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THE UNIVERSITY OF ALBERTA

Micropropagation of cultivars of the Saskatoon, *Amelanchier alnifolia* Nutt.

by

Krzysztof Pruski

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Horticulture

Department of Plant Science

EDMONTON, ALBERTA

Spring 1987

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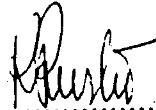
(SIGNED) 

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Micropropagation of cultivars of the Saskatoon, *Amelanchier alnifolia* Nutt. submitted by Krzysztof Pruski in partial fulfilment of the requirements for the degree of Master of Science in Horticulture.

R. H. Knowles
Supervisor

Date Nov. 13/86

Abstract

The propagation of four cultivars of the Saskatoon *Amelanchier alnifolia* Nutt. by *in vitro* techniques was investigated. The cultivars used were: 'Smoky', 'Pembina', 'Thiessen' and 'Northline'.

Aseptic cultures were established using shoot tips, dormant buds and active buds as explants. Shoot tips proved to be the most practical, giving the highest number of active cultures. Cultivar 'Thiessen' was the most vigorous.

Four media, Murashige and Skoog-MS (1962), Linsmaier and Skoog-LS (1965), Gamborg *et al.*-B5 (1968) and Nitsch-Nc (1968) were used to determine which, if any were the best. MS and LS media supplemented with 6-benzylamino purine (BAP) at 4.44 μM and with indole-3-butyric acid (IBA) at 0.49 μM gave the most satisfactory results. In subsequent studies only MS medium was used. Cultures were well established after 3 weeks incubation at 24/22°C day/night temperature with 16h photoperiod at 55 $\mu E m^{-2} s^{-1}$.

During the multiplication stage the influence of BAP on the length and number of shoots per culture was investigated. The BAP levels of 8.88 μM and 13.30 μM were found optimal for shoot proliferation, giving 8 to 10 good quality shoots (15-25 mm long) per culture. Experimental conditions for multiplication were the same as those used for establishing cultures. Again, cultivar 'Thiessen' was the most vigorous.

Preliminary rooting studies, involving *in vitro* and *in vivo* techniques were conducted with cv.'Smoky'. Rooting of *in vitro* derived shoots in *in vivo* conditions, was more successful and less complicated and was used in experiments involving all 4 cultivars. Experiments were conducted on a bottom heated (25°C) greenhouse bench with intermittant mist. Rockwool blocks 3.5x3.5x3.5 cm soaked with 1/3 strength of MS salts were used over perlite. The IBA concentration of 2.45 μM in the medium was found optimal for rooting of most cultivars. The percentage of rooted plants varied from 24.4% to 68.9%, depending on the cultivar. The best response was from cultivar 'Smoky'. Satisfactory results were also obtained using commercial rooting powder 'Rootone F'. The percentage of rooted plants varied from 21.1% to 60.0%, with cv.'Smoky' giving the highest number of rooted plants. Most plants were

rooted after 4 weeks.

Foliar application of BAP and gibberellic acid (GA_{4+7}) successfully overcame summer dormancy in newly *in vivo* rooted plants. All plants treated with the growth regulators broke dormancy and began active growth. The combined, BAP + GA_{4+7}, treatment applied to cvs. 'Smoky' and 'Thiessen' was more effective than single BAP or single GA_{4+7} treatments. Plants treated with BAP + GA_{4+7} had the highest number of stems and subterranean buds per plant.

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ABA	abscisic acid
AdS	adenine sulfate
ATN & HC	Alberta Tree Nursery & Horticulture Centre
B5	Gamborg *et al.* medium
BA	benzyladenine
BAP	6-benzylamino purine
2,4-D	2,4, dichlorophenoxyacetic acid
GA_3	gibberellic acid 3
GA_{4+7}	gibberellic acid 4+7
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
LS	Linsmaier and Skoog medium
MS	Murashige and Skoog medium
NAA	naphthalenacetic acid
Nc	Nitsch medium
PG	phloroglucinol
RH	relative humidity
RWC	relative water content

Note: All temperatures are in °C.

I. Introduction

The selection of Saskatoon (*Amelanchier alnifolia* Nutt.) plant types was started in 1918 by W.D. Albright at the Experimental Farm, Beaverlodge, Alberta. In 1944 three valuable cultivars from this program, 'Northline', 'Sturgeon' and 'Forestburg' were released. Albright's work was carried on by Mr. J.A. Wallace at Beaverlodge and in 1952 this led to the selection of cultivars 'Smoky' and 'Pembina'. In 1969 Dr.A.J. Porter of Parkside, Sask., introduced the cultivar 'Thiessen'.

The Saskatoon has considerable potential as a commercial fruit crop since it can be readily cultivated and can be mechanically harvested (Grainger, 1980). Fruits are sweet and juicy, containing 10-11% sugar and are suitable for food processing as jam and jelly (Mazza, 1979).

There have been problems with plant production. Seedlings rarely are true to type showing variation of up to 30%, and with grafted stock a high percentage of incompatability has been encountered (Grainger, 1980). The Saskatoon can be propagated by softwood cuttings (Harris, 1976; Bishop and Nelson, 1980), however, dependable rooting of cuttings seems to be a problem since the percentage of rooted plants varies from 10 to 100% (Harris, 1980).

One additional and even more serious problem has been noted with softwood cuttings. When rooting of these has been completed, the new plants, almost invariably go into summer dormancy. Because newly rooted cuttings have a limited leaf surface when summer dormancy occurs the new plants are seldom able to accumulate sufficient reserves to survive the winter. Scott (1975), and Wallace and Graham (1976) have reported that extension of day length following propagation tended to prolong active growth and increase the probability of winter survival. Under these conditions newly rooted plants were able to accumulate sufficient reserves of carbohydrate to assure winter survival and to promote vigorous growth the following year (Bishop and Nelson, 1980).

It has been found that propagation by tissue culture methods offers some prospect for the rapid and successful propagation of the Saskatoon (Harris, 1980; Struve and Lineberger, 1985). It has also been noted that the dormancy problem with newly rooted cuttings can be overcome by using certain growth regulators. Grainger (1980) was succesful in promoting extension growth of dormant terminal cuttings with GA_3, but growth was from the terminal bud only. Ali (1984) showed that two growth regulators, BA and GA_{4+7} can act together in the promotion of breaks from lateral buds and subsequent growth of shoots in summer dormant Saskatoon cuttings.

In this study, objectives were, 1) to find the optimal conditions for the propagation of Saskatoon by tissue culture methods, 2) to overcome the summer dormancy condition that occurs right after rooting of plantlets, and 3) to develop a bushier plant with an active leaf surface large enough to provide the plant with sufficient reserves for the following season.

II. Literature Review

A. Plant Tissue Culture Techniques

Introduction

Propagation of plants by tissue culture methods is gaining popularity throughout the world. Hussey (1978) has listed a number of reasons for using plant tissue culture techniques. These include:

-Rapid multiplication of new hybrid cultivars, which arise as single plants, for testing and eventual commercial production

-Elimination of viruses from infected stocks

-Multiplication of difficult to propagate species

-Year around propagation of clones

-Propagation of genetically uniform parent plants in large numbers for large-scale hybrid seed production.

Plant tissue technology can be divided into five classes based on the type of materials used (Gamborg and Shyluk, 1981):

1. Callus Culture. The culture of callus cell masses on agar media.
2. Cell Culture. The culture of cells in liquid media in vessels which are usually aerated by agitation.
3. Organ Culture. The aseptic culture on nutrient media of embryos, anthers, ovaries, roots, shoots or other plant organs.
4. Meristem Culture. The aseptic culture of shoot meristems on nutrient media for the purpose of growing a complete plant.
5. Protoplast Culture. The aseptic isolation and culture of protoplasts from cell cultures or other plant sources.

The Source Plant

Explant Source

The tissue or organ used as a source of explants has an influence on the degree of success to be expected from plant tissue culture. Early work with tobacco cell cultures led to the concept that plant cells were totipotent i.e. had the capability for the regeneration of a whole plant from a single cell.

Somatic embryos are generally considered to originate from single cells (Ammirato, 1984). This is still questionable, somatic embryos have been observed to arise from single isolated cells or protoplasts, but very often a mass of undifferentiated tissue forms prior to embryo development (Kameya and Uchimiya, 1972). Hughes (1981) noticed that in practice plant tissues which have embryogenic competence are limited and the ability to produce embryos varies from tissue to tissue and from one species to another. Plant cells may lose embryogenic competence but still retain the ability to form plant organs, a process known as determination. The degree of determination varies from cell type to cell type. Some cells may retain only a degree of morphogenic ability. Callus derived from *Nigella sativa* roots for instance produced only roots, while callus derived from stem and leaf tissues produced only shoots (Banergee and Gupta, 1976). Within any plant, tissues can differ in their degree of determination and their ability to undergo morphogenesis. Takayama and Misawa (1979) examined the ability of different explants of *Lilium auratum* and *Lilium speciosum* to produce bulbs *in vitro*. Explants were taken from leaves, penduncles, bulb scales, petals, anthers and from scales of bulbs grown in culture. Explants from anthers produced neither bulbs nor roots. Leaf explants did not survive the culture conditions but 53% of the penduncle explants, 75% of the petal explants, and 95% of the bulb scale explants produced bulbs. When the explant was taken from the bulb grown in culture, the success rate was 100%.

Shoot production may be initiated from organized centers of cell division, derived from the initial explant. These are identified as meristemoids by Torrey (1966). Meristemoids

have relatively small size, dense cytoplasm, thin cell walls, minimal vacuolation and large nuclei. In regeneration of the whole plant the presence of meristemoids or cells which can readily develope into meristemoids, are necessary. Such groups of cells have been found in shoot tips and buds of many species. Thus shoot tips and buds are highly valued as explants in tissue culture procedures where the regeneration of a whole plant is the objective (Seabrook, 1980).

Murashige (1977) noticed that often there is considerable variability associated with the genotype of plants used as explants. Within a species, some genotypes appear to propagate easily while others fail to respond. Genotype-specific effects have been reported for both *Anthurium andraeanum* (Pierik et al.,1974) and for *Anthurium scherzerianum* (Pierik and Steegmans, 1976). Only one third of the *Anthurium andraeanum* genotypes were capable of forming callus but three fourths of the *Anthurium scherzerianum* genotypes formed callus and subsequently produced plants.

Seabrook (1980) noticed that the ease of organ regeneration *in vitro* is usually related to the ease of vegetative propagation by traditional methods. Plants which are difficult to propagate vegetatively are also often difficult to regenerate *in vitro*.

Explant size

It is commonly observed, that the larger the explant, the greater the likehood of microbial contaminants in the tissue. There is, however, a minimum effective size for explants. Very small explants do not grow as fast as larger explants and have low survival rates in culture (Seabrook, 1980; Hughes, 1981). In studies with carnation, shoot apices measuring 0.09 mm were incapable of morphogenesis. Apices up to 0.2 mm were capable of slight growth. Apices 0.35 mm produced the largest number of normal shoots. When the explant was as large as 0.5 mm, shoot production was again reduced, possibly due to the presence of excess subapical tissue (Gukasyan *et al.*, 1977). Some workers suggest using small 0.2 - 0.5 mm shoot apices, while others prefer much larger explants including shoot tips 3 - 4

cm long. Hedtrich (1980), propagating raspberry cultivars, used 0.3 mm meristems successfully as explants while James (1980) used 1 cm long shoot tips in the micropropagation of raspberry cv.'Malling Jewel' and obtained satisfactory results.

Explant size is rarely a problem unless the purpose of the culture system is to obtain virus-free plants. At one time the apical meristem area was believed to be virus-free, but more recent studies have demonstrated that viruses may even be found here (Hughes, 1981). Hughes suggested, that virus-free plants may be obtained by excising very small stem apices and culturing these in combination with heat treatment to eliminate residual viruses. Pyott (1981) proved that heat treatment of a source plant can reduce the microorganism content of the explant in culture. He propagated raspberries using heat treatment on the source plant and followed this with micropropagation. Treatment involved taking well established, healthy plants grown in pots, pruning them and placing them in a growth room for 8 weeks at 37° with light of 10 000 lux for 16 hours a day. Plants were watered with a 25% Hoagland's solution. After 8 weeks he harvested 2 - 3 cm shoots and following surface sterilization removed apices and axillary buds (0.2 - 0.3 mm with 2 - 4 primordia) and used them as explants. Plants regenerated from these explants were virus-free. Seabrook (1980) noticed that explants free of microorganisms can be obtained by pretreating the source plant with antibiotics, systemic fungicides or antiviral agents i.e.Virozol.

Juvenility and the initiation of the culture

Juvenile plants usually provide a greater number of regenerative explants than do adult plants, however, this varies with plant species (Seabrook, 1980). The youngest and least differentiated tissues are found in plant meristems and culture of this plant tissue has been successful in a wide range of species. Weiler and Emershad (1977) studying micropropagation of *Iris* observed that shoots can be produced from stem segments but only from the youngest tissue near the bud. Similar patterns of differentiation were observed in *Gladiolus* (Ziv, 1970). Pierik and Steegmans (1975) observed that the ability of *Rhododendron* stem segments to

produce roots, decreased with increasing age of the stem. In the *Pinaceae*, excised embryos and parts of the young seedlings (cotyledons, hypocotyl, and epicotyl) have all been successfully used as sources of explant for the initiation of adventitious buds in culture (Sommer and Brown, 1979). High rooting percentages (e.g.85%) of adventitious shoots and the successful transfer of plantlets to the nursery bed have been obtained with *Pinus radiata* (Horgan and Aitken, 1981).

A wide variation in morphogenic response has been found in juvenile conifer cultures. Mott *et al.*(1977) and Wochok and Abo El-Nil (1978) found that cotyledon cultures of *Pinus taeda* and *Pseudotsuga menziesii* respectively yielded averages of 21 and 97 buds per bud-forming embryo with maxima of 42 and 264. With embryo cultures of *Pinus radiata*, a variation of 1 to 200 buds per embryo has been observed (Reilly and Washer, 1977). Aitken *et al.* (1981) examined the influence of explant selection upon the shoot forming capacity of juvenile tissue of *Pinus radiata*. They obtained more than 1300 shoots from the cotyledons of one germinated seed. To date, no other conifer tissue culture method has been reported to give such a large number of adventitious shoots from a single clone.

Media

Although whole plants have relatively simple requirements for growth, plant tissue cultures have more complex needs. The *in vitro* plant tissue cultures require mineral salts (macro- and micronutrients), a source of carbon (usually sucrose), vitamins and growth factors. The media formulations such as those of Murashige and Skoog (1962), White (1963) and Gamborg *et al.*(1968) are suitable for a wide range of plants in the promotion of organogenesis in cultures. The MS formula is commonly used when morphogenesis is required. The B5 medium, particularly in combination with 2,4-D is used when rapid cell proliferation is desirable (Skirvin, 1981).

Murashige (1977) has suggested that the use of vitamins in plant tissue culture media has been a matter of custom rather than of necessity. He noted that thiamine HCl was the

only vitamin which was consistently required for growth of plant tissues *in vitro*. Recently, some researchers have found that thiamine HCl, nicotinamide and pyridoxine were essential for their cultures (Skirvin, 1981).

The most important factors determining organ formation in tissue culture are the relative quantities of auxins and cytokinins (Skoog, 1957). Skoog in his work with Tobacco noticed that stem segments produced callus when the medium was supplied with auxin and cytokinin of approximately equal concentrations. If the auxin level was raised relative to the cytokinin concentration roots were induced. When the auxin concentration was lower than that of cytokinin, shoots were formed on Tobacco tissue. Similar results were obtained by Thorpe (1968).

The effects of the cytokinin/auxin ratio on organogenesis appears to be typical for all cultures. James (1980) noticed that for shoot proliferation of red raspberry the optimal concentration of cytokinin (BAP) was 1 mg/l and the concentration of auxin (IBA) was 0.1 mg/l. When cytokinin was removed from the medium and the concentration of IBA increased to 1 mg/l, root formation resulted. Harris (1982) obtained similar results with *Vitis*. The best shoot formation was achieved when the concentration of cytokinin (BAP) in the medium was 3 mg/l in the absence of auxin. The best rooting, on the other hand, was observed when BAP was removed from the medium and the concentration of IAA was 0.1 mg/l.

Materials such as PG and AdS have been added to media to enhance shoot and root formation. James *et al.* (1980) studying the micropropagation of red raspberry observed that 162 mg/l of PG significantly increased shoot number at all auxin/cytokinin concentrations. PG promoted also the number of roots per rooted culture but did not significantly increase the percentage of rooting. James (1983) reported that PG consistently synergized IAA-induced rooting of *Malus pumila* rootstocks in the dark for contact periods up to 13 days with the highest rooting being recorded at 9 days. Cultures were maintained in the dark for periods ranging from 1h to 13 days before transfer to hormone-free medium in the light for root elongation. When AdS is added to the medium, it can often enhance growth and shoot

formation. Harris (1980) observed that the addition of 80 mg/l AdS to the medium increased the yield of shoots per culture of *Amelanchier alnifolia*.

Natural materials such as coconut milk, yeast extract, and casein hydrolisate can also be used in media, however, it is almost impossible to control the quality of these products. A more effective way of supplementing media with organic constituents is to add various known chemicals once it has been determined that they can improve growth of cultures (Seabrook, 1980).

Environmental Factors Influencing Tissue Cultures

Light

In general, the characteristics of light which normally influence plant development are also those which affect the growth of plant tissues in culture. These factors of light include intensity, spectral quality and the length of the daily exposure period (Murashige, 1974).

Tissue cultures normally do not grow autotrophically and therefore do not require the same level of light intensity required by the whole plant. However, several studies have demonstrated that light plays an important role in inducing organogenesis. In the *Iridaceae*, callus cultured in the dark produced shoots when the cultures were later transferred to the light (Simonsen and Hildebrandt, 1971; Hussey, 1976). Constant darkness reduced the number of spears produced in cultures of *Asparagus* (Hasegawa *et al.*, 1973). Experiments with *Nicotiana tabacum* indicated that light was a critical factor in the initiation of shoots. Thorpe and Murashige (1968, 1970) have shown that there is a significant accumulation of starch in tobacco callus cells which ultimately gives rise to bud primordia. The accumulation was observed in both light and dark-grown cultures but was much higher in light-grown tissues.

Murashige (1974) has reported that optimum light intensity for cultured plant tissues in stage I and II, initiation and multiplication respectively, is around 1 000 lux whereas for stage III- rooting, light intensity between 3 000 and 10 000 lux is required. Boxus *et al.* (1977)

examined the influence of light intensity on growth of strawberry plantlets and noticed that plantlets grew as well under a low light intensity of 1 000 lux as they did at higher intensities of 2 000 - 5 000 lux. In contrast, Wainwright and Flegmann (1984) found that the rate of proliferation of black currant *in vitro* cultures was very low under low light intensity of 0.9 W/m^2 (about 300 lux) and much higher at 18.5 W/m^2 (about 6 000 lux). Gautheret (1969), however, noticed that adventitious root formation in tuber sections of *Helianthus tuberosus* was severely reduced by intensities greater than 7 000 lux.

Liu *et al.* (1983) used explants of apple seedlings maintained in light or in the dark to assess morphogenic responses. Leaf and cotyledon explants were cultured in the dark for the initial 3 weeks, and then transferred to the light for 4 weeks. They produced 5 to 20 times more adventitious shoots than those cultured for 7 weeks in light. However, light did not significantly influence the number of shoots formed on hypocotyl explants. It has also been found that a daily 5-minute exposure of leaf explants to red light (651 nm) supressed adventitious shoot formation by 80% while a 5-minute exposure to far-red light (729 nm) immediately following the red light, counteracted the red light suppression (Liu *et al.*, 1983)

Morphogenic responses that are wavelength-specific are common with *in vivo* grown plants and are also observed when plants are cultured *in vitro*. The major photosynthetic pigments, *chlorophyll a* and *chlorophyll b*, have absorbtion spectra in both blue and red wavelengths. *Chlorophyll a* has absorbtion peaks at 440 and 680 nm. *Chlorophyll b* has absorbtion peaks at 470 and 650 nm. Both wavelengths are therefore necessary for photosynthesis. These wavelengths are required by tissue cultures as well.

The role of light of different wavelengths on bud formation in *Heloniopsis orientalis* was investigated by Kato (1978). White, red and blue light were effective in inducing bud formation but green light was not. Bud induction of pine cotyledons was maximized at 660 nm, while purple and near UV light had no effect (Katkade *et al.*, 1978). These experiments indicate that photomorphogenesis does occur *in vitro*, possibly by interacting with phytochrome, the chlorophylls and other photo-absorbing pigments (Hughes, 1981).

Early studies with ***Bryophyllum tubiflorum*** (Kalanchoë) indicated that photoperiod affected bud formation (Sironval, 1956). Sironval selected two series of young Kalanchoë plants; one was grown during short days (8 h), the other during long days (16 h). After four months, only the leaves of the 16 h plants had initiated adventitious buds.

The most common light cycle for tissue cultures is 16 hours of light followed by 8 hours of darkness. However, the effective photoperiod for morphogenesis varies between taxa. Gautheret (1969) found that a 12 h photoperiod was optimal for shoot production from *Helianthus* tuber sections. Maximum bud formation was induced in geranium callus with a photoperiod of 15 to 16 h (Pillai and Hildebrandt, 1969). Alleweldt and Radler (1961) investigated the effect of photoperiod on growth of a short-day-sensitive grape. They found that the cultivar produced roots under short days but only callus under long days. Hussey and Falavigna (1980) working with cultures of *Allium cepa* found that more adventitious shoots were formed in 16 h than in 8 h days. With shoot cultures of *Solanum tuberosum*, however, maximum node formation took place in continuous light with fewer nodes and weaker shoots being formed in 8 h days (Hussey and Stacey, 1981).

Temperature and pH

Studies on the *in vitro* growth of plant cells have indicated that the optimum temperature for growth generally lies within the range of 21 to 30°. Harris (1980) found that a temperature of about 22° appears close to the optimum for establishment and proliferation of the Saskatoon (*Amelanchier alnifolia*) shoot-tip cultures. Many authors have found that the optimum temperature for raspberry cultures is about 25° (Snir,1980; Pyott,1981). Similar results were obtained with *Narcissus* (Seabrook and Cumming, 1978). The best induction of roots on *Rhododendron* explants was also at 25° (Pierik and Steegmans, 1975). On the other hand, Takayama and Misawa (1979)in studies with *Lilium auratum* found that the best bulb formation was at 20° and decreased progressively as the temperatures were increased to 30°. Lower temperatures were not tested in this experiment. Harris (1982) noticed that the

optimum temperature for adventitious shoot formation of *Vitis* was 23 ± 2°. He also compared four different temperatures for rooting: 20, 25, 30 and 35°. The best rooting was observed at 30°.

Plant tissue cultures may be affected by alternating day/night temperature cycles. Gautheret (1969) observed that root morphogenesis from *Helianthus tuberosus* tuber explants was best with alternating temperatures of 25°, day and 20° night.

In general, the most favorable pH for the growth of plant tissue cultures lies between pH 5 and 6 (Seabrook, 1980). Nesius and Fletcher (1973) found that the optimum pH for Paul's Scarlet Rose cell cultures was 5.2 - 5.4. Skirvin (1981) noticed that most fruit tissue cultures are grown at pH 5.6 to 5.8 e.g. strawberry pH 5.6 (Boxus, 1977), *Vitis* pH 5.7 (Harris, 1982), blackberry and tayberry pH 5.7 (Harper, 1978; Bromme and Zimmerman, 1978), apples pH 5.8 (James, 1983). On the other hand, acid-loving plants such as *Vaccinium* and *Rhododendron* appear to grow best at pH 4.5 (Anderson, 1975).

B. The Micropropagation of Woody Species

The use of *in vitro* techniques for clonal or asexual mass propagation is the most advanced application of plant tissue culture technology (Thorpe, 1983). Asexual multiplication has been achieved by 1)enhancing axillary bud break 2)production of adventitious buds and 3)somatic embryogenesis.

Adventitious bud stimulation *in vitro* is much more common than somatic embryogenesis and has much more potential for mass clonal propagation of plants than multiplication from axillary buds (Thorpe, 1983). In softwoods, no examples of somatic embryogenesis leading to plantlet formation have been reported, while in hardwoods, only a few cases have been documented (Biondi and Thorpe, 1982).

In general, four stages leading to plantlet formation are recognized (Biondi and Thorpe, 1982):

1. induction of shoot buds

2. development and multiplication of these buds
3. rooting of shoots
4. preparation of plantlets for the field

Adventitious buds can be produced directly on the explants or on callus derived from primary explants. Thorpe (1983) noticed that the direct production of buds is preferred and recommended, because when organs are formed from callus, abnormalities may occur. With callus cultures, there have been numerous reports of cytological irregularities leading to mutations in the regenerated plants. This has been found with plants from calluses of *Populus* spp. (Lester and Berbee, 1977). Hussey (1982) noticed that mutation in totipotent cells may be enhanced as a result of the possible mutagenic action of media constituents such as NAA, 2,4-D or BAP which are used when callus growth is desired. *Asparagus*, multiplied by axillary shoot enhancement. (Murashige *et al.* 1972) showed no genetic aberrations while those regenerated from callus (Malnassy and Elisson 1970) included up to 70% polyploids.

In woody plants, age is a critical factor (Thorpe, 1983). Many authors have reported that the success is relatively easy with juvenile tissue, and progressively more difficult with adolescent and mature tissues. Excised embryos and seedling parts (cotyledons, hypocotyls) from aseptically germinated seeds are the most commonly used explants in woody species. In hardwoods increasing use is being made of axillary buds and shoot tips (Thorpe, 1983).

In plant tissue culture techniques various types of media are used. Several nutrient formulations are available (Gamborg *et al.*, 1976; Gamborg, 1984). The key phytohormone for shoot formation is cytokinin. However, Biondi and Thorpe (1982) noticed that sometimes, low levels of auxins are also needed for shoot formation.

Culture establishment is the first step in *in vitro* propagation. Its purpose is to allow the explant to survive under artificial conditions and free of microorganisms. Many authors report that larger explants survive better than smaller ones and retain more functionally organized tissues. However, as previously discussed, the elimination of pathogens becomes a greater problem with increased explant size.

Shoot proliferation is the second step in micropropagation. It is also called the multiplication stage. In Angiosperms rapid shoot proliferation has been achieved with a wide range of species with initial explants being taken from shoots of field-grown trees or shrubs (Chalupa, 1979; Cheng, 1979; Zimmerman and Bromme, 1980; Harris, 1980; Snir, 1980; Jones 1982).

The use of explants from naturally rejuvenated tissues has been advantageous with some angiosperms, for example Christie (1978) observed that explants from suckers of *Populus* spp. began to grow and proliferate on the nutrient medium immediately, whereas those from tree shoots frequently did not begin to grow for several months. Generally, shoot proliferation from explants of gymnosperms (explants taken from normal aerial shoots of nursery or field-grown plants) has been less rapid than from explants of angiosperms taken from a similar source. With gymnosperms the use of rejuvenated tissue seems to be essential for the achievement of shoot proliferation (Jones, 1982; Thorpe, 1983).

Many researchers report that rooting is very often the critical point in micropropagation of woody plants when aseptic cultures of shoot tips or buds are used. Successful results, however, were obtained with different types of woody plants. Generally, the rooting of angiosperm shoots in culture has been relatively easy, while gymnosperms have presented more difficult problems (Thorpe, 1983). A reduction in the concentrations of both sucrose and nutrient salts in the medium generally enhances rooting of shoots grown *in vitro*. Seabrook (1976) and Biondi and Thorpe (1982) noted that lowering both the sucrose concentration from 3% to 1% and reducing the concentration of salt solution by 50% gave better rooting.

As far as hormonal treatments for rooting are concerned, auxins represent the critical factor. They are beneficial in inducing roots. The optimum concentration varies with species. Abbott and Whiteley (1976) successfuly rooted up to 80% of their apple cultures by dipping the young shoots in IBA (1 mg/l) for 15 min. The shoots were then placed on a filter paper support (Heller bridge) in liquid medium containing Linsmaier and Skoog salts, where rooting

occurred. Hasegawa (1980) lowered the MS salt concentration by 75% and using an IAA concentration of 1 mg/l in the medium obtained 100% rooting of *Rosa hybrida* shoots. Harris (1980) successfuly rooted 100% of his saskatoon cultures using 0.1 mg/l IAA concentration in the MS medium.

Several researchers noticed an interaction between hormones in rooting. The addition of GA_3 to the rooting media (which already contained auxin) is reported by Button and Borman (1971) to give enhanced rooting of 'Washington Navel' orange *in vitro*. On the other hand, Futz (1971) reported that GA_3 inhibited rooting of raspberry. Thorpe (1983) found that sometimes a low concentration of cytokinin (0.01 to 0.1 mg/l) added to the medium can be beneficial in rooting.

Rooting of gymnosperms *in vitro* has been generally much more difficult than that for angiosperms. Biondi and Thorpe (1982) noted that often the use of two auxins combined (IAA + IBA or IBA + NAA), or dipping of shoots overnight in a concentrated solution of IAA or IBA at 120 mg/l gave satisfactory results. They also found that once root primordia were formed transfer to a hormone-free medium was necessary to allow root elongation to take place. The use of these methods greatly enhanced rooting of such species as *Pinus Taeda*, *Tsuga heterophylla*, *Pseudotsuga menziesii*, *Thuja plicata*, *Pinus radiata*, *Sequoia sempervirens* and *Pinus pinaster* (Biondi and Thorpe, 1982). A further problem that has been noted with gymnosperms was that a proportion of rooted plantlets have been plagiotropic and therefore unsuitable as forest trees. This has been especially serious with *Pseudotsuga menziesii* (Bonlay, 1980).

Biondi and Thorpe (1982) found that rooting in non-sterile conditions may be equivalent or even superior to rooting aseptically. Rooting of *in vitro* derived shoots in non-sterile conditions has some advantages:

1. The root system which develops *in vitro* is usually non-functional in a normal substrate because of the lack of root hairs on agar grown roots. When shoots are rooted *in vivo*, root hairs are developed (Biondi and Thorpe, 1982; Maene and Debergh, 1983).

2. The transfer of *in vitro* rooted plantlets to the greenhouse bench is often a problem because the vascular connection between shoot and root is incomplete and there is usually a large amount of callus formed between root and shoot (Biondi and Thorpe, 1982; Thorpe, 1983). This does not occur when plants are rooted *in vivo*.

3. Optimal root formation requires higher auxin concentration for root initiation but these levels are often inhibitory for root elongation (McGuire *et al.*, 1969; Maene and Debergh, 1983). McGuire *et al.* (1969), observed that a high concentration of auxin could inhibit rooting . A 10 sec, basal dip of *Ilex crenata* cv. 'Convexa' cuttings in 1% IBA lowered the number of rooted plants compared to 0.25% IBA treatment or even to the untreated control. Rooting *in vivo* using rockwool blocks has the possible advantage of having the auxin concentration decrease under mist during the root elongation stage (Maene and Debergh, 1983).

4. Often, the root system produced *in vitro* is damaged during transplanting and microorganisms readily enter the wounds and cause disease. This problem can be overcome using *in vivo* rooting (Debergh and Maene, 1981).

5. The cost is reduced if rooting is carried out *in vivo*. This factor is very important when producing plants on a commercial scale (Debergh and Maene, 1981; Thorpe, 1983; Maene and Debergh, 1983).

The rooting of *in vitro* derived shoots in non-sterile conditions has been successful with many species including *Pseudotsuga menziesii* (Cheng, 1976), *Pinus radiata* (Aitken, 1981), *Rubus idaeus* (Snir, 1981), *Cordyline* and *Begonia* (Maene and Debergh, 1983). It offers favorable probabilities for the propagation of difficult-to-root species.

C. Hardening of Plantlets and Transplanting to the Field

Many valuable plantlets can be lost if special care is not taken when transferring them to soil. Sommer (1981) made some essential points concerning the preparation of plantlets for transfer to soil:

1. There should be a reasonable balance between root and shoot. An unbalanced root-shoot system, especially an inadequate root system, can lead to poor growth and eventually the risk of death.
2. The plantlet must undergo a gradual transition (over two to three weeks) from a constant high humidity regime to a low humidity regime.
3. All traces of agar must be removed as it provides a substrate for pathogen growth. Treatment with a fungicide (125 mg/l Benlate) may be a good practice.
4. Some shading is necessary to prevent leaf burn during the transition period from laboratory conditions to full sunlight.
5. Containers in which plants are rooted and grown must also be carefully selected. Pots which do not allow root growth through the sides and bottom cause root malformation and spiraling. Jiffy pots, peat pots, trays or special rooting supports are recommended.

Both acclimation and acclimatization are terms which describe the process of adaptation of an organism to an environmental change. Acclimation is a process regulated by nature; acclimatization is a process regulated by man. These definitions were accepted by the Environmental Conditioning Symposium of 1977 in Chicago, Ill. Aseptically cultured plants grown in high RH often sustain severe water stress when the RH is lowered. Water stress is a major cause of transplanting shock (dieback or death) of aseptically cultured plants when transferred to the greenhouse (Brainerd *et al.*, 1981). In their study of leaf anatomy and water stress of aseptically cultured 'Pixy' plum grown under different environments they observed that leaves of tissue-cultured plants differ anatomically from those of greenhouse plants. The aseptically cultured plant leaves had less epicuticular wax, smaller palisade cells and larger mesophyll air space. Sutter and Langhaus (1979) also found that water stress on high RH grown plants when transferred to a lower RH can be attributed to poorly developed epicuticular and cuticular waxes. Brainerd and Fuchigami (1981) determined that stomatal functioning also is involved in acclimatization to lower humidity. They measured the RWC and stomatal closure when rooted cultures of *Malus* cv. 'Mac 9' were exposed to 30-40% RH

for 0, 6, 12, 24, 48, and 96 hours. Cultures were left in the agar and the culture jar lids were removed. Ten ml of distilled water was added daily to the open jars to prevent drying of the medium. Leaves were acclimatized within 4 to 5 days to 30-40% RH. Struve and Lineberger (1985) developed an acclimatization method for *Amelanchier laevis*. Transplanted plantlets were immediately placed on a shaded bench under mist for 3 days (6 sec mist every 6 min), then humidity was lowered and plants were grown under standard greenhouse conditions. The same method was used by Lineberger (1983) for tissue cultured *Prunus* cv. 'Hally Jolivette'. He obtained up to 100% survival.

Field transplanting survival levels of nearly 100% for *Amelanchier laevis* were achieved by Struve and Lineberger (1985). Plants acclimatized in the greenhouse, approximately 15 cm tall were kept for 0, 5, 10, and 15 days under 60% shade outdoors before planting to the field. Plants exposed to a period of outdoor acclimatization under shade prior to planting appeared to have less transplant shock. Plants acclimatized for 5 and 10 days exhibited the greatest increase in stem elongation and survival was 99%. Such high survival percentages are very important when producing plants on an commercial scale.

D. Dormancy Problems with Newly Propagated Plants

Poor winter survival of rooted cuttings is a common problem when shoot extension growth ceases immediately following rooting. Harris (1975) observed that softwood Saskatoon cuttings behave in this fashion. Similar behavior was observed with summer-rooted cuttings of Japanese Holly (*Ilex crenata*) cv. 'Helleri' (Gilliam and Wright, 1977). Harris (1980) noted that *in vitro* rooted Saskatoon plantlets cease growth and tend to lose their leaves right after transplanting them to the greenhouse bench. Bishop and Nelson (1980) observed that Saskatoon cuttings began to defoliate on the mist bench even before rooting was completed. They found that the normal reduction of day-length from the end of July onward contributed to the leaf loss.

Dormancy is a commonly recognized phase in the annual life cycle of all woody plants in the north temperate zone. In general, most temperate zone plants exhibit three distinct phases of inactivity. These are described as summer dormancy, true dormancy and post dormancy (Vegis, 1964). In most deciduous trees and shrubs summer dormancy begins about mid-summer and long before leaf fall. It occurs, when new shoots have completed extension growth (Berrie, 1984). The condition that developes becomes deeper with time, the plant eventually entering the stage of true dormancy (Salisbury and Ross, 1978).

Bud dormancy can be induced in many species by a shortened day-length especially if ambient temperatures remain high. With several deciduous trees studied at Beltsville (Downs and Borthwick, 1956), a short-day treatment resulted in the formation of a terminal bud and the cessation of internode elongation and leaf expansion. The day-length response was observed at temperatures of 21 to 27°. At temperatures of 15 to 21° the effect was less noticable.

Downs and Bevington (1981) looked at the effect of day length and night temperature on the dormancy of *Betula papyrifera* seedlings from different localities. Seedlings of Alaskan origin (collected at latitudes 64-67°N) and from the continental USA (collected at latitudes 44-48°N) were exposed to different photoperiods. Under a 14-h day, only Alaskan material was dormant. Under a 9-h day all material was dormant if the night temperature was low (day temp. 30°, night temp. 14°). At a day/night temperature regime of 30°/26° only 40% of the Alaskan material and none of the other, were dormant.

Several researchers noted changes in levels of growth regulators that could be correlated with the onset of bud dormancy. Investigation of endogenous levels of hormones in some plants showed a decrease in growth promoters such as gibberellins and cytokinins and an increase in the growth inhibitor ABA during the development of dormancy (Eagles and Wareing, 1963; 1964; Wareing and Sanders, 1971; Wright, 1975).

With the breaking of dormancy Taylor and Dumbroff (1975) found an increase in cytokinin activity in plant extracts. Experiments with exogenously applied hormones have

shown that bud dormancy can be overcome by the use of both, cytokinins and gibberellins. Luckwill (1968) observed that in young apple trees cv.'Stark Earliest' gibberellic acid caused all lateral buds to break and all lateral shoots to grow. Apical dominance was apparently destroyed by the GA application. Concentrations of 50, 100, 200, and 500 ppm were equally effective.

Kender and Carpenter (1972) observed stimulation of lateral bud growth of apple trees by BAP. Gilliam and Wright (1977) found that a BA application of 600 ppm promoted bud break of *Ilex crenata* cv.'Helleri'. BA increased the number of breaks and suppressed stem elongation, while GA_3 at 400 ppm decreased the number of breaks and increased stem length. Grainger (1983) showed that the application of GA_3 on dormant Saskatoon seedlings broke dormancy and induced growth from terminal buds. Plants obtained after such treatment were single stemmed. McConnel and Herman (1980) investigated the effect of both, GA_3 and BA on inducing bud break of rooted softwood cuttings of *Salix pentandra* L. and *Viburnum Lantana* L. They found that GA_3 caused bud break and increased shoot length in *S. pentandra* and *V. Lantana* but reduced their overwintering ability. BA stimulated adventitious bud initiation, reduced average shoot length and increased winter survival of both plants.

Several workers have observed that the combined treatment of cytokinin plus gibberellin can induce growth from dormant buds (Baldini *et al.*, 1973; Forshey, 1982; Ali, 1984). Williams and Billingsley (1970) found that the application of BAP in combination with GA_{4+7} caused a significant increase in the number of buds breaking dormancy in "Delicious" apple trees compared to those from trees treated with either BAP or GA_{4+7}. Total shoot growth per tree was increased when the BAP and GA_{4+7} were combined. BAP or GA_{4+7} used alone did not have such an effect. Forshey (1982) found a significant increase in the numbers of shoots and in shoot length, when both hormones were applied together to dormant buds of apple trees. Ali (1984) found that lateral buds of *Amelanchier alnifolia* responded positively by breaking dormancy and making some extension growth on all plants when BA was applied as a foliar spray. GA_{4+7} when it was used in treatment with BA, not only contributed to the

release of additional buds from the dormant condition but also contributed significantly to shoot length.

Bud break can also be influenced by photoperiod. As mentioned earlier, short days induce cessation of extension growth while long days promote bud activity and vegetative growth. Wallace and Graham (1976) reported greater survival of rooted Saskatoon cuttings when they were transplanted and kept under long day conditions just after root emergence. Scott (1975) has suggested that additional hours of supplemental light as well as a higher temperature (up to 30°) in the mist bed may be the way to activate more photosynthesis in newly rooted *Cornus alba* and *Viburnum opulus* cuttings.

III. Materials and Methods

The objectives of this study were to develop a method of micropropagation for *Amelanchier alnifolia* and for post propagation procedures that would promote growth and reduce plant stress for successful field transplanting.

A. Plant Material - Explant Sources

In this study three types of explants were compared: 1) shoot tips, 2) dormant buds and 3) active buds- in the silver tip stage in order to determine which, if any, was more practical for initiation of cultures.

Explants were taken from mature shrubs (approximatelly 3m tall) from field #11 at the Alberta Tree Nursery and Horticulture Centre, Edmonton. Four cultivars were used in the experiments: 'Smoky', 'Thiessen', 'Pembina' and 'Northline'. Shoot-tip explants were taken from actively growing branches in June. Dormant-bud explants were taken from dormant branches in November. Active-bud explants were taken from branches in April. Terminal buds were used in all experiments.

In 1984/85, preliminary experiments were carried out with the cv.'Smoky' only. In 1985/86 studies were made with cvs.:'Smoky','Thiessen','Pembina' and 'Northline'.

B. Sterilization Procedures

Shoot Tips.

After harvesting, shoot tips were taken to the laboratory. Fully opened leaves were removed and the apical portions were rinsed in a stream of tap water for 30 min. Shoot tips were then sterilized for 10 min by placing them in a 7% calcium hypochlorite solution containing 0.1% Tween 80 (surfactant). The sterilant was stirred constantly on a magnetic stirrer. The shoot tips were then washed 3 times with sterilized, distilled water and the outer layer of leaves covering the shoot tip was removed in a Laminar Flow Bench. Using a sterile

scalpel, shoot tips were shortened to 6-8 mm and placed on a solid agar medium in 25x150 mm culture tubes (one shoot tip per tube). Culture tubes were closed with plastic caps-'Bellco- Kap-Uts' (Carolina Biological Supply Company, North Carolina, USA).

Dormant and Active Buds.

Dormant branchlets were removed from field grown plants and taken to the laboratory, where terminal buds were separated from twigs and the first layer of bud scales excised and discarded. Buds were then sterilized using the same technique as for shoot tips, except the time of sterilization in the calcium hypochlorite solution was increased to 20 min. After sterilization the basal portions of buds were shortened with a sterile scalpel to give better contact with the medium. The buds were then placed on the medium in culture tubes. The sterilization procedure used with dormant buds was also used with active buds. All procedures involving transfer of cultures from one test tube to another or from one culture vessel to another (i.e. transfering fresh media) were carried out in sterile air flow in the Laminar Flow Bench. All instruments used in preparing the cultures were autoclaved for 15 min at 121° before using. In addition, the sterilized instruments were flamed before each use.

C. Media and Culture Vessels

The degree of success in initiation and growing of cultures depends also on the type of medium on which cultures are grown. Media used in all experiments were prepared in the laboratory at the Alberta Tree Nursery & Horticulture Centre. Media were sterilized in the autoclave at 121° for 10 to 15 min. All media were solidified with 0.6% agar. No liquid media were used.

The media formulations such as those of Murashige and Skoog (1962) and Gamborg *et al.* (1968) are suitable for a wide range of plants including woody species from the family *Rosaceae* to which *Amelanchier alnifolia* belongs. In this study the above formulations were tested as well as two others, that of Linsmaier and Skoog (1965) and Nitsch (1968). For

media formulations see Appendix 6.

The pH of media were adjusted to 5.7 for MS, 5.8 for LS and 5.5 for both B-5 and Nc using 0.2 N KOH and 0.2 N HCl. Agar was added later.

The culture vessels used were 25x150 mm culture tubes containing 10 ml of medium and 100 ml glass jars ("baby food jars") containing 20 ml of medium. Unless otherwise indicated, culture tubes were used for establishing aseptic cultures (stage I) and for rooting of cultures (Stage III). For shoot multiplication (Stage II) the food jars were used.

D. Experimental Conditions

Light

As revealed by the literature, the light intensity plays an important role with plants grown *in vitro*. Usually, for the establishment and multiplication of cultures the light intensity required is lower than for rooting (Murashige, 1977).

Establishment and Multiplication of Cultures.

The standard environmental light condition for both establishment and multiplication of shoots consisted of a 16h photoperiod at an irradiance of 55 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$. Cultures were incubated in the "Conviron S 10h" growth cabinet with mixed fluorescent and incandescent light sources.

Rooting in vitro.

Rooting of shoots was carried out in the same "Conviron" growth cabinet with 16h light period at an irradiance of 70 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$. To determine the influence of light quality on rooting *in vitro*, broad band blue (B), yellow (Y), and red (R) filters were used over the growing cultures. A clear plastic (W) filter was used as control. These filters were constructed out of 20x20 cm plexiglass pieces, taped together with plastic tape to form cubes with one side

open. Twenty five culture tubes were placed under each cube. Lamp wattage as well as **distance between cultures and** lamps was adjusted so that each cube had a spectral maximum of 10 $\mu Em^{-2}s^{-1}$. The light spectrum for each filter was measured using a Quanta Spectrometer QSM-2500 (Techtum Instruments). The spectra used are shown in Figure 1. Rooting studies were also carried out in the dark in a 'Percival I-35L' growth cabinet.

Temperature

All lighted cultures were maintained in a 24/22° day/night temperature regime except for cultures under the blue filter which were kept in 27/22° regime due to the heat radiating from lamps in the growth cabinet (the blue filter was placed as close as possible to the light source to permit the highest irradiance). Cultures in constant darkness were held at 23 ± 1°.

Rooting in Non-Sterile Conditions - *in vivo*.

The objective of this study was to develop a rooting technique appropriate to the production objectives of the ATN & HC. Rooting of shoots in non-sterile condition permitted the use of ordinary nursery methods and seemed to be more practical. Rooting of shoots derived from aseptic cultures was carried out on the greenhouse bench under mist. To maintain high humidity the bench was covered with a transparent plastic tent. The bench was bottom heated using a lead-covered, thermostatically controlled heating cable set at 25°.

E. Establishing Aseptic Cultures- Stage I

In studies with shoot tips of the 4 cultivars used, the 4 media- MS, LS, B5 and Nc were employed. The growth regulator concentrations in all media were: 0.49 μM (0.1 mg/l) of IBA (Fisher Scientific CO., Fair Lawn, N.J.) and 4.44 μM (1 mg/l) of BAP (Alfa Products, Danvers, MA). Explants were incubated in standard white light conditions for 3 weeks in the growth chamber. After incubation,the length and number of shoots obtained from each explant were recorded as well as the number of living and active cultures.

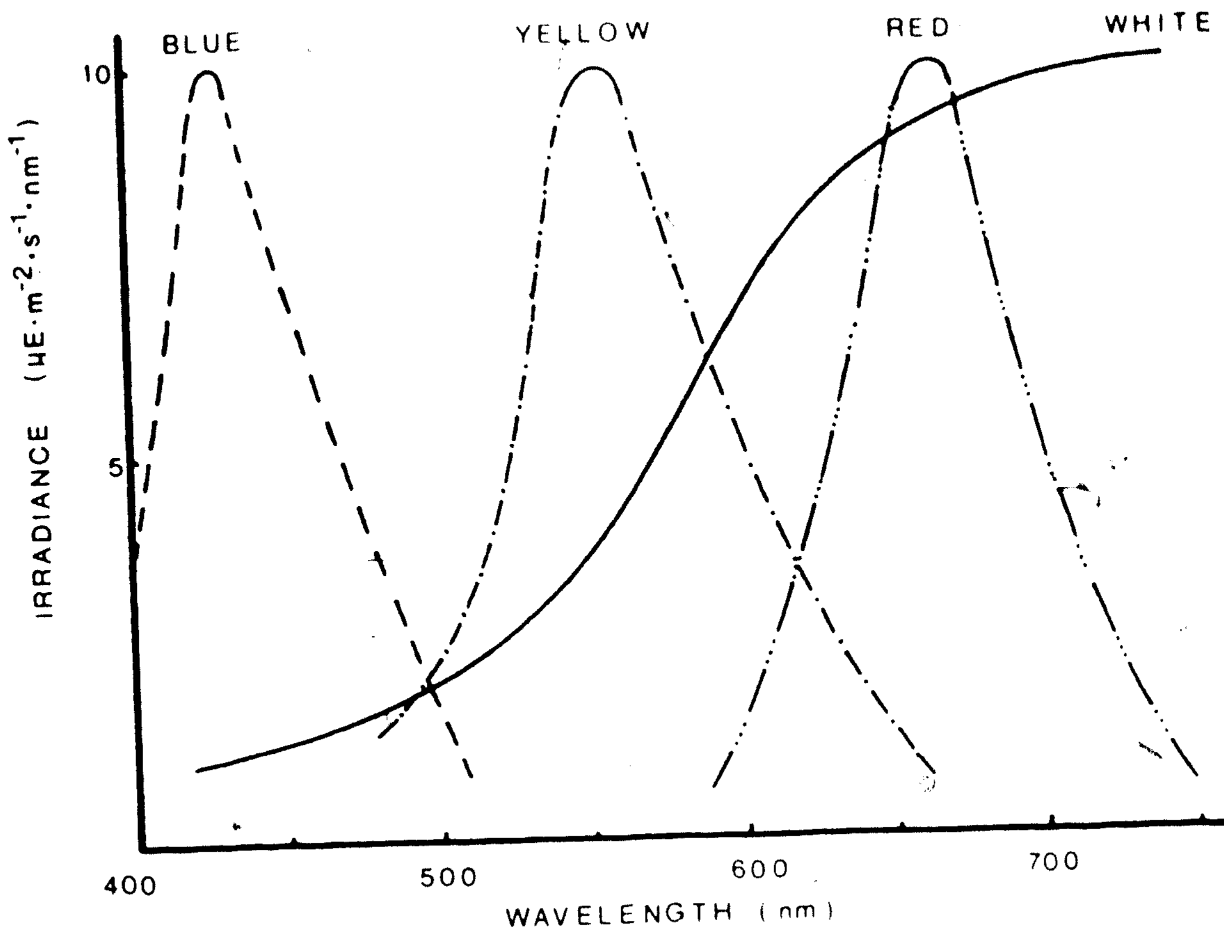


Figure 1. Spectra of the 4 light filters used in Rooting Studies.

In experiments with dormant buds the same 4 cultivars were used. The effects of 3 types of media were examined: MS, B-5 and Nc. Because MS and LS formulations were very similar to each other, the LS medium was not used. The growth regulator concentrations in all media were: 0.49 μM of IBA and 4.44 μM of BAP. All cultures were incubated under standard conditions for 3 weeks. The number of cultures alive and active after 3 weeks of incubation was recorded as well as length of shoots from growing buds.

In experiments with active buds, only the cultivar 'Smoky' was used. Explants were cultured for 3 weeks on MS, B-5 and Nc media each containing 0.49 μM of IBA and 4.44 μM of BAP. Length of shoots and the number of buds producing only callus, was recorded after the 3 week incubation period.

F. Shoot Multiplication- Stage II

In these experiments only the MS medium was used. All 4 cultivars were included in this study.

Shoots approximately 25 mm long were placed horizontally in 100 ml jars containing 20 ml of the basic MS medium. Horizontal placement was used to obtain maximum proliferation. No auxin was used, however, five different concentrations of BAP were added to the medium: 0.00, 8.88, 13.30, 17.70, and 22.20 μM (0, 2, 3, 4, 5 mg/l respectively) to provide 5 treatments.

Cultures were incubated for 3 weeks and then were sub-cultured onto fresh media. At the time of sub-culturing the following measurements were taken: number of shoots per culture and shoot length. All shoots longer than 3mm were harvested and the sub-cultures were grown for another 3 weeks. Measurements were again taken at the end of this period using 10 sub-cultures from each treatment selected at random. Sub-culturing was done every 3 weeks and measurements were taken at the time of sub-culturing.

G. Rooting Studies- Stage III

Rooting in vitro

The effect of growth regulators, IBA and BAP in the medium on the rooting of shoots of cultivar 'Smoky' was studied. Preliminary experiments had shown that the best rooting occurred when the concentration of IBA in the medium was 2.45 μM (0.5 mg/l) in the absence of BAP.

In this study, MS salts at 1/3 strength were used and 20 g/l of sucrose was included in the medium. Four concentrations of IBA were used: 0.00 , 0.49 , 2.45 and 4.90 μM (0.0, 0.1, 0.5, 1.0 mg/l respectively). Thirty shoots, each 15-25 mm long, were cultured in each of the four treatments. Shoots were placed in jars (one shoot per vessel) containing 20 ml of medium. Cultures were incubated for 4 weeks. After incubation the number of cultures that had rooted was recorded.

Light Quality on Rooting in vitro

In this study the influence of light quality as well as the effect of a 2 week dark period followed by 2 week light period was examined on the rooting response of shoots of cv.'Smoky'. The medium used contained 1/3 strength of MS salts, 20 g/l of sucrose and 2.45 μM IBA. Shoots were placed in culture tubes (one shoot per culture tube) and broad band filters were placed over t[illegible]owing cultures to achieve light qualities required (see 'Experimental Conditions'). After a 4 week incubation period 24/22° day/night temp. at 10 $\mu E \cdot m^{-2} s^{-1}$, the number of rooted shoots was recorded. Under the white filter the influence of irradiance of 70 $\mu E \cdot m^{-2} s^{-1}$ on rooting was also investigated.

In studies where a dark period was followed by a light period, cultures were incubated for 2 weeks in the dark at 23±1° and then for 2 weeks in the light under standard conditions. After incubation the number of rooted cultures was recorded.

Rooting in vivo

The rooting of *in vitro* derived shoots from cultivars: 'Smoky', 'Thiessen', 'Pembina' and 'Northline' in non-sterile conditions was also investigated. Shoots were stuck individually in 3.5 x 3.5 x 3.5 cm rockwool cubes in trays and were placed under mist on the greenhouse bench. Preliminary experiments with cv.'Smoky' had shown that the best rooting occurred when the concentration of IBA in the medium was 0.49 or 2.45μM in the absence of BAP.

In this experiment, rockwool blocks were saturated with a nutrient solution containing MS salts at 1/3 strength and 20 g/l of sucrose and one of four concentrations of IBA : 0.00, 0.49 , 2.45 , 4.90 μM. Media were prepared using tap water and the pH was adjusted to 5.7. Rockwool cubes were saturated by immersing them in the solutions 24 hours before use. Shoots 15-25 mm long were stuck in the rockwool cubes and then transferred to the greenhouse bench under mist. The misting system was set to provide 30 sec of mist every hour from 6 AM to 6 PM and every two hours from 6 PM to 6 AM for the first week. During the next two weeks the mist treatment was changed to provide 30 sec of mist every two hours from 6 AM to 6 PM and every four hours from 6 PM to 6 AM. Plantlets were watered once a week with a nutrient solution containing 0.50 g/l of KH_2PO_4, 0.25 g/l of KNO_3, 0.30 g/l of $Ca(NO_3)_2$,0.10 g/l of $MgSO_4$ and Chelated Mixed Trace Elements(Plant-Green) containing:

Iron (Fe)............... 2.1 ppm

Manganese (Mn)...... 0.6 ppm

Zinc (Zn).............. 0.12 ppm

Copper (Cu).......... 0.03 ppm

Boron (B).............. 0.39 ppm

Molybdenum (Mo).. 0.018 ppm

The use of commercial rooting compounds Stimroot #2 (0.4% IBA) and Rootone F (0.057% IBA + 0.067% NAA) were also tested. Shoots were dipped in the dry preparations and then were stuck in rockwool cubes that had previously been saturated with nutrient medium before being transferred to the mist bench. The nutrient medium consisted

of 1/3 MS salts containing 20 g/l of sucrose.

Plants were grown for 4 weeks and when roots were noted, they were transferred to Hillson Trays. The growing medium used was a mixture of peat moss, sand and vermiculite, 1:1:1. Irradiance under the plastic tent in the greenhouse, measured at noon, was 340 $\mu E \cdot m^{-2} \cdot s^{-1}$.

H. Dormancy Studies

Dormancy Studies with Newly Rooted Plants

The effect of GA_{4+7} and BAP on breaking the dormancy condition of freshly rooted plants was investigated. Two cultivars, 'Smoky' and 'Thiessen' were used in this experiment. After planting to Hillson Trays the rooted plantlets were kept for an additional week on the bench with 30 sec mist every two hours. Following this, the plastic tent used over the mist frame was removed and plants were kept for three days under mist, 30 sec every 2 hours. Plants were then transferred to a greenhouse bench with[illegible]ist. About 90% of newly rooted plants were dormant.

GA_{4+7} at 100 ppm and BAP at 400 ppm were used to break dormancy of newly rooted plants. Chemicals were applied separately by spraying three times allowing the plants to dry between sprayings. The same procedure was applied to the control plants except they were sprayed with water only. In the combined, BAP+GA_{4+7} treatment, plants were first sprayed with BAP (three applications were made allowing the plants to dry between sprayings) and then, after 4 hours they were sprayed with GA_{4+7} (the same procedure was followed as for BAP application). In all cases Tween 80 at 0.1% concentration was used as the surfactant. One week after these applications were completed all plants were sprayed with nitrogen at 200 ppm. Commercial water-soluble fertilizer 20-20-20 was used as a source of nitrogen.

Use of nitrogen was continued at weekly intervals during the 8 week growing period. Plants were grown in the growth cabinet at 24/18° day/night temperature. After 8 weeks, the

total length of shoots per plant, the number of stems, the number of subterranean buds and fresh weight of plants was recorded. Plants were then oven-dried for 48 hours at 70° and the dry weight recorded.

Dormancy Studies with Young Established Plants

Six month old, vigorously growing cv.'Smoky' plants were selected for this study. Plants were uniform (approximatelly 20 cm tall), single stemmed and were growing in 1 gallon pots (one plant per pot) in the greenhouse. The separate and combined effects of the four treatments, namely BAP, GA_{4+7}, truncation (removal of the terminal bud) and non-truncation on the dormancy of lateral buds, were examined.

Chemicals were applied using the same procedure as for newly rooted plants. Concentrations used: BAP at 400 ppm and GA_{4+7} at 100 ppm. Plants from truncated and non-truncated controls were sprayed only with water. One week after treatments were made all plants were sprayed with nitrogen at 200 ppm (from the commercial fertilizer 20-20-20). Use of nitrogen was continued at weekly intervals during the 8 week growing period. After 8 weeks the number of stems, the number of subterranean buds and the total length of shoots from laterals were recorded.

I. Field Performance of Tissue-Cultured Plants.

The survival of cv.'Smoky' plants when transplanted to the field was examined. Actively growing, tissue-cultured plants, acclimatized to the greenhouse conditions were used in this study. Plants were approximately 10 cm tall. They were acclimatized for 0, 7, and 14 days under 50% shade outdoors. The lath-house #3 at the Alberta Tree Nursery, Edmonton was used for this purpose. The first field planting was made June 8, 1985. These plants were transferred from the greenhouse directly to the field. The second planting was made June 15, 1985. This group of plants after being removed from the greenhouse was kept in the lath-house under 50% shade for one week and then planted to the field. The irradiance in the

lath-house measured at noon was 960 $\mu E \cdot m^{-2} s^{-1}$; the irradiance on the open field measured at noon was 2 000 $\mu E \cdot m^{-2} s^{-1}$. The third planting was made June 22, 1985. These plants were kept in the lath-house for 2 weeks before planting them to the field. At the time of field planting, plant height and number of shoots were recorded for all treatments. By September 15, 1985, terminal winter buds had been already formed and plants were again measured. Plants were again examined on May 16, 1986, to check winter survival.

IV. Results and Discussion

The literature has revealed that there is no universally acceptable system for the *in vitro* propagation of plants. Those that do well on one medium can respond poorly on others and what appears to be the wrong type of explant for one species may be ideal for another.

In this study the effects of 3 variables were considered on the *in vitro* propagation of the Saskatoon: cultivar, explant type and culture medium. Since *in vitro* propagation involves 3 different stages the effect of these variables at each stage was also examined.

A. Establishing Aseptic Cultures - Stage I.

Aside from the importance of sterile technique, the establishment of aseptic cultures is very much dependent on explant type. In this study it has been demonstrated that all explant types are not equally suited to this method of propagation. In the establishment of aseptic cultures it was found that a 3 week incubation period was adequate. It was also noted that while it was important to have an active culture it was also important to have a culture that produced a shoot with several nodes. The best active cultures are obviously those that produce the longest shoots. Therefore, shoot length comparisons were also made.

Preliminary studies with cv.'Smoky' showed the successful use of shoot tip and dormant bud explants in establishment of cultures. Those obtained from active buds produced mostly callus instead of shoots, and these explants were not used in further experiments. In detailed studies with the four *Amelanchier alnifolia* cultivars only shoot tips and dormant buds were used as explants.

Shoot Tip Explant

No difficulty was encountered in producing aseptic cultures when the method outlined for sterilization of shoot tips was followed. In all cases less than 1% of the cultures showed contamination.

It can be seen in Figure 2 that the number of active cultures was affected by both, the type of medium used and by cultivar. While the number recorded for each of the four cultivars varied only slightly, the statistically significant differences noted can be attributed to the effect of the medium on which the cultures were grown. The cultivar 'Thiessen' displayed no preference for medium, whereas cvs.'Pembina' and 'Northline' performed better on the MS and LS media (Fig. 2). The cultivar 'Smoky' showed a preference for MS as well as for Nc medium.

When shoot length was examined (Table 1) the superiority of the MS and LS media for establishing cultures of *Amelanchier alnifolia* was quite apparent as was the cultural vigour of the cv.'Thiessen'. Length of shoots of cultures from MS and LS media almost doubled shoot length of cultures from B5 and Nc media.

Table 1. The effect of media on the promotion of shoot growth from shoot tip cultures of 4 cultivars of *Amelanchier alnifolia*.

	Shoot Length					
	Medium					
Cultivar	MS	LS	B5	Nc	Means	
Thiessen	35.5	41.3	24.3	15.2	29.1	a
Northline	29.5	34.0	18.2	14.3	24.0	b
Pembina	29.8	29.0	16.9	12.8	22.1	b
Smoky	28.0	22.9	17.2	19.3	22.0	b
Means	30.7 a	31.8 a	19.2 b	15.5 b		

All measurements in [mm].
Values not followed by the same letter are significantly different at the 5% level as determined by LSD.
Data based on 30 cultures per treatment, per cultivar (3 replicates with 10 cultures per replicate).

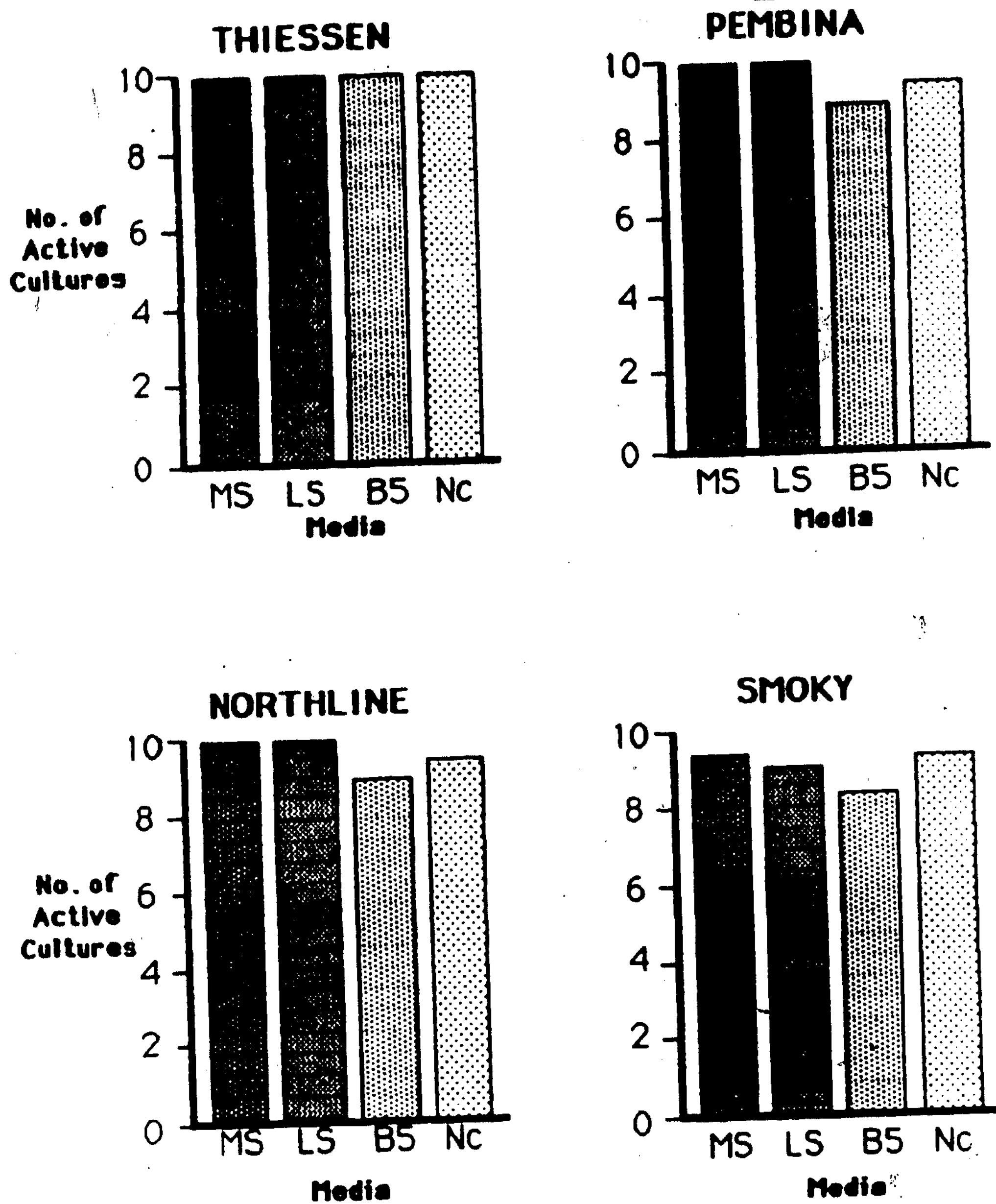


Figure 2. The effect of media on the number of active cultures obtained from shoot tip explants of 4 cultivars of *Amelanchier alnifolia*. Data based on 30 cultures per treatment (10 cultures per replicate). Values represent means from 3 replicates.

The much higher nutrient level, especially nitrogen in both the MS and LS media might be responsible for the better establishment of cultures. For details see Appendix 1 (tables I - IIa).

Dormant Bud Explant

In experiments with dormant bud explants three media, namely: MS, B5 and Nc were tested. The LS medium was not used because the results obtained on this medium with shoot tip explants did not differ significantly from results obtained on MS medium. Furthermore, both MS and LS formulations are very similar to each other. For media formulations see Appendix 6.

Some difficulty was encountered in producing aseptic cultures when the method outlined for sterilization of dormant buds was followed. Approximatelly 20% of dormant bud cultures showed contamination after incubation. This was likely due to the presence of pathogens beneath the bud scales where the sterilant was unable to penetrate.

The number of active cultures obtained from dormant bud explants was strongly affected by the type of medium used (Figure 3). All cultivars responded in a similar way showing a preference for MS medium. Dormant bud explants appeared to be much more sensitive to the type of medium used than were shoot tip explants. Dormant bud explants, however, produced fewer active cultures than shoot tip explants.

When shoot length from dormant bud cultures was examined (Table 2) the results again reinforced those observations made of the number of active cultures. The superiority of MS medium was apparent. Shoots from cultures grown on B5 and Nc media were significantly shorter. As had been the case when shoot tips were used as explants, cv. 'Thiessen' gave the longest shoots. Shoots from the other three cultivars were significantly shorter. For details see Appendix 1 (tables III - IVa).

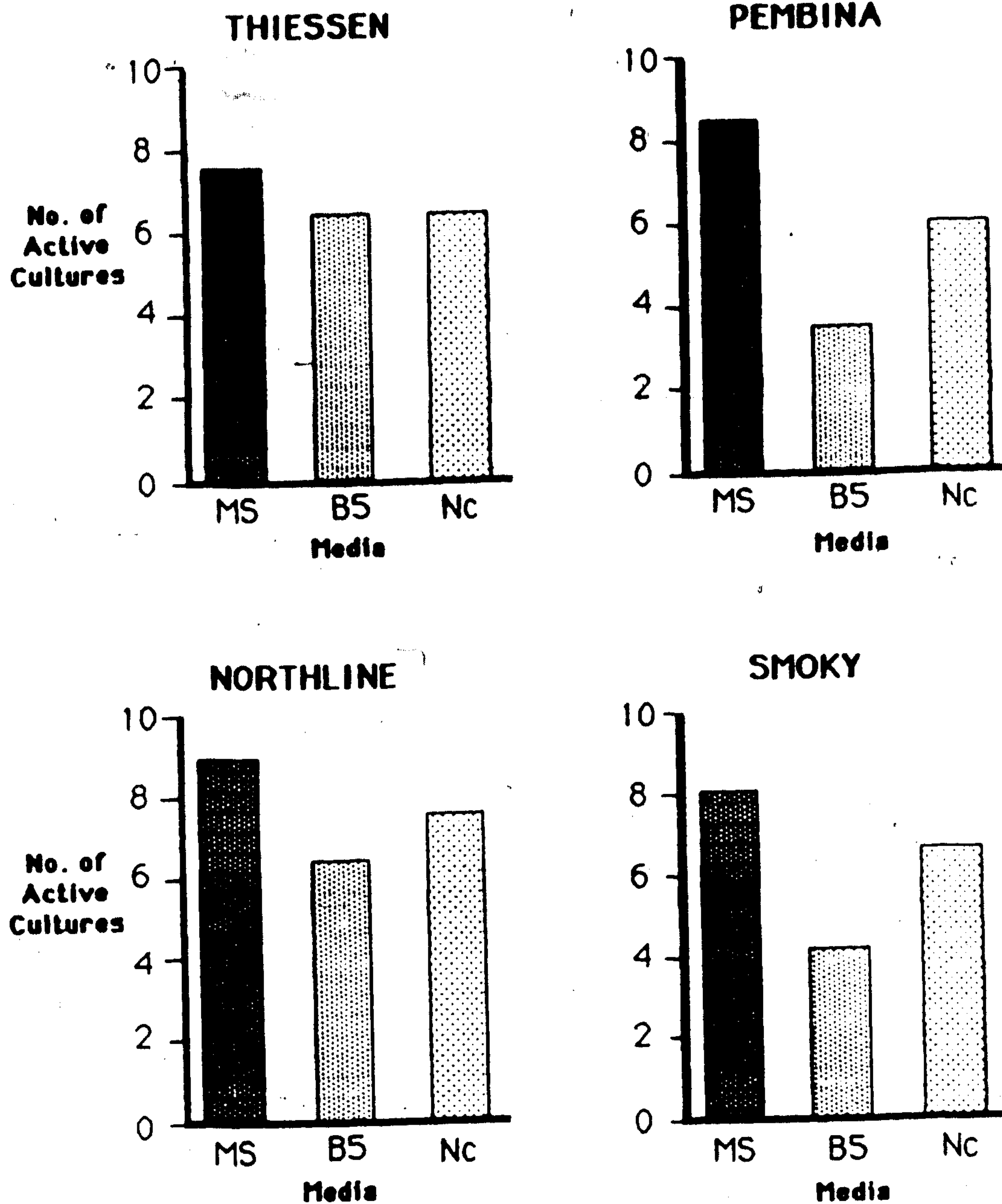


Figure 3. The effect of media on the number of active cultures obtained from dormant bud explants of 4 cultivars of *Amelanchier alnifolia*. Data based on 20 cultures per treatment (10 cultures per replicate). Values represent means from 2 replicates.

Table 2. The effect of media on shoot length of cultures from dormant bud explants of 4 cultivars of *Amelanchier alnifolia*.

	Shoot Length			
	Medium			
Cultivar	MS	B5	Nc	Means
Thiessen	15.1	12.0	10.9	12.7a
Smoky	12.3	11.4	10.7	11.5b
Northline	11.1	10.6	10.4	10.7c
Pembina	10.8	8.9	10.0	9.9c
Means	12.3 a	10.7 b	10.5 b	

All measurements in [mm].
Values not followed by the same letter are significantly different at the 5% level as determined by LSD.
Data based on 20 cultures per treatment (2 replicates with 10 cultures per replicate).

Comparing data from Table 2 with that for shoot tip explants (Table 1) it can be seen that shoots obtained from dormant buds were half as long as shoots from shoot tip explants. When dormant buds are used as explants, dormancy of the bud must be broken prior to shoot growth, whereas shoot tip explants start to grow immediately after being placed on the medium. This could be a reasonable explanation for the difference in shoot length between cultures obtained from shoot tips and from dormant buds following incubation, which in both cases was of 3 weeks duration.

Active Bud Explant

Preliminary experiments with active buds of cv.'Smoky' showed that cultures obtained from these buds tended to produce callus instead of shoots. Active buds were therefore not used in further studies with other cultivars, however, the comparison between the number of active cultures obtained from shoot tips, dormant buds and active buds of cv.'Smoky' was made and is shown in Table 3. The percentage of active cultures produced from active buds was the lowest on all media, yet as with the other two explants, active bud explants showed a preference for MS and Nc media.

Additionally, some difficulty was encountered in producing aseptic cultures when the method outlined for surface sterilization of active buds was followed. About 20% of active bud cultures showed contamination following the incubation period which was likely due to the presence of pathogens under the bud scales where the sterilant was unable to penetrate. For details see Appendix 1 (tables V and Va).

Table 3. Percentage of active cultures from cv.'Smoky' produced from three types of explant on three different media.

	Active Cultures %		
	Medium		
Explant Type	MS	B5	Nc
Active Bud	56.7	46.7	56.7
Dormant Bud	86.7	60.0	73.4
Shoot Tip	97.0	83.4	97.0

B. Multiplication of Shoots - Stage II

In the multiplication stage it was also found that a 3 week incubation period was adequate. At this stage it was important to obtain the highest number of shoots per culture and to have shoots of good quality and large enough for rooting. Because of this, the best cultures were those which produced many, long fleshy shoots.

The shoot length and the number of shoots per culture with all cultivars, were affected by the concentration of BAP in the nutrient medium. The MS medium was chosen for this study, because its superiority in establishment of cultures had already been demonstrated. Figure 4 illustrates the influence of BAP concentration on shoot length and the number of shoots per culture of the four cultivars. On the medium lacking BAP each culture produced only one or two, 20mm long shoots with well developed leaves (Plate 1). When 8.88 μM BAP was used in the medium, cultures of all cultivars produced many more shoots and the length of shoots increased slightly, except for cv.'Northline' where shoots were slightly shorter than in the control treatment. Leaves of all cultures were well developed and light green in color. With increasing amounts of BAP in the medium, more shoots were produced but they were shorter. At the BAP concentration of 17.70 μM the highest number of shoots per culture was observed [illegible] cultivars but in all cases shoots were shorter and more delicate, and had slightly deformed leaves. When BAP was increased to 22.20 μM there was a marked decrease in the number of shoots produced and a very significant decrease in shoot length of cultures from all cultivars (Fig.4a,b). At this BAP concentration all cultures produced fragile, short shoots with poorly developed, dark green leaves. Thus, the BAP concentration of 8.88 - 13.30 μM appeared optimal for shoot multiplication, producing eight to ten sturdy, 20 mm shoots per culture, with well developed leaves.

Differences in cultivar response to BAP concentration were also noted. Cultures of cv.'Thiessen', at all concentrations, produced more shoots than either of other cultivars, and these shoots were the longest as well (Fig.4a,b). Cultures of cv.'Northline' produced fewer shoots than any of the other three, and in all cases shoots were shorter.

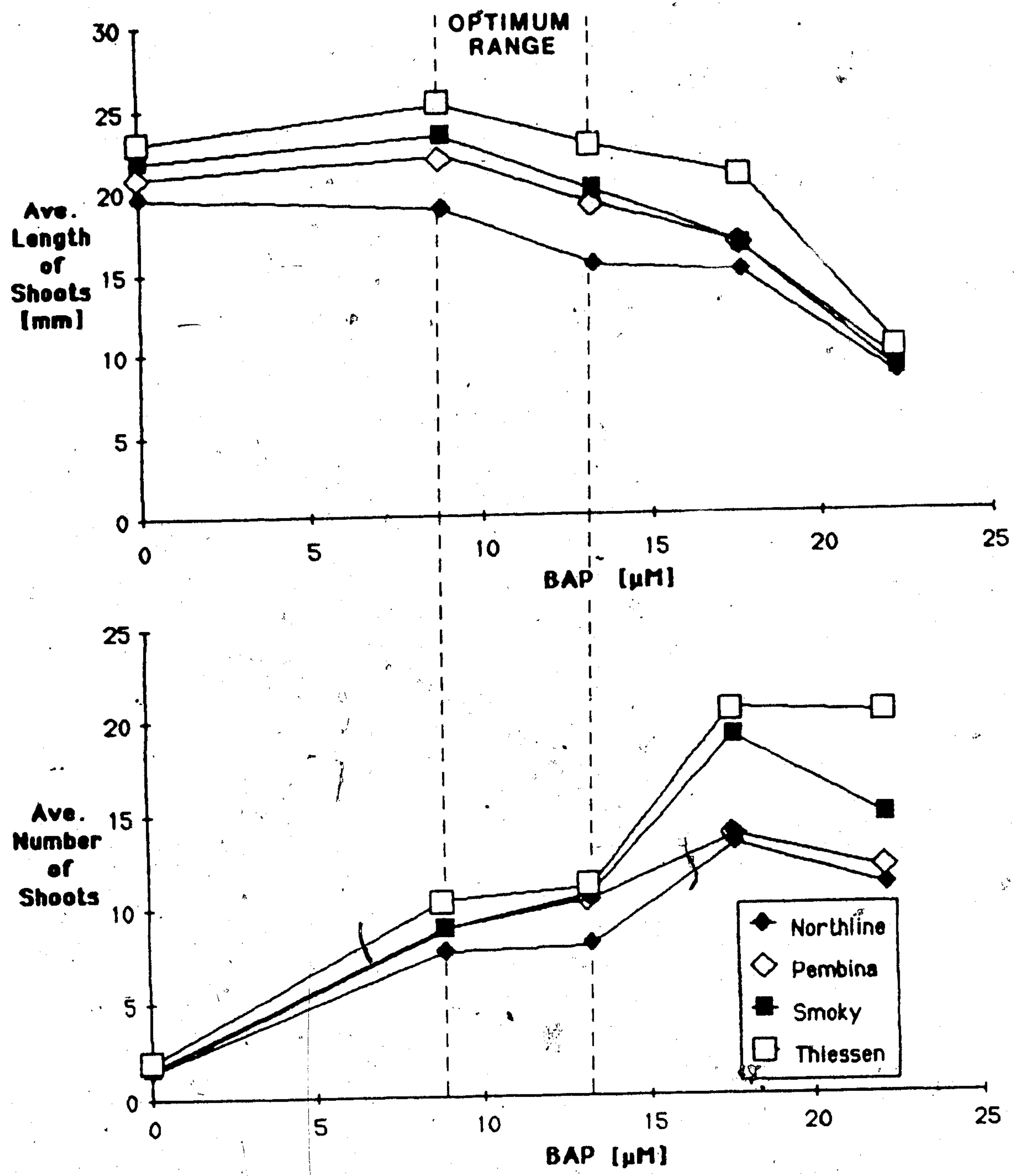


Figure 4. The influence of BAP concentration in the medium on the shoot length and number of shoots per culture of 4 cultivars of *Amelanchier alnifolia*.
Data based on 30 cultures per treatment (10 cultures per replicate). Values represent means from 3 replicates.

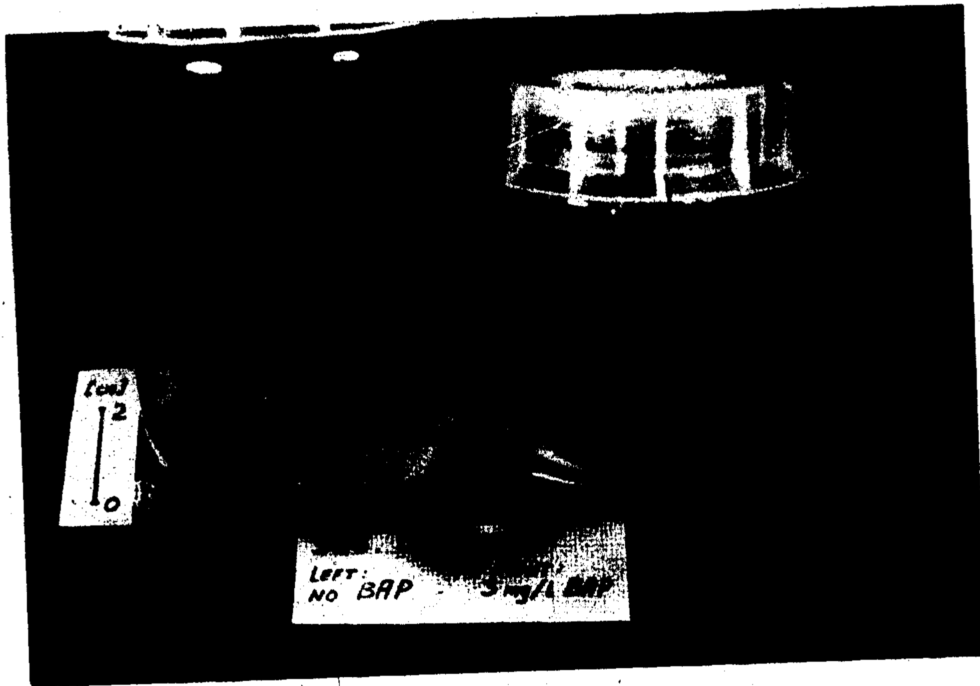


Plate 1. The influence of BAP concentration in the medium on multiplication of cultivar 'Thiessen' cultures after the 3 week incubation period.

In further work with multiplication and production of shoots for rooting, only the optimal BAP concentration was used. All shoots longer than 15mm were harvested every 3 weeks and used to start new cultures on fresh, stage II medium. It was observed that when culturing was continued for a period of 18-20 weeks, changes occurred in the basic material. Shoots were shorter and leaves were smaller and not well developed. These symptoms became more pronounced with each sub-culturing with shoots ultimately exhibiting characteristics similar to those obtained from cultures grown on media containing 22.20 μM of BAP. From the behaviour of these sub-cultures it appeared as though cytokinin (BAP) was accumulating in the tissues and when further sub-culturing was done on fresh media supplied with cytokinin there was a further BAP accumulation. When BAP was removed from the medium for one or two sub-culturings, it was found that shoot deformation could be eliminated. Such sub-cultures produced fewer but normal shoots. After a second sub-culturing on the BAP-free medium the number of shoots was even lower, but then, if the medium was again supplied with the optimal cytokinin concentration, shoot production from cultures returned to normal. For details see Appendix 2 (tables VI-VIIa).

C. Rooting Studies - Stage III

The rooting of *in vitro* produced shoots is a problem with some species. With *Vitis*, Gifford and Hewitt (1961) rooted only 2% of such shoots; Galzy (1972) was able to root 40% of *in vitro* produced shoots of *Vitis* but only succeeded in producing normal plantlets from 21% of these; Harris (1980) stated that he had occasionally rooted up to 100% of *in vitro* produced Saskatoon shoots, but more often the percentage of rooted plantlets in his experiments had been less than 10%.

One objective of this study was to find practical ways for rooting of *Amelanchier alnifolia*. In the beginning, *in vitro* rooting experiments were carried out with cv.'Smoky' only. Since rooting of shoots *in vitro* required more work and special lab equipment it seemed an impractical technique for the production of plants on a commercial scale. For this reason the later rooting studies with the 3 additional cultivars, were carried out *in vivo* on the greenhouse bench, under mist.

Rooting in Sterile Conditions - *in vitro*.

Preliminary experiments with cv.'Smoky' had shown that the best rooting occurred when 2.45 μM of IBA was included in the MS medium (at 1/3 strength) and in the absence of cytokinin - BAP (Table 4). Concentrations higher than 2.45 μM IBA inhibited rooting as did BAP in the medium. With increasing BAP levels the percentage of rooted plantlets decreased dramatically despite the presence of auxin. When BAP concentration was higher than 0.44 μM no rooting occurred (Table 4). It was observed that with increasing BAP concentration the amount of callus formed at basal portions of shoots also increased. All shoots exposed to any BAP concentration between 2.22 μM and 6.65 μM and to IBA at any concentration produced callus only. Similar observations were made by Lineberger (1983) with *Prunus* x 'Hally Jolivette'.

Detailed studies confirmed 2.45 μM of IBA in the medium to be the optimal for *in vitro* rooting of Saskatoon shoots (Appendix 3, table VIII), however, plantlets rooted in agar produced two or three 'rat-tail' type roots and were difficult to establish in soil.

Table 4. The influence of IBA and BAP concentration in the medium on percentage of *in vitro* rooted cv.'Smoky' shoots following 4 weeks incubation under standard conditions.

	% Rooting					
	IBA μM					
BAPμM	0.00	0.25	0.49	2.45	4.90	7.37
0.00	17.0	7.7	47.3	100.0	31.0	28.6
0.44	11.1	8.3	16.0	9.1	1.5	3.6
2.22	0.0	0.0	0.0	0.0	0.0	0.0
4.44	0.0	0.0	0.0	0.0	0.0	0.0
6.65	0.0	0.0	0.0	0.0	0.0	0.0

Twenty five shoots were used in each treatment.
Medium used: 1/3 strength of MS salts + 20g/l of sucrose.

The Effect of Light Quality on Rooting *in vitro*.

Several researchers have reported that morphogenic responses which are wavelength specific may be observed when plants are cultured *in vitro* (Kato, 1978; Katkade *et al.*, 1978). Murashige (1974) noted that light intensity also plays an important role in culturing plants *in vitro* and generally should be higher during the rooting stage than in the establishment and multiplication stages.

In this study, the light intensity used was low. The dark blue filter used in this experiment permitted a maximum irradiance of only 10 $\mu E \cdot m^{-2} \cdot s^{-1}$, so the other filters were adjusted to permit the same irradiance as the blue one.

During the rooting phase it was found that while it was important to have the highest percentage of rooted plantlets it was also important to have rooted plantlets with well developed, long roots. This was impossible to achieve under these low light conditions, however, data in Table 5 show that some differences occurred in rooting under different light qualities.

Table 5. The influence of light quality and level of irradiance on the rooting of cv.'Smoky'.

Irradiance	Type of Filter	% Rooted Shoots	Roots per Rooted Shoot	Length [mm] of Roots per Rooted Shoot
$10 \mu E \cdot m^{-2} \cdot s^{-1}$	W	28.0	1.57	9.1
	B	28.0	1.85	15.2
	Y	28.0	1.63	9.4
	R	44.0	1.90	19.2
$70 \mu E \cdot m^{-2} \cdot s^{-1}$	W	86.0	3.29	85.3

Data collected after the 4 week incubation period.
There were 25 shoots grown under each filter.

At 10 $\mu E \cdot m^{-2} \cdot s^{-1}$ the best rooting ocurred under red - R light, 660 nm (Table 5). The highest percentage of rooted shoots was achieved under these conditions and the roots produced were the longest as well. The percentage of rooted shoots under the three other filters was much lower than that for R. Root length and the number of roots per rooted shoot were also lower. However, the length of roots under the blue filter was higher than under either yellow or white (Table 5).

The irradiance of white light obviously plays a key role in rooting of shoots. When the irradiance under this filter was increased to 70 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$, the percentage of rooted plantlets, the length and the number of roots per rooted plantlet all increased dramatically. There was a 3-fold increase in the percentage of rooted shoots, a 2-fold increase in the number of roots and almost a 10-fold increase in length of roots per rooted plantlet (Table 5).

The rooting studies in which shoots were incubated for the first two weeks in the dark and then for another two weeks in light under standard conditions ($70\mu E{\cdot}m^{-2}{\cdot}s^{-1}$) did not give any positive results. No rooting was observed. All shoots produced large amounts of callus, which did not differentiate into roots.

Rooting in Non-Sterile Conditions - *in vivo*.

It was stated earlier that rooting of shoots *in vitro* requires more work and more equipment than rooting *in vivo*. In addition, when *in vitro* rooted plantlets are transferred to a conventional substrate, growth usually stops and does not continue until a new root system is developed. Despite the care taken, many *in vitro* rooted plantlets were lost during the transfer from agar to soil.

Rooting of *in vitro* derived shoots under non-sterile conditions is a valuable alternative for micropropagation. The rooting period in the greenhouse is spent on the mist bench hence plantlets do not need a period of acclimatization to greenhouse conditions.

In this study attempts were made to find optimal conditions for *in vivo* rooting of *in vitro* derived shoots. A 4 week rooting period was found adequate. Preliminary experiments with rooting of cv.'Smoky' had shown that the best results were achieved with small amounts (up to 2.45 μM) of IBA in the medium in the absence of BAP (Table 6). The presence of higher IBA concentrations suppressed rooting as did higher concentrations of BAP.

Table 6. The influence of IBA and BAP concentrations on the percentage of *in vivo* rooted shoots of cv.'Smoky' following a 4 week rooting period.

	% Rooting				
	IBA μM				
BAPμM	0.00	0.49	2.45	4.90	7.37
0.00	33.3	83.3	87.0	40.0	50.0
0.44	20.0	20.0	23.0	20.0	33.3
2.22	13.3	16.7	16.7	17.7	30.0
4.44	20.0	23.3	24.3	30.0	36.7

Thirty shoots were grown in each treatment.
Rockwool blocks were soaked with 1/3 strength of MS salts + IBA and BAP.

However, results in Table 6 show that the inhibitory effect of BAP on rooting was not as marked as it was when rooting was carried out *in vitro* (Table 4). Data in Table 4 had shown that no rooting occurred when the BAP concentration in the medium exceeded 0.44 µM, whereas data recorded with rooting *in vivo* (Table 6) showed that rooting occurred even at the highest concentrations of BAP. The percentage of rooted plantlets was lower than in treatments without BAP, but it was possible to root more than 36% of shoots in presence of 4.44 µM BAP and 7.37 µM IBA. The higher concentrations of IBA in the medium tended to counter the obvious inhibitory effect of BAP.

An experiment conducted later confirmed the 2.45 µM of IBA in the medium to be the optimal concentration for rooting of most *Amelanchier alnifolia* cultivars (Table 7). The use of commercial rooting powder 'Rootone F' also gave satisfactory results and the mean percentage of rooted plantlets did not differ significantly from that for 2.45 µM IBA. The use of the other commercial rooting powder 'Stimroot 2' did not give positive results in this experiment and the highest percentage of rooted shoots achieved with this treatment was 40% with cv.'Thiessen'.

When the optimum concentration of IBA (2.45 µM) was used, differences in percentages of rooted plantlets were observed between cultivars. Cultivars 'Smoky' and 'Pembina' gave the best rooting, 68.9% and 53.3% respectively. 'Thiessen', however, showed a marked decline and 'Northline', which was the most difficult to root, came close to achieving its best performance at the optimum IBA concentration. Surprisingly, the highest number of rooted plantlets of cv.'Thiessen' was observed in the control treatment (Table 7). For details see Appendix 3 (tables IX and IXa).

Table 7. The effect of treatment with root promoting chemicals on the percentage rooting of 4 cultivars of *Amelanchier alnifolia*.

	% Rooting				
	Cultivar				
Treatment	Smoky	Pembina	Thiessen	Northline	Means
0.00[μM]IBA	43.3	37.7	50.0	0.0	26.3c
0.49[μM]IBA	52.2	50.0	24.4	12.2	35.0b
2.45[μM]IBA	68.9	53.3	25.5	24.4	45.8a
4.90[μM]IBA	57.7	33.3	36.6	25.5	34.4b
Rootone F	60.0	52.2	21.1	26.6	44.7a
Stimroot 2	37.7	25.5	40.0	18.9	27.7c
Means	53.3 a	42.0 b	32.9 c	17.9 d	

Means not followed by the same letter are significantly different at the 5% level as determined by LSD.
Data based on 90 plantlets per treatment per cultivar (3 replicates with 30 shoots per replicate).
All treatments contained 1/3 MS salts.

Generally, cultivar 'Smoky' was easy to root under all treatments whereas cv.'Northline' was very difficult. With cv.'Northline' it was not unusual to obtain up 50% rooting in 4 weeks in some tests but little or no rooting when tests were repeated. Cultivar 'Smoky' on the other hand, responded consistently to treatments each time they were applied. Plate 2 illustrates an *in vivo* rooted plantlet of cv.'Smoky'.

A problem with the use of rockwool blocks was encountered in the course of this experiment. Algae built up on the surface of the blocks, as a result of using a nutrient solution. To avoid this problem, in later studies, shoots were rooted directly in soil mixture, in rooting containers (Hillson Trays). The surface of the media was covered with a thin layer of coarse sand and nutrients and mist were applied as in previous experiments. The percentage rooting was similar or sometimes even higher than that in rockwool blocks, however the quality of rooted plantlets was much improved.

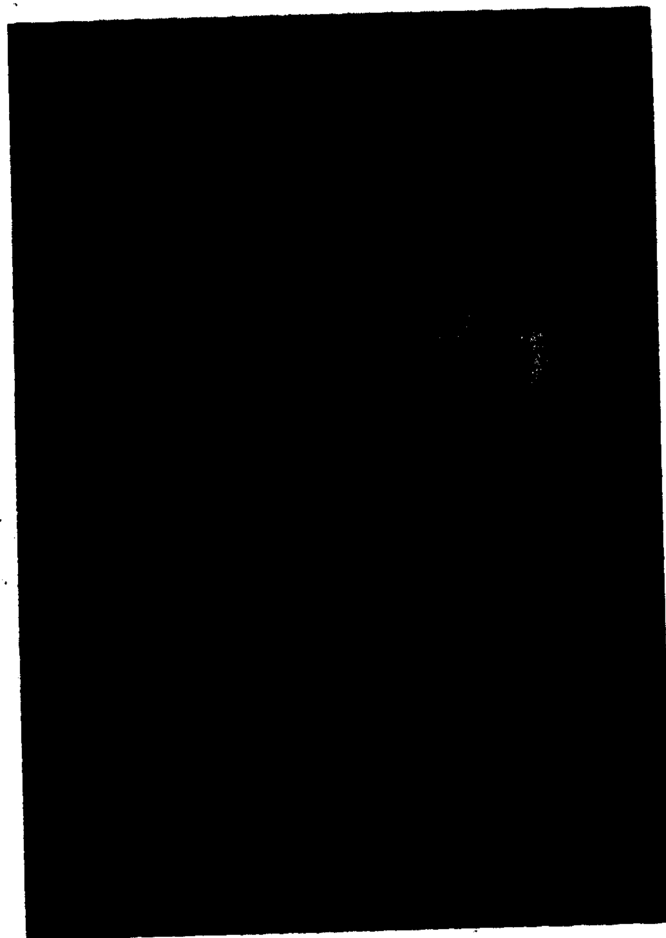


Plate 2. An *in vivo* rooted cv.'Smoky' plantlet after the 4 week rooting period on the greenhouse bench under mist. Vigorous roots grew through the rockwool block into perlite medium which filled the lower portion of each rooting compartment in the Hillson Tray.

D. Dormancy Studies

Summer dormancy of newly rooted plants is a major problem with *Amelanchier alnifolia*. Often, such plants enter summer dormancy even before rooting is completed. This study has confirmed the above observations. It has been found that about 80 - 90% of newly *in vivo* rooted plants were dormant by the time rooting was completed.

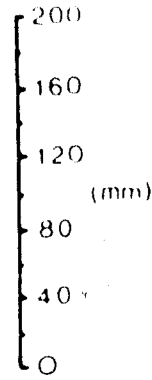
In experiments reported here, the effect of various growth regulator treatments on the breaking of dormancy of *in vivo* rooted *Amelanchier alnifolia* plantlets was examined. It was important to have vigorous, bushy plants with healthy foliage in order to survive the winter. Ideal plants would be those with more than one stem and with stems of good size. The number of subterranean buds formed on the basal portion of each plant was also important, since in the following year those buds would have the potential to grow into shoots.

Dormancy Studies with Newly Rooted Plants

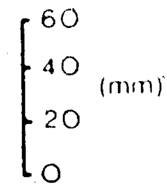
All plants treated with growth regulators broke dormancy and started active growth. Generally, plants from both cultivars, treated with GA_{4+7} overcame the dormancy condition within 48 hours after treatment, but grew only from terminal buds giving, in effect, single stemmed plants with elongated internodes and small leaves (Plate 3A).

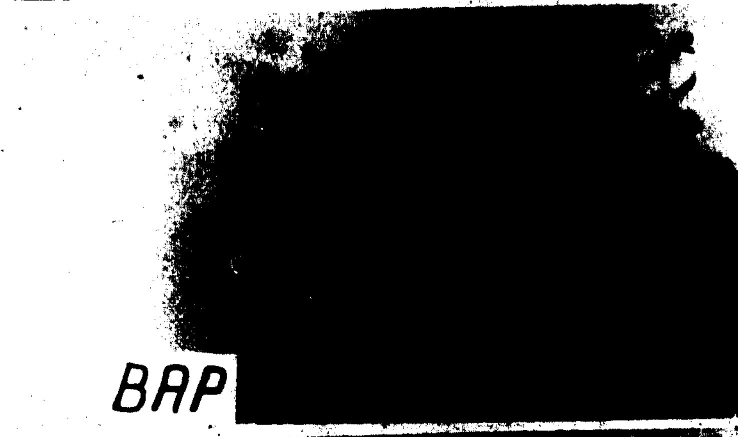
In plants treated only with BAP the formation of new buds on the basal portion of the stem was observed within one week after treatment. Plants then grew not only from terminal buds but also from new formed buds. In effect, compact bushy plants were obtained after 8 weeks (Plate 3B) when treated with BAP, though, growth from terminal buds was slightly stronger than that from the new formed buds.

Plants treated with BAP+GA_{4+7} behaved similarly to those treated with BAP and the formation of new buds on the basal portion of the stem was observed within one week after treatment. Following this, plants grew from all buds and the rapid elongation of all shoots was noticed. After the 8 week growing period all shoots were equally long but thin, with smaller leaves (Plate 3C).



A





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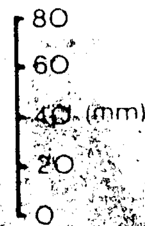

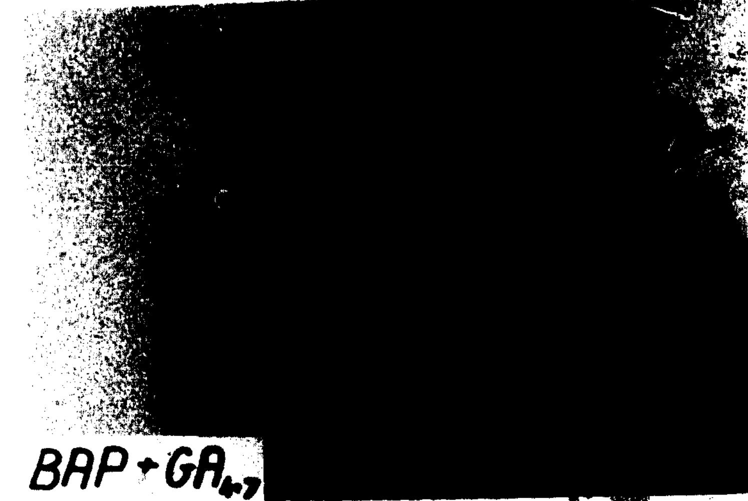

C

Plate 3. Effects of growth regulators on breaking dormancy of newly rooted cv.'Thiessen' plants; A) the effect of GA_{4+7}, B) the effect of BAP, C) the effect of BAP+GA_{4+7}. Pictures were taken 8 weeks after the growth regulator applications.

Data taken 8 weeks after growth regulator application are shown in Table 8. In both cultivars significant differences were observed between total shoot length, dry weight and number of stems of plants treated with GA_{4+7} and those treated with BAP.

Table 8. The influence of growth regulator treatments on growth of cultivars 'Smoky' and 'Thiessen'.

Cultivar	Treatment	Stems /Plant		Total Shoot Length [mm]		Number of Subterr. Buds		Dry Weight [g]	
Smoky	Control	0.83	c	13.5	e	0.33	c	0.020	c
	BAP	1.66	b	39.9	d	2.50	a	0.607	b
	GA_{4+7}	1.00	c	162.7	a	2.24	a	2.557	a
	BAP+GA_{4+7}	2.29	a	109.4	b	2.66	a	1.048	b
Thiessen	Control	1.00	c	25.0	d	0.83	c	0.162	c
	BAP	1.62	b	35.6	d	1.95	b	0.846	b
	GA_{4+7}	1.04	c	123.6	b	2.16	ab	2.289	a
	BAP+GA_{4+7}	1.58	b	62.9	c	3.04	a	1.189	b

Values not followed by the same letter are significantly different from each other at the 5% level as determined by LSD.
Data based on 24 cultures per treatment (3 replicates with 8 cultures per replicate).

As anticipated, GA_{4+7} caused a marked increase in shoot length of treated plants but did not increase the number of stems produced by those plants. Shoots of plants treated with GA_{4+7} were the longest compared to shoots of plants from other treatments (Table 8; Plate 3a). Both cultivars, 'Thiessen' and 'Smoky' responded in similar fashion, however, there was a significant difference in shoot length per plant. 'Smoky' appears to be more vigorous than 'Thiessen'.

The BAP treatment caused an increase in the number of stems per plant in both cultivars and in case of cv.'Smoky' produced a 3-fold increase in the total length of shoots

over the control. With cv.'Thiessen' the increase in total shoot length attributable to BAP was much less (Table 8). Comparing these data with results from the GA_{4+7} treatment, it is apparent that the effect of BAP on the length of shoots was much smaller than that of GA_{4+7}. Shoots of BAP treated plants were only 1/4 the length of those from GA_{4+7} treatment. As indicated by dry weights, plants treated with BAP showed much less growth than GA_{4+7} treated plants. On the other hand, shoots of BAP treated plants were short but of good quality and had larger leaves.

The most interesting results were obtained when both treatments were combined. The BAP+GA_{4+7} combination caused a marked increase in the number of stems and in the total shoot length per plant in both cultivars. Shoots from the combined treatment were shorter than those from the GA_{4+7} treatment but significantly longer than those treated with BAP alone (Table 8; Plate 3C). Differences between cultivars were also observed in the combined treatment. 'Smoky' continued to show a more vigourous response than cv.'Thiessen'. A marked increase in growth of plants treated with BAP+GA_{4+7} over those from the control treatment was also observed. However, as indicated by dry weight, growth of plants from the combined treatment was much less than that for plants treated with GA_{4+7} alone. It was also noted that the combined treatment showed only a small increase in dry weight of treated plants over those from the BAP treatment (Table 8).

All treatments with growth regulators caused an increase in the number of subterranean buds over those of the control treatment (Table 8). The number of subterranean buds in both cultivars was the highest in the combined BAP+GA_{4+7} treatment. The importance of subterranean buds was described earlier. For details see Appendix 4 (tables X - XIIIa).

Dormancy Studies with Young Established Plants

The effect of various treatmen[illegible]aking dormancy of lateral buds of single stemmed, 6-month old tissue-cultured pla[illegible] cv.'Smoky' is shown in Table 9.

Table 9. The influence of chemical treatment and of stem truncation on growth from lateral buds and on the initiation of subterranean buds on plants of cv.'Smoky'.

Shoot Length [mm]		Number of Lateral Bud Breaks		Number of Subterr. Buds	
Treatment		Treatment		Treatment	
T[1]+BAP+GA_{4+7}	289 a	T+BAP+GA_{4+7}	6.7 a	T+BAP	4.8 a
BAP+GA_{4+7}	273 a	BAP+GA_{4+7}	6.2 a	T+BAP+GA_{4+7}	4.4 ab
T+GA_{4+7}	204 ab	T+BAP	5.5 a	BAP	3.2 bc
GA_{4+7}	169 bc	BAP	4.8 a	BAP+GA_{4+7}	3.0 bc
T+BAP	74 cd	T	2.8 b	T	2.4 cd
BAP	63 d	T+GA_{4+7}	2.3 b	T+GA_{4+7}	2.0 cd
T	52 d	GA_{4+7}	1.2 b	GA_{4+7}	1.4 de
Control	6 d	Control	1.2 b	Control	0.6 de

Data based on 5 plants per treatment.
Values not followed by the same letter are significantly different at the 5% level as determined by LSD.
[1]T=Truncation

All treatments promoted lateral bud break. In all cases truncation was beneficial, supplementing the effect of applied growth regulators. The average shoot length from lateral buds, the number of bud breaks and the number of subterranean buds initiated, were higher in truncated plants treated with growth regulators than in plants treated with growth regulators only. Truncation itself, caused a 2-fold increase in the number of breaks, a 4-fold increase in the number of subterranean buds and an almost 10-fold increase in the length of shoots obtained from laterals of treated plants over those from the control.

In most cases apical dominance was destroyed by truncation and the effect of various growth regulators was enhanced. When GA_{4+7} was applied to truncated plants, however, the number of bud breaks increased because of truncation but in most cases growth was observed only from the most distal lateral bud. In other words the GA_{4+7} treatment quickly re-established apical dominance (Plate 4).

The BAP treatment caused a marked increase in the number of lateral bud breaks but shoots obtained from such laterals were short yet quite uniform in length. However, when GA_{4+7} was combined with BAP, shoot length was noticably enhanced (Plate 4). Subterranean bud initiation was highest in truncated plants treated with BAP. GA_{4+7} appeared to suppress initiation of subterranean buds but when it was combined with BAP this suppression was counteracted. For details see Appendix 4 (tables XIV - XVIa).

E. Field Performance of Tissue-Cultured Plants

Plants of the cultivar 'Smoky' that had been tissue-cultured and then acclimatized in the greenhouse were used in this study to examine field transplant survival. Growth of these plants was vigorous. Plants were either directly transplanted from the greenhouse to the field or were exposed to periods of outdoor acclimatization under 50% shade in the lath-house prior to field planting. It was found that plants kept under 50% shade for either 7 or 14 days before planting showed the least amount of transplant shock. Data taken 3 months after transplanting showed that plants which received the 7 or 14 day period of outdoor acclimatization had longer shoots than plants directly transplanted from the greenhouse to the field (Table 10). Significant differences in shoot length and the number of shoots were not detected between control plants and those exposed to acclimatization treatments, due to a high variability within replicates (Appendix 5; tables XVII-XVIIIa). On the other hand, outdoor acclimatization caused an increase in the number of shoots per plant compared to those which were transplanted directly from the greenhouse to the field (Table 10).

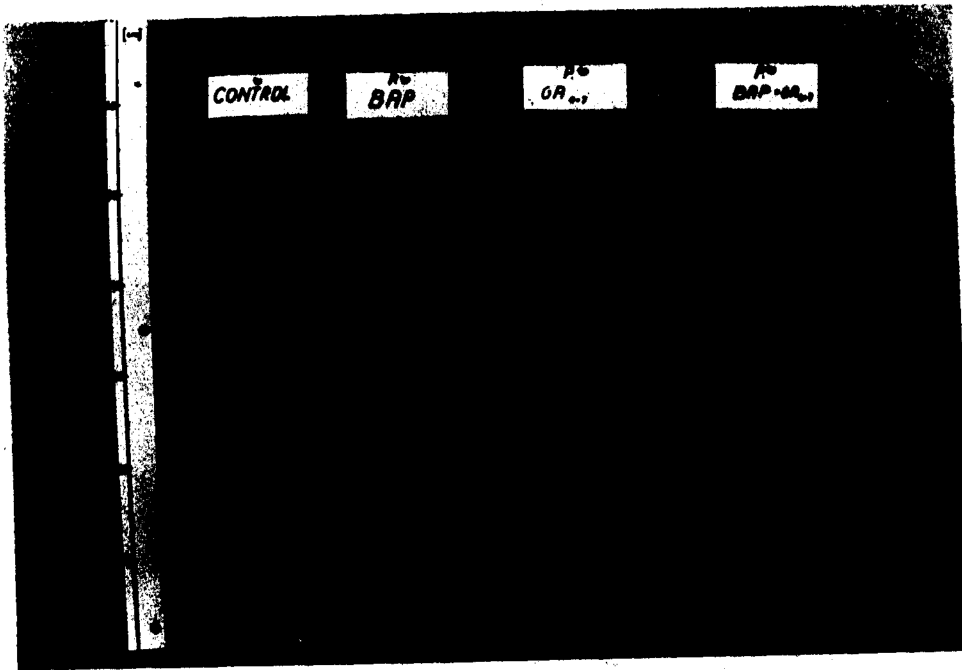

Plate 4. The effect of BAP and GA_{4+7} on truncated cv.'Smoky' plants.
P = Truncation

Plate 5. Tissue-cultured cv.'Smoky' plants one year after transplanting to the field.

Table 10. The effect of outdoor acclimatization periods on shoot growth and percentage survival of field transplants of *Amelanchier alnifolia* cv.'Smoky', 3 months after transplanting.

Acclimatization Period [days]	Shoot Length Initially [mm]	Shoot Length after 3mo [mm]	Number of Shoots Initially	Number of Shoots after 3mo.	% Survival
0	73.6	122.0	1.27	1.83	86.11
7	82.9	133.7	1.22	2.07	94.44
14	80.9	153.4	1.27	2.57	97.22

Data based on 36 plants per treatment (6 replicates with 6 plants per replicate).

The percentage survival of plants was also higher with these exposed to the outdoor acclimatization than with those which were not. The 14 day period in the lath-house before field planting, increased the percentage survival of plants from 86 to 97% (Table 10). Similar results for *Amelanchier laevis* were obtained by Struve and Lineberger (1985).

One additional study was conducted to examine how field transplants would survive winter. The survival data was taken in May the following year when it was noted that all had survived the winter and had begun active shoot growth. Plate 5 shows tissue-cultured cv.'Smoky' plants following a winter in the field.

This study has demonstrated excellent survival of acclimatized tissue-cultured *Amelanchier alnifolia* cv.'Smoky' plants. When transplanted to the field, survival levels of nearly 100% can be achieved with 2 week outdoor acclimatization under shade. The 2 week acclimatization in the lath-house prior to field planting is obviously a practical one, since it has been shown to give a 10% increase in transplant survival.

V. Summary and Conclusions

These studies have demonstrated the usefulness of tissue culture techniques in the propagation of the Saskatoon, *Amelanchier alnifolia* Nutt.

In the micropropagation of Saskatoon, shoot-tip explants were found to be the most satisfatory type to use for all cultivars tested for two reasons; they were fairly easy to surface sterilize, they were non-dormant and therefore responded readily to growing conditions. Dormant buds were usable but were less satisfactory because it was more difficult to obtain aseptic cultures with these explants and they did not respond quickly to growing conditions because of the dormancy factor. The active bud explant was found unsuitable for the micropropagation because of its tendency to produce masses of callus tissue rather than differentiated growth from primary meristems.

The cultivar 'Thiessen' was found to be the one that responded most favourably to establishment conditions out of the 4 cultivars tested. It consistently produced the greatest number of active cultures and in addition the longest shoots.

Of the 4 media tested for culture establishment, all cultivars showed a preference for MS and LS when shoot tips were used as the explants. With the exception of 'Thiessen', which showed no preference for media, cultivars were consistently less responsive to the B5 medium. The difference in these media lies in the concentration of nutrients and levels of sucrose which in MS and LS media are higher than those of B5 and Nc.

The response of dormant bud explants to media was similar to that of shoot tip explants. All cultures of all cultivars had a preference for MS medium. Growth of dormant bud cultures on B5 and Nc media was much slower, and on B5 medium especially some tendency to callus formation was observed. The MS and LS formulations were recommended for micropropagation of Saskatoon, since cultures obtained on these media from all types of explant were vigorous.

In the multiplication of shoots (stage II), the BAP concentrations of 8.88 and 13.30 μM in the medium were found optimal for all cultivars tested. When the medium was supplemented with these amounts of BAP the shoots produced were long, well formed and suitable for rooting. Higher BAP concentrations were not recommended since shoots obtained from cultures when BAP exceeded 13.30 μM, tended to become deformed. Deformation of shoots, associated with higher concentrations of BAP in the medium, was observed in all cultivars tested. Lineberger (1983) noted deformation of shoots of *Prunus* x 'Hally Jolivette' when the level of BAP in the medium was 22.2 μM. These shoots were stunted, indicating the deleterious effect of higher levels of cytokinin.

In multiplication studies, the cultivar 'Thiessen' once again demonstrated its cultural vigour. The greatest proliferation was observed with this cultivar and its cultures produced longer shoots than those of other cultivars.

The aging of cultures was noticable in the multiplication stage. Cultures started to degenerate after 18 - 20 weeks. Shoots became thinner and shorter, with poorly developed leaves. Subculturing on BAP-free medium was beneficial and shoot production from these cultures returned to normal after two subculturings (6 weeks). To assure that shoots produced during the multiplication stage were normal, new cultures from actively growing shoot tips or dormant buds had to be initiated semi-annually.

It was found that rooting of *in vitro* derived shoots of *Amelanchier alnifolia* in non-sterile conditions was possible and gave more satisfactory results than rooting *in vitro*. The hormonal treatment played a key role. An IBA concentration of 2.45 μM in MS medium, used at 1/3 strength was optimal for most cultivars. The commercial rooting powder 'Rootone F' also gave satisfactory results, a point worth considering when propagating plants on a commercial scale as rooting powders are easy to use.

Large differences in response to rooting were observed between cultivars. While an average of 69% rooting with cv.'Smoky' was relatively easy to achieve, cv.'Northline' gave only 26% rooting. The consistent response of cv.'Smoky' to applied tr[illegible] rooting

studies was not observed with cv.'Northline'. The cultural vigour of cv.'Thiessen' recorded in both the establishment and multiplication stages was not observed in the rooting stage. An average of only 50% rooting was achieved with this cultivar.

The rooting of *in vitro* derived Saskatoon shoots in non-sterile conditions permits the use of ordinary nursery methods and facilities, and therefore is more practical. It does not involve the complicated laboratory procedures of *in vitro* rooting. The big advantage of the *in vivo* rooting of *Amelanchier alnifolia* plants is that these plants do not require acclimatization to greenhouse conditions as do plants rooted *in vitro*. The danger of losing plants during the acclimatization period is reduced to a minimum when plants are rooted directly in the greenhouse. Survival of such plants rooted in non-sterile conditions was often 100%.

Rockwool blocks were suitable for rooting, but algae built-up on the rockwool surface. Later studies showed that *in vitro* derived shoots could be rooted directly in containers (Hillson Trays) filled with soil mixture without using rockwool. The algae problem was minimized by using this method and the quality of rooted plants improved. The percentage rooting was similar to that for plants rooted in rockwool.

The summer dormancy problem that occurs in newly rooted plants can be overcome by the use of the growth regulators BAP and GA_{4+7}. Both proved to be effective in breaking dormancy in all newly rooted plants. GA_{4+7} was quite obviously involved in mobilizing the plant nutrients to the terminal bud on intact shoots and to the most distal lateral on truncated shoots (Plate 3A). Smaller leaf size from the more proximal laterals also seemed to support this conclusion.

BAP was not only capable of breaking dormancy of lateral buds but it was also involved in the initiation of new buds on shoots in culture and on basal portions of stems in newly rooted plants. It can therefore be concluded that the ability of BAP to enhance adventitious bud formation on the explant in culture, can be extended to the whole plant. Shoots produced from BAP initiated buds are short but they produce leaves that are larger than normal. It has been observed (Horgan, 1984) that cytokinins, including BAP, can cause

an increase in size of a leaf by a process involving only cell enlargement. This could explain the larger than normal size of leaves in BAP treated plants (Plate 3B).

The two growth regulators BAP and GA_{4+7} appeared to act in an additive way in promoting growth from dormant plants (Plate 3C). When GA_{4+7} was applied to BAP treated plants the effect on plant form (bushiness) was greatly enhanced. The additive effects were also evident when truncated plants were treated with BAP+ GA_{4+7}. The effects were manifested by bud break and the initiation of subterranean buds.

The progressive improvement in field performance (shoot length and percentage survival) noted with increase in length of outdoor acclimatization period, was interesting, though not unexpected.

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VII. Appendix

A. Appendix 1

Establishing Aseptic Cultures - Stage I

Table I. Average shoot length per culture of *Amelanchier alnifolia* cultivars obtained from shoot tip explants after the 3 week incubation period under standard conditions.

		Length of Shoots			
		Medium			
Cultivar	Repl.	MS	LS	B5	Nc
Smoky	1	27.6	18.0	17.1	19.3
	2	34.9	29.9	14.0	19.4
	3	21.6	20.9	20.5	20.5
	Mean	28.03	22.93	17.20	19.73
Pembina	1	40.0	30.3	24.7	12.6
	2	30.3	32.6	13.3	12.7
	3	19.0	24.2	12.7	11.3
	Mean	[illegible]	29.03	16.90	12.80
Thiessen	1	36[illegible]	46.7	19.2	17.4
	2	32.1	36.7	24.8	15.3
	3	37.3	40.4	28.9	12.8
	Mean	35.47	41.27	24.30	15.17
Northline	1	28.5	31.7	16.0	19.2
	2	27.5	36.0	20.9	11.8
	3	32.5	34.0	17.8	11.8
	Mean	29.50	34.00	18.23	14.27

All measurements in [mm].
There were 10 cultures per replicate per treatment.

Table Ia.Anova for shoot length.

	DF	SS	MS	F	5%	1%
Replicates	2	48.40	24.20	1.04	3.32*	5.39
Cultivars	3	393.45	131.15	5.66**	2.92	4.51
Media	3	2415.01	805.00	34.76**	2.92	4.51
Med.x Cvs	9	434.38	48.26	2.08	2.21	3.06
Error	30	694.97	23.16			
Total	47	3986.21				

LSD = 4.01 - for both, cultivars and media

Table II. The effect of media on the number of active shoot tip cultures of *Amelanchier alnifolia* cutivars after the 3 week incubation period under standard conditions.

		No. of Active Cultures			
		Medium			
Cultivar	Repl.	MS	LS	B5	Nc
Smoky	1	10	9	8	10
	2	8	9	8	9
	3	10	9	9	9
	Mean	9.33	9.00	8.33	9.33
Pembina	1	10	10	9	10
	2	10	10	9	10
	3	10	10	9	9
	Mean	10.00	10.00	9.00	9.6[illegible]
Thiessen	1	10	10	10	10
	2	10	10	10	[illegible]
	3	10	10	10	10
	Mean	10.00	10.00	10.00	10.00
Northline	1	10	10	9	10
	2	10	10	9	10
	3	10	10	9	9
	Mean	10.00	10.00	9.00	9.66

There were 10 cultures per replicate per treatment.

Table IIa. Anova for number of active cultures.

	DF	SS	MS	F	5%	1%
Replicates	2	0.30	0.15	0.83	3.32	5.39
Cultivars	3	6.34	2.11	11.72**	2.92	4.51
Media	3	4.17	1.39	7.72**	2.92	4.51
Med.x Cvs	9	1.83	0.17	0.94	2.21	3.06
Error	30	5.33	0.18			
Total	47	17.67				

LSD = 0.35 - for both, cultivars and media

Table III. Average shoot length per culture of ***Amelanchier alnifolia*** cultivars obtained from dormant bud explants after the 3 week incubation period under standard conditions.

		Length of Shoots		
		Medium		
Cultivar	Repl.	MS	B5	Nc
Smoky	1	12.22	12.00	10.16
	2	12.43	11.40	10.71
	Mean	12.33	11.70	10.44
Pembina	1	11.25	8.00	8.57
	2	10.33	9.80	11.40
	Mean	10.79	8.90	9.99
Thiessen	1	15.14	10.85	9.83
	2	15.12	13.16	12.0[illegible]
	Mean	15.13	12.00	10.92
Northline	1	11.00	10.83	10.00
	2	11.12	10.42	10.77
	Mean	11.06	10.63	10.36

All measurements in [mm].
There were 10 cultures per replicate per treatment.

Table IIIa. Anova for shoot length.

	DF	SS	MS	F	5%	1%
Replicates	1	3.24	3.24	4.20	4.84	9.65
Cultivars	3	25.52	8.50	11.03**	3.59	6.22
Media	2	16.12	8.66	11.24**	3.98	7.20
Med.x Cvs	6	10.81	1.80	2.33	3.22	5.39
Error	11	8.56	0.77			
Total	23	64.25				

LSD = 1.12 - for cultivars
LSD = 0.97 - for media

Table IV. Number of active cultures of *Amelanchier alnifolia* cultivars obtained from dormant bud explants after the 3 week incubation period under standard conditions.

		No. of Active Cultures		
		Medium		
Cultivar	Repl.	MS	B5	Nc
Smoky	1	9	3	6
	2	7	5	7
	Mean	8.0	4.0	6.5
Pembina	1	8	3	7
	2	9	4	5
	Mean	8.5	3.5	6.0
Thiessen	1	7	7	6
	2	8	6	7
	Mean	7.5	6.5	6.5
Northline	1	10	6	8
	2	8	7	7
	Mean	9.0	6.5	7.5

There were 10 cultures per replicate per treatment.

Table IVa. Anova for the number of active cultures.

	DF	SS	MS	F	5%	1%
Replicates	1	0.83	0.83	0.76	4.84	9.65
Cultivars	3	10.34	3.44	3.15	3.59	6.22
Media	2	39.09	19.54	17.92**	3.98	5.07
Med.x Cvs	6	9.08	1.51	1.38	3.09	5.07
Error	11	12.00	1.09			
Total	23	71.34				

LSD = 2.29 - for interaction Media x Cultivars

Table V. Frequency of callus production rather than shoot production from 3 types of explant of *Amelanchier alnifolia* cv.'Smoky'.

Explant	Medium	No. of Callus Cultures Replicates 1	2	3	Mean
Active Bud	MS	5	4	4	4.33
	B5	6	6	4	5.33
	Nc	4	3	6	4.33
Dormant Bud	MS	1	2	1	1.33
	B5	3	5	4	4.00
	Nc	2	3	3	2.66
Shoot Tip	MS	0	1	0	0.33
	B5	1	2	2	1.66
	Nc	0	0	1	0.33

There were 10 cultures per replicate per treatment.

Table Va.Anova for callus growth.

	DF	SS	MS	F	5%	1%
Replicates	2	0.96	0.48	0.62	3.63	6.23
Explants	2	68.07	34.03	44.19**	3.63	6.23
Media	2	13.40	6.70	8.70**	3.63	6.23
Med.x Expl.	4	2.82	0.70	0.90	3.01	4.77
Error	16	12.37	0.77			
Total	26	97.62				

LSD = 0.88 - for both, explant type and media

B. Appendix 2

Multiplication of Shoots - Stage II

Table VI. The effect of BAP concentration in the MS medium on the average shoot length per culture of *Amelanchier alnifolia* cultivars after the 3 week incubation period under standard conditions.

		Shoot Length				
		Replicates				
Cultivar	BAP [μM]	1	2	3	Mean	SE[1]
Smoky	0.00	20.8	23.4	21.2	21.80	0.80
	8.88	21.0	25.7	23.1	23.27	1.36
	13.30	19.3	21.0	19.1	19.80	0.60
	17.70	15.8	16.6	16.8	16.40	0.30
	22.20	8.3	8.8	8.9	8.67	0.18
Pembina	0.00	20.1	21.5	20.9	20.83	0.40
	8.88	22.4	21.6	22.2	22.07	0.24
	13.30	19.4	18.4	19.1	18.97	0.29
	17.70	16.9	16.3	16.0	16.41	0.26
	22.20	9.6	9.6	8.9	9.37	0.23
Thiessen	0.00	22.7	23.8	22.4	22.97	0.42
	8.88	24.9	26.0	24.4	25.10	0.47
	13.30	24.5	21.1	22.2	22.60	1.00
	17.70	21.0	21.3	19.9	20.73	0.42
	22.20	10.7	10.0	9.5	10.07	0.34
Northline	0.00	20.1	19.7	19.1	19.63	0.29
	8.88	18.9	18.4	18.8	18.70	0.15
	13.30	15.9	14.0	15.8	15.23	0.64
	17.70	15.5	14.2	14.6	14.77	0.38
	22.20	7.9	8.5	8.9	8.43	0.29

All measurements in [mm]
There were 10 cultures per replicate per treatment.
[1]SE = Standard error

Table VIa: Anova for shoot length.

	DF	SS	MS	F	5%	1%
Replicates	2	1.64	0.82	0.94	3.25	5.21
Cultivars	3	184.68	61.56	16.20**	3.49	5.95
BAP	4	1318.41	329.60	86.74**	3.26	5.41
BAPxCvs	12	45.61	3.80	4.37**	2.02	2.69
Error	38	33.01	0.87			
Total	59	1583.35				

LSD = 0.69 - for cultivars
LSD = 0.77 - for BAP concentration
LSD = 1.54 - for interaction Cultivars x BAP

Table VII. The effect of BAP concentration in the MS medium on the average number of shoots per culture of *Amelanchier alnifolia* cultivars after the 3 week incubation period under standard conditions.

		No. of Shoots				
		Replicates				
Cultivar	BAP [μM]	1	2	3	Mean	SE[1]
Smoky	0.00	1.5	1.7	1.4	1.53	0.09
	8.88	11.3	7.1	8.4	8.93	1.24
	13.30	10.1	12.4	9.6	10.70	0.86
	17.70	15.9	19.5	21.5	18.97	1.64
	22.20	15.0	14.5	14.7	14.73	0.14
Pembina	0.00	1.4	1.6	1.5	1.50	0.08
	8.88	8.8	9.1	8.9	8.93	0.09
	13.30	9.5	10.6	11.2	10.43	0.49
	17.70	13.3	14.3	13.5	13.70	0.30
	22.20	12.7	11.6	11.1	11.80	0.50
Thiessen	0.00	1.7	2.1	1.9	1.90	0.10
	8.88	9.8	10.6	10.8	10.40	0.30
	13.30	10.6	11.2	11.3	11.03	0.18
	17.70	18.0	21.0	22.6	20.53	1.35
	22.20	20.9	20.1	19.7	20.23	0.28
Northline	0.00	1.6	1.3	1.4	1.43	0.09
	8.88	7.9	7.7	7.4	7.67	0.14
	13.30	8.2	7.5	8.4	8.03	0.27
	17.70	13.3	13.5	12.9	13.23	0.17
	22.20	11.5	10.5	10.9	10.97	0.29

There were 10 cultures per replicate per treatment.
[1]SE = Standard error

Table VIIa. Anova for number of shoots.

	DF	SS	MS	F	5%	1%
Replicates	2	1.05	0.53	0.43	3.25	5.21
Cultivars	3	179.82	59.94	11.53**	3.49	5.95
BAP	4	1680.84	420.21	84.04**	3.26	5.41
BAPxCvs	12	62.35	5.20	4.26**	2.21	2.69
Error	38	46.28	1.22			
Total	59	1970.34				

LSD = 0.82 - for cultivars
LSD = 0.91 - for BAP concentration
LSD = 1.82 - for interaction Cultivars x BAP

C. Appendix 3

Rooting Studies - Stage III

Rooting *in vitro*

Table VIII. Number of rooted plantlets of cv.'Smoky' grown on 1/3 strength MS salts with differing concentrations of IBA.

IBA [μM]	Replicates 1	2	3	Mean	%
0.00	2	3	3	2.66	26.6
0.49	5	4	7	5.33	53.3
2.45	7	10	9	8.66	86.6
4.90	6	4	3	4.33	43.3

There were 10 plants per replicate per treatment.

Rooting *in vivo*

Table IX. Number of *in vivo* rooted plants of *Amelanchier alnifolia* cultivars subjected to various treatments after the 4 week rooting period in the greenhouse.

		No. of Rooted Plants			
		Replicates			
Cultivar	Treatment	1	2	3	Mean
Smoky	0.00μM IBA	17	10	12	13.00
	0.49μM IBA	16	12	19	15.67
	2.45μM IBA	19	17	26	20.67
	4.90μM IBA	16	21	15	17.33
	Rootone F	19	14	21	1[illegible].00
	Stimroot 2	13	11	11	11.66
Pembina	0.00μM IBA	8	16	10	11.33
	0.49μM IBA	12	19	14	15.00
	2.45μM IBA	12	21	15	16.00
	4.90μM IBA	7	12	11	10.00
	Rootone F	17	16	14	15.67
	Stimroot 2	6	9	7	7.33
Thiessen	0.00μM IBA	6	9	7	7.33
	0.49μM IBA	9	7	7	7.67
	2.45μM IBA	13	8	12	11.00
	4.90μM IBA	4	8	7	6.33
	Rootone F	10	16	10	12.00
	Stimroot 2	7	8	10	8.33
Northline	0.00μM IBA	0	0	0	0.00
	0.49μM IBA	2	5	4	3.67
	2.45μM IBA	11	5	6	7.33
	4.90μM IBA	12	4	7	7.67
	Rootone F	5	9	10	8.00
	Stimroot 2	6	6	5	5.67

There were 30 plants per replicate per treatment.

Table IXa.Anova for the number of rooted plantlets.

	DF	SS	MS	F	5%	1%
Replicates	2	6.25	3.12	0.36	3.20	5.10
Cultivars	3	1156.26	385.42	44.92**	2.81	4.24
Treatments	5	362.46	72.49	8.44**	2.42	3.44
Treat.xCvs	15	200.16	13.34	1.55	1.91	2.50
Error	46	394.87	8.58			
Total	71	2120.00				

LSD = 1.97 - for cultivars
LSD = 2.41 - for treatments

D. Appendix 4

Dormancy Studies

Dormancy Studies with Newly Rooted Plants

Table X. Total length of shoots per plant of *Amelanchier alnifolia* cultivars subjected to various hormonal treatments.

		Shoot Length			
		Replicates			
Cultivar	Treatment	1	2	3	Mean
Smoky	Control	0.00	7.66	33.00	13.55
	BAP	36.37	36.87	46.25	39.83
	GA_{4+7}	162.12	170.50	155.62	162.74
	BAP+GA_{4+7}	103.75	93.00	131.62	109.45
Thiessen	Control	19.50	31.50	24.00	25.00
	BAP	24.75	41.50	40.75	35.66
	GA_{4+7}	104.37	146.87	119.75	123.66
	BAP+GA_{4+7}	53.12	63.62	72.12	62.95

All measurements in [mm]
There were 8 plants per replicate per treatment.

Table Xa. Anova for shoot length.

	DF	SS	MS	F	5%	1%
Replicates	2	952.22	476.11	3.38*	3.24	6.51
Cultivars	1	2176.96	2176.96	1.82	10.13	34.12
Treatment	3	55435.49	18448.50	15.46*	9.28	29.46
Treat.xCvs	3	3580.68	1193.56	8.47**	3.34	5.56
Error	14	1972.03	140.86			
Total	23	64027.38				

LSD = 12.73 - for replicates
LSD = 14.70 - for treatments
LSD = 20.79 - for interaction cultivars x treatments

Table XI. Number of shoots produced per plant of ***Amelanchier alnifolia*** cultivars subjected to various hormonal treatments.

		No. of Shoots			
		Replicates			
Cultivar	Treatment	1	2	3	Mean
Smoky	Control	0.00	1.00	1.50	0.83
	BAP	1.75	1.50	1.75	1.66
	GA_{4+7}	1.00	1.00	1.00	1.00
	BAP + GA_{4+7}	2.37	2.50	2.00	2.29
Thiessen	Control	1.00	1.00	1.00	1.00
	BAP	1.50	1.75	1.62	1.62
	GA_{4+7}	1.12	1.00	1.00	1.04
	BAP + GA_{4+7}	1.62	1.62	1.50	1.58

There were 8 plants per replicate per treatment.

Table XIa. Anova for the number of shoots.

	DF	SS	MS	F	5%	1%
Replicates	2	0.09	0.05	0.71	3.24	6.51
Cultivars	1	0.11	0.11	0.31	10.13	34.12
Treatment	3	4.34	1.45	4.14	9.28	29.46
Treat.x Cvs	3	1.06	0.35	5.00*	3.34	5.56
Error	14	0.93	0.07			
Total	23	6.53				

LSD = 0.46 - for interaction cultivars x treatments

Table XII. **The number of subterranean buds formed on plants of** ***Amelanchier alnifolia*** **cultivars subjected to various hormonal treatments.**

		No. of Subterranean Buds			
		Replicates			
Cultivar	Treatment	1	2	3	Mean
Smoky	Control	0.00	0.00	1.00	0.33
	BAP	2.00	2.50	3.00	2.50
	GA_{4+7}	1.87	3.00	1.67	2.24
	BAP + GA_{4+7}	2.87	2.00	3.12	2.66
Thiessen	Control	1.00	1.50	0.00	0.83
	BAP	2.12	1.87	1.87	1.95
	GA_{4+7}	1.62	2.25	2.62	2.16
	BAP + GA_{4+7}	3.37	2.37	3.37	3.04

There were 8 plants per replicate per treatment.

Table XIIa. Anova for the number of subterranean buds.

	DF	SS	MS	F	5%	1%
Replicates	2	0.27	0.13	0.38	3.24	6.51
Cultivars	1	0.03	0.03	0.08	4.60	8.86
Treatment	3	16.91	5.63	16.55**	3.34	5.56
Treat.x Cvs	3	1.02	0.34	1.00	3.34	5.56
Error	14	4.82	0.34			
Total	23	23.05				

LSD = 0.72 - for treatments

Table XIII. Dry weight of plants of *Amelanchier alnifolia* cultivars subjected to various hormonal treatments.

Cultivar	Treatment	Total Dry Weight of Plants — Replicates 1	2	3	Mean
Smoky	Control	0.000	0.000	0.060	0.020
	BAP	0.634	0.684	0.504	0.607
	$GA_{4,7}$	1.872	3.422	2.378	2.557
	BAP + $GA_{4,7}$	0.966	0.962	1.216	1.048
Thiessen	Control	0.038	0.258	0.189	0.162
	BAP	0.944	0.514	1.080	0.846
	$GA_{4,7}$	1.996	2.312	2.558	2.289
	BAP + $GA_{4,7}$	0.824	0.980	1.762	1.189

All measurements in [g].
There were 8 plants per replicate per treatment.

Table XIIIa. Anova for dry weight.

	DF	SS	MS	F	5%	1%
Replicates	2	0.415	0.208	1.61	3.24	6.51
Cultivars	1	0.024	0.024	0.19	4.60	8.86
Treatment	3	17.448	5.816	45.09**	3.34	5.56
Treat.xCvs	3	0.190	0.063	0.49	3.34	5.56
Error	14	1.800	0.129			
Total	23	19.877				

LSD = 0.455 - for treatments

Dormancy Studies with Young Established Plants

Table XIV. Total length of shoots from lateral buds of *Amelanchier alnifolia* cv.'Smoky' plants subjected to truncation and various hormonal treatments.

Treatment	Total Length of Shoots Repeats 1	2	3	4	5	Mean
Control	0	0	0	20	9	5.8
BAP	85	57	28	68	79	63.4
GA_{4+7}	360	375	0	0	110	169.0
BAP + GA_{4+7}	230	293	229	351	262	273.0
T[1]	72	60	28	18	81	51.8
T + BAP	111	122	74	69	94	74.0
T + GA_{4+7}	165	270	157	172	255	203.8
T + BAP + GA_{4+7}	313	233	293	218	389	289.2

All measurements in [mm].
Data based on 5 plants per treatment.
[1]T = Truncation

Table XIVa. Anova for total length of shoots from laterals.

	DF	SS	MS	F	5%	1%
Between tr.	7	392605	56086	9.55**	2.32	3.25
Within tr. (Error)	32	187862	5870			
Total	39	580467				

LSD = 97

Table XV. Number of lateral bud breaks from truncated and non-truncated plant of *Amelanchier alnifolia* cv.'Smoky' when subjected to various hormonal treatments.

	No. of Bud Breaks					
	Repeats					
Treatment	1	2	3	4	5	Mean
Control	0	0	0	4	2	1.20
BAP	8	5	2	4	3	4.83
GA_{4+7}	1	1	2	1	1	1.20
BAP+GA_{4+7}	8	8	5	8	8	6.16
T[1]	3	3	5	3	2	2.83
T+BAP	8	8	4	6	7	5.50
T+GA_{4+7}	3	2	2	3	4	2.33
T+BAP+GA_{4+7}	9	10	7	6	8	6.66

Data based on 5 plants per treatment.
[1]T = Truncation

Table XVa. Anova for number of lateral bud breaks.

	DF	SS	MS	F	5%	1%
Between tr.	7	316.8	45.25	19.93 **	2.32	3.25
Within tr. (Error)	32	72.8	2.27			
Total	39	389.6				

LSD = 1.9

Table XVI. Number of subterranean buds from truncated and non-truncated *Amelanchier alnifolia* cv.'Smoky' plants subjected to various hormonal treatments.

	No. of Subterranean Buds					
	Repeats					
Treatment	1	2	3	4	5	Mean
Control	1	1	0	0	1	0.60
BAP	2	4	4	3	3	3.20
GA_{4+7}	1	1	2	1	2	1.40
BAP+GA_{4+7}	2	3	3	3	4	3.00
T[1]	3	4	3	0	2	2.40
T+BAP	5	6	4	4	5	4.80
T+GA_{4+7}	2	2	1	2	3	2.00
T+BAP+GA_{4+7}	4	5	4	5	4	4.40

Data based on 5 plants per treatment.
[1]T = Truncation

Table XVIa. Anova for number of subterranean buds.

	DF	SS	MS	F		5%	1%
Between tr.	7	61.22	8.57	7.35	**	2.32	3.25
Within tr. (Error)	32	38.18	1.19				
Total	39	99.40					

LSD = 1.4

E. Appendix 5

Field Performance of Tissue-Cultured Plants

Table XVII. The effect of length of outdoor acclimatization period on the average shoot length of *Amelanchier alnifolia* cv.'Smoky' plants.

	Ave. Shoot Length						
	Replicates						
Days outdoors	1	2	3	4	5	6	Mean
0	103.00	118.00	118.33	126.00	123.75	143.33	122.06
7	208.00	158.33	166.67	75.20	90.67	103.33	133.70
14	139.17	180.00	116.67	168.33	158.00	158.00	153.36

All measurements in [mm].
There were 6 plants per replicate per treatment.

Table XVIIa. Anova for shoot length.

	DF	SS	MS	F	5%	1%
Replicates	5	2301.18	460.23	0.32	3.33	5.64
Treatment	2	3002.30	1501.15	1.04	4.10	7.56
Error	10	14497.97	1449.79			
Total	17	19801.45				

No significant difference between treatments.

Table XVIII. The effect of length of outdoor acclimatization period on the average number of shoots of ***Amelanchier alnifolia*** cv.'Smoky' plants.

	No. of Shoots						
	Replicates						
Days outdoors	1	2	3	4	5	6	Mean
0	1.80	2.20	2.00	1.33	1.20	2.50	1.83
7	3.20	2.00	2.00	2.33	1.60	1.33	2.07
14	1.83	2.33	2.50	2.80	3.67	2.33	2.57

There were 6 plants per replicate per treatment.

Table XVIIIa. Anova for number of shoots.

	DF	SS	MS	F	5%	1%
Replicates	5	0.03	0.006	0.01	3.33	5.64
Treatments	2	1.66	0.83	1.56	4.10	7.56
Error	10	5.29	0.53			
Total	17	6.98				

No significant difference between treatments.

F. Appendix 6

Table XIX.Code for salts and vitamin mixtures.

	Gamborg [B5]	Linsmaier & Skoog [LS]	Murashige & Skoog [MS]	Nitsch [Nc]
Macroelements [mg/l]				
$(NH_4)_2SO_4$	134	x[1]	x	x
NH_4NO_3	x	1650	1650	720
KNO_3	2500	1900	1900	950
$CaCl_2 \cdot 2H_2O$	150	440	440	166
$MgSO_4 \cdot 7H_2O$	250	370	370	185
KH_2PO_4	x	170	170	68
$NaH_2PO_4 \cdot H_2O$	150	x	x	x
Na_2EDTA	33	37.3	37.3	37.3
$FeSO_4 \cdot 7H_2O$	25	27.8	27.8	27.8
Microelements [mg/l]				
$MnSO_4 \cdot H_2O$	10	x	x	x
$MnSO_4 \cdot 4H_2O$	x	22.3	22.3	25
$ZnSO_4 \cdot 7H_2O$	3.0	8.6	8.6	10
H_3BO_3	3.0	6.2	6.2	10
KI	3.0	0.83	0.83	x
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.25	0.025	0.025	0.025
$CoCl_2 \cdot 6H_2O$	0.25	0.025	0.025	x
Vitamins [mg/l]				
Folic acid	x	x	x	0.5
Thiamine HCl	10.0	0.4	0.1	0.5
Nicotinamide	1.0	x	0.5	5.0
Pyridoxine HCl	1.0	x	0.5	0.5
Others [mg/l]				
myo-Inositol	100	100	100	100
Glycine	x	x	2.0	2.0
Sucrose	20,000	30.000	30,000	20,000
pH	5.5	5.8	5.7	5.5

[1]x = not added