{-1} GA_3. Similar findings were also observed by Gulsen and Dumanoglu (1991) they observed that shoot tips of quince cultivar established best on MS medium supplemented with 3.0mg l^{-1} BA, 0.1 mg l^{-1} IAA, 0.1mg l^{-1} GA_3.

5.3 Culture proliferation

Much attention has been focused on achieving high proliferation/multiplication rates through manipulation of growth regulators in the culture medium (Al-Maarri *et al.,* 1986; Morini and Sciutli, 1991; Vinterhalter and Neskovic, 1992). In the present studies, proliferation parameters of SKAU-016 selection was markedly affected by the concentration of different growth regulators.

Cytokinins are known to promote cyto-differentiation (Fukuda and Komamine,1985) and eliminate apical dominance (Wickson and Thimann, 1958). Per cent proliferation of established cultures of quince selection was found to be affected by both the application of BAP and IBA. Maximum proliferation of 72.49 per cent was observed when MS medium was supplemented with BAP at

0.50 mg l^{-1} and IBA at 0.01 mg l^{-1}. A slight deviation in the concentration of the growth regulators results in the decrement of the proliferation and the lowest percentage of proliferation was observed when MS medium was supplemented with BAP 0.25 mg l^{-1}. These findings are in close conformity with Vinter halter and Neskonic (1992) who observed that the best multiplication/proliferation medium was MS supplemented with 4.5 um BA and 0.1 mm IBA. Al-Maarri *et al.* (1986) reported that the best rate of shoot multiplication was obtained with 2 mg l^{-1} 6-benzylamino purine.

Growth regulators had a significant influence upon the shoot growth of explants. Highest number of shoots per explant were observed in BAP at 0.50 mg l^{-1} plus IBA 0.01mg l^{-1}. The lowest number of shoots per explant were observed when MS medium was supplemented with 0.25 mg l^{-1} of BAP plus 0.01 mg l^{-1} of IBA. The observations related to proliferation parameters are in close conformity with number of workers who used combinations of cytokinin (BA) and auxin (IBA) for shoot proliferation and multiplication in quince.(kaupinen 2001,Erig & Schuch 2004a, Cosac & Frasin 2007). Morini and Sciutli (1991) reported that 13-14 shoots per explant were produced in MS medium fortified with 0.8 mg l^{-1} BA & 0.08mg l^{-1} IBA after 3-weeks of culture period.

Shoot multiplication rate of 8.5 in a four week period with 66.3 per cent of shoots being longer than 10 mm was observed by Vinterhalter and Neskovic (1992) on MS medium supplemented with 4.5 mg/l BA and 0.1 mg/l IBA. Erig and Schuch (2004a) also recorded maximum proliferation of quince on MS medium supplemented with 10mg l^{-1} myo Inositol, BAP 13.3 µm, IAA 0.6 µm and GA_3 0.3 µm. Best shoot proliferation and shoot development with 30g sucrose and 5g agar l^{-1} at pH 5.5 was observed by Gulsen and Dumanoglu (1991) on MS medium supplemented with 3 g ml^{-1} BA, 0.1 mg l^{-1} IAA, 0.1mg l^{-1} GA_3.

Maximum shoot length was achieved in MS medium using different concentration of BAP and it was found that maximum shoot length of 5.49 cm was achieved when MS medium was supplemented with BAP 0.50 mg l^{-1} plus

IBA 0.01 mg l^{-1}.This is because the requirements of growth hormones & salt concentration is specific for different crops / genotypes.

5.4 *In vitro* rooting

Highest mean number of roots per shoot (5.0 cm) were obtained after 10 days of transfer of root induced shoots to the hormone free MS basal medium. Root initiation in micro shoots is a crutial step in the development of *In vitro* propagation protocol for any fruit crop. Two phase root induction procedure was followed for inducing roots in microshoots of quince-SKAU-016 selection, as this procedure has been found effective in rooting microshoots of apple and peach (Modgil *et al.*, 1994 and Hammerschlag 1980). Micro shoots were kept in auxin supplemented medium for 10 days for root initiation and then transferred to hormone free medium for root development and elongation. Maximum rooting percentage was observed when MS medium was supplemented with IBA (1.25 mg l^{-1}). Root number and mean root length were significantly influenced by auxin treatment. The shoots inoculated in root induction medium (MS+ IBA at 1.25 mg l^{-1} gave not only maximum percentage and mean number of roots per shoot but also the maximum mean length of the roots where as increment/ decrement of the IBA concentration resulted in decline of these parameters. These results are in close conformity with Erig and Schuch (2004b) and Giorgota *et al.* (2009) in quince, Dumanoglu and Gulsen (1994) and Anirudh *et al.* (2008) in wild pear.

5.5 Micro-propagation protocol for quince

Taking an over all view of the various studies conducted in course of the present investigation, a scheme for the micro propagation protocol of quince has been formulated which is presented as under :

There are two options for obtaining explants. These can be obtained from forced stock plants or field grown mature unforced stock plants. Forced ones

should be preferred. In the event of availability of forced stock plants as explant source, proceed as under :

Step 1 : Collect 15-20 cm dormant cuttings in the month of November-December

Step 2 : Treat the cuttings with 0.2 per cent captan and kept them in cold store at 4±3 ^{o}C for 40 days.

Step 3 : Take the cuttings from cold store and place them in jars containing distilled water and keep the jars in growth chamber (temperature 24^{o}C ± 1^{o}C, light intensity:3.5 Klu, photoperiod: 16 hour light and 8 hour darkness).

Step 4 : Forced cuttings will put forth vegetative flush within 15-20 days in growth chamber.

If the option of obtaining explants from forced stock plants is not available and instead the only source is field grown mature stock plants proceed as follow :

Step 5 : Collect explants from field grown mature stock plants.

Step 6 : Treat the explants with the sterilization regime of 0.1% Mercuric chloride.

Step 7 : The cultures are established in Murashige and skoog's (1962) full strength medium and kept in the establishment medium for 4±1 weeks.

Step 8 : The established cultures are sub cultured on the same medium (MS full strength) for another 4±1 weeks.

Step 9 : After 4±1 weeks, the established cultures are subcultured to proliferation medium (MS + BAP 0.50 mg l^{-1} IBA 0.01 mg l^{-1}).

Step 10 : After 4±1 weeks, subculture shoots of 10 mm length in the rooting medium (MS + IBA 1.25 mg l^{-1}).

Step 11 : Transfer root initiated shoots to MS blank (with out growth regulator) medium for complete plantlet development. Complete plantlet shall be ready within 5±1 weeks.

Chapter – 6

SUMMARY AND CONCLUSION

The present investigations on the "*In vitro* propagation studies in quince" were carried out in the Biotechnology Laboratory of the Division of Fruit Science, SKUAST-Kashmir, Shalimar campus, Srinagar. The investigations were carried out under different experiments on SKAU-016 selection quince.

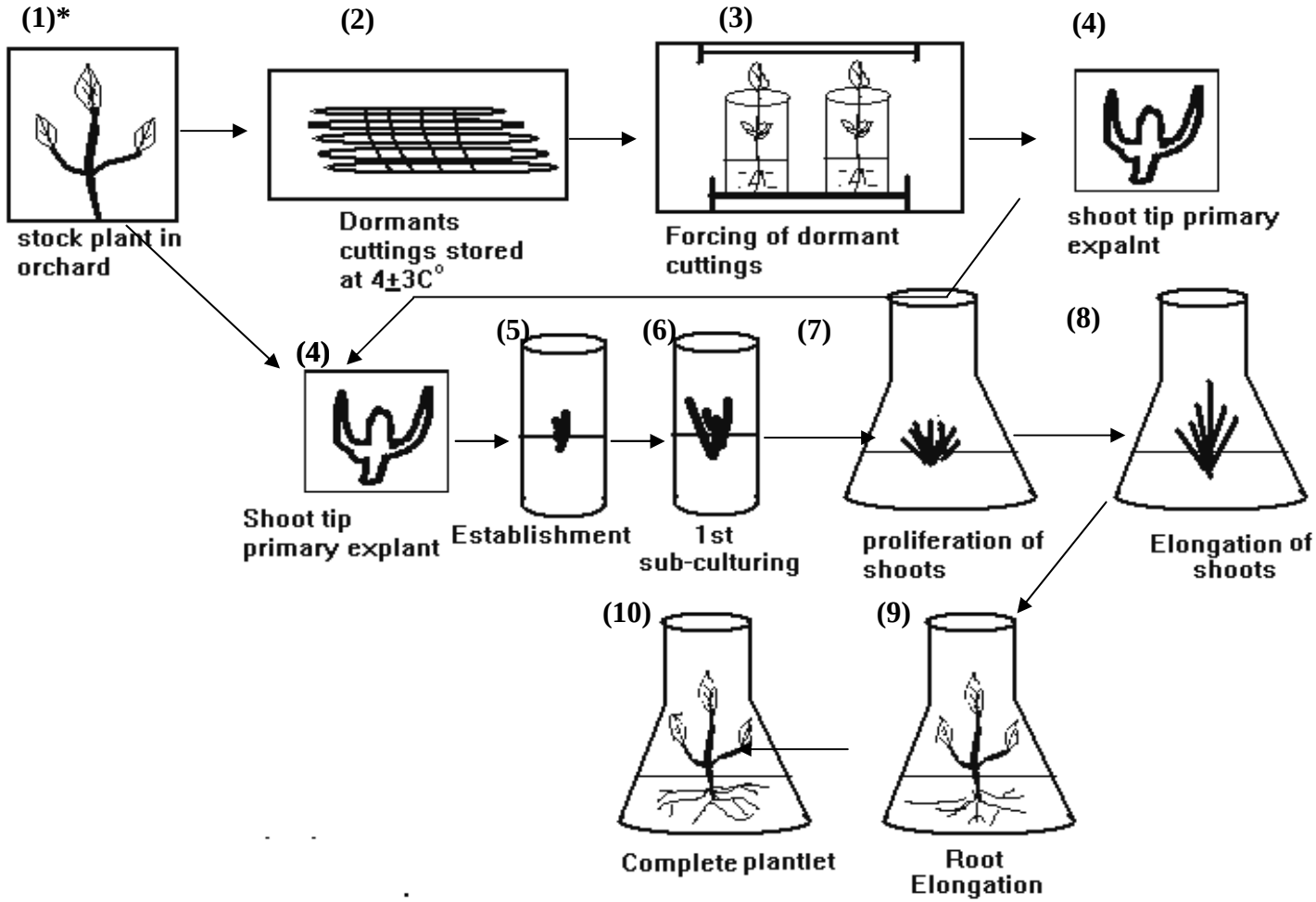
The results obtained during the course of this study are summarized as under :

- All the sterilization treatments yielded aseptic cultures but the maximum percentage of aseptic cultures were achieved by using 0.1 per cent mercuric chloride for 10 minutes followed by 70 per cent ethyl alcohol for 10 seconds.
- The maximum percentage of surviving explants were achieved when the explants were surface sterilized with 0.1 per cent mercuric chloride only as the sterilization treatment of 0.1 per cent mercuric chloride followed by 70 per cent ethyl alcohol for 10 seconds, no doubt gave maximum aseptic cultures but results in more mortality of the explants.
- *In vitro* establishment of the explants was high in MS medium supplemented with BAP (0.50 mg l^{-1}) plus IBA (0.01 mg l^{-1}) in SKAU-016 selection.
- Microbial contamination of the explants originating from the forced stock plants was relatively lesser than those from field grown unforced stock plants, thus resulting in higher percentage of aseptic cultures.
- Explants obtained from the forced stock plants of SKAU-016 selection gave maximum percentage of *In vitro* explant survival than the unforced ones.

- Multiple shoot formation was found to be maximum on MS medium supplemented with BAP (0.50 mg l^{-1}) plus IBA (0.01 mg Sl^{-1}) in SKAU-016 selection.
- Highest multiplication efficiency in terms of number of shoots per explant and maximum shoot length was achieved in Murashige and Skoog's medium supplemented with BAP (0.50 mg l^{-1}) plus IBA (0.01 mg l^{-1}) in SKAU-016 selection.
- IBA concentration of 1.25mg l^{-1} in MS medium not only gave the maximum mean number of roots in forced explants but also gave maximum length of roots per rooted shoots in SKAU-016 selection.
- MS medium was found superior to WPM medium in all the parameters studied.

CONCLUSION

- The sterilization regime of 0.1 per cent mercuric chloride for 10 minutes followed by 70 per cent ethyl alcohol for 10 seconds gave maximum percentage of aseptic cultures.
- Murashige and Skoog's full strength medium supplemented with 0.5 mg l^{-1} BAP and 0.01 mg l^{-1} IBA gave the highest percentage of established cultures.
- It was found that forced explants obtained from artificial forcing of the dormant cuttings gave maximum percentage of established cultures.
- The growth regulator regime of BAP (0.50 mg l^{-1}) and IBA (0.01 mg l^{-1}) in MS medium gave the highest proliferation percentage.
- MS medium supplemented with IBA at 1.25 mg l^{-1} gave the highest percentage of rooting.
- On the basis of these studies various features of micro-propagation protocol have been developed (Fig. 1).



*Figures in parenthesis refer to the sequential steps of the protocol

Fig. 1 : Micropropagation protocol of Quince

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. V.K. Wali, Professor & Head, Division of Fruit Science, SKUAST-Jammu, during Viva-Voce examination held on 9th of June, 2012 have been incorporated in the manuscript entitled **"*In vitro* propagation studies in Quince"** submitted by **Ms. Yusra Ali Basu (Regd. No. 2010-A-857-M)**.

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