STUDIES ON PROPAGATION OF PLUM (*Prunus* spp.) BY CUTTINGS AND TISSUE CULTURE

[redacted]

THESIS

By

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Submitted to Dr. Y. S. Parmar University of Horticulture and Forestry
in partial fulfilment of the requirements for the
Degree of

DOCTOR OF PHILOSOPHY

in

HORTICULTURE
(POMOLOGY AND FRUIT TECHNOLOGY)



40581

DEPARTMENT OF POMOLOGY AND FRUIT TECHNOLOGY
COLLEGE OF HORTICULTURE
SOLAN (H.P.)

1988

Affectionately

Dedicated

to my

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C E R T I F I C A T E - I

This is to certify that the thesis entitled "Studies on propagation of plum (Prunus spp.) by cuttings and tissue culture" submitted in partial fulfilment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY in HORTICULTURE (POMOLOGY AND FRUIT TECHNOLOGY) of Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan is a faithful record of the bonafide research work carried out by Shri N.Benjongliba Aier under my guidance and supervision and no part of this thesis has been submitted for any other degree or diploma.

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

Dated, Solan
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CERTIFICATE II

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ACKNOWLEDGEMENTS

It is my proud privilege to express my deep sense of gratitude and sincere thanks to Dr. Som Dutt Sharma, Chief Scientist, Horticultural Regional Research Station, Sharbo (District Kinnaur) of the Dr Yashwant Singh Parmar University of Horticulture and Forestry and Chairman of my Advisory Committee for his worthy guidance, keen interest, untiring help, constructive criticism and constant encouragement throughout the course of these investigations and during the preparation of this manuscript.

I owe my profound thanks to Dr.S.A.Ananda, Horticulturist, Dr.R.C.Sharma, Assistant Plant Pathologist, Dr.S.K.Sharma, Assistant Professor of Plant Physiology and Dr.P.S.Thakur, Assistant Scientist, Plant Physiology, who as members of my Advisory Committee had always been giving me generous help, valuable suggestions and guidance during the course of these investigations and critical review of this manuscript.

I am thankful to Dr.R.P.Awasthi, Professor and Head, Department of Fruit Culture and Orchard Management, College of Horticulture, Solan for his encouragement and providing the necessary facilities.

My sincere thanks are due to Dr.J.M.Singh, Professor of Horticulture, Dr.V.P.Bhutani, Professor of Horticulture, Dr.S.K.Chopra, Head, Department of Post Harvest Technology, Shri R.P.Singh and Dr.H.S.Rana for their generous help and timely encouragement.

I wish to express my special thanks and gratitude to Dr.O.P.Sehgal, Head, Department of Floriculture and Landscaping and Dr.D.R.Sharma, Head, Department of Biotechnology for their constant help and providing me the laboratory facilities for conducting the present course of investigations in tissue culture studies and for their creative and valuable suggestions given from time to time.

I am extremely grateful to Dr.S.P.Dhall, Associate Professor of Statistics and Shri Rajeev Raina for helping me in statistical analysis of data.

I am thankful to the Department of Agriculture, Government of Nagaland, for sending me on deputation for my Ph.D. programme and I am highly grateful to the North East Council for awarding me NEC Fellowship for this study.

I also extend my heartfelt thanks to Shri Kanshi Ram for his assistance in laboratory and field work.

I avail this opportunity to thank my friends Bist, Joginder, Belie, Ben Yanthan, Bongti, Sashi, Rakesh, Dubey, Kamal, Suresh and all others for their timely encouragement and sincere help.

I am also thankful to Shri Hansraj Abrol and Shri Ramesh Chand Sharma for their painstaking efforts in typing this manuscript at the cost of their personal convenience.

Last but not the least, I will be failing in my duty if I do not put on record my deep sense of gratitude to my respectable parents and family members, whose constant encouragement, love and affection was a source of inspiration for me to continue my studies.

(N.Benjongliba Aier)

Dated: Solan
30th September, 1988.

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C H A P T E R - I

I N T R O D U C T I O N

INTRODUCTION

The commercialization of fruits in Himachal Pradesh has changed the economy of the people. The sub-temperate zone of the state (1200-1500 metre a.m.s.l) is ideally suited for the cultivation of stone fruits. Under mid-hill conditions, because of the unsuitability of the agro-climatic conditions for apple cultivation, stone fruits, particularly plum, have a tremendous scope. This can be visualized from the fact that the area under stone fruits increased from 6872 ha in 1970-71 to 24,940 ha during 1985-86 (Azad and Sharma, 1987) and plum occupies about 50 per cent of this area.

In India, most of the commercial cultivars of plum are generally grafted or budded on seedling rootstocks. The plants thus raised are not uniform and in some cases external symptoms of delayed graft incompatibility are also found. Thus, the use of cuttings for clonal propagation offers immense opportunities for raising uniform materials for commercial plantations as well as for experimental studies.

Propagation of plum species through stem cuttings is the most convenient, simple, easy, rapid and less expensive method as compared to other conventional methods of propagation. At the same time, the problem of

incompatibility due to poor graft union does not arise in this method.

The plum species have shown wide variations in their rooting ability. Most of the Japanese plum (Prunus salicina Lindl.) cultivars can be easily propagated by cuttings whereas European plum (P.domestica L.) is difficult to propagate through cuttings even if factors like nutrition of the stock plants, time of taking cuttings, flowering conditions of the plant and growth regulator treatments are taken care of (Bartolini and Roselli, 1979). The difficulty to root in European plum by stem cuttings is due to the synthesis of an inhibitory substance by its foliage which prevents its rooting even under ideal conditions although, latent factors responsible for rooting are present in it.

The rooting response of plum species is also influenced by seasonal changes which not only modify the cambial activity but also change the morpho-physiological status of the trees which in turn raises the levels of endogenous hormones and nutrients in favour of rooting (Nanda and Kochhar, 1985).

The propagational potentials of difficult-to-root plum cultivars can be improved by treating the cuttings with suitable growth promoting hormones like Indole butyric acid (Chauhan and Reddy, 1974 and Hartmann and Kester, 1983).

The regeneration of difficult-to-root species of plum through semi-hardwood cuttings can also be improved by planting them in a mist chamber where their leaves can be kept in a turgid condition under intermittent mist until the roots are produced (Hartmann and Kester, 1983).

Recently, in addition to propagation through cuttings, some advances have been made in multiplication of difficult-to-root and not-to-root at all plant species through a new technique of plant regeneration in vitro under aseptic conditions known as micropropagation or tissue culture. This method has been extensively used to ensure a rapid multiplication of genetically uniform and true-to-type plant materials. Such material is in great demand not only for research workers but also for the high density plantations of stone fruit plants, a concept which has gained favour all over the world (Pannell, 1982). However, in India, this technique is still at an initial stage and only a limited work has been done so far in propagating planting materials by this method.

Keeping in view the problems enumerated above and considering the advantages of propagating plum species through stem cuttings and tissue culture over the conventional methods of propagation, the present studies

were carried out to achieve the following objectives:-

1. To find out the best season of planting under different planting conditions and optimum IBA concentrations in four plum cultivars, two each from Japanese plum (Prunus salicina Lindl.) and European plum (P.domestica L.) for successful rooting of cuttings and their establishment in the field for raising clonal rootstocks.

2. To study the effect of preconditioning and wounding treatments, size of the cuttings and optimum concentrations of IBA on rooting behaviour of Santa Rosa plum during dormant season.

3. To study the endogenous biochemical and physiological factors in relation to rooting of stem cuttings in plum species.

4. To standardize a tissue culture technique for rapid multiplication of plant materials in plum species.

C H A P T E R - II

R E V I E W O F L I T E R A T U R E

REVIEW OF LITERATURE

Although the propagation of fruit plants through cuttings and tissue culture has been the subject of considerable research in different countries of the world, yet this subject received attention in India only recently. As a result, the information on propagation through stem cuttings and tissue culture of different fruit species in general and of plum species in particular are quite limited. It was, therefore, necessary to bring together the relevant literature published in various parts of the world to evaluate and placed them in proper prospective. The findings of various research workers on the propagation of plum species by means of stem cuttings and tissue culture are reviewed under the following headings:

1. Propagation through stem cuttings

1.1. Synthetic growth substances in relation to rooting

Zimmerman and Wilcoxon (1935) were the first to use chemicals that induce rooting. Since then, the synthetic auxins like indole-3-acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) and indole propionic acid (IPA) have been widely used for inducing the rooting of stem cuttings of many plant species (Sen and Bose, 1966 and Farmer, 1966).

However, many workers (Singh, 1956; Dikshit, 1956; Pope, 1960; Sircar, 1971 and Worrall, 1976) have reported that IBA was found to be more effective in inducing rooting than other synthetic growth regulators due to its non-toxicity over a wide range of concentrations and to its wide adaptability.

According to Nanda (1975), auxins initiate cell division and elongation in the meristematic tissues, differentiation of cambial initials into primordia and in the mobilization of reserve food materials by enhancing the activity of hydrolyzing enzymes.

The effectiveness of 500 ppm alcoholic solution of IBA in Myrobalan B was established by Hatcher and Garner (1947, 1950). Dikshit (1957) succeeded in multiplying difficult-to-root plum cultivars by stem cuttings when treated with 30 ppm IBA. Nigam (1966) conducted a trial on rooting of four plum varieties and observed that the basal treatment with hormone was necessary for the rooting of stem cuttings. Konarli (1968) found that propagation of Can and Myrobalan B plum varieties using hardwood cuttings resulted in 30.6 per cent and 18.6 per cent rooting, respectively whereas their leafy cuttings when treated with IBA at 3000 ppm and planted under mist resulted in 66.1 and 57.5 per cent rooting, respectively.

Howard and Nahlawi (1968) observed that application of 5000 ppm IBA enhanced the rooting percentage in hardwood cuttings of Myrobalan B, St.Julien A and some other clonal

rootstocks of plum. Fontanazza (1969) obtained better rooting with 79.9 per cent in the hardwood cuttings of plum cv. Methley on treating with 3000 ppm IBA. Kapetanovic et al. (1972), working on plum cultivars Banjalucka and Ruzica found that IBA at 1000 ppm was suitable for softwood cuttings and its 2000 ppm was optimum for hardwood cuttings.

Nicotra (1971) in his trials on rooting of hardwood cuttings of St.Julien A found that the optimum IBA concentration for one year old cuttings was 2000 ppm whereas its higher concentrations depressed rooting. Nahlawi and Howard (1973) found that hardwood cuttings of Myrobalan B, St.Julien A, Brompton and EA 16 plum rootstocks responded better to a single pretreatment with 5000 ppm IBA than the repeated applications at various concentrations. Cappellini and Nicotra (1973) reported better rooting when the hardwood cuttings of Myrobalan B were treated with different IBA concentrations. Breen and Muraoka (1973) observed enhanced rooting in softwood cuttings of 'Marianna 2624' plum when treated with 4000 ppm IBA. Chauhan and Reddy (1974) reported that the best treatment for rooting in hardwood cuttings of Santa Rosa plum was their treatment with IBA at 1000 ppm, which produced 25.77 per cent rooting against no rooting under control treatment.

Kapetanovic and Hanic (1975) treated hardwood cuttings of three plum cultivars and Myrobalan in November and February with different concentrations of IBA varying between 500 to 3000 ppm and found that IBA at 2500 ppm in February

was optimum for rooting of plum cultivars Ruzica, Statikulja and Myrobalan whereas IBA concentration at 1500 ppm was the best for plum cultivar Banjalucka. Ivanicka and Pastyrik (1978) observed increased rooting following post-dormancy application of 2500 ppm IBA in hardwood cuttings of some plum rootstocks.

Nicotra and Moser (1978) obtained better rootings in different plum cuttings when treated with IBA at 2000 ppm. Jawanda et al. (1980) observed best rooting from semi-hardwood cuttings of plum cultivars Kataro Chak and Lalri when treated with IBA at 100 ppm producing 80.0 and 63.0 per cent rooting, respectively. Rathore (1983) reported that planting of Santa Rosa plum semi-hardwood cuttings in soil:sand (1:4) mixture after their treatment with 6000 ppm IBA for one minute resulted in 100.0 per cent rooting followed by 87.0 per cent rooting with IBA at 3000 ppm.

Hitchcock and Zimmerman (1932, 1936, 1939) observed that optimum concentration of growth regulator for optimum rooting of cuttings was influenced by the form and kind of regulators, the kind of carriers, the species of the plants, the age and relative activity of the shoots from where the cutting materials were obtained, the season of taking cuttings, treatment administered and the method of application of growth regulators to the cuttings. Several reviews on the effectiveness of different synthetic growth substances on the induction of rooting have also been published by several workers (Audus, 1959; Hartmann and Kester, 1983 and Nanda

and Kochhar, 1985).

1.2. Seasonal and varietal variations in relation to rooting

Season or time of the year when cuttings are taken and planted has a dramatic influence on their rooting. In deciduous species, hardwood cuttings root better in dormant season while leafy semi-hardwood cuttings root better during the growing season (Brainerd and Evert, 1980). However, the best season for rooting, even in easy-to-root plants varies considerably (Sandved, 1964; Morishita, 1964 and Hansen and Hartmann, 1966).

The increased biosynthesis of auxins in the active season and decreased levels along with the inhibitory substances and the morpho-physiological conditions of the mother plants determine the season for their rooting (Hartmann and Loreti, 1965; Fadl and Hartmann, 1967 and Nanda, 1975). Nanda and Kochhar (1985) reported that spring and rainy seasons are the most suitable seasons for the regeneration of plants from cuttings. They further observed that cuttings taken during the season of active growth root better than those taken at any other time of the year.

Briscoe (1963), Deolle and Mitchell (1964) and Hartmann and Kester (1983) also reported that the best results were obtained if the cuttings were taken in the spring as soon as the leaves are fully expanded and the shoots have attained some degree of maturity. Hatcher and Garner (1950) reported that the hardwood cuttings of Myrobalan B plum rooted

satisfactorily when planted from mid-October to early February. Hartmann and Hansen (1958) found best rooting in the hardwood cuttings of Marianna plum when the cuttings were collected in mid-November. Doran and Bailey (1957) reported that softwood cuttings of beach plum (Prunus maritima) rooted better when planted between June 15 to July 15, whereas Paunovic (1966) obtained the best rooting in September planted cuttings from four European plum varieties and four selections of Myrobalan plum rootstocks.

Hutchinson (1968) reported successful rooting in hardwood cuttings of the clonal rootstocks, Myrobalan B, Brompton, St.Julien A, Ackermann and Marianna when their cuttings were planted in the early autumn by treating with 0.05 per cent IBA or 0.3 per cent commercial IBA powder. Chauhan and Reddy (1974) reported that the hardwood cuttings of Santa Rosa plum planted during December proved to be superior for rooting and shoot growth over those planted in November. Gnezdilov (1982) in trials with plum and cherry plum clonal rootstock cuttings obtained the best rooting (ranged from 63.0 to 92.0 per cent) from the cuttings which were planted in autumn.

Krupnov and Zitkov (1968) and Takahashi et al. (1982) recorded over 90.0 per cent rooting in semi-hardwood cuttings of 22 varieties of plum, Myrobalan and peach when these were planted in June after IBA treatments. Larsen (1976) found that the application of IBA at 500 to 2000 ppm and planting the cuttings in April was conducive for better rooting with

plum rootstock Myrobalan B and Marianna plum. Spycher (1976) observed 90.0 per cent rooting in Prunus mariana and P.mehalab, when the cuttings were taken between mid-June and mid-September. Osipov and Morozova (1976) observed the best rooting when the cuttings of Prunus cerasus were planted in June. Singh (1978) observed highest rooting success (76.0 per cent) with plum cultivar Alubukhara when its basal stem cuttings were taken and compared with four other plum cultivars. Fontanazza and Rugini (1980) obtained very good rooting in semi-hardwood cuttings of plum cultivar Methley when its cuttings were taken on August 13 and 2000 ppm IBA was applied to these before planting.

Kofler et al. (1986) reported that softwood and hardwood cuttings of Pixy and Kozlienka plum cultivars greatly improved rooting when planted in June and August instead of November after treating the cuttings with IBA at 5000 ppm and 3000 ppm, respectively. Nahlawi (1973) observed better rooting in hardwood cuttings of two Syrian plum cultivars when these were planted during November, December and January. Ivanicka and Barborka (1976) also reported that the hardwood cuttings of 'Myrobalan 4040' and 'St.Julien 4048' plums rooted best when taken in early December and treated with 2500 ppm IBA as compared to cuttings taken in early February. They observed that 'Greengage 4045' and 'St.Julien 4047' gave very poor rooting. Bartolini and Roselli (1979) and Bartolini et al. (1982) found that eight Japanese plum cultivars had rooted better than nine others of European group with or without IBA treatments.

Hartmann (1982) observed poor rooting of softwood cuttings of plum cultivar German Prune when they were planted in May and June under mist. But very good rooting was obtained when the same were taken in spring from four forced plants grown from suckers. Grzyb (1982) while working on European plum (Prunus domestica L.) hardwood cuttings taken in spring obtained rooting upto 88.8 per cent after treating these cuttings with 2500 ppm IBA.

The ability of cuttings to regenerate also varies considerably with the plant species. While the cuttings of some plum species regenerate easily, the others regenerate with great difficulty even after treatment with growth substances (Bartolini et al., 1982). Kosina (1981, 1983) collected softwood cuttings of some clonal plum rootstocks in the second half of June and treated with different concentrations of IBA. The best results were shown by Myrobalan clones SE 4041, SE 4040, SE 4044 and EMLA Brompton at 2500 ppm IBA as compared to 4000 ppm IBA which showed rooting averages of 94.4, 92.0, 84.0 and 78.4 per cent, respectively over three years. Myrobalan clones also rooted better than Damas C clones.

1.3. Size of cuttings in relation to rooting

Size of the cuttings, especially their length is also important in rooting of the stem cuttings. Longer cuttings have been reported to root better than the shorter ones (Nanda and Kochhar, 1985). Fontanazza (1969) obtained 86.7 per cent rooting with 30 cm long cuttings of some plum varieties

after their treatment with IBA. Nahlawi (1973) obtained the highest rooting in two Syrian plum cultivars Ajami Janirek and Tephahi Janirek when their 60 cm long cuttings were treated with 5000 ppm IBA and planted from November to January. Pathak et al. (1975) working on hardwood cuttings of plum also found that the highest rooting percentage, root numbers and length were recorded when 60 cm long cuttings were treated with IBA at 2500 ppm.

Chauhan and Reddy (1974) have recorded maximum rooting (25.77 per cent) coupled with higher number of primary roots, average root length and diameter by using 23 cm long Santa Rosa plum cuttings which were treated with 1000 ppm IBA and planted during November and December. Long dip of softwood cuttings of 8-10 cm long with 25-100 ppm IBA for 16 hours and planting these cuttings in coarse sand:compost medium under intermittent mist resulted in maximum rooting of eight plum cultivars (Merganov, 1978) whereas quick dip treatment of plum and prune hardwood cuttings (15-20 cm long) with 2000-4000 ppm IBA resulted in higher rooting (50.0 to 60.0 per cent) when these cuttings were planted during October.

2. Field survivability of rooted cuttings

Mroczkowski et al. (1974) obtained highest field survival of some plum hardwood cuttings when they were treated with IBA at 4000 ppm and planted the cuttings during November and transplanted the rooted cuttings in the early spring. Pathak et al. (1975), however, obtained highest survival

percentage from hardwood cuttings of plum when treated with IBA at 2000 ppm.

Grzyb (1975) and Hartmann (1982) in separate experiments obtained best results on field survival of softwood cuttings of some plum rootstocks after their treatment with IBA at 4000 ppm. Bartolini and Roselli (1975) in their studies with six European plum cultivars found the best degree of establishment in the field with Precoce di Ebersucier and Claudia di Bavay after treating with IBA at 2000 ppm.

Hartmann (1982) observed that IBA at 4000 ppm was optimum for rooting and field survival of cuttings from the base, 2000 ppm for cuttings from the middle part and 500 ppm for cuttings from the apical shoot portion of plum cultivar German Prune. Sinha et al. (1983) obtained the best rooting and field survival in Myrobalan A and B cuttings when they were treated with IBA at 1000 ppm as compared to 500 or 2000-5000 ppm IBA.

3. Effect of preconditioning and wounding treatments on rooting of cuttings

3.1. Forcing

Forcing referred to the method of heading back the main branches of the mother trees during dormant season so as to induce active shoot growth that are ready to be planted in the next season.

Rooting in cuttings of jackfruit has been successfully induced through etiolation of forced shoots treated with 5000 ppm IBA under mist resulting in 84.0 per cent rooting and 75.0 per cent field survival of the rooted cuttings by Mukherjee and Chatterjee (1979). Hartmann (1982) working on softwood cuttings of plum cultivar German Prune under mist also obtained better rootings from four forced mother plants grown from suckers.

3.2. Etiolation

This refers to the development of plants or plant parts in the absence of light (Hartmann and Kester, 1983). Etiolation of shoots before the cuttings are taken, promotes better rooting as was observed by many workers (Teaotea and Pandey, 1961 and Herman and Hess, 1963).

According to Kawase (1965), etiolation of plant parts causes a decrease in starch content, decreases the cell wall thickness and total amount of vascular tissues in comparison with non-etiolated stems. Etiolated stems, thus had more parenchyma cells and more tissues in a less differentiated condition, which react strongly to the exogenously applied auxins and rooting cofactors which are necessary for root formation. Etiolation also resulted in a higher level of endogenous auxin (IAA) at the site of etiolation which favoured root initiation. The etiolation of shoots stimulates the formation and development of root primordia in the cuttings. The effect of etiolation is also enhanced by

ringing the cuttings (Nanda and Kochhar, 1985).

Hartmann and Kester (1983) have summarized the promotive influence of etiolation on tissues during root initiation as the photoinactivation of an essential component in a complex required for the root initiation and formation of root initials.

3.3. Ringing

Ringing of shoots by disrupting the downward movement of metabolites through phloem of the stem with thin wire has resulted in increased root formation (Hartmann and Kester, 1983).

Stoltz and Hess (1966) observed that the promotion of rooting by ringing stimulates the accumulation of carbohydrates above the ringed portion of the shoot and enhanced the rooting of difficult-to-root plant species. They also observed that the girdling causes substantial increase in rooting cofactors above the girdled portion which promoted root initiation.

Allan et al. (1980) observed improvement in rooting of pecan cuttings from 45.0 to 75.0 per cent when the shoots were ringed one month before taking the cuttings. The rooting of such cuttings were further increased if the ringed shoots were subjected to cold temperature for two weeks before planting under mist. Fontanazza and Rugini (1980) found that the ringing of semi-hardwood cuttings of plum cultivar Methley at 4 cm from the base resulted in blocking the downward movement of the metabolites from the leaves and thus enhanced root formation.

3.4. Wounding

Hartmann and Kester (1983) stated that the wounding of cuttings permitted greater absorption of applied growth regulators by disrupting cambium tissues at the base of the cuttings. Wounding of cuttings thus facilitated the outward penetration of the new developing roots. Fontanazza (1969) reported that the longitudinal incisions at the bases of 30 cm long cuttings of plum cv. Florentia in combination with IBA at 3000 ppm induced 86.7 and 24.4 per cent rooting in basal and apical halves, respectively. Nahlawi and Howard (1970, 1971) observed rooting improvement in hardwood cuttings by ringing the shoots while attached to the mother plants and wounding-cuts given to the cuttings at their base prior to IBA treatment between 500-5000 ppm.

Nicotra (1971) observed improved rooting in St.Julien A plum following a longitudinal 3 cm cut at the base of the cutting before their treatment with 2000 ppm IBA. Nanda (1975) observed that roots from the cuttings emerged in a linear manner following longitudinal slits given at the base of the cuttings to break complete ring of collenchymatous hypodermal tissue which hinders the penetration of developing root initials.

4. Biochemical factors affecting rooting

The physiological and biochemical differences in the plants determine to a greater extent the ability of fruit trees to regenerate from cuttings. The role of various

biochemical factors in the rooting of cuttings are briefly reviewed in the following pages.

4.1. Carbohydrates-Nitrogen ratio

The extent of regeneration of roots from the cuttings largely depends upon the differences in internal nutritional conditions. But the available evidences on the effect of carbohydrates and nitrogen ratio of the mother plants on rooting behaviour of the cuttings is somewhat controversial.

The rooting propensity of cuttings is favoured by low and medium levels of nitrogen and a high level of carbohydrates in the stock plants (Basu and Ghosh, 1974 and Hartmann and Kester, 1983). Kraus and Kreybill (1918) showed that high carbohydrates content was essential for rooting of stem cuttings. Increase in the total carbohydrates and protein contents was also detected at the basal ends of the cuttings after IBA treatments (Strydom and Hartmann, 1960). The beneficial effect of high C/N ratio on rooting of cuttings was observed by various workers (Pearse, 1943; Sen and Basu, 1960 and Hartmann and Kester, 1983).

A number of workers ascribed better rooting of cuttings with etiolation due to the increase in the levels of sucrose, glucose, fructose and proteins and decrease in the level of starch (Ali and Westwood, 1966 and Herman and Hess, 1966). They also demonstrated that a proper balance between nutrition and auxin is necessary for optimal production of roots and that even starch can be used as carbon source in the formation of

adventitious roots on cuttings. Smith (1926) however, reported that the starch reserves in cuttings were more important for rooting than the reducing sugars. Starch has been reported to increase the rooting in filberts by Perez et al. (1984) whereas neither starch nor total carbohydrates were statistically related to the proportion of successful rooted cuttings in grape (Treeby and Considine, 1982).

Robinson and Schwabe (1977) observed that maximum accumulation of carbohydrates in shoots occurred in autumn (November) which resulted in the highest regeneration and survival of the cuttings. Hosoi et al. (1972) reported that the soluble nitrogen content (per cent dry weight) gradually decreased in both upper and lower half of stems throughout the propagation period. On the other hand, Basu et al. (1972) found that in mango cuttings, soluble nitrogen increased during pre-callusing and callusing periods but showed a gradual decline at the time of root emergence.

4.2. Phenolic compounds

Phenolic compounds play a paramount role in rooting of stem cuttings. They form a part of 'rhizocaline' as well as 'rooting cofactors', the action of which partly protect the root inducing naturally occurring auxin-IAA from destruction due to enzyme IAA oxidase. The effect of phenolic compounds on IAA oxidase activity, however, differs with the type of phenols. Monophenols were found to act as cofactors of IAA oxidase, while o- and p- dihydric and poly-phenols are

inhibitors of IAA oxidase (Schneider and Wightman, 1974). However, according to Nanda and Kochhar (1985), the extent of synergism between mono-, di- and poly-phenols is limited. They ascribed this differential effect of phenolic compounds due to the differences in their structural configuration in relation to the position of hydroxyl groups. No such relationship appears to hold good in many cases (Nanda, 1975). Although, the exact mode of action of phenols is still a controversy and remain to be elucidated, yet it is seen that cuttings with high phenolic contents have more rooting potential (Nanda and Kochhar, 1985).

Basu et al. (1969) reported that tannic acid in combination with IBA and NAA promoted rooting but its combination with IAA proved ineffective. Mokashi et al. (1982) while studying the biochemical constituents of difficult-to-root Thompson Seedless grapes observed that tnese cuttings contained a lower amount of phenolic compounds besides other factors. However, the presence of ferulic, p-hydrobenzoic and p-coumaric acids extracts and salicylic acid was related with difficult-to-root behaviour of some ornamental plants (Nanda and Kochhar, 1985).

4.3. Rooting cofactors

Cooper (1938) suggested that occurrence of some rooting cofactors facilitated the rooting of cuttings in some plant species. Thimann and Delisle (1939) also suggested that some unknown factors other than auxins are involved in root

initiation.

Hess (1957, 1959) postulated the concept of rooting cofactors which he considered as the substances that occur endogenously in plants and were capable of acting synergistically with indole-3-acetic acid (IAA) in promoting rooting in Mung bean cuttings. He identified four such cofactors and found that the amount of such rooting cofactors was more in easy-to-root than in difficult-to-root plant species. Hess (1962) isolated these cofactors from the cuttings of Hedera, Chrysanthemum and Hibiscus rosasinenfis and identified and designated them as cofactors 1, 2, 3 and 4 on the basis of their position on the chromatogram. Later on, Hess (1964) further divided the cofactors '4' into four active fractions A, B, C and D and higher rooting was found to be associated with higher content of fraction A. Hess (1965) identified cofactors 3 and 4 as iso-chlorogenic acid and oxygenated terpenoids, respectively. He also studied the synergism of other phenolic compounds with IAA and found that the presence of two hydroxyl groups at ortho-position with a free para-position was necessary to synergise the action of IAA on rooting (Hess, 1968).

Lanphear and Meahl (1966) while working on Juniperus horizontalis reported that environmental influences on rooting were accompanied by specific changes in the levels of rooting cofactors. While comparing the levels of root promoting and inhibiting substances in three clones of Rhododendron, Lee et al. (1969) found that the highest level of four rooting cofactors were found in its stem and leaf tissues. Fadl (1967)

and Fadl and Hartmann (1967) isolated rooting cofactors from pear cuttings with intact buds treated with IBA which were identified as a complex of indolic and phenolic compounds. These cofactors could not be isolated either from dormant cuttings or those cuttings without buds, even when these were treated with IBA.

Taylor and Odom (1970) reported that three to four areas of endogenous rooting cofactors' activity corresponding to specific Rf values were located in basal extracts of all cuttings by paper chromatography of the Mung bean rooting bioassay. Many workers have lent support to the concept of rooting cofactors (Herman and Hess, 1963; Challenger et al., 1964 and Girgouard and Hess, 1964, 1966).

4.4. Growth inhibitors

Much attention has been given recently to the possible role of abscisic acid (ABA) in the rooting of cuttings (Lipecki and Dennis, 1972). Ting-Yun Chin et al. (1969) and Basu et al. (1970) have shown that ABA stimulated rooting of cuttings of Phaseolus aureus Roxb., Lycopersicon esculentum Mill. and Hedera helix L. when applied exogenously with or without auxin. Cornforth et al. (1966) isolated an inhibitor from Myrobalan B plum which was shown to contain abscisic acid and was found to be active in promoting rooting in Mung beans. Van der Lek (1934) found that buds which stimulated rooting in summer and autumn inhibited rooting in winter with inhibition decreasing progressively by late winter. The low rooting of cuttings

with buds in winter could be due to the accumulation of inhibitors. Such inhibitors may not only arrest bud development but also inhibit the formation of endogenous root promoting substances (Spiegel, 1954).

The inhibitor isolated from plum cuttings by Barlow and Hancock (1961) has been identified as (+) abscision II (Milborrow, 1967). Gasto et al. (1967) and Mendez et al. (1968) found that while both inhibitor and promoter zones were present in easy- as well as difficult-to-root species, the inhibitor was much stronger in difficult-to-root than in easy-to-root plants. Fadl and Hartmann (1967) worked with cuttings of easy-to-root 'Old Home' and difficult-to-root 'Bartlett' pears and found that while the content of promoter was high in 'Old Home', the content of inhibitor was high in 'Bartlett' pear. Lee et al. (1969) found the presence of an inhibitor in three clones of Rhododendron but it appeared less responsible for difference in their rooting than variation in the levels of rooting cofactors.

Taylor and Odom (1969) reported that two consistent areas of inhibiting activity corresponding to specific Rf values were evident in stem cuttings of pecan from all the preconditioning treatments. Shirad and Miles (1977) working with cuttings of apple rootstock M.14 found that root inhibiting activity of beta-inhibitors at Rfs. 0.5-0.8 remained active throughout the year except in October and November.

5. Tissue culture in fruit plants

The basic idea of tissue culture was formulated by Haberlandet in 1902. However, Robbins (1922) and Kotte (1922) are credited with the first successful attempts on plant culture. Stewards et al. (1958) proved the totipotency of higher plant cells and since then a great deal of attention has been devoted to plant cells, tissues and organ cultures in understanding the various problems associated in propagation of fruit plants through tissue culture (Reinert and Bajaj, 1977 and Bassi, 1984).

The regeneration of plants from cell and callus culture is a wide field but the need for tissue culture techniques in fruit plants has been picked up only recently when a rapid multiplication of plants was necessitated in a short time. The works on the development of upto-date techniques in tissue culture as well as for the aseptic culture for plum explants in vitro havebeen reviewed by various workers (Nitsch, 1974; Garland and Stoltz, 1981; Loreti et al., 1982; Marino, 1983; Pietropaolo and Reisch, 1984 and Ruzic and Cerovic, 1985).

Shoot proliferations and root formation in tissue culture are controlled by a number of factors. A brief review of works done on different aspects of tissue culture has been enumerated as under:-

5.1. Type of explants

Type of explants has been reported to influence the survival of the regenerated plants in vitro (Hammerschlag, 1982).

Apical meristems withpout leaf primordia had higher potentials for shoot proliferation whereas explants of shoot tips having one or two buds had a higher survival rate in culture (Jones et al., 1977).

5.2. Culture environment

Hammerschlag (1982) reported that the most important culture environment for tissue culture are the incubation temperatures and light. Constant incubation temperatures between 24°C and 27°C both during day and night and light intensities of 2-4 Kilolux under cool white fluorescent lamp with a photo-period of 14 to 18 hours day length has been favoured by most of the workers for callus formation and shoot proliferation in most of the plant species. However, for the root initiation, incubation of the culture under dark conditions for two weeks followed by incubation under 16-hour photoperiod is generally followed in many Rosaceous plants (Nanda and Kochhar, 1985).

Hammerschlag (1982) reported 100.0 per cent rooting of Myrobalan shoots cultured in vitro when they were incubated under dark conditions for two weeks followed by incubation under 3 Klx cool white fluorescent lamp at 26°C for 16-hour photoperiod.

5.3. Culture media

The compositions of the culture media is very important in the establishment of shoot proliferation and the rooting of

plantlets in tissue culture. Different culture media have been developed by various workers (Gautheret, 1955; Murashige and Skoog, 1962; White, 1963 and Gamborg and Wetter, 1975). Earlier, the nutrient medium for meristem culture was based on that developed by White (1943) to which minor elements were added according to Heller (1953). Since then, many improvements in the nutrient media have been made. A useful basic medium devised by Murashige and Skoog (1962) has been widely used by many workers to study tissue cultures in plum species (Loreti et al., 1982; Marino, 1983 and Ruzic and Cerovic, 1985).

5.4. Synthetic growth regulators

The balance of different levels of growth substances control the initiation and development of callus tissues, shoots, plantlets and roots in many plant species. Higher concentration of cytokinins in the medium favours rapid shoot proliferations whereas higher concentration of auxins stimulates root initiation in tissue culture (Abbott and Whiteley, 1976).

Marino (1983) reported that dormand buds excised from woody shoots of Santa Rosa plum cultivar in late winter proliferated at a rate of 8:1 to 13:1 when grown on MS media containing NAA, BA and GA_3 at 0.05, 1.0-2.0 and 0.1 mg/l, respectively. He also obtained best rooting (80.0 to 100.0 per cent) in MS media containing 0.5 mg/l IBA or 0.2 mg/l NAA.

Nemeth (1979) reported that MS medium containing 0.5 mg/l IBA was the most active in rooting in several plum species. However, Ivanicka and Pretova (1980) obtained better root formation with 0.2 mg/l NAA when compared with IBA containing 1.0 mg/l in the medium. Rosati et al.(1980) reported that shoot tips of Japanese plum cultivar Calita proliferated at a rate of 10:1 to 20:1 per month when grown on modified MS medium containing 1.0 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3. The best rooting occurred with 2.0 mg/l IBA at 21°C.

Hammerschlag (1982) cultured shoot tips of Myrobalan plum in MS medium containing 0.01 mg/l IBA and 0.2 mg/l BA and obtained ten-fold multiplication rate, every 4-6 weeks when in vitro proliferated shoots were transferred to MS medium containing 2.5-5.0 mg/l IBA and incubated in the dark for 2 weeks at 26°C.

Loreti et al.(1982) obtained good shoot proliferation and rooting in 'Mr.S.2/5' plum rootstock using actively growing 1-2 cm long tips cultured in MS medium containing 0.1 mg/l BA and 1.0 mg/l IBA, respectively. The addition of 0.5 mg/l IBA, 1.5-2.5 mg/l BA and 1 mg/l GA_3 in MS medium promoted shoot proliferation in European plum cv. Prune d' Agen Clone 707 at a rate of 12:1 in 3-4 weeks and also obtained the best rooting with 0.8 mg/l IBA (Bassi, 1984).

Pietropaolo and Reisch (1984) obtained successful shoot proliferations in 1.1 mg/l BA and rooting with

0.5-2.5 mg/l IBA when they were added to MS medium. Ruzic (1984) also achieved rapid shoot proliferation from shoot tips (1.5-3.0 cm long) of plum cv. Pozegaca on MS medium enriched with 1.0 mg/l BA, 1.0 mg/l IBA and 0.1 mg/l GA_3. He also obtained best rooting (50.0 to 100.0 per cent) with 2.0 mg/l IBA and 0.1 mg/l GA_3.

The transfer of proliferated shoots of plum cv. Pozegaca to a supplemented MS medium containing 0.1 to 5.0 mg/l IBA or NAA resulted in a moderate rooting of explants but the best rooting response of 90.9, 80.0 and 75.0 per cent were obtained when this medium was supplemented with 1.0 mg/l NAA, 0.5 mg/l NAA and 0.5 mg/l IBA, respectively.

C H A P T E R -III

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

The present investigations on the studies on propagation of plum (*Prunus* spp.) by cuttings and tissue culture were carried out in the Department of Pomology and Fruit Technology, College of Horticulture, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan (HP) during 1985-88.

Location and Climate

Solan is situated between 30°50'-30°52' North Latitude and 77°08'-77°11' East Longitude. The altitude of the University Campus ranges from 1000-1500 metre (a.m.s.l.). The soils are loam to clay loam in texture, greyish brown in colour and well drained. The weather conditions prevailing during the period of present trials are given in Appendix I.

The details of experimental materials used, techniques applied and methods of analysis followed during the course of different experiments were as given below:-

Experiment-I

Effect of IBA concentrations and seasons of planting under different conditions on rooting of cuttings in different plum cultivars.

1.1. Collection and preparation of stem cuttings

One year old hardwood cuttings ranging from 0.5 to 1.0 cm in diameter and about 25 cm in length were collected from healthy, uniform and moderately vigorous plum trees for dormant and autumn plantings in the nursery under field condition. The semi-hardwood cuttings of the same diameter and length with 4-6 leaves were taken from the same trees from which the hardwood cuttings were collected, for summer planting under mist condition. While preparing the cuttings, care was taken to give a straight cut slightly below a node on the basal end and a slanting cut above a node on the apical end.

1.2. Cultivars used

i) Santa Rosa
ii) Beauty
iii) Greengage
iv) Early Transparent Gage

1.3. Season of planting of cuttings

i) Dormant season (in nursery) : January, 14.
ii) Summer season (under mist) : July, 14.
iii) Autumn season (in nursery) : October, 14.

1.4. Growth regulator concentrations

i) 0 ppm IBA (dipping in distilled water only)
ii) 2000 ppm IBA
iii) 3000 ppm IBA
iv) 4000 ppm IBA

1.5. Replications

Each treatment was replicated four times and one replication consisted of 20 stem cuttings.

1.6. Experimental Design

Split-split Plot Design.

1.7. Application of growth regulator

Indole butyric acid (IBA) at 0, 2000, 3000 and 4000 ppm concentrations was used for treating the cuttings. The stock solution of IBA was prepared in 95.0 per cent ethanol and distilled water in an equal ratio and later on desired concentrations were prepared with distilled water. Before dipping the cuttings in different concentrations of IBA, two longitudinal wounding-cuts of about 2 cm were made in the basal ends of the freshly prepared cuttings. The basal ends of about 2.5 cm were then dipped for about 10 seconds. The treated cuttings were taken out of the solutions and were allowed to dry by keeping them under shade and were planted directly in a well prepared nursery bed under field condition in case of dormant and autumn seasons planting and in polythene bags placed on wooden boxes in the case of summer planting. The beds for summer planted cuttings were misted under intermittent mist system for 15 seconds at an interval of 10 minutes.

1.8. Planting and cultural operations for dormant and autumn season cuttings in nursery

The prepared cuttings were planted 10 cm apart with two-third of its length inside the soil. Thereafter, the beds were mulched with dried grasses and irrigations were given at frequent intervals. Hoeing and weeding were also done whenever needed to give proper aeration to the cuttings.

1.9. Preparation of rooting medium and beds for planting semi-hardwood leafy cuttings during summer under mist

The rooting medium of river sand was prepared by sterilizing the sand with formalin solution containing 35.0 per cent active ingredient and water in the ratio of 1.5: 10 (v/v). The sand was thoroughly drenched with the prepared solution on a black alkathene sheet. The heap was then covered with the sheet and left as such for 72 hours. After this period, the cover was removed and the medium was raked often for about 15 days to ensure complete removal of formalin vapours. This sterilized sand was filled in polythene bags of 12 x 22 cm size with sufficient basal perforations. These bags were place in wooden boxes already kept on the benches in the mist chamber. The prepared semi-hardwood cuttings were planted in these polythene bags and each treatment was replicated four times and each replication consisting of 20 cuttings.

1.10. Application of foliar nutrition to leafy cuttings

The leafy cuttings of plum cultivars were sprayed with

Hoagland and Arnon's nutrient solution at every alternate days. The sprouted dormant cuttings were also sprayed in a similar manner with the same nutrient solution in the field.

1.11. Hardening of rooted cuttings under mist

The hardening of rooted cuttings under mist was done for about one month by gradually decreasing the misting time and increasing the misting interval before transplanting them to the field.

Experiment-II

Effect of preconditioning treatments, size of cuttings and IBA concentrations on rooting behaviour of dormant season cuttings of Santa Rosa plum.

The preconditioning treatments comprised the forcing of stock plants and etiolation and ringing of the forced shoots before the cuttings are detached from the mother trees. For forcing the stock plants, the scaffold branches of the mother trees were headed back during the month of February so as to induce active shoot growth. Etiolation of a part of shoot protion was done by wrapping 3 cm wide black tape around the shoot at the base and the ringing was done by binding the base of the trunk of the forced shoots with thin matallic wires when the shoots attained a height of about 70 cm. The preconditioned shoots were removed from the mother plants on 15th January during both

the years and three cutting sizes of 20 cm, 30 cm and 40 cm length were prepared and planted in the field on the same day after giving basal wounding-cuts and IBA treatments.

The following treatment combinations were made to study the effect of preconditioning treatments, size of cuttings and IBA concentrations on the rooting in Santa Rosa plum:

T_1 F + 0 ppm
T_2 F + 2000 ppm
T_3 F + 3000 ppm
T_4 F + 4000 ppm
T_5 F + W + 0 ppm
T_6 F + W + 2000 ppm
T_7 F + W + 3000 ppm
T_8 F + W + 4000 ppm
T_9 F + W + E + 0 ppm
T_{10} F + W + E + 2000 ppm
T_{11} F + W + E + 3000 ppm
T_{12} F + W + E + 4000 ppm
T_{13} F + W + R + 0 ppm
T_{14} F + W + R + 2000 ppm
T_{15} F + W + R + 3000 ppm
T_{16} F + W + R + 4000 ppm

F = Forced; W = Wounding-cut; E = Etiolated; R = Ringed.

The treated cuttings were planted in well prepared

beds in the nursery area by adopting Randomized Block Design. The treatments were replicated four times, each replication consisting of twenty stem cuttings. The beds were mulched with dried grasses and light watering with hose on alternate days was done. Partial shade to the cuttings was also provided with thatches during the hot summer months.

3. Observations recorded

The following data on different parameters were recorded 90 days after planting of cuttings in all the seasons.

3.1. Sprouting of cuttings

When the first bud sprouted and unfolded into the first leaf on a cutting, the cutting was taken to have sprouted. Such cuttings were counted and the data were expressed in per cent.

3.2. Percentage of rooted cuttings

The number of rooted cuttings in different treatment combinations were recorded and the results obtained were expressed in per cent.

3.3. Average length of main shoots

The average length of the newly emerged shoots was measured from ten randomly selected main shoots of the rooted cuttings in each treatment. The distance between the point

of emergence of the main shoot at the base and the shoot tip was considered as the length of the shoot.

3.4. Average diameter of shoots

The ten main shoots which were randomly selected for measuring their shoot length were also used for recording the shoot diameter of the cuttings under each treatment. The diameter was measured 2 cm away from the point of emergence of shoot and average values were calculated.

3.5. Average number of primary roots per cutting

The number of roots which were directly arising from the cuttings were counted from five randomly selected rooted cuttings as primary roots and their average numbers per cutting in each treatment was calculated.

3.6. Average length of primary roots

The length of individual root was measured from five randomly selected rooted cuttings and average length was calculated.

3.7. Average diameter of primary roots

The diameter of five randomly selected primary roots was measured from each rooted cutting. This measurement was taken at three different points and their average diameter was expressed in centimeter in each treatment.

3.8. Average number of secondary roots per cuttings

The number of secondary roots were counted from five randomly selected rooted cuttings in each treatment and the number of secondary roots per cutting was ascertained.

3.9. Per cent survival of rooted cuttings

The rooted semi-hardwood cuttings obtained in the mist chamber from summer planting were kept for hardening treatment for about a month in the mist chamber till the weather conditions become favourable for transplanting of deciduous fruit crops in the field. The field survival of these cuttings was recorded 90 days after they were transplanted in the field.

The data on the field survival of the rooted hardwood cuttings which were planted during dormant and autumn seasons under field conditions were recorded after one growing season.

4. Statistical analysis of the data

Data on the percentage of sprouting and rooting, number, length and diameter of primary roots, number of secondary roots, the length and diameter of shoots and the survival percentage of the rooted cuttings were statistically analysed as per standard methods adopted by Snedecor and Cochran (1961). In the cases where the percentages cover a wide range of values, angular transformation was used to convert the data to degrees before subjecting it to

statistical analysis as suggested by Fisher (1938) and Fisher and Yates (1963). The data in parentheses showed the angular transformed values.

5. Biochemical analysis

5.1. Collection, preparation and storage of samples

In order to study the levels of total nitrogen, carbohydrates, phenolic compounds and endogenous growth promoters and inhibitors in the cuttings, a random sample of ten basal segments measuring upto 2.5 cm was taken from the cuttings of each type during each season of planting.

For the analysis of total nitrogen, carbohydrates and phenolic compounds, the samples were chopped finely and dried in an oven at 80°C. Oven dried samples were then ground in a Willy's mill with one mm sieve and were stored in stoppered specimen tubes.

For analysing growth promoters and inhibitors, fresh samples at zero day of planting cuttings and rooted cuttings collected after 60 days of planting were washed and preserved in 80 per cent ethanol and stored in a refrigerator at 0°C.

5.2. Extraction of plant material in alcohol

Oven dried sample weighing one gram was boiled on a water bath with 20 ml of 80 per cent ethanol for about ten minutes. After cooling, the supernatant was decanted and the residue was again extracted 2-3 times by thoroughly mascerating with further addition of 80 per cent hot ethanol.

The decanted alcoholic extract was filtered through Whatman No.1 filter paper and the final volume was made to 25 ml by reducing through evaporation. This extract was used for the estimation of total and Ortho dihydric phenols.

5.3. Determination of total phenols

The contents of total phenols in the cuttings were estimated by using 'Folin Ciocalteu reagent' as per method given by Bray and Thorpe (1954). The reagent was diluted with equal volume of distilled water before use.

One hundred microliter of alcoholic extract of the cuttings was boiled in a 25 ml graduated test tube till free from alcohol. To this, one ml of Folin reagent and two ml of sodium carbonate (20 per cent) were added. The mixture was shaken well and heated over boiling water bath for exactly one minute and then cooled with running water. The blue solution so obtained was diluted to 25 ml with distilled water. The optical density of the solution was read at 650 nm after 30 minutes on Bausch and Lomb Spectronic-20 colorimeter. Blank was also run simultaneously to adjust zero absorbance. Total phenols were calculated using a standard curve prepared from graded concentrations of caffeic acid and were expressed as mg/g dry weight basis.

5.4. Estimation of Ortho dihydric phenols (O.D.phenols)

The Ortho dihydric phenols in the cutting samples were determined in accordance with the method as suggested by

Arnow (1937).

Arnow's reagent

Ten grams each of sodium nitrite and sodium molybdate were dissolved in distilled water and the final volume was made to 100 ml.

To 100 microliter of alcoholic extract of the cuttings, one ml of 0.5 N HCl, one ml of Arnow's reagent and ten ml of distilled water were added followed by an addition of two ml of 1 N NaOH. Soon after the addition of the alkali solution, a pink colour developed. Absorbance of this solution was measured in a Bausch and Lomb Spectronic-20 photocolorimeter at a wavelength of 515 nm. Contents of O.D. phenols were calculated from a standard curve prepared from caffeic acid and were expressed as mg/g dry weight basis.

5.5. Estimation of total nitrogen

The digestion of samples for nitrogen was carried out in concentrated sulphuric acid (AR grade) by adding digestion mixture in one gram of oven dried cutting sample as described by Jackson (1967). The final volume of the clear solution thus obtained was made to 100 ml with distilled water.

The total nitrogen from cutting samples was estimated by Micro-Kjeldahl's method (A.O.A.C., 1965) and the distillation was done by taking 5 ml of aliquot with 20 ml of 40 per cent NaOH in a Micro-Kjeldahl's distillation

apparatus. The ammonia thus evolved was absorbed in 10 ml of boric acid containing mixed indicator. After complete distillation, the distillate was titrated against N/100 potassium-bi-iodate. The blank was also run simultaneously with the samples. The nitrogen content was calculated and expressed as percentage on dry weight basis.

5.6. Estimation of carbohydrates

5.6.1. Extraction

Sugars and starch contents of plum cuttings were extracted and separated by the procedure given by Clegg (1956). To one gram of ground sample, 12 ml of 80 per cent ethanol was added and boiled on a water bath for 5 minutes. The supernatent was decanted off and the residue was further extracted 3-4 times with hot 80 per cent ethanol. It was then cooled and filtered through cheese cloth. The residues left on the cloth were used for the determination of starch and the filterates were used to determine total sugars.

5.6.2. Estimation of total sugars

Total sugars in the cutting samples were estimated with phenol-sulphuric acid method as suggested by Dubois et al. (1951). One ml of the alcoholic extract of the cutting was taken in a test tube, in which one ml of 5 per cent phenol solution was added. Five ml of the concentrated sulphuric acid was added to this by allowing the chemical to run down by the sides of the test tube and mixed thoroughly.

Thereafter, the tube was cooled under the running tap water. The intensity of the yellow-orange colour was measured at 490 nm wave length with a Spectronic-20 colorimeter. The amount of total sugars was calculated using a standard curve prepared from glucose and expressed in per cent on dry weight basis.

5.6.3. Estimation of starch

The starch contents in the cutting samples were estimated as per procedure given by Clegg (1956). The residues left on the cheese cloth were hydrolysed overnight with 5 ml of distilled water followed by 6 ml of 52 per cent perchloric acid. Then the contents were shaken for 30 minutes and filtered after adding 20 ml of distilled water. The final volume of the extract was made to 100 ml with distilled water. The starch content was determined by using the conversion factor 0.9 to convert the values of glucose to starch.

Total carbohydrate content was calculated by adding up total sugars and starch percentages and expressed in per cent on dry weight basis of the cuttings. C/N ratio was calculated by dividing carbohydrates percentage with nitrogen percentage for every treatment.

6. Determination of endogenous growth promoters and abscisic acid-like substances

6.1. Extraction

The extraction procedure adopted by Abdel-Rahman *et al.* (1974) was used in the present study.

Twenty gram basal 2.5 cm segments of fresh (zero day of planting) and rooted cuttings (after 60 days) from four different plum cultivars under study were collected. Accordingly, each sample was mascerated in a blender with a small quantity of cold methanol at 3°C and the homogenates were extracted twice with eight volumes of cold methanol. The extracted material along with plant tissue was kept in a freezer for 24 hours and then vacuum filtered. The extract after filteration was reduced to a small aqueous volume by evaporating the methanol under reduced pressure in the dark at 40°C. The pH of the aqueous extract was adjusted to 2.5 with 5 per cent HCl and was partitioned four times with ethyl acetate. The final ethyl acetate fraction contained all ethyl acetate soluble acidic substances including those showing auxin-like growth promoters and growth inhibitors activity. The final acidic ethyl acetate fraction was reduced to dryness and finally dissolved in two ml of methanol, thus, representing one gram cutting material in 100 microliter of extract. It was then stored in vial at -10°C.

6.2. Paper chromatography

The descending chromatographic techniques with Whatman No.1 chromatography paper was followed. Spotting was done by strip loading of 100 microliter of the methanolic extract and the chromatograms (in triplicate) were developed by the descending method at a temperature at 24°C until the solvent front had travelled approximately 20 cm from the

starting line. Chromatograms were run in the isopropanol: ammonia:water (10:1:1 v/v) solvent system for 18 hours in dark and then dried at room temperature. The chromatograms were cut into ten equal pieces, parallel to the line drawn on chromatogram. Each piece was then taken in a 2 x 4 cm specimen-tube and eluted overnight with water (five times the weight of the applied tissue extract) at 8-10°C. For bioassay, each chromatogram elute was subsequently transferred to a small vial with the exhausted chromatogram paper placed along its inside wall.

6.3. Bioassay for auxin-like substances

The Mung bean (Phaseolus aureus Roxb.) bioassay technique described by Hess (1964) was followed. Mung bean seeds were soaked for three minutes in 0.33 per cent solution of sodium hypochlorite and then kept under running tap water for 24 hours. The seeds were then sown in moist pre-sterilized sand for germination. Cuttings were made from 7 days old uniform seedlings by cutting off the root system, 3 cm below the cotyledonary node when their primary leaves had fully expanded but the trifoliate leaf bud has not expanded. These cuttings consisted of 3 cm of hypocotyl plus the epicotyl, the primary leaves and the unexpanded trifoliate leaf buds. To avoid wide variations between individual cuttings in the same vial, cuttings of uniform shape and size were selected. Five cuttings of Mung bean were placed in 6.8 x 2.0 cm size vials which also contained elutes from the chromatogram strip and 5 ml distilled water.

No synthetic auxins were added in the vials. The Mung bean cuttings were placed for rooting. Occassionally, distilled water was added in the vials to maintain the original water level. Root numbers on each cutting were counted after one week. The results were expressed on the basis of increase or decrease in root numbers over control and graphed in the form of histograms. Each histogram represents the activity of root promoters in one gram of the cutting material on fresh weight basis.

6.4. Bioassay for ABA-like substances

The presence and activity of growth inhibitory substances in the extracts was determined by cress seed (Lepidium sativum var. Curled) germination inhibition bioassay test as described by Masuda (1962), which is as follows:-

The pieces of chromatogram strips (each corresponding to Rf value) were placed in petri dishes (5 cm diameter) and were moistened with 2 ml distilled water. In each of these petri dishes, 50 cress seeds were placed and allowed to germinate in darkness at 25 $\pm$ 2^{o}C. The number of cress seeds germinated were counted after 72 hours and the per cent inhibition of germination was calculated from triplicate tests. The results were given on the basis of inhibition of cress seed germination over control. The data were plotted in the histograms. Each histogram represents the activity of inhibitors in one gram of the cutting material on fresh weight basis.

Experiment-III

Study on the response of tissue culture in respect of shoot proliferations and root initiations.

The tissue culture experiments were conducted at the Tissue Culture Laboratory for initiating callus and shoot proliferation in vitro from actively growing apical meristems and shoot tips with a lateral bud and inducing rootings in the proliferated shoots under artificial conditions. The materials and specific procedures followed are given as under:-

Aseptic manipulations

An aseptic area in the laboratory was created by using a Laminar Flow Transfer Cabinet fitted with a Bactericidal Ultra-Violet tube at the ceiling. The working bench of the cabinet was thoroughly washed with ethanol and whole of the internal surfaces were disinfected with formalin (35 per cent active ingredient). The UV tube and the inflow of filtered air was then switched on and the room closed for at least 30 minutes before the experiment was started.

Culture environment

The environmental conditions for the growth of the tissues consisted a light intensity of about 3.5 Kilolux (Klx) of cool white fluorescent lamps at the top of the culture tubes and flasks. Photoperiods of 16-hour day lengths was maintained. The temperature of the room was kept at

24 ± 3°C for the whole experiment. The growing callus and the proliferated shoots were transferred every four weeks on to the fresh culture medium.

Preparation of culture medium for calliusing and shoot proliferation

The basal medium consisted of macro and micro nutrients of MS medium (Appendix 2) as suggested by Murashige and Skoog (1962) with the following additions as adjuvants (in mg/l): sucrose (30,000), agar (7000), myo-inositol (100), thiamine HCl (0.5), pyridoxine HCl (0.5), nicotinic acid (0.5), glycine (2.0), IBA (0.1), BAP (1.0) and GA_3 (0.1). The composition of the medium was kept the same under all the treatments and was based on the preliminary studies conducted during the course of present investigations. The pH of the culture medium was kept between 5.5 and 5.8. Twenty five ml of the culture medium was poured into 25 x 150 mm glass culture tubes and the tubes were capped with polypropylene closures.

Sterilization of culture apparatus and culture media

All the glasswares and the culture apparatus used for the experiment were soaked in Laboline, a lab wash, for a few hours followed by thorough washing in tap water and finally with distilled water. The culture media and the glasswares were then autoclaved at 15 lb/in^2 (121°C) for 20 minutes. Dry glasswares were sterilized by heating in an oven at 150°C for about an hour. All the required instruments (scalpels,

forceps, scissors, etc.) were wrapped carefully in aluminium foil and then autoclaved. They were again dipped in alcohol and flamed in a spirit lamp at the time of conducting the experiment.

Initiation of shoot proliferation in vitro

Selection and preparation of experimental materials

About 5 cm long apices of newly emerged shoots from grown up healthy plum trees of four selected cultivars, namely, Santa Rosa, Beauty, Greengage and Early Transparent Gage were collected along with their leaves from the orchard in polythene bags and immediately brought to the laboratory. They were kept in running water for 30 minutes to remove the extranous particles and other microbes. They were then dipped in 0.01 per cent Teepol (Shell Chemicals) for a few seconds followed by a quick dip in absolute alcohol and then immersed in 10 per cent solution of hydrogen peroxide (H_2O_2) for 15 minutes. This surface sterilizing agent had given better results under preliminary studies as compared to mercuric chloride and sodium hypochlorite solutions. The materials were given three thorough washings in autoclaved sterile glass distilled water for the complete removal of the sterilizing agent and kept them there until dissection.

Dissection of apical meristems and its transfer to culture medium

Dissections of apical buds were carried out under sterile conditions of Laminar Flow Transfer Cabinet.

Meristematic portion of the apex was uncovered by holding the shoot in one hand and then the immature leaves and leaf primordia are snapped off with a slight pressure from the forcep held in the other hand. The exposed meristem tip measuring about 1 cm long appeared like a shiny dome and was gently severed from the shoot with a razor blade. They were gently placed vertically on the MS medium in the culture tubes with the help of sterile forceps. The remaining portion of the shoot was used to prepare shoot tip as explant having a lateral bud, one cm above the basal cut and 0.5 cm below the apical cut which was also gently placed vertically on the MS medium in the culture tube with the help of sterile forcep. Both basal and apical ends were trimmed off before placing in the culture medium. The forceps and razor blades required for the transfer of explant into the culture tubes were frequently disinfected with ethanol to prevent any contamination. The culture tubes were capped with polypropylene closures in front of a spirit lamp in the Laminar Flow Transfer Cabinet. The contamination of the explants if any, became apparent within two weeks. In five to six weeks, the surviving explants were sufficiently developed so as to transfer them to a fresh medium.

Transfer of explants to culture medium

The explants were first transferred under aseptic conditions to the MS culture medium containing only 3.0 per cent sucrose and 0.7 per cent agar. After 48 hours, they

were transferred to the growing medium containing adjuvants. The transfer of explants was necessitated to avoid the initial interference of phenolic substances oozing out into the medium which hindered the growth of the explants. Any contamination of the explants could be observed within two weeks of their transfer. In 8-10 weeks, explants made sufficient growth and development and were ready for the first sub-culture and their transfer to fresh medium.

Estimation of phenols in the culture medium

Some brownish substances, later identified as phenolic compounds were seen oozing out from the explants into the culture medium, which resulted in growth inhibition (Feucht, 1973). The explants were therefore, transferred to the fresh medium after 48 hours of their planting in the culture tubes. Thus, the transfer of explants to fresh medium every 48 hours prevented the oozing out of phenolics into the medium. The culture medium thus discarded were used for the estimation of total phenols.

Total phenols of the medium were extracted in 70 per cent ethanol as suggested by Johnson and Schall (1957). Out of this extract, 0.2 ml was pipetted into a 25 ml graduated test tube containing 3 ml of glass distilled water. To this, one ml of Folin Ciocalteu reagent and two ml of 20 per cent sodium carbonate solution were added and the final volume was made to 20 ml. The blue colour was allowed to develop for one hour and its per cent transmittance was read in a

Bausch and Lomb Spectronic-20 photocolorimeter using 660 millimicron filter. The values were expressed as mg/g of phloroglucinol of the medium.

Multiplication

The initial MS medium and its adjuvants used for the callus initiation and shoot proliferation was also used for shoot multiplication. The culture could be routinely divided and subcultured after every four weeks. At each transfer, explants were encouraged to produce shoots by clipping off their leaves. The rate of multiplication varied with different cultivars.

Rooting

For the induction of roots, shoots were transferred to rooting medium in conical flasks in which cytokinin (BAP) was omitted and auxins were added. The flasks were kept for 2 weeks in complete darkness followed by 4 weeks incubation under 3.0 Klx cool white fluorescent lamps for 16-hour photoperiod. The amount of sucrose was also lowered down to 20 g/l. In 6-7 weeks, roots were developed sufficiently to enable the plantlets to be weaned.

Weaning

For the successful establishment of plantlets in compost after rooting, rooted plantlets were subsequently weaned for two weeks in rooting medium consisted of soil, sand and compost in the ratio of 1:1:1 and under conditions

of high relative humidity, shade and adequate heat. After two weeks, the plantlets were ready for transfer to the glass house in the field.

Observations recorded

1. Callus growth after 4 weeks, 8 weeks and 12 weeks.
2. The number of shoots proliferated after every 4 weeks.
3. Growth of proliferated shoots at the time of transfer to the rooting media.
4. Rooting behaviours such as rooting percentage, number of roots per plantlets and their root length.

Experimental Design: Completely Randomized Design.

Statistical analysis of the data

Data on the number, length and diameter of the proliferated shoots, percentage of rooting, number and length of the roots per plantlet were statistically analysed as per standard method suggested by Snedecor and Cochran (1961).

CHAPTER - IV

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The data on the propagation of plum by cuttings and tissue culture along with their biochemical aspects were recorded in the present studies and the results thus obtained on various aspects are presented under the following headings:-

1. Seasonal rooting behaviour of plum cuttings as influenced by IBA treatments

1.1. Percentage of sprouting of cuttings

The data recorded on the sprouting per cent of cuttings of four plum cultivars as affected by different planting seasons and IBA treatments during the years 1986 and 1987 are presented in Table 1.

It is evident from the data in Table 1 that the effects of cultivars, planting seasons and IBA treatments on sprouting were found to be significant at 5 per cent level of significance whereas there were no significant differences in sprouting per cent of cuttings of different cultivars. However, the highest success in both the years was found in the cuttings of Santa Rosa with 78.1 and 79.1 per cent success followed in the descending order by the Greengage, Early Transparent Gage and Beauty in both the years.

The season of planting the cuttings had a profound influence on their sprouting. The summer cuttings gave

Table 1. Effect of IBA concentrations and planting seasons under different conditions on sprouting of cuttings (in per cent) in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	70.5	80.0	85.0	77.5	72.5	82.5	87.5	75.0
Summer	80.5	90.0	86.0	82.0	75.5	95.0	85.0	77.5
Autumn	67.5	72.5	75.0	70.5	71.0	75.0	80.0	72.5
C_2 Beauty								
Dormant	65.5	70.0	80.5	75.0	70.0	80.0	77.5	75.0
Summer	75.0	87.5	82.5	80.0	72.5	90.0	85.0	82.5
Autumn	72.5	75.0	67.5	70.0	65.0	70.0	75.0	67.5
C_3 Greengage								
Dormant	68.0	75.0	80.5	75.0	70.0	80.0	87.0	75.5
Summer	78.0	82.5	78.0	80.0	75.0	85.0	80.0	77.5
Autumn	75.0	80.0	77.5	75.0	70.0	75.0	78.5	72.5
C_4 Early Transparent Gage								
Dormant	66.0	72.5	82.5	75.0	72.5	75.0	85.0	77.5
Summer	75.0	85.0	80.0	82.5	70.0	87.5	80.0	72.5
Autumn	72.5	75.0	77.5	70.0	67.0	70.0	75.5	72.0

Means of treatments

		1986	1987
a) Cultivars:	C_1	78.1	79.1
	C_2	75.1	75.4
	C_3	77.0	77.1
	C_4	76.1	75.9
	C.D. (P=0.05)	5.47	5.49
b) Seasons :	Dormant	74.9	77.5
	Summer	81.5	80.7
	Autumn	73.4	72.2
	C.D. (P=0.05)	4.21	4.59
c) IBA concentrations:	0 ppm	72.2	71.0
	2000 ppm	78.7	80.4
	3000 ppm	79.4	81.3
	4000 ppm	76.0	74.8
	C.D. (P=0.05)	4.54	5.42

significantly higher sprouting than dormant and autumn cuttings. Maximum sprouting of 81.5 per cent was recorded during 1986 when the cuttings were planted during summer. The cuttings planted during dormant and autumn seasons were statistically at par with each other in their sprouting behaviour although the minimum sprouting was obtained in autumn planting. Similar results on the influence of season of planting on the sprouting of cuttings were also found during the year 1987.

The effect of different IBA treatments on sprouting were also found to be significantly different from one another during both the years of study. Maximum sprouting of 79.4 and 81.3 per cent was recorded with 3000 ppm IBA treatment during 1986 and 1987, respectively. During the year 1986, all the IBA treatments were at par with each other in respect of their effect on the sprouting but they were statistically superior to control. More or less similar effect of different IBA treatments was also observed during 1987 on the mean sprouting percentage.

A perusal of data in Table 2 revealed that the interaction between IBA treatments and time of planting of cuttings was found to be significant during 1986 and 1987. Maximum sprouting (86.3 per cent) was obtained when the cuttings were treated with 2000 ppm IBA and planted during summer. This treatment was closely followed by 3000 ppm IBA concentration during dormant season and 3000

Table 2. Main effects and interactions between planting seasons and IBA concentrations on sprouting of cuttings (in per cent) in plum

	1986					1987				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
S_1	67.4	74.4	82.1	75.6	74.9	71.3	79.4	84.3	75.6	77.6
S_2	77.1	86.3	86.2	81.1	82.5	73.3	89.4	82.5	77.5	80.7
S_3	71.9	75.6	76.1	71.3	73.7	68.3	72.5	77.3	71.1	72.3
Mean	72.1	78.8	79.3	76.0	-	70.9	80.4	81.3	74.8	-

C.D.(P=0.05)

Season (S)	= 4.21	4.69
Concentrations(T)	= 4.64	5.42
Interaction (Season x Concentrations)	= 8.04	8.07

ppm and 4000 ppm IBA in summer season during 1986. The lowest sprouting (67.4 per cent) was obtained with untreated cuttings planted during dormant season. During the year 1987, the maximum sprouting percentage of 89.4 per cent was recorded from the cuttings treated with 2000 ppm IBA concentration and planted during summer season. However, the minimum sprouting of 68.3 per cent was observed from the cuttings under control and planted during autumn season.

1.2. Percentage of rooting

The data on rooting percentage of cuttings of different plum cultivars as influenced by different planting seasons and IBA treatments during the year 1986 and 1987 are presented in Table 3 and depicted in Fig.1.

The data presented in Table 3 revealed that cultivars, planting seasons and IBA concentrations exerted significant influence on the rooting percentage of plum cuttings.

During the year 1986, highest rooting of 59.2 per cent was obtained in Santa Rosa cuttings which was statistically superior to the remaining cultivars. Beauty cultivar was also significantly better than Greengage and Early Transparent Gage. However, the rooting percentage in the cuttings of Greengage and Early Transparent Gage was statistically at par with each other. A similar trend on the rooting per cent of different plum cultivars was recorded during the year 1987.

Table 3. Effect of IBA concentrations and planting seasons under different conditions on rooting of cuttings (in per cent) in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	38.0 (38.1)	65.0 (53.7)	68.0 (55.5)	60.0 (51.1)	35.0 (36.3)	62.5 (52.2)	70.0 (56.8)	57.5 (49.3)
Summer	47.5 (43.6)	85.0 (67.2)	72.5 (58.4)	67.5 (55.2)	45.0 (42.1)	87.5 (69.3)	77.5 (61.7)	70.0 (56.8)
Autumn	32.5 (34.8)	55.0 (47.9)	62.5 (52.2)	57.5 (49.3)	30.0 (32.2)	60.0 (50.8)	67.5 (55.2)	52.5 (46.4)
C_2 Beauty								
Dormant	23.0 (28.7)	57.5 (49.3)	60.0 (50.8)	47.5 (43.5)	30.0 (33.2)	50.0 (45.0)	56.0 (48.4)	45.0 (42.1)
Summer	30.0 (33.2)	65.0 (53.7)	57.5 (49.3)	50.0 (45.0)	37.5 (37.8)	67.5 (55.2)	62.0 (51.9)	52.5 (46.4)
Autumn	20.0 (26.6)	47.5 (43.6)	52.5 (46.4)	40.0 (39.2)	27.5 (31.6)	45.0 (42.1)	50.0 (45.0)	42.5 (40.7)
C_3 Greengage								
Dormant	17.5 (24.7)	42.5 (40.7)	45.0 (42.1)	37.5 (37.8)	22.5 (28.3)	37.5 (37.8)	46.0 (42.7)	40.0 (39.2)
Summer	22.5 (28.3)	50.0 (45.0)	47.5 (43.6)	42.5 (40.7)	27.0 (31.3)	53.0 (46.7)	50.0 (45.0)	45.0 (42.1)
Autumn	15.0 (22.8)	37.5 (37.8)	40.0 (39.2)	30.0 (33.2)	18.0 (25.1)	35.0 (36.3)	42.5 (40.7)	32.5 (34.8)
C_4 Early Transparent Gage								
Dormant	12.5 (20.7)	35.0 (36.3)	40.0 (39.2)	42.5 (40.7)	16.0 (23.6)	40.0 (39.2)	45.0 (42.1)	35.0 (36.3)
Summer	20.0 (26.6)	55.0 (47.9)	45.0 (42.1)	37.5 (37.8)	18.5 (25.5)	52.5 (46.4)	47.5 (43.6)	40.0 (39.2)
Autumn	10.0 (18.4)	27.5 (31.6)	35.0 (36.3)	32.5 (34.8)	12.5 (20.7)	32.5 (34.8)	40.0 (39.2)	25.0 (30.0)
Means of treatments								
a) Cultivars :	C_1			59.2(50.6)				59.6(50.8)
	C_2			45.9(42.4)				47.1(43.3)
	C_3			35.5(36.5)				38.1(37.7)
	C_4			32.7(34.4)				34.0(35.5)
	C.D. (P=0.05)			6.12				5.92
b) Seasons :	Dormant			43.2(40.8)				43.1(40.9)
	Summer			49.7(44.8)				52.5(46.2)
	Autumn			37.2(37.5)				38.4(38.3)
	C.D. (P=0.05)			5.30				5.13
c) IBA Concentrations:	0 ppm			24.0 (29.3)				27.1(31.0)
	2000 ppm			51.2(46.1)				52.1(46.3)
	3000 ppm			52.3(46.2)				54.4(47.6)
	4000 ppm			45.2(42.4)				46.0(41.9)
	C.D. (P=0.05)			5.30				5.92

Figures in parentheses are angular transformed values

PLATE I

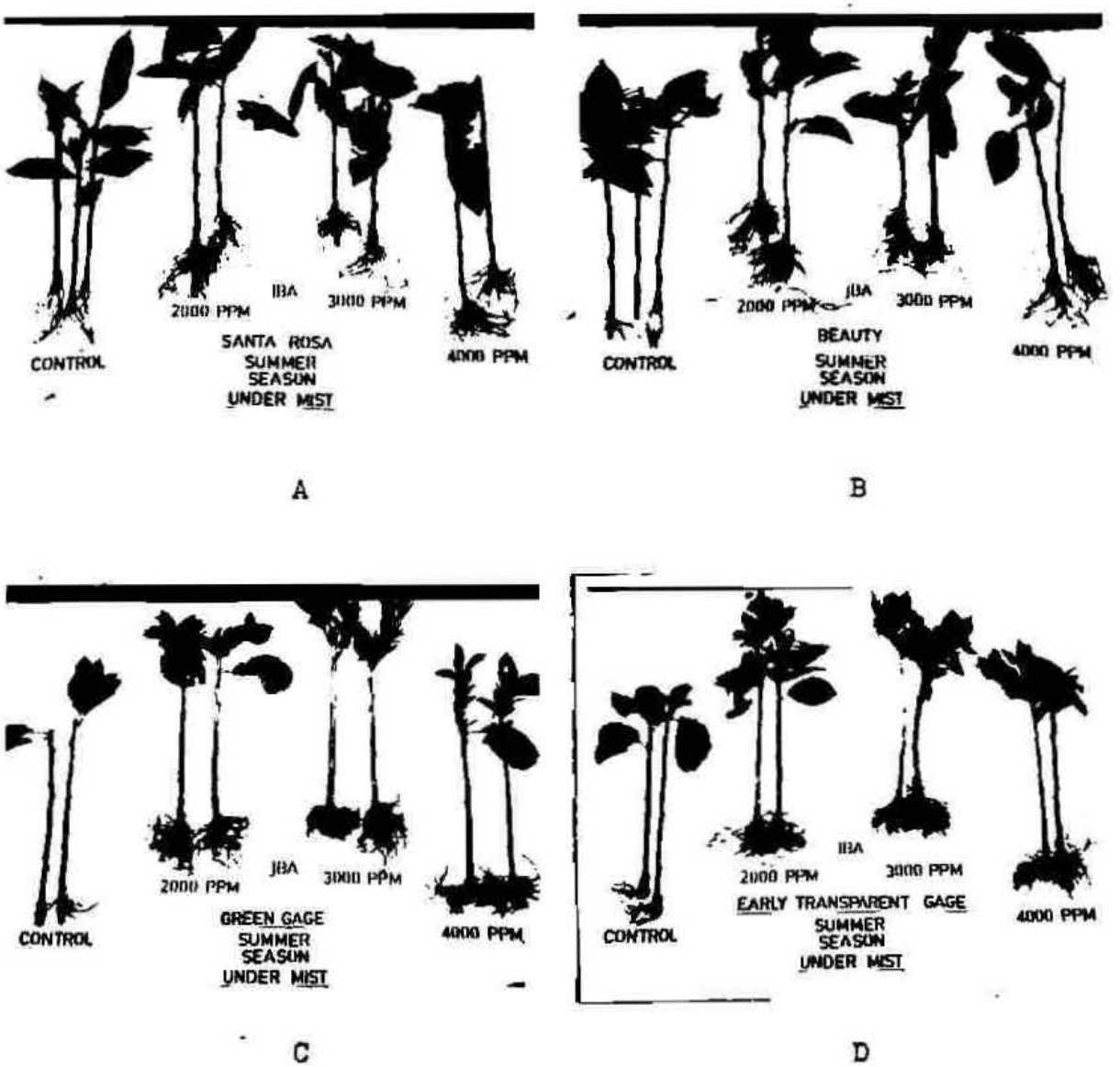


Plate 1. A-D. Rooting behaviour in summer planted semi-hardwood cuttings of plum as influenced by IBA treatments.

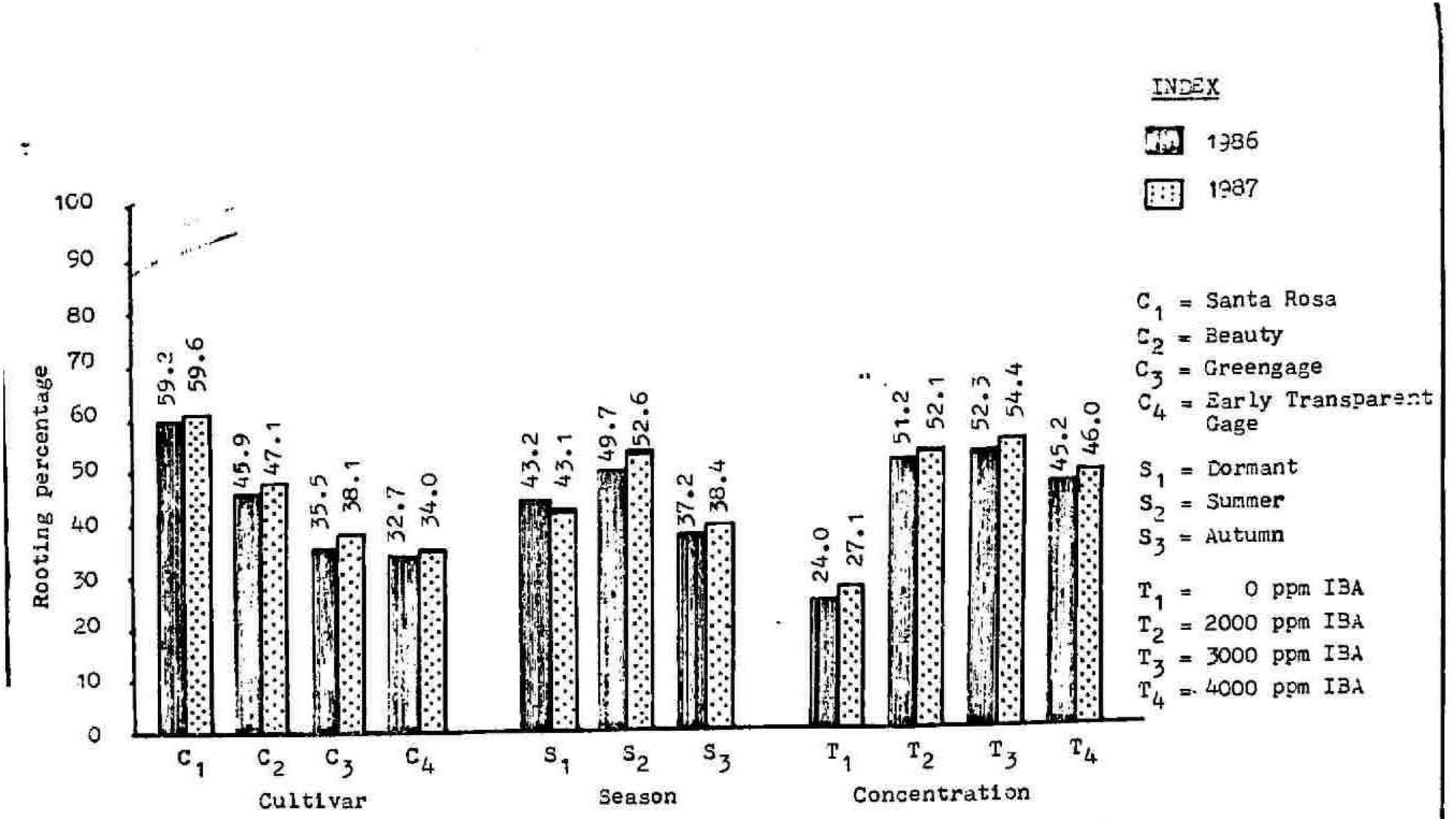


Fig.1. Effect of IBA concentrations and planting seasons under different conditions on rooting of cuttings in plum.

The lowest rooting per cent was recorded in Early Transparent Gage in both the years under study.

Seasons of planting had a remarkable effect on the rooting of cuttings in both the years. Planting of plum cuttings during summer season recorded the highest rooting of 49.7 per cent in 1986 and 52.6 per cent in 1987. The autumn planting gave the minimum rooting of 37.2 and 38.4 per cent during 1986 and 1987, respectively. The cuttings planted during summer season were statistically superior to cuttings planted during dormant or autumn seasons of both the years.

Applications of IBA also influenced the rooting behaviour of plum cuttings. Maximum rooting of 52.3 and 54.4 per cent was recorded in 3000 ppm IBA during 1986 and 1987, respectively. However, this treatment was statistically at par with IBA 2000 ppm but both the treatments were much superior to IBA 4000 ppm. Minimum rooting was, however, recorded in control during both the years.

The interactions between IBA treatments and season of planting of cuttings were also found to be significant during both the years under study. IBA treatment with 2000 ppm concentration gave the maximum rooting of 64.4 per cent when the cuttings were planted during summer while the minimum rooting (19.4 per cent) was recorded in control when



planted during autumn in the year 1986. During the year 1987 also the maximum rooting (65.6 per cent) was recorded with 2000 ppm IBA application when planted during summer season. This treatment was closely followed by planting of cuttings during summer with 3000 ppm IBA and in dormant with 3000 ppm IBA application. The minimum average percentage of rooting (22.5 per cent) was recorded when the cuttings were planted during autumn season and under control treatment (Table 4).

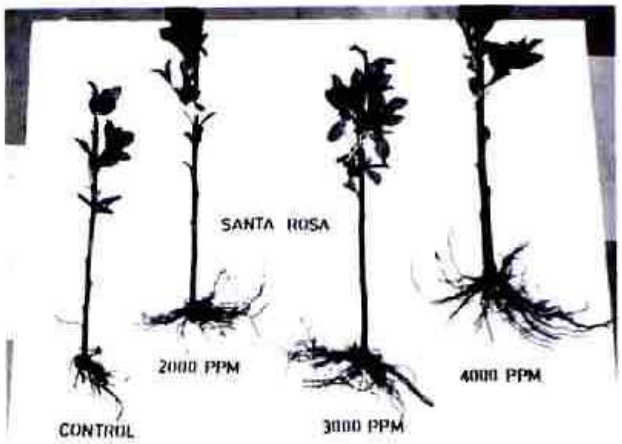
1.3. Length of shoots

The observations on the shoot length produced by the cuttings of four plum cultivars, as influenced by different concentrations of IBA are presented in Table 5.

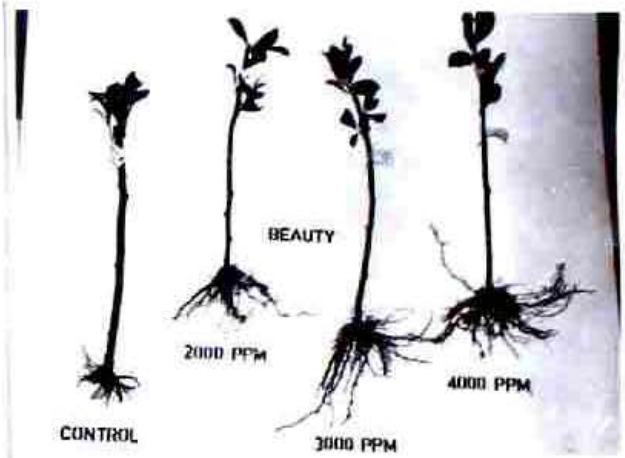
A perusal of Table 5 revealed that the shoot growth of the cuttings showed significant differences at the varietal level during both the year. Maximum average shoot length (11.3 and 12.2 cm) were recorded in the plum cv. Santa Rosa which was significantly higher than Beauty, Greengage and Early Transparent Gage, in both the years under study.

Season of planting was also found to be highly effective in influencing the average shoot length of all the plum cultivars in both the years. Cuttings from summer season produced maximum average shoot length of 12.2 cm in 1986, followed by dormant (8.9 cm) and autumn (7.9 cm). More or less similar trend was also evident during the year 1987.

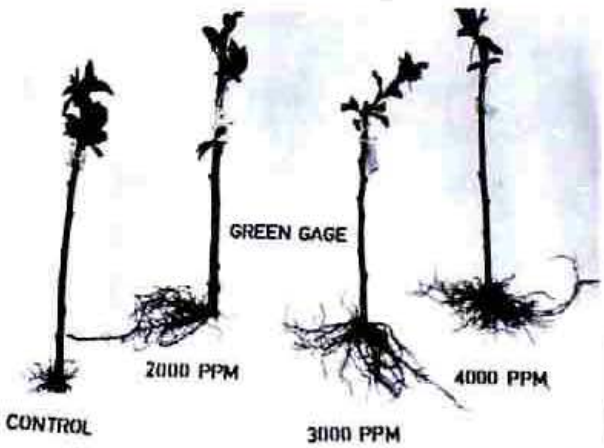
PLATE 2



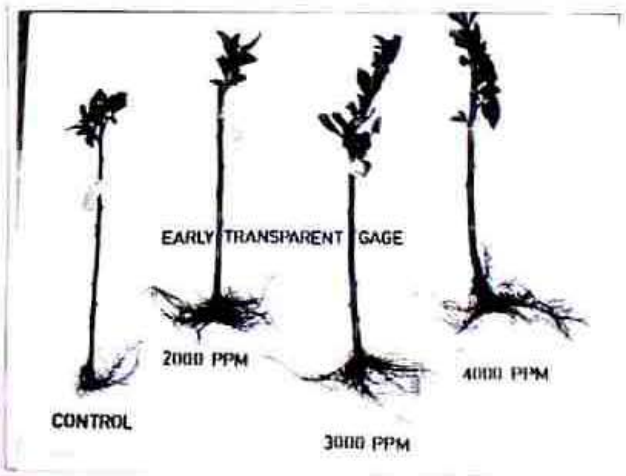
A



B



C



D

Plate 2.A-D. Rooting behaviour in autumn planted hardwood cuttings of plum as influenced by IBA treatments.

Table 4. Main effects and interactions between planting seasons and IBA concentrations on rooting of cuttings (in per cent) in plum

	1986					1987				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
S_1	22.8	50.0	53.9	46.3	43.2	25.9	47.5	54.4	44.4	43.1
S_2	30.0	64.4	55.6	49.4	49.9	33.1	65.6	59.3	52.5	52.6
S_3	19.4	41.9	47.5	40.0	40.0	22.5	43.1	49.4	38.8	38.4
Mean	24.0	53.1	52.3	45.2		27.2	52.1	54.1	45.2	

C.D.(P=0.05)

Season (S)	= 5.30	5.12
Concentrations (T)	= 5.29	5.92
Interaction (Season x Concentrations)	= 9.18	10.27

Table 5. Effect of IBA concentrations and planting seasons under different conditions on main shoot length (cm) from cuttings in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	7.0	10.0	13.3	11.8	8.0	11.2	14.8	12.5
Summer	10.0	16.5	15.0	13.8	9.3	17.5	16.3	15.0
Autumn	6.2	9.0	12.5	10.8	7.5	10.3	13.5	11.5
C_2 Beauty								
Dormant	6.3	8.7	11.0	9.7	5.8	10.2	12.3	10.8
Summer	8.5	15.0	13.5	12.5	9.0	15.7	14.2	13.3
Autumn	5.2	8.0	10.0	8.5	6.3	9.0	11.7	9.8
C_3 Greengage								
Dormant	5.5	8.3	10.2	9.0	6.0	9.5	11.2	9.7
Summer	7.8	14.5	12.8	11.5	8.8	15.5	13.5	12.5
Autumn	4.7	7.2	9.3	8.0	5.3	8.2	10.0	9.0
C_4 Early Transparent Gage								
Dormant	5.9	8.5	9.0	8.8	5.2	9.0	9.8	9.2
Summer	7.0	13.8	12.0	10.7	8.3	14.5	13.0	11.9
Autumn	4.3	7.0	8.5	8.2	5.0	8.3	9.7	8.8

Means of treatments

		1986	1987
a) Cultivars :	C_1	11.3	12.2
	C_2	9.8	10.7
	C_3	9.1	9.9
	C_4	8.6	9.4
	C.D. (P=0.05)	0.93	1.19
b) Seasons :	Dormant	8.9	9.7
	Summer	12.2	13.0
	Autumn	7.9	9.0
	C.D. (P=0.05)	0.72	1.03
c) IBA Concentrations:	0 ppm	6.5	7.0
	2000 ppm	10.5	11.6
	3000 ppm	11.4	12.8
	4000 ppm	10.3	11.2
	C.D. (P=0.05)	0.84	0.90

Statistical analysis of the data clearly indicated that summer cuttings of plum was much superior over dormant season. On the other hand, dormant season was significantly better than autumn season during both the years.

A perusal of data given in Table 5 also showed that the treatments with different IBA concentrations had a profound effect on the average shoot length from the cuttings of all the four plum cultivars in both the years. During the year 1986, the maximum average shoot length of 11.4 cm was recorded in the cuttings treated with 3000 ppm IBA concentrations which was found to be significantly superior to IBA at 2000 ppm and IBA at 4000 ppm. Cuttings under control had minimum average shoot length. The effect of different concentrations of IBA remained identical during the year 1987 with maximum shoot length producing in the cuttings treated with 3000 ppm IBA and minimum average shoot length obtained from the cuttings under control treatment.

The inter-action between season of planting and IBA treatments was found to be significant in both the years and is presented in Table 6. The interaction between season of planting and IBA concentrations resulted in maximum average shoot length of 14.9 cm and 15.8 cm from the cuttings treated with 2000 ppm IBA and planted during summer season against the minimum average shoot length of 5.1 cm and 6.0 cm produced by the cuttings under control

Table 6. Main effects and interactions between planting seasons and IBA concentrations on average shoot length (cm) from cuttings in plum

	1986					1987				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
S_1	6.1	8.9	10.9	9.8	8.9	6.3	10.0	12.0	10.6	9.7
S_2	8.3	14.9	13.3	12.1	12.2	8.8	15.8	14.3	13.1	13.0
S_3	5.1	7.8	10.0	8.9	7.9	6.0	8.9	11.3	9.7	9.0
Mean	6.5	10.5	11.4	10.3		7.0	11.6	12.5	11.1	

C.D.(P=0.05)

Season (S)	= 0.72	1.03
Concentrations (T)	= 0.84	0.80
Interaction (Season x Concentrations)	= 1.45	1.39

planted during autumn season in 1986 and 1987, respectively.

1.4. Diameter of shoots

The data recorded on the shoot diameter of cuttings from four plum cultivars as influenced by planting seasons and IBA treatments during 1986 and 1987 are presented in Table 7.

It is quite clear from Table 7 that the average shoot diameter was significantly influenced by the type of cultivars from which the cuttings were obtained. During 1986, the maximum average diameter of shoot (0.49 cm) was recorded in Santa Rosa which was statistically superior to Beauty, Greengage and Early Transparent Gage (0.43 cm). But Beauty, Greengage and Early Transparent Gage were found to be statistically at par with each other in respect of shoot diameter. A similar trend was also observed during 1987 which recorded the maximum average shoot diameter of 0.46 cm and the minimum with 0.42 cm.

During the year 1986, summer planted plum cuttings gave the best results producing 0.51 cm thick shoots which was followed by dormant and autumn planted cuttings. Similarly, in the year 1987, cuttings planted during summer season exhibited maximum average diameter of shoots (0.49 cm, whereas the autumn planted cuttings produced minimum average shoot diameter of 0.41 cm. Statistical analysis of the data had shown that shoot produced by summer planted cuttings was found to be significantly superior to other

Table 7. Effect of IBA concentrations and planting seasons under different conditions on main shoot diameter (in cm) from cuttings in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	0.43	0.45	0.50	0.48	0.40	0.47	0.51	0.47
Summer	0.50	0.59	0.55	0.54	0.49	0.60	0.58	0.51
Autumn	0.41	0.46	0.48	0.45	0.42	0.45	0.50	0.46
C_2 Beauty								
Dormant	0.40	0.42	0.46	0.45	0.41	0.44	0.48	0.42
Summer	0.46	0.56	0.53	0.51	0.44	0.54	0.50	0.48
Autumn	0.39	0.41	0.46	0.48	0.37	0.40	0.44	0.42
C_3 Greengage								
Dormant	0.38	0.45	0.45	0.44	0.40	0.41	0.46	0.43
Summer	0.43	0.49	0.54	0.50	0.45	0.53	0.48	0.46
Autumn	0.39	0.45	0.47	0.41	0.36	0.40	0.44	0.39
C_4 Early Transparent Gage								
Dormant	0.38	0.43	0.44	0.43	0.36	0.41	0.45	0.43
Summer	0.46	0.53	0.49	0.46	0.41	0.50	0.46	0.48
Autumn	0.34	0.36	0.44	0.42	0.35	0.41	0.43	0.40

Means of treatments

		1986	1987
a) Cultivars :	C_1	0.49	0.46
	C_2	0.46	0.44
	C_3	0.45	0.43
	C_4	0.43	0.42
	C.D. (P=0.05)	0.030	0.031
b) Seasons :	Dormant	0.44	0.43
	Summer	0.51	0.49
	Autumn	0.42	0.41
	C.D. (P=0.05)	0.026	0.027
c) IBA Concentrations :	0 ppm	0.41	0.41
	2000 ppm	0.48	0.46
	3000 ppm	0.48	0.47
	4000 ppm	0.46	0.45
	C.D. (P=0.05)	0.030	0.031

two seasons. However, the average shoot diameter from dormant and autumn planted cuttings were statistically at par with each other.

Treatments of plum cuttings with different IBA concentrations also resulted in a significant increase on average shoot diameter. Data in Table 7 clearly shows that IBA concentration at 3000 ppm produced significantly bigger shoots with 0.48 cm closely followed by 2000 ppm and 4000 ppm IBA treatments during 1986. More or less similar effect on the average diameter of shoots was recorded during 1987 with 3000 ppm IBA treated cuttings giving maximum average diameter of shoots with 0.47 cm closely followed by 2000 ppm and 4000 ppm IBA treatments. The minimum shoot diameter was obtained from the cuttings under control in both the years under study.

The interaction among cultivars, seasons of planting and IBA concentrations were not consistent and were found to be non-significant in both the years.

1.5. Number of primary roots per cutting

The data on number of primary roots per cutting for the year 1986 and 1987 are presented in Table 8 and depicted in Fig.2.

It is evident from Table 8 that during the year 1986, the cuttings of Santa Rosa produced maximum number of primary roots (17.8) per cutting which was statistically

Table 8. Effect of IBA concentrations and planting seasons under different conditions on the number of primary roots per cutting in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	7.8	19.7	24.0	21.3	9.0	21.7	25.8	22.5
Summer	12.7	29.5	26.5	24.0	13.5	28.5	26.0	25.5
Autumn	6.0	12.3	15.7	14.2	5.3	13.5	17.0	14.8
C_2 Beauty								
Dormant	6.8	17.7	22.3	20.2	7.5	19.5	23.7	22.0
Summer	10.8	24.0	23.5	19.5	11.7	25.8	21.5	22.5
Autumn	4.0	12.0	13.8	13.3	4.8	12.7	15.0	13.8
C_3 Greengage								
Dormant	6.0	16.8	20.5	19.0	7.5	18.2	21.8	19.5
Summer	8.8	21.5	21.2	18.8	9.7	23.0	22.5	20.2
Autumn	4.5	11.2	12.8	12.0	4.0	12.0	14.7	13.3
C_4 Early Transparent Gage								
Dormant	6.8	17.2	19.0	18.5	5.5	16.3	20.8	19.5
Summer	8.3	21.3	20.4	19.0	9.0	22.2	21.3	19.0
Autumn	3.8	11.7	13.3	12.8	4.3	11.0	12.5	12.2

Means of treatments

a) Cultivars :	C_1	17.8	18.7
	C_2	15.6	16.7
	C_3	14.5	15.8
	C_4	14.2	14.6
C.D. (P=0.05)		1.24	1.43
b) Seasons :	Dormant	16.5	17.6
	Summer	19.3	20.2
	Autumn	10.8	11.3
C.D. (P=0.05)		1.07	1.24
c) IBA Concentrations:	0 ppm	7.2	7.6
	2000 ppm	18.0	18.8
	3000 ppm	19.5	20.9
	4000 ppm	17.7	18.9
C.D. (P=0.05)		1.24	1.43

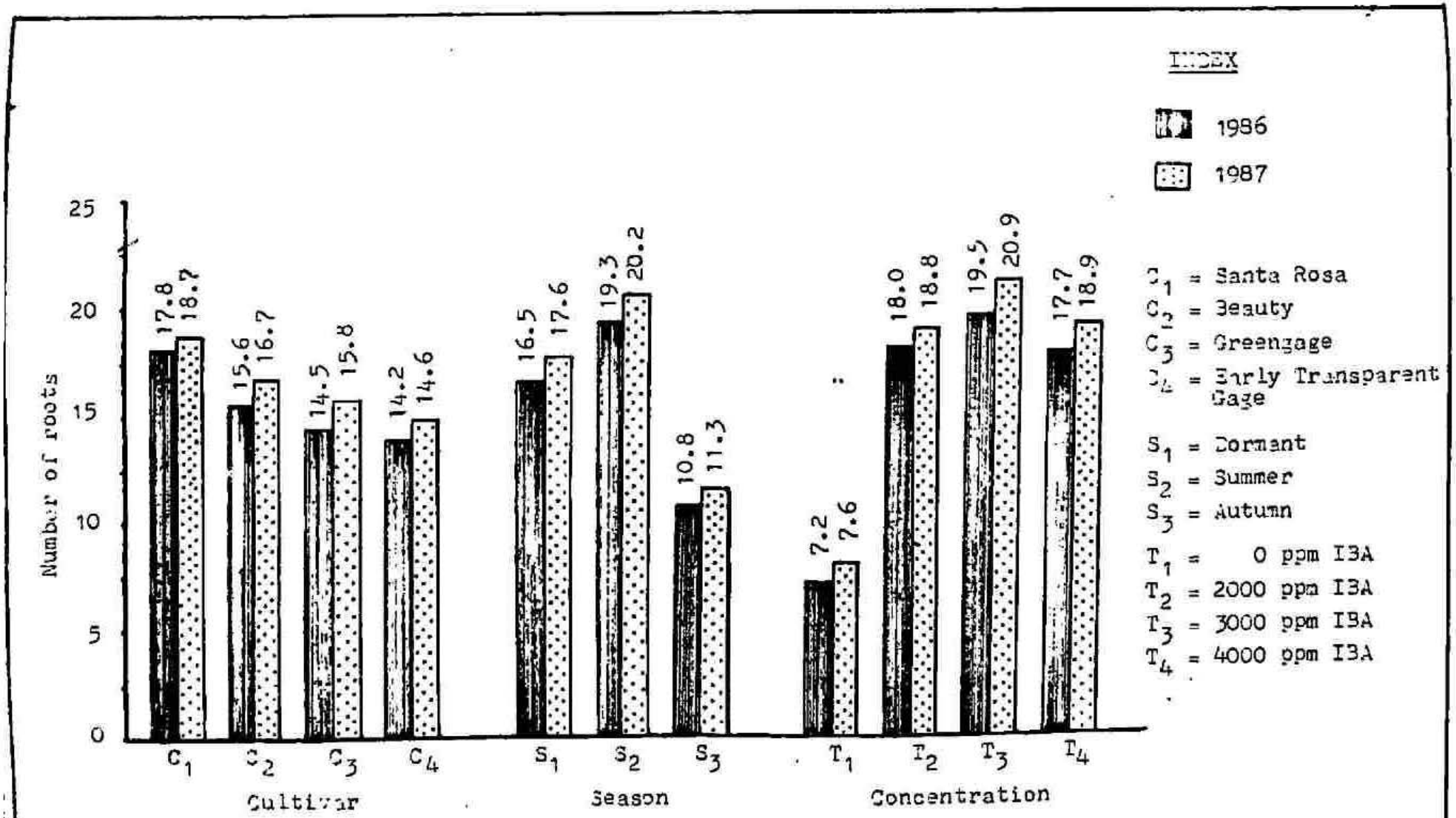


Fig.2. Effect of IBA concentrations and planting seasons under different conditions on the number of primary roots per cutting in plum.

superior to Beauty, Greengage and Early Transparent Gage. The cuttings of Beauty were at par with those of Greengage but were significantly superior to Early Transparent Gage in number of primary roots. The cuttings from Early Transparent Gage produced minimum number of primary roots (14.2) per cutting but were statistically at par with Greengage.

Similarly during 1987, maximum number of primary roots per cutting (18.7) were obtained from Santa Rosa plum cuttings which was significantly different from all the three cultivars. The cuttings of Beauty and Greengage as well as the cuttings of Greengage and Early Transparent Gage were at par with each other. However, the cuttings of Beauty were significantly better than Early Transparent Gage which produced the minimum number of primary roots (14.6) per cutting during 1987.

Season of planting had shown a remarkable effect on increasing number of primary roots per cutting in both the years of study. Cuttings planted during summer produced maximum number of primary roots with 19.3 during 1986 and 20.2 during 1987 followed by dormant and autumn season plantings in both the years. The summer season was thus found to be significantly superior over dormant and autumn planting seasons. However, the dormant planted cuttings were significantly better than autumn planted cuttings in production of maximum number of primary roots per cutting.

The lowest number of primary roots per cutting in both the years were recorded under control during autumn season.

The results presented in Table 8 also revealed that during 1986, 3000 ppm IBA treated cuttings produced maximum number (19.5) of primary roots per cutting followed by 2000 ppm and 4000 ppm IBA treatments. Cuttings under control produced minimum number of primary roots (7.2) per cutting. The IBA at 3000 ppm was found to be significantly superior to the remaining IBA treatments. More or less identical results were recorded during 1987, in which maximum primary roots per cutting was produced by cuttings treated with 3000 ppm IBA and the minimum was obtained from control treatment. The treatment of cuttings with 2000 ppm and 4000 ppm IBA did not show any significant difference with each other. However, it was noticed that the cuttings planted during 1987 produced more number of primary roots per cutting as compared to those cuttings planted during the year 1986.

It is evident from Table 9 that the interaction between season of planting and IBA treatments had shown significant difference on the number of primary roots per cutting in both the years under study and a combination of summer planting with 2000 ppm IBA treatment was judged best in producing maximum number of 24.3 and 25.2 primary roots per cutting in the year 1986 and 1987, respectively. Minimum number of primary roots per cutting were recorded in a combination when untreated (control) cuttings were planted

Table 9. Main effects and interactions between planting seasons and IBA concentrations on number of primary roots per cutting in plum

	1986					1987				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
S_1	6.8	17.9	21.6	19.7	16.5	7.3	18.9	23.0	21.3	17.6
S_2	10.0	24.3	22.7	20.3	19.3	11.5	25.2	22.8	21.9	20.2
S_3	4.6	11.8	13.9	13.1	10.8	4.6	12.3	14.8	13.5	11.3
Mean	7.8	18.0	19.3	17.7		7.6	18.8	20.2	18.9	

C.D.(P=0.05)

Season (S)	=	1.07 1.24
Concentrations (T)	=	1.24 1.43
Interaction (Season x Concentrations)	=	2.14 2.48

in autumn of both the years.

1.6. Length of primary roots per cutting

The data recorded on the length of the primary roots per cutting as affected by different cultivars, planting seasons and IBA concentrations are presented in Table 10.

It was observed that the results in respect of the cultivars, planting seasons and IBA concentrations were significant during both the years of study.

The data presented in Table 10 indicated that in both the years of study, different types of plum cultivars had shown considerable effect on the root length produced by the rooted cuttings. Longest primary root per cutting (11.9 cm during 1986 and 12.5 cm during 1987) were produced by Santa Rosa cuttings which was significantly superior over all other cultivars. However, plum cultivars Greengage and Early Transparent Gage were statistically at par with each other and Early Transparent Gage produced minimum length of primary roots per cutting in both the years with 8.7 cm and 9.3 cm, respectively.

In both the years, cuttings planted during summer season showed maximum root length (12.3 cm in 1986 and 12.9 cm in 1987) while the cuttings planted during dormant and autumn seasons of both the years had relatively lower root length. Statistical analysis of the data showed that

Table 10. Effect of IBA concentrations and planting seasons under different conditions on primary root length (in cm) from cuttings in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	6.3	13.5	11.9	11.3	7.1	14.5	12.5	12.0
Summer	10.1	19.0	17.1	15.2	10.8	18.2	17.9	16.0
Autumn	5.5	11.7	10.8	10.0	6.5	13.2	11.8	9.4
C_2 Beauty								
Dormant	5.2	12.3	11.8	10.1	6.0	13.1	11.0	10.8
Summer	7.8	15.5	13.9	12.1	8.8	14.4	14.6	13.1
Autumn	4.9	10.5	9.4	9.0	5.6	11.6	10.1	8.6
C_3 Greengage								
Dormant	4.7	11.2	10.5	9.2	5.5	12.1	11.1	10.1
Summer	6.4	13.8	12.6	11.5	7.4	14.1	13.2	12.4
Autumn	5.4	8.9	8.4	7.4	4.5	10.1	9.3	8.1
C_4 Early Transparent Gage								
Dormant	5.0	10.8	10.2	8.4	4.5	11.7	9.8	9.1
Summer	5.8	12.7	12.0	11.3	6.5	13.8	13.1	12.0
Autumn	4.3	8.6	8.3	7.0	4.7	9.5	9.4	7.8
Means of treatments								
a) Cultivars :		C_1		11.9			12.5	
		C_2		10.2			10.7	
		C_3		9.2			9.8	
		C_4		8.7			9.3	
	C.D. (P=0.05)			0.89			0.94	
b) Season :		Dormant		9.5			10.1	
		Summer		12.3			12.9	
		Autumn		8.1			8.8	
	C.D. (P=0.05)			0.78			0.81	
c) IBA Concentrations:		0 ppm		5.9			6.5	
		2000 ppm		12.4			13.0	
		3000 ppm		11.4			12.0	
		4000 ppm		10.2			10.8	
	C.D. (P=0.05)			0.89			0.94	

summer planted cuttings had significantly higher root length than dormant and autumn plantings. However, cuttings planted during dormant season were also superior to autumn planted cuttings in both the years of study.

The effect of IBA treatments on the root length per cutting were also significantly different from one another. Maximum root length (12.4 cm) was found in cuttings treated with 2000 ppm IBA followed by IBA at 3000 ppm, 4000 ppm and control during the year 1986. Similar results on the effect of different IBA treatments on average root length were observed in 1987. Minimum average root length per cutting were observed under control in both the years.

All the interactions between cultivars, season of planting and IBA treatments were found to be non-significant during both the years.

1.7. Diameter of primary roots

The observations on the diameter of primary roots produced in the cuttings of four plum cultivars during the year 1986 and 1987 are presented in Table 11. It was observed from the data recorded in Table 11 that the results obtained in respect of cultivars, seasons of planting and IBA treatments were significant in both the years 1986 and 1987.

It is evident from Table 11 that during the year 1986, the maximum diameter of primary root (0.23 cm) obtained from Santa Rosa was significantly superior to Beauty,

Table 11. Effect of IBA concentrations and planting seasons under different conditions on the diameter (in cm) of primary root from the cuttings in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	0.16	0.24	0.23	0.21	0.18	0.25	0.24	0.22
Summer	0.21	0.29	0.27	0.26	0.23	0.31	0.29	0.28
Autumn	0.15	0.23	0.21	0.22	0.16	0.25	0.24	0.21
C_2 Beauty								
Dormant	0.15	0.23	0.22	0.21	0.16	0.24	0.23	0.20
Summer	0.19	0.25	0.24	0.20	0.20	0.26	0.25	0.23
Autumn	0.14	0.22	0.21	0.20	0.14	0.23	0.21	0.21
C_3 Greengage								
Dormant	0.15	0.21	0.20	0.20	0.14	0.24	0.22	0.20
Summer	0.18	0.24	0.23	0.21	0.18	0.26	0.25	0.20
Autumn	0.14	0.21	0.20	0.19	0.15	0.22	0.21	0.21
C_4 Early Transparent Gage								
Dormant	0.14	0.21	0.20	0.19	0.15	0.23	0.21	0.20
Summer	0.16	0.23	0.21	0.18	0.18	0.24	0.23	0.21
Autumn	0.14	0.20	0.20	0.19	0.14	0.21	0.22	0.20
Means of treatments								
a) Cultivars :		C_1		0.23			0.24	
		C_2		0.20			0.21	
		C_3		0.19			0.21	
		C_4		0.19			0.20	
	C.D. (P=0.05)			0.019			0.020	
b) Seasons :		Dormant		0.20			0.21	
		Summer		0.22			0.24	
		Autumn		0.19			0.20	
	C.D. (P=0.05)			0.016			0.017	
C) IBA Concentrations:		0 ppm		0.16			0.17	
		2000 ppm		0.23			0.25	
		3000 ppm		0.22			0.23	
		4000 ppm		0.21			0.21	
	C.D. (P=0.05)			0.019			0.019	

Greengage and Early Transparent Gage. The last three cultivars were statistically at par with each other. During the year 1987, the maximum root diameter (0.24 cm) was attained in the cuttings of Santa Rosa which was significantly higher than the remaining cultivars for this character. The cuttings of Beauty and Greengage were, however, at par with each other but were significantly superior to Early Transparent Gage.

In both the years under study, summer planting of cuttings produced maximum diameter of root (0.22 cm during 1986 and 0.24 cm during 1987), which was significantly superior to those planted during dormant and autumn seasons. The data also showed that the average diameter of roots from cuttings planted during summer season was significantly higher than those in dormant and autumn seasons in both the years of study.

The data given in Table 11 has also indicated that the treatment of cuttings in IBA at 2000 ppm gave the best result with 0.23 cm thick roots in 1986 and 0.25 cm in 1987, which was found to be significantly superior to other concentrations during both the years under study. The minimum diameter of primary roots was, however, observed in the untreated cuttings with 0.16 cm and 0.17 cm diameter in 1986 and 1987, respectively.

The results in respect of interaction were not consistent and found to be non-significant in both the years with respect to this character.

1.8. Number of secondary roots per cutting

The data recorded on the number of secondary roots per cutting are presented in Table 12. It was found that the results in respect of cultivars, planting seasons and IBA concentrations were significant during both the years.

It is evident from the data given in Table 12 that during 1986, maximum number of secondary roots (47.2) per cutting was recorded in Santa Rosa which was significantly superior to the remaining cultivars. The cuttings of Beauty and Greengage were statistically at par with each other. Similarly, the cuttings of Greengage and Early Transparent Gage were at par with one another. However, Beauty cultivar was significantly superior to Early Transparent Gage in this respect. More or less similar effect were found during 1987 in which the cuttings from Santa Rosa produced the maximum number of secondary roots (49.7) per cutting showing its superiority to all the other cultivars under study. However, no significant difference were observed between the cuttings of Beauty and Greengage and also between the cuttings of Greengage and Early Transparent Gage. But its was observed that Beauty was significantly superior to Early Transparent Gage which gave the minimum average number of secondary roots per cutting with 40.3.

All the cuttings planted during summer season showed higher number of secondary roots per cutting with 48.1 roots during 1986 and 50.2 roots during the year 1987, which

Table 12. Effect of IBA concentrations and planting seasons under different conditions on the number of secondary roots per cuttings in plum

Cultivar/ Season	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
C_1 Santa Rosa								
Dormant	22.2	45.8	58.2	51.5	24.5	47.7	61.2	53.5
Summer	29.8	56.5	76.3	63.5	28.5	60.5	80.2	67.2
Autumn	19.7	41.5	53.2	47.8	21.5	44.5	57.0	49.5
C_2 Beauty								
Dormant	21.0	43.0	54.0	49.0	20.0	46.3	57.0	50.2
Summer	22.3	48.7	65.5	53.8	24.5	50.8	63.7	57.0
Autumn	18.3	38.5	49.3	44.5	19.5	42.3	51.8	45.7
C_3 Greengage								
Dormant	18.3	41.2	50.7	46.2	20.0	44.2	55.5	49.3
Summer	20.7	45.2	61.2	52.0	23.5	48.7	56.8	52.5
Autumn	16.8	36.0	45.3	41.0	18.5	39.0	48.2	42.0
C_4 Early Transparent Gage								
Dormant	17.2	38.5	45.7	43.2	19.3	40.2	50.0	46.2
Summer	19.0	47.7	57.0	49.2	21.2	44.3	60.5	53.5
Autumn	15.7	35.2	42.0	40.3	17.8	36.5	46.0	42.0

Means of treatments

a) Cultivars :	C_1	47.2		49.7
	C_2	42.3		44.2
	C_3	39.6		42.5
	C_4	38.0		40.3
	C.D. (P=0.05)	3.41		3.00
b) Seasons :	Dormant	40.5		43.0
	Summer	48.1		50.2
	Autumn	36.5		39.1
	C.D. (P=0.05)	2.95		2.60
c) IBA Concentrations:	0 ppm	20.1		21.7
	2000 ppm	43.2		45.5
	3000 ppm	55.0		58.0
	4000 ppm	48.5		51.0
	C.D. (P=0.05)	3.41		3.00

was significantly higher than those planted in dormant and autumn seasons. The data given in Table 12 further revealed that the dormant season planting was significantly superior to autumn season planting in both the years of study.

It is evident from the data recorded in Table 12 that the maximum number of secondary roots per cutting of 55.0 and 58.0 were recorded during 1986 and 1987, respectively, which were significantly superior to other IBA concentrations. Cuttings under control treatment produced the minimum number of secondary roots per cutting in both the years with 20.1 and 21.7 roots, respectively.

The influence of cultivars, time of planting and IBA treatments was found to be non-significant during both the years.

1.9. Field survival percentage of the cuttings

The data in respect of survival percentage of cuttings are presented in Table 13 and depicted in Fig.3. It was found that the results in respect of cultivars, seasons of planting and IBA treatments were significant both in 1986 and 1987.

During the year 1986, maximum percentage of filed survival (65.3 per cent) was recorded in the cuttings of Santa Rosa, which was significantly superior to all the other cultivars under study. There were no significant differences among the cuttings of Beauty, Greengage and Early Transparent Gage which were at par with each other

Table 13. Effect of IBA concentrations and planting seasons under different conditions on field survival of rooted cuttings (in per cent) in plum

Cultivar/ Season	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
C_1 Santa Rosa								
Dormant	49.6	62.7	63.3	52.1	53.0	70.9	69.0	64.8
Summer	52.0	83.5	78.3	75.7	55.6	85.2	77.6	73.0
Autumn	54.2	68.8	72.7	70.9	56.3	74.1	76.3	74.2
C_2 Beauty								
Dormant	44.9	57.4	51.6	50.4	47.4	60.8	67.4	61.5
Summer	50.7	72.1	70.0	67.8	52.8	75.7	72.4	73.1
Autumn	47.6	60.7	63.6	60.4	50.0	65.8	69.7	66.2
C_3 Greengage								
Dormant	41.7	54.7	58.4	54.2	43.8	56.4	61.9	58.5
Summer	46.8	68.7	66.2	64.7	50.0	73.1	71.4	66.0
Autumn	45.7	56.7	60.4	58.3	46.6	61.7	65.9	62.7
C_4 Early Transparent Gage								
Dormant	44.2	51.7	59.2	48.9	42.8	54.2	64.8	57.9
Summer	47.4	66.6	63.5	59.5	46.7	71.3	67.7	65.2
Autumn	48.3	52.5	61.7	55.4	45.3	57.4	65.0	60.3

Means of treatments

a) Cultivars :	C_1	65.3	69.2
	C_2	58.9	63.5
	C_3	56.4	59.8
	C_4	54.9	58.2
	C.D. (P=0.05)	6.28	6.04
b) Seasons :	Dormant	53.4	58.4
	Summer	64.5	67.3
	Autumn	58.6	62.3
	C.D. (P=0.05)	4.21	4.59
c) IBA Concentrations :	0 ppm	47.7	49.2
	2000 ppm	63.0	67.2
	3000 ppm	64.9	69.1
	4000 ppm	59.9	65.3
	C.D. (P=0.05)	5.09	5.45

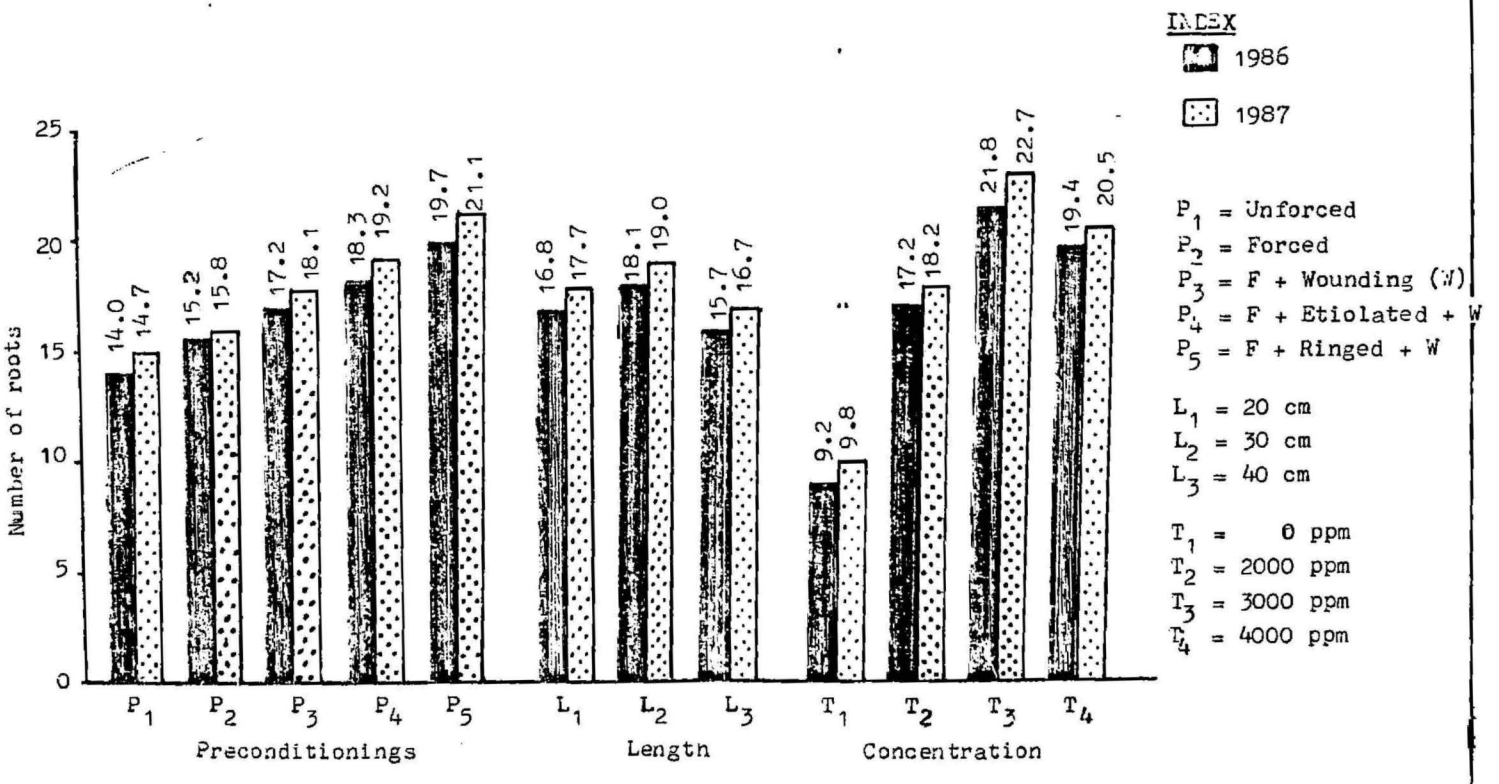


Fig. 13. Effect of preconditioning treatments, size of cuttings and IBA concentrations on number of primary roots per cuttings of plum cv.Santa Rosa

in this respect. The minimum survival percentage was shown by Greengage with 54.9 per cent.

More or less similar trend was observed during 1987 in which the maximum field survival of 69.2 per cent was obtained in Santa Rosa. This was significantly superior to the cuttings of the remaining cultivars. Beauty, Greengage and Early Transparent Gage cultivars did not differ significantly from each other in this character. However, the cuttings of Early Transparent Gage had shown minimum field survival with 58.2 per cent.

During both the years under study, season of planting had also shown significant effect on survival rate of the cuttings. Planting of cuttings during summer season was found best exhibiting 64.5 per cent field survival in 1986 and 67.3 per cent in 1987 which were significantly better than both dormant and autumn season plantings. Statistical analysis of the data further showed that dormant and autumn season planting were at par with each other in this respect.

During the year 1986, maximum field survival of 64.9 per cent was recorded when the cuttings were treated with 3000 ppm IBA whereas during the year 1987, the maximum field survival (69.1 per cent) was observed in the cuttings treated with 3000 ppm IBA. This was closely followed by IBA treatments at 2000 ppm and 4000 ppm, respectively. These IBA concentrations showed an identical effect on the average field survival of the plants raised through cuttings in both

the years. The different IBA treatments were significantly superior to control treatment, which recorded the minimum survival percentage with 47.7 and 49.2 per cent during 1986 and 1987, respectively.

The differences due to interactions among all the factors were not consistent and found to be non-significant. This implies that these factors, that is, cultivars, seasons of planting and IBA treatments did not influenced field survival of different plum cuttings.

2. Biochemical status of plum cuttings in relation to rooting behaviour

The data in respect of different biochemical status of plum cuttings including nitrogen, carbohydrates, carbohydrates/nitrogen ratio, total phenols and ortho dihydric phenols are presented in Table 14.

2.1. Total nitrogen

It is evident from Table 14 that maximum nitrogen content of 0.75 per cent was recorded in Beauty plum cuttings during January whereas the minimum content of 0.53 per cent was recorded in Early Transparent Gage during July in the year 1986. During tne year 1987, nitrogen contents ranged between 0.52 and 0.78 per cent with the maximum nitrogen content recorded from January planted cuttings in Beauty plum and the minimum was recorded in Early Transparent Gage during July.

Table 14. Mean values of some biochemical constituents (on dry wt.basis) and their relationship with rooting of hardwood and semi-hardwood cutting of plum

Cultivars	Year/date of planting cuttings	Mean rooting (%)	Total nitrogen (%)	Total carbohydrate (%)	C/N ratio	Total phenols (mg/g)	O.D. phenols (mg/g)
Santa Rosa	1986						
	Jan., 14	57.8	0.73	21.1	28.9	28.4	8.1
	July, 14	68.1	0.60	19.7	32.8	25.3	7.8
	Oct., 14	51.9	0.64	17.5	27.3	22.3	7.4
	Correlation coefficient(r)	-	-0.44	+0.47	+0.91**	+0.35	+0.43
	1987						
	Jan., 14	56.3	0.70	22.3	31.9	31.6	8.4
	July, 14	70.0	0.57	20.2	35.4	27.8	8.0
	Oct., 14	52.5	0.60	16.8	28.0	25.5	6.5
	(r)	-	-0.51	+0.34	+0.88**	+0.07	+0.72**
Beauty	1986						
	Jan., 14	47.0	0.75	20.8	27.7	32.8	9.2
	July, 14	50.6	0.61	17.7	29.0	30.9	7.8
	Oct., 14	40.0	0.72	19.0	26.4	27.0	6.3
	(r)	-	-0.61*	+0.24	+0.87**	+0.63*	+0.66*
	1987						
	Jan., 14	45.3	0.78	20.1	26.8	33.2	9.1
	July, 14	54.9	0.63	19.1	30.3	32.4	8.4
	Oct., 14	41.3	0.74	18.6	25.1	29.5	7.2
	(r)	-	-0.83**	+0.02	+0.89**	+0.57*	+0.43
Greengage	1986						
	Jan., 14	35.6	0.72	17.2	23.9	31.0	9.4
	July, 14	40.6	0.61	15.5	25.4	23.3	8.3
	Oct., 14	30.6	0.65	14.9	22.9	26.8	7.5
	(r)	-	-0.39	+0.21	+0.86**	-0.35	+0.42
	1987						
	Jan., 14	36.5	0.77	18.9	24.5	32.5	10.7
	July, 14	43.8	0.56	16.2	28.9	25.2	9.6
	Oct., 14	32.0	0.69	15.7	22.8	29.3	8.0
	(r)	-	-0.70**	+0.01	+0.84**	-0.37	+0.47*
Early Transparent Gage	1986						
	Jan., 14	32.5	0.70	17.7	25.3	30.7	8.8
	July, 14	39.4	0.53	15.4	29.1	22.6	6.2
	Oct., 14	26.3	0.64	13.5	21.1	27.6	7.0
	(r)	-	-0.65*	+0.42	+0.88**	-0.63*	+0.62*
	1987						
	Jan., 14	34.0	0.76	20.6	27.1	28.8	8.6
	July, 14	39.6	0.52	16.3	31.3	25.7	8.2
	Oct., 14	27.5	0.65	15.8	24.3	27.5	7.0
	(r)	-	-0.49	+0.14	+0.86**	-0.54	+0.3

* Significant at 5 per cent

** significant at 1 per cent

The nitrogen content had a negative response with the seasonal rooting percentage of plum cuttings as was shown by the significant negative correlation coefficient values in both the years. The highest negative correlation coefficient (r=-0.65) was recorded in the basal cuttings of Early Transparent Gage whereas the lowest (r=-0.39) was recorded in Greengage cuttings during 1986. During the year 1987, Beauty cuttings had shown the maximum response with the seasonal rooting ability of the cuttings as was exhibited by its significant negative correlation coefficient (r=-0.83) while the minimum (r=-0.49) was recorded in Early Transparent Gage.

2.2. Total carbohydrates

Maximum carbohydrates content of 21.1 per cent was recorded in the cuttings of Santa Rosa in January whereas the minimum content of 13.5 per cent was recorded in Early Transparent Gage plum cuttings during 1986. During 1987, the maximum contents of carbohydrates was recorded in Santa Rosa cuttings with 22.3 per cent while the minimum value of 15.7 per cent was obtained from Greengage plum cuttings during October.

The contents of carbohydrates showed a positive associations with the rooting ability of the cuttings as was exhibited by the positive correlation coefficient in both the years. The highest positive correlation coefficient (r=+0.47 and +0.34) were recorded in Santa

Rosa plum cuttings while the minimum positive associations (r=+0.21 and +0.01) were recorded from Greengage during 1986 and 1987, respectively.

2.3. Carbohydrates/Nitrogen ratio (C/N ratio)

The data presented on Table 14 revealed that maximum C/N ratio of 32.8 was exhibited in Santa Rosa plum cuttings collected during July while the minimum ratio of 21.1 was recorded in October planted cuttings of Early Transparent Gage during 1986. Similarly, the highest C/N ratio of 35.4 was recorded in Santa Rosa cuttings during July and the lowest C/N ratio (22.8) was observed in October planted cuttings during the year 1987.

The C/N ratios were found to be positively correlated with the average rooting percentage of the cuttings as was shown by the significant positive correlation coefficient in both the years under study. During 1986, the maximum positive correlation coefficient (r=+0.91) of C/N ratio in relation to their rooting ability of the cuttings was obtained from Santa Rosa plum cuttings whereas the minimum (r=+0.86) was obtained from Greengage cuttings. However, during 1987, the maximum positive correlation coefficient (r=+0.89) was recorded in Beauty plum cuttings while the minimum (r=+0.84) was recorded from Greengage plum cuttings.

2.4. Total phenols

The perusal of Table 14 indicated that maximum total phenols contents of 32.8 mg/g was obtained in Beauty plum

cuttings during January and the minimum content of 22.3 mg/g was obtained from October planted cuttings of Santa Rosa plum during the year 1986. Similarly, the maximum content of total phenols (33.2 mg/g) was recorded in Beauty but the minimum of 25.2 mg/g was recorded in July collected Greengage plum cuttings during the year 1987.

In both the years, the contents of total phenols showed a positive associations with respect to the rooting ability of the cuttings in case of Santa Rosa and Beauty plum. However, in case of Greengage and Early Transparent Gage, total phenols contents showed a negative correlations with respect to the rooting of cuttings in both the years.

2.5. Ortho dihydric phenols (O.D. phenols)

It is evident from the data presented in Table 14 that maximum O.D. phenols (9.4 mg/g) was recorded in the Greengage cuttings collected during January whereas the minimum content of 6.3 mg/g was recorded in October planted cuttings of Beauty plum during the year 1986. During 1987, the contents of O.D. phenols ranged from 6.5 to 10.7 mg/g with maximum O.D. phenols recorded from the cuttings of Greengage collected during January while the minimum O.D. phenols content was recorded from the cuttings of Santa Rosa which were collected during October.

The contents of O.D. phenols were positively correlated with the rooting propensity of plum cuttings in both the years under study as was shown by their positive

correlation coefficient in Table 14. During 1986, the highest positive correlation coefficient (r=+0.66) was recorded from Beauty plum cuttings while the minimum (r=+0.42) was recorded from Greengage cuttings. However, the highest positive correlation coefficient (r=+0.72) was recorded from Santa Rosa plum which was found to be significant whereas the minimum (r=+0.31) was recorded from Early Transparent Gage during 1987.

3. Status of endogenous promoters and inhibitors in the cuttings at zero and 60 days after planting

In order to elucidate the influence of physiological status of plum cultivars on their rooting propensity, the endogenous levels of promoters and inhibitors were determined in both the hardwood and semi-hardwood cuttings under study. The histograms relating to the endogenous levels of promoters and inhibitors present in the semi-hardwood and hardwood cuttings at zero and 60 days after their planting are presented in Figs. 4-11.

3.1. Status of endogenous promoters

The growth promoting and inhibiting activities in the extracts of basal segments of fresh (zero day) and rooted cuttings (60 days) of different plum cultivars as detected through Mung bean bioassay are depicted in figures 4-7 in the form of histograms. Each histogram represents the activity of one gram (fresh weight) of basal segments of cuttings. Each column represents a chromatogram section, the origin

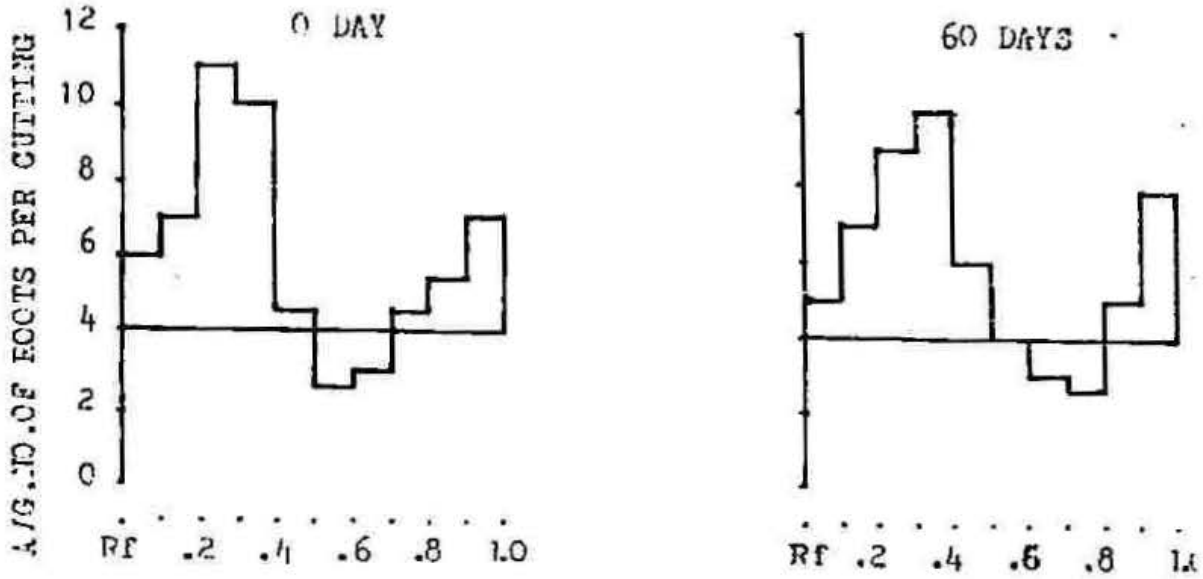
being on the left. The columns above the horizontal lines indicated the promotive bioactivities of rooting while the columns below the same line represented the root inhibition activities. The promotion or inhibition obtained from each chromatogram strip was entirely due to the material present on that strip, since the vials contained no additives other than glass distilled water.

It is evident from the figure 4 that in Santa Rosa plum, the Mung bean bioassay of the extracts obtained at zero day from fresh semi-hardwood cuttings showed the presence of root promoting factors at Rf 0.2-0.4 as well as 1.0 and the inhibitory activity appeared at Rfs 0.6-0.7. After 60 days, the basal extracts of rooted cuttings showed the peak zones of promotions at Rfs 0.2-0.5 and 0.9-1.0 and the inhibitory activity was located at Rfs 0.7-0.8.

Mung bean bioassay of the extract of fresh hardwood cuttings showed the presence of peak zones of promotion at Rfs 0.1-0.4 and 0.8-1.0. However, the strongest promotive activity was obtained at Rfs 0.3-0.4, 0.8 and 1.0 while the inhibitory activity was observed at Rfs 0.5-0.7. After 60 days, the activities of rooting promoters were decreased at Rfs 0.2-0.4 and 0.9-1.0 while the inhibitory activity was conspicuous only at Rfs 0.6-0.7.

In Beauty plum the bioactivity of extracts obtained from fresh semi-hardwood cuttings at the time of planting at zero day revealed that the zones of promotion were found

SEMI-HARDWOOD CUTTINGS OF PLUM cv. SANTA ROSA



HARDWOOD CUTTINGS OF PLUM cv. SANTA ROSA

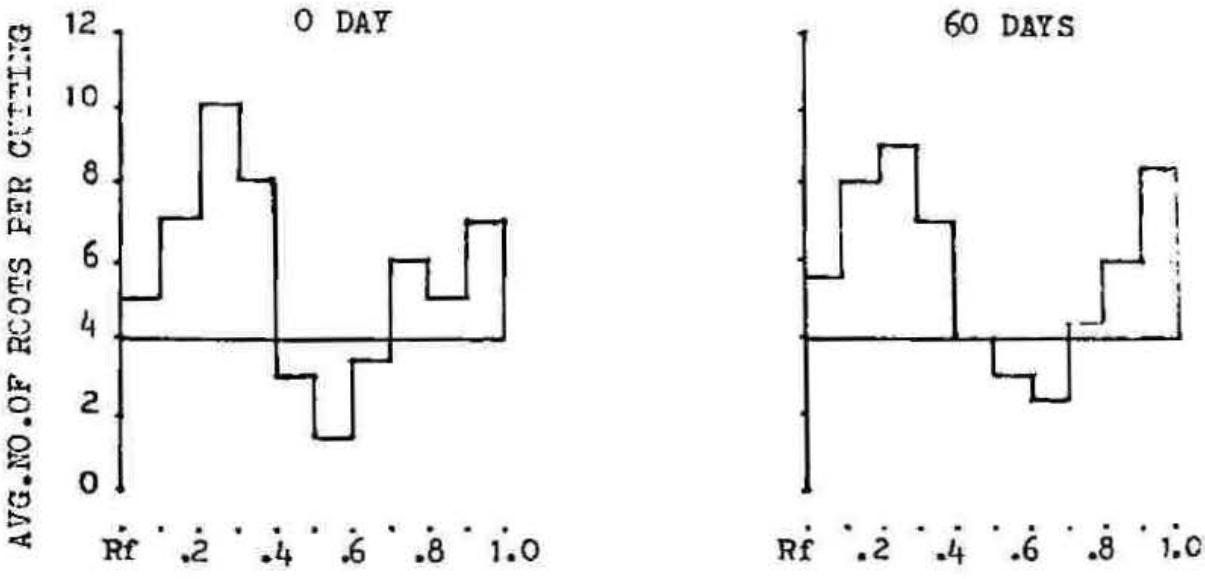


Fig. 4. Activity of endogenous rooting promoters extracted from basal segments of rooted (after 60 days) cuttings of Santa Rosa plum. Each histogram represents the activity of 1.0 g (fresh weight).

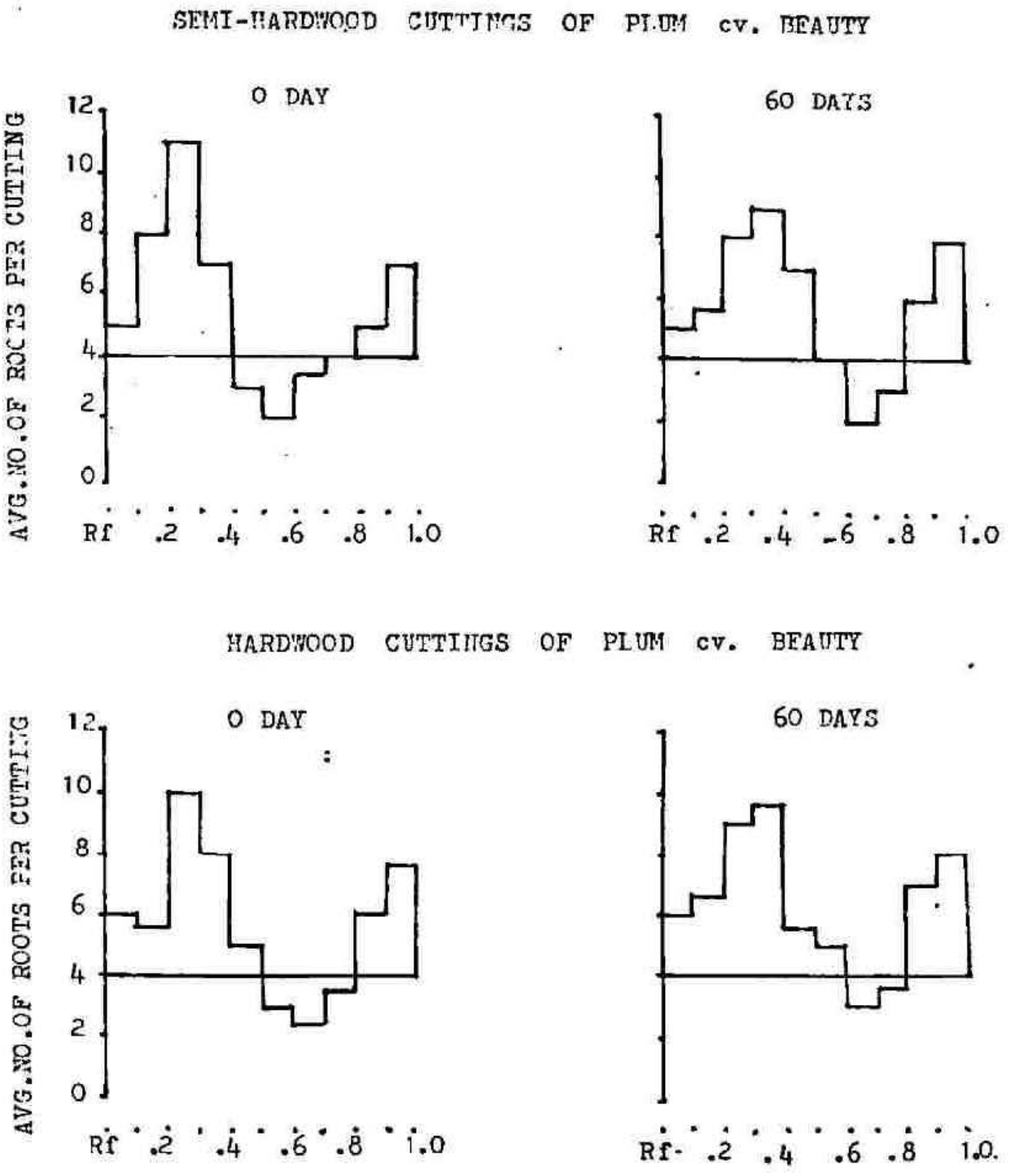


Fig.5. Activity of endogenous rooting promoters extracted from basal segments of rooted (after 60 days) cuttings of Beauty plum.

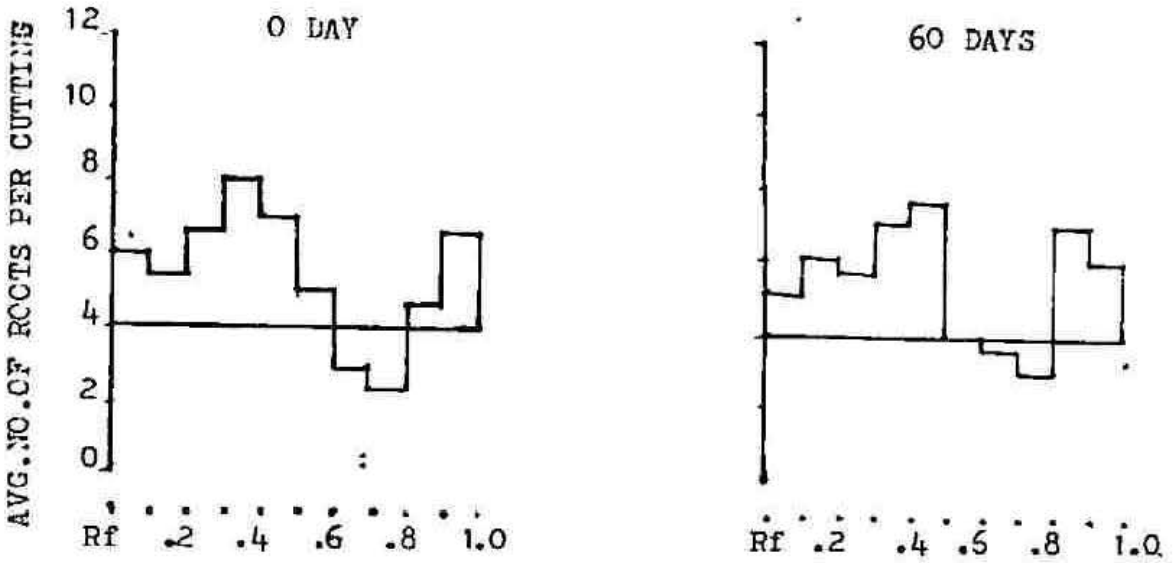
at Rfs 0.2-0.4 and 1.0 whereas for rooting inhibition, they were obtained at Rfs 0.5-0.7 (Fig.5). Sixty days after planting, the extracts of the basal rooted cuttings showed a considerable reduction in the activity of root promotion at Rfs 0.1-0.4 but this activity was increased at Rf position 0.9-1.0 and the inhibitory activities were also simultaneously increased at Rfs 0.7-0.8.

In case of the basal segments of hardwood cuttings, an appreciably higher promoter activities was found at Rfs 0.1, 0.3-0.4 and 0.9-1.0 while a slight inhibitory zones were observed at Rfs 0.6-0.8. After 60 days in the rooted cuttings, the activities of the promoters were found very less at Rfs 0.5-0.6, the peak promotive activities were at Rfs 0.3-0.4 and 0.9-1.0 and the inhibitory activity was observed at Rfs 0.7-0.8.

Bioassay of the basal cutting extracts of semi-hardwood fresh cuttings of Greengage plum revealed that the zones of promotion were at Rfs 0.1, 0.3-0.5 and 1.0 (Fig.6). The inhibitory activity was also found in the cuttings at Rfs 0.7-0.8. Similar zones of activity were also observed after 60 days of planting but the promotive activity at Rf 0.6 was reduced considerably whereas that of Rf position at 0.9 was appreciably higher in the extracts of basal segments of the cuttings.

In case of basal extracts from fresh hardwood cuttings, the zones of root promotion were seen at Rfs 0.4-0.5 and 1.0 while the inhibitory activity was found at Rfs 0.6-0.9.After

SEMI-HARDWOOD CUTTINGS OF PLUM cv. GREENGAGE



HARDWOOD CUTTINGS OF PLUM cv. GREENGAGE

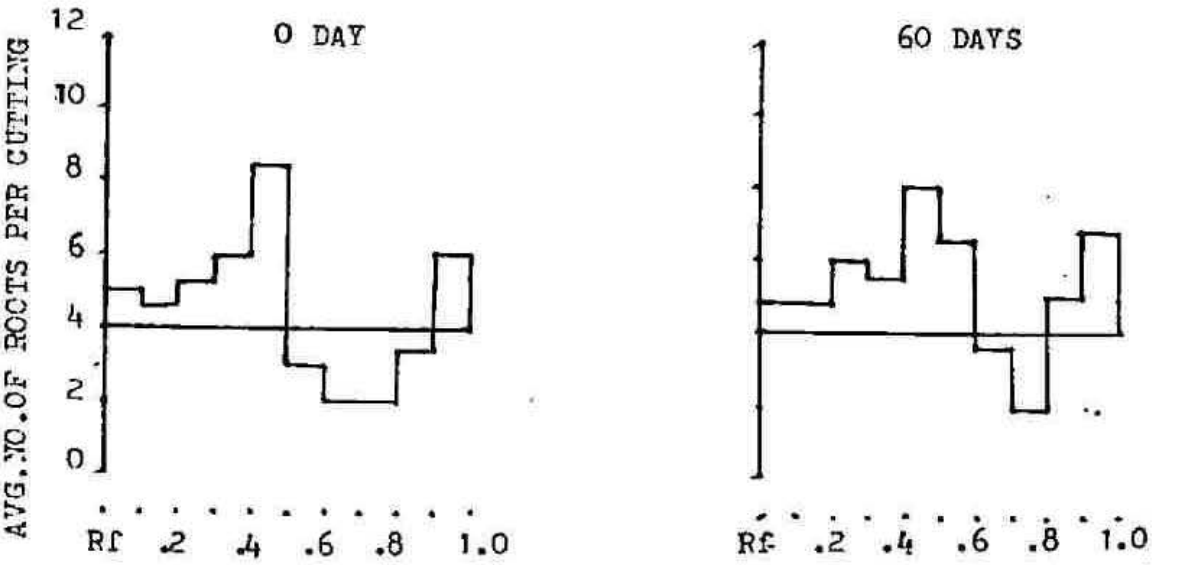
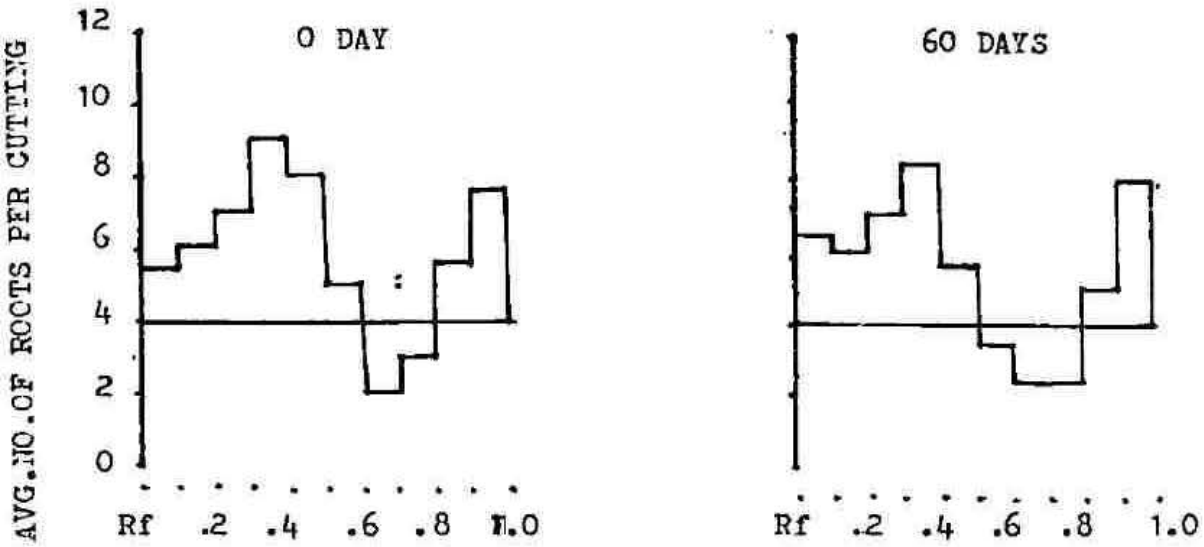


Fig.6. Activity of endogenous rooting promoters extracted from basal segments of rooted (after 60 days) cuttings of Greengage plum.

SEMI-HARDWOOD CUTTINGS OF PLUM cv.
EARLY TRANSPARENT GAGE



HARDWOOD CUTTINGS OF PLUM cv. EARLY TRANSPARENT GAGE

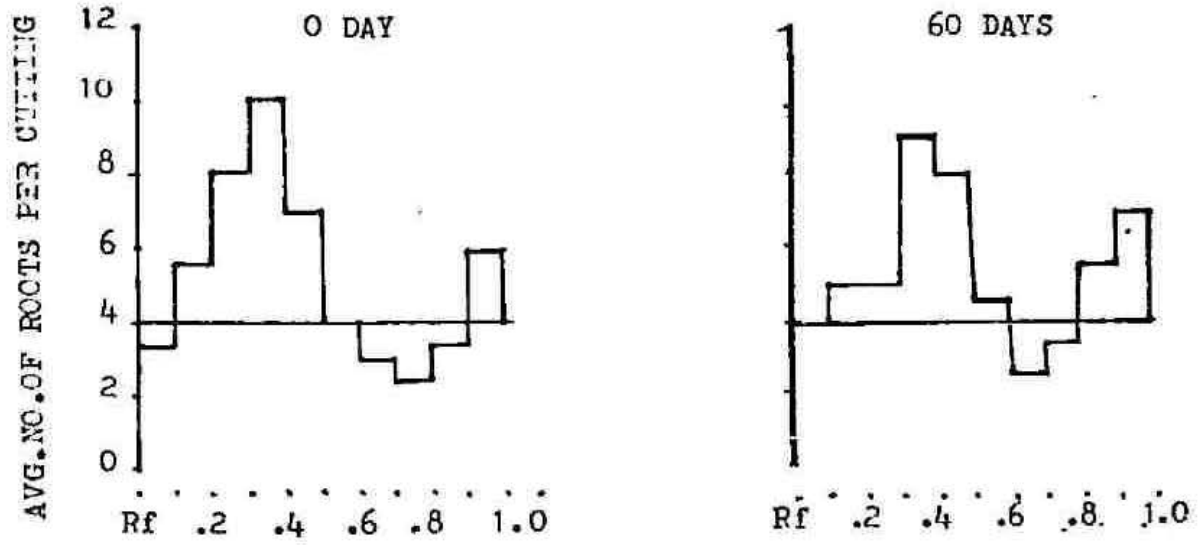


Fig.7. Activity of endogenous rooting promoters extracted from basal segments of fresh and rooted (after 60 days) cuttings of Early Transparent Gage plum.

60 days, in the basal extracts of rooted cuttings, the peak zones of promotion were seen at Rfs 0.3-0.6 and 1.0 while the inhibitory activity was seen at Rfs 0.7-0.8.

In the Early Transparent Gage, the Mung bean bioassay of the extracts obtained from basal segments of the semi-hardwood cuttings at zero day exhibited the zones of promotion at Rfs 0.1-0.6 and 0.9-1.0 while the inhibiting activity was also seen at Rfs 0.7-0.8 (Fig.7). The basal extract from sixty days old rooted cuttings exhibited root promotive activity at Rfs 0.1-0.5 and 1.0 while the zones of inhibitory activity were observed at Rfs 0.6-0.8.

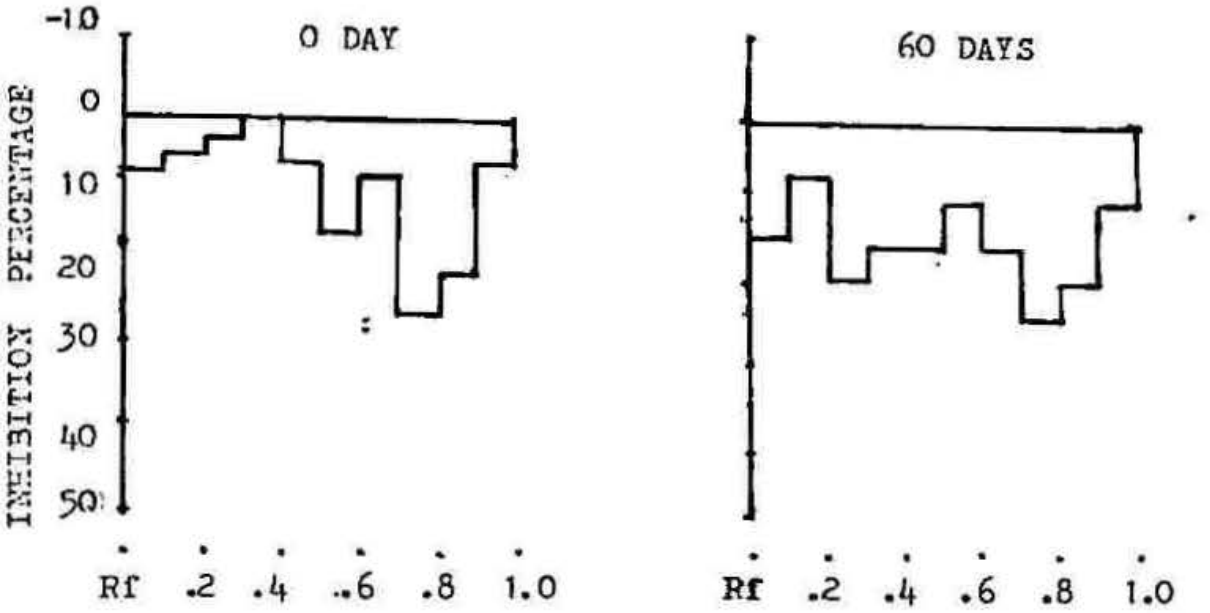
Bioassay of the extracts obtained from the basal segments of hardwood cuttings taken during dormant and autumn seasons revealed that the zones of root promotion were located at Rfs 0.2-0.5 and 1.0 while the inhibitory activities were simultaneously observed at Rfs 0.7-0.9. After 60 days of planting in the rooted cuttings, these zones of promotion were observed at Rfs 0.3-0.4 and 0.9-1.0.

3.2. Status of endogenous inhibitors

The data on the relative activity of inhibitory substances present in the cuttings' extract of plum cultivars as was estimated by Cress Seed germination method are embodied in Figs. 8, 9, 10 and 11.

Cress seed germination bioassay of the basal segments of fresh semi-hardwood cuttings of Santa Rosa showed the presence of inhibitory factors at Rfs 0.6-0.9 (Fig.8). Sixty

SEMI-HARDWOOD CUTTINGS OF PLUM cv. SANTA ROSA



HARDWOOD CUTTINGS OF PLUM cv. SANTA ROSA

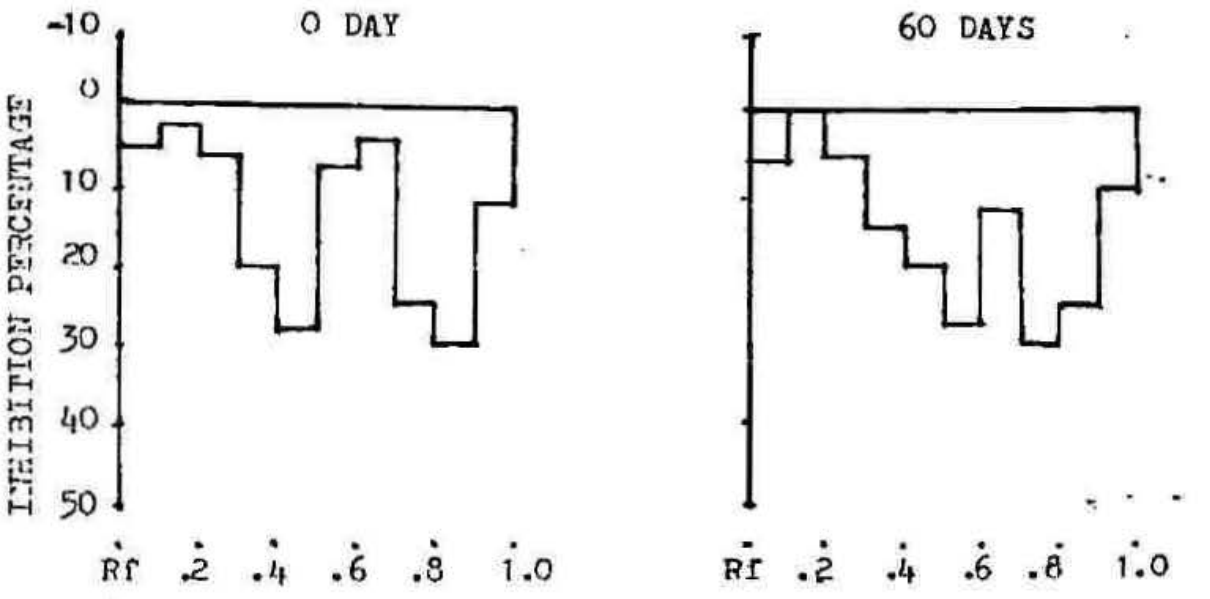


Fig.8. Activity of endogenous rooting inhibitors extracted from basal segments of fresh and rooted (after 60 days) cuttings of Santa Rosa plum.

days after planting the rooted cuttings showed a considerable reduction in the activities and only a slight inhibition was recorded at Rfs 0.1 and 0.3-0.5.

In case of hardwood cuttings, the extracts from the basal segments of fresh cuttings showed the peak zones of inhibition at Rf 0.5 and 0.9. After 60 days of planting in the rooted cuttings, reduced inhibitory activities were found at Rfs 0.4-0.6 and 0.8-1.0.

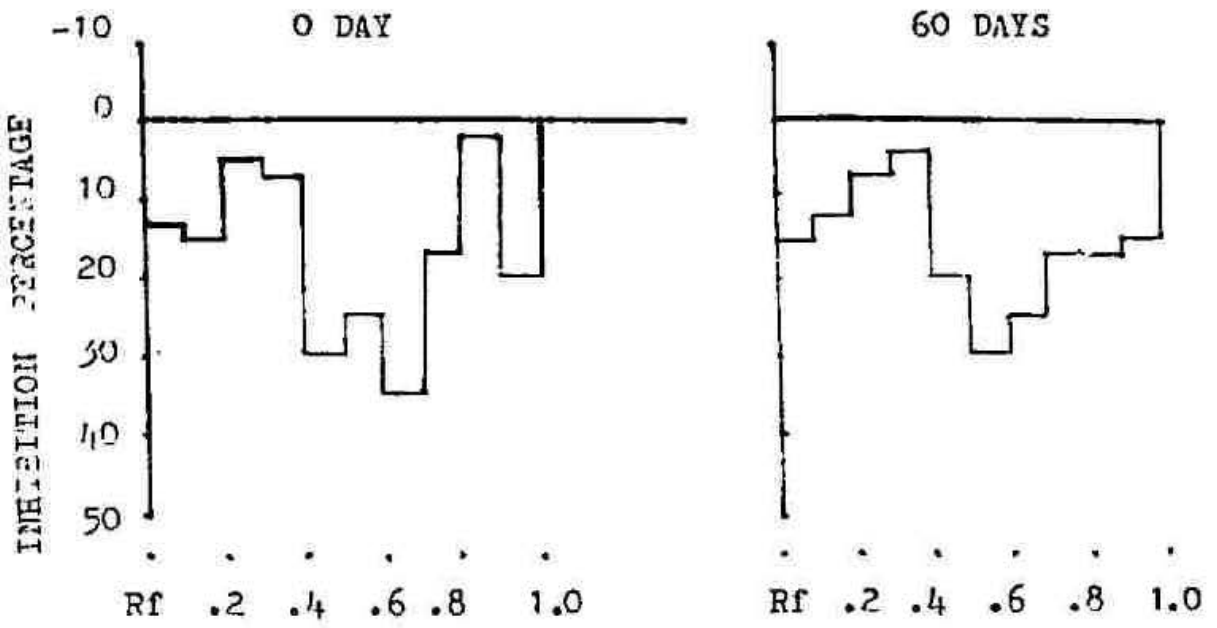
The bioactivity of the extracts of the fresh basal segments of semi-hardwood cuttings of Beauty plum exhibited the presence of inhibitory factors at most of the Rf zones but their activities were more conspicuous at Rfs 0.5-0.8 and 1.0 (Fig.9). However, the extracts obtained from rooted cuttings had shown reduced inhibitory activity at Rfs 5-1.0.

Basal extracts from the fresh hardwood cuttings showed zones of inhibitory activities at Rfs 0.1 and 0.6-0.8, the peak zone being at Rf 0.8. In rooted cuttings the activities of inhibitors were found to have reduced at 0.6-0.9.

The bioactivity of extracts obtained from fresh semi-hardwood cuttings of Greengage plum revealed zones of inhibition at Rfs 0.6-1.0 at a decreasing order (Fig.10). After 60 days, in the basal extract of the rooted cuttings, zones of inhibition were conspicuous at Rfs 0.5-0.9.

The inhibitory activity of the extract obtained from fresh hardwood cuttings was apparent at Rfs 0.6-1.0 but in

SEMI-HARDWOOD CUTTINGS OF PLUM cv. BEAUTY



HARDWOOD CUTTINGS OF PLUM cv. BEAUTY

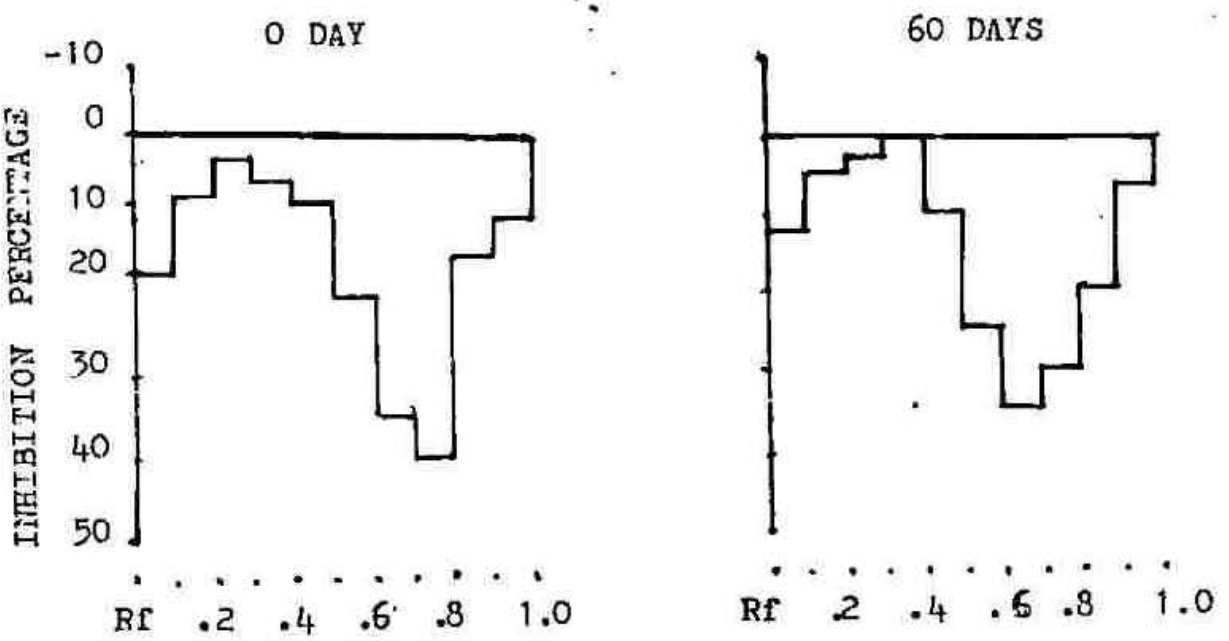
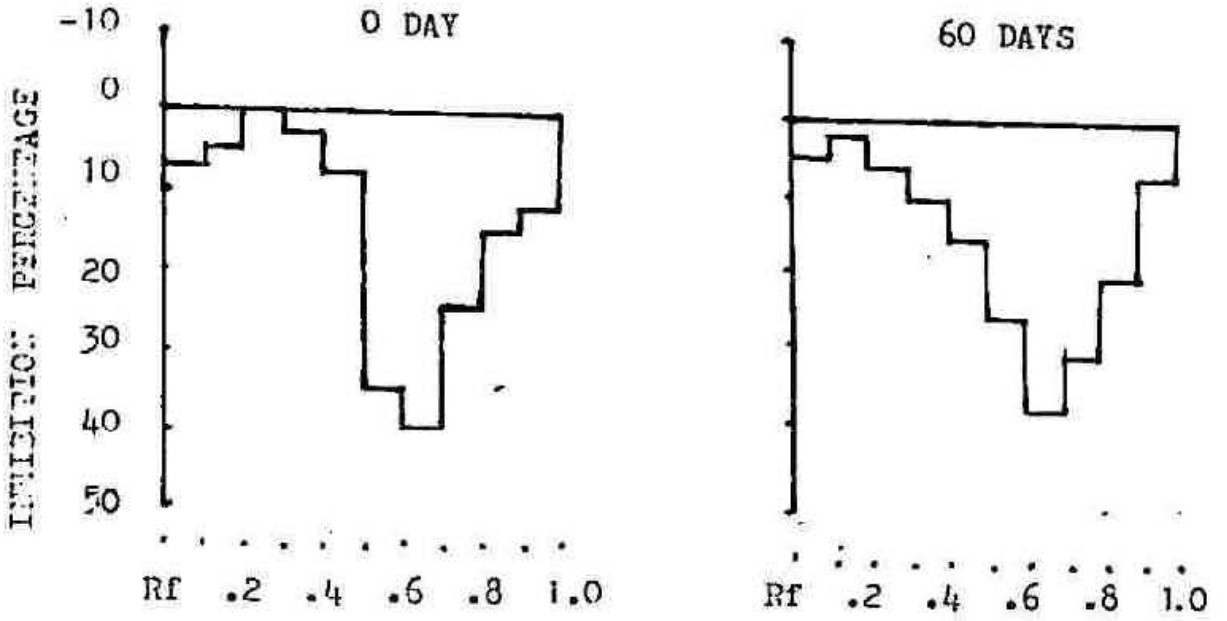


Fig.9. Activity of endogenous rooting inhibitors extracted from basal segments of fresh and rooted (after 60 days) cuttings of Beauty plum.

SEMI-HARDWOOD CUTTINGS OF PLUM cv. GREENGAGE



HARDWOOD CUTTINGS OF PLUM cv.GREENGAGE

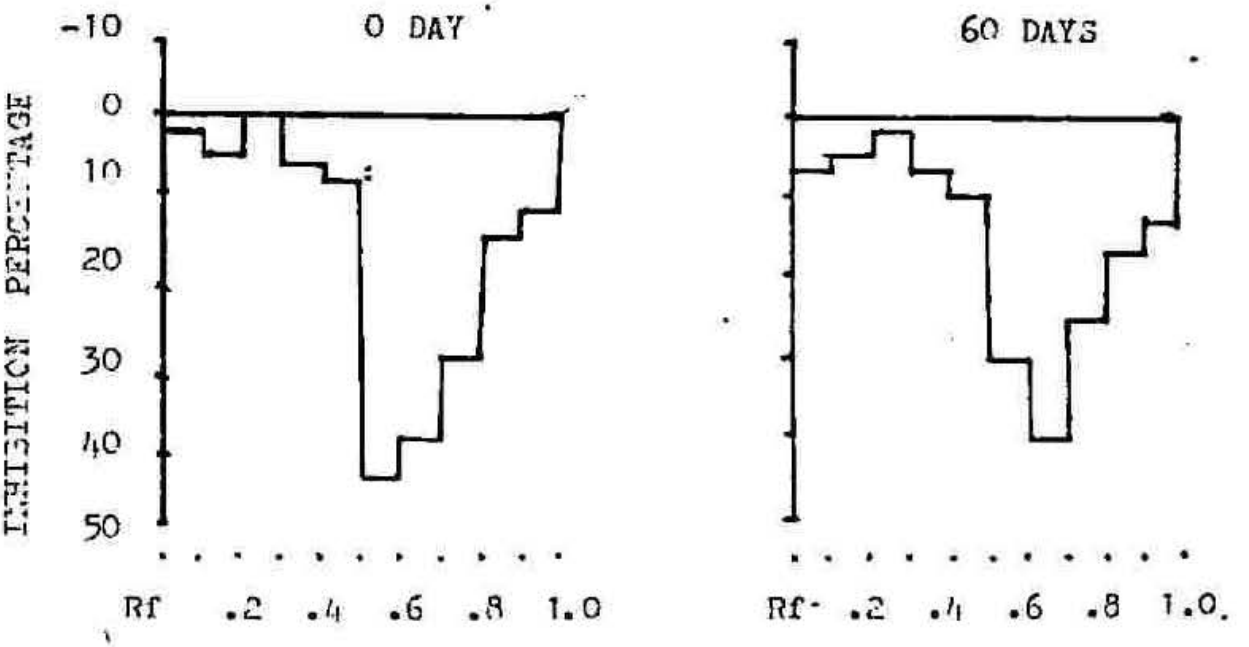
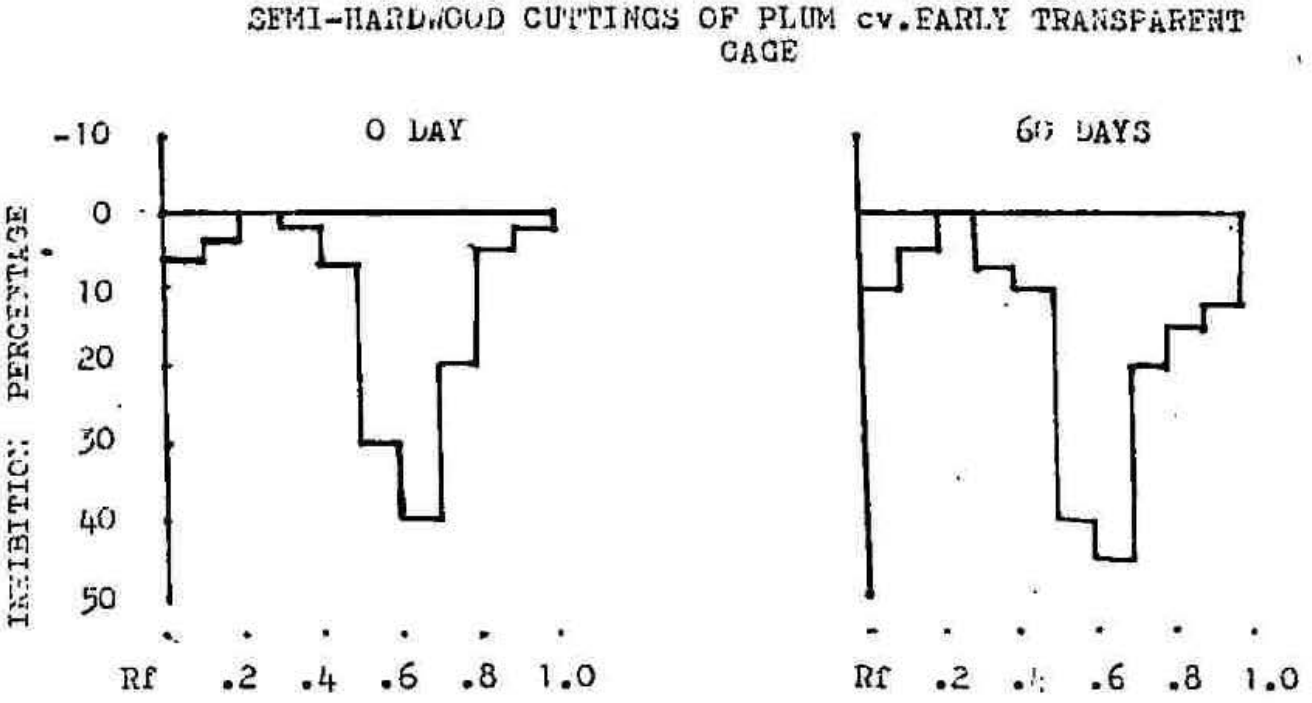


Fig. 10. Activity of endogenous rooting inhibitors extracted from basal segments of fresh and rooted (after 60 days) cuttings of Greengage.



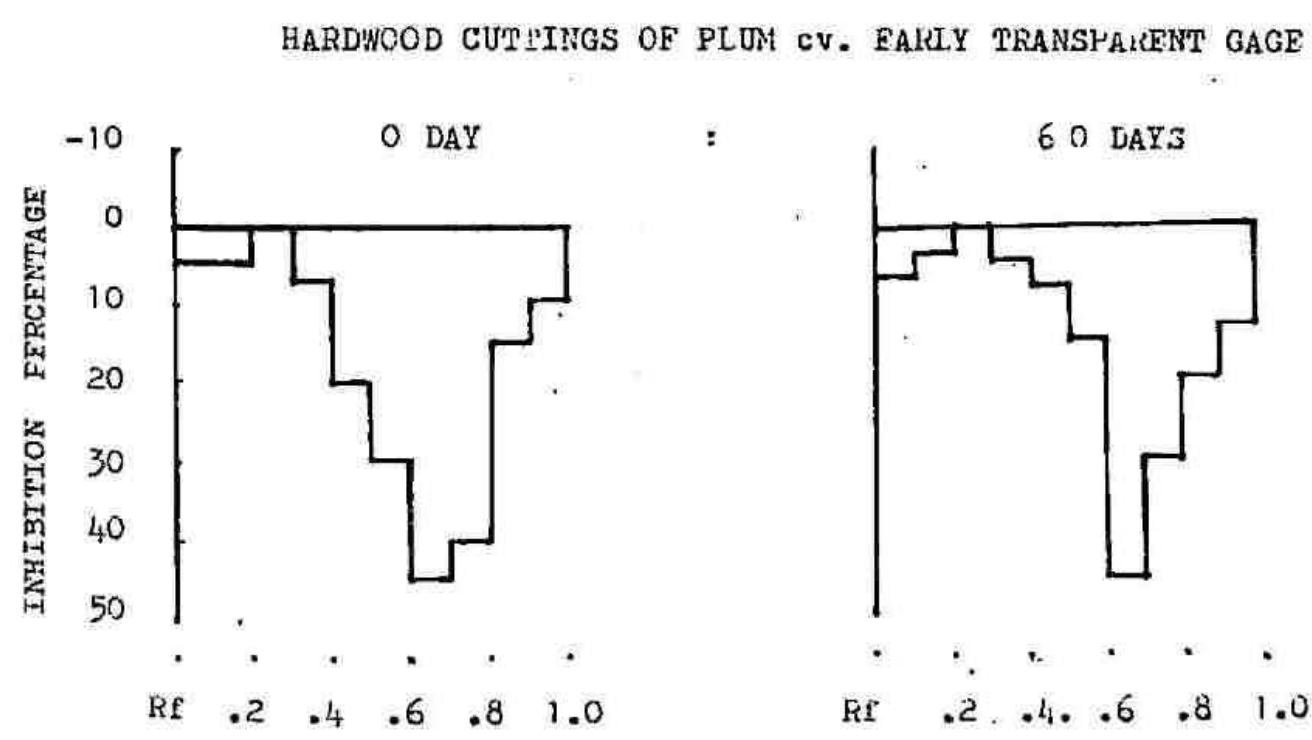


Fig.11. Activity of endogenous rooting inhibitors extracted from basal segments of fresh and rooted (after 60 days) cuttings of Early Transparent Gage plum.

a descending order. Similar zones of reduced inhibitory activity were also found in the basal extracts of the rooted cuttings at Rf positions 0.6-1.0.

In the basal extracts of fresh semi-hardwood cuttings of Early Transparent Gage, the zones of inhibition were found at Rfs 0.6-0.8 but in the rooted cuttings after 60 days of planting, the extracts from the rooted basal segment recorded the inhibitory substances at Rfs 0.4-0.9 in a reduced quantity. Some more inhibitors accumulated in the cuttings during rooting process at Rfs 0.9-1.0.

The bioactivity of the extracts of basal segments of fresh hardwood cuttings showed the presence of inhibitory activities at Rfs 0.5-0.6 and it was very intense at Rfs 0.7-0.8 whereas after 60 days of planting the inhibitory factors were found at Rfs 0.6-1.0 the peak zone being at Rf 0.7 in the basal extracts of the rooted cuttings.

4. Effect of preconditioning treatments, size of cuttings and IBA treatments on rooting behaviour of dormant season cuttings of plum cv. Santa Rosa.

4.1. Percentage of sprouting of cuttings

The data recorded on the sprouting percentage of Santa Rosa plum cuttings as influenced by various preconditioning treatments, size of the cuttings and IBA treatments are presented in Table 15.

It is evident from the data in Table 15 that during the year 1986, maximum sprouting of 83.6 per cent was recorded

Table 15. Effect of preconditioning treatments, size of cuttings and IBA concentrations on sprouting of cuttings (in per cent) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
P_1 Unforced								
20	58.5	62.0	65.5	64.0	61.0	60.0	70.0	67.0
30	63.5	70.0	72.5	68.5	65.5	69.5	75.0	71.5
40	61.0	65.0	67.5	66.0	60.0	67.5	72.0	65.8
P_2 Forced								
20	62.5	67.0	65.5	70.0	65.0	70.0	72.5	74.0
30	66.0	73.5	75.0	72.5	70.0	77.7	80.0	74.5
40	64.0	69.0	72.0	67.5	68.5	75.0	78.0	72.5
P_3 Forced + Wounding								
20	65.0	70.0	75.0	67.5	70.0	73.5	77.5	72.0
30	70.0	77.5	80.0	75.0	72.5	75.5	82.0	77.0
40	72.5	80.0	74.0	70.0	75.0	77.5	80.0	76.0
P_4 Forced + Etiolated + Wounding								
20	67.5	71.5	77.5	75.0	73.5	76.5	80.0	75.0
30	74.0	80.0	83.5	78.0	75.5	78.5	85.0	80.0
40	70.5	75.0	80.0	73.5	76.0	80.5	83.0	77.5
P_5 Forced + Ringed + Wounding								
20	77.5	80.0	85.5	82.0	75.0	82.5	88.5	84.0
30	82.0	85.0	90.0	87.5	85.5	89.5	84.5	83.0
40	79.5	82.5	87.0	85.0	81.5	85.0	83.5	82.5
Means of treatments								
a) Preconditionings :	P_1		65.3				67.1	
	P_2		68.7				73.1	
	P_3		73.0				73.1	
	P_4		75.5				78.4	
	P_5		83.6				85.8	
C.D. (P=0.05)			8.94				7.80	
b) Size of cuttings :	20 cm.		70.5				73.4	
	30 cm		79.7				81.2	
	40 cm		73.1				75.9	
C.D. (P=0.05)			5.20				4.50	
c) IBA concentrations:	0 ppm		68.9				71.6	
	2000 ppm		73.9				75.9	
	3000 ppm		76.7				79.4	
	4000 ppm		73.5				76.5	
C.D. (P=0.05)			5.80				5.01	

in the cuttings under P_5 treatment followed by P_4, P_3, P_2 and P_1. During 1987, P_5 treatment had produced maximum sprouting of 85.8 per cent while the minimum sprouting was shown by P_1 treatment with 67.1 per cent. During both the years, P_5 was observed to be significantly better than all the preconditioning treatments except with P_4, which was found to be statistically at par with P_5 in this character.

Size of cuttings has also shown remarkable effect on sprouting of cuttings in both the years under study. Maximum sprouting of 79.7 per cent in 1986 and 81.2 per cent in 1987 recorded in 30 cm long cuttings was significantly superior to 20 cm and 40 cm long cuttings in both the years. However, the difference between 20 cm and 40 cm cutting sizes were found to be non-significant in both the years under study.

The effect of different concentrations of IBA in sprouting were statistically at par with each other in both the years under study. During 1986, 3000 ppm IBA had given maximum sprouting of 76.7 per cent followed by 2000 ppm and 4000 ppm IBA treatments. Cuttings under control treatment resulted in the minimum sprouting of 68.9 per cent. Similarly, during 1987, maximum sprouting of 79.4 per cent was recorded with the cuttings under 3000 ppm IBA concentration while the minimum sprouting of 71.6 per cent was observed under control treatment. Statistical analysis of the data show that treatment under 3000 ppm IBA was significantly superior over control treatment but was at par with 4000 ppm and 2000 ppm IBA treatments during both the years.

The results in respect of interaction among different factors were not consistent and were found to be non-significant.

4.2. Percentage of rooting

The data on the rooting of Santa Rosa plum cuttings as influenced by the preconditioning treatments, size of the cuttings and IBA treatments are given in Table 16 and depicted in Fig.12.

It is quite apparent from Table 16 that in different preconditioning treatments, although maximum rooting of 62.3 per cent was recorded in P_5 yet this treatment was statistically at par with P_4. In the remaining treatments, P_3 and P_2 did not differ significantly from each other in respect of their rooting. Minimum rooting was recorded in P_1 treatment, in both the years.

It is also clear from Table 16 that the size of the cuttings gave significant influence on rooting percentage in both the year. Thirty cm long cuttings showed the highest percentage of rooting with 54.3 and 55.0 per cent during 1986 and 1987, respectively which was significantly better than 40 cm and 20 cm long cuttings in both the years. The minimum rooting was observed in 40 cm long cuttings with 45.2 and 47.0 per cent during 1986 and 1987, respectively.

Among different IBA treatments, highest rooting of 61.3 and 64.0 per cent were recorded in IBA 3000 ppm in 1986 and 1987, respectively which was followed by 4000 ppm and

Table 16. Effect of preconditioning treatments, size of cuttings and IBA concentrations on rooting of cuttings (in per cent) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
P_1 Unforced								
20	21.5 (27.6)	42.5 (40.7)	50.0 (45.0)	45.0 (42.1)	20.0 (26.6)	37.5 (37.8)	52.5 (46.4)	40.0 (39.2)
30	25.0 (30.0)	47.5 (43.6)	55.0 (47.9)	52.0 (46.1)	28.0 (31.9)	50.0 (45.0)	57.5 (49.3)	48.0 (43.8)
40	18.0 (25.1)	35.0 (36.3)	45.0 (42.1)	37.5 (37.8)	22.5 (28.3)	40.0 (39.2)	48.0 (43.8)	43.5 (41.3)
P_2 Forced								
20	23.5 (29.0)	45.0 (42.1)	55.0 (47.8)	50.0 (45.0)	24.0 (29.3)	47.5 (48.6)	57.5 (49.3)	46.5 (43.0)
30	27.5 (31.6)	52.5 (46.4)	60.0 (50.8)	55.0 (47.9)	30.0 (33.2)	55.0 (47.9)	62.5 (52.2)	52.5 (46.4)
40	20.0 (26.6)	40.0 (39.2)	52.5 (46.4)	47.5 (43.6)	22.5 (28.3)	45.0 (42.1)	52.5 (46.4)	49.0 (44.4)
P_3 Forced + Wounding								
20	25.0 (30.1)	50.0 (45.0)	60.0 (50.8)	52.5 (46.4)	27.5 (31.6)	47.5 (43.6)	62.5 (52.2)	50.0 (45.0)
30	30.0 (33.2)	57.5 (49.3)	65.0 (53.7)	55.0 (47.9)	32.5 (34.8)	50.5 (45.3)	67.5 (55.2)	57.0 (49.0)
40	22.5 (28.3)	45.0 (42.1)	57.5 (49.3)	50.0 (45.0)	24.0 (29.3)	42.5 (40.7)	60.0 (50.8)	47.5 (43.6)
P_4 Forced + Etiolated + Wounding								
20	26.0 (30.7)	57.5 (49.3)	64.0 (53.1)	60.0 (50.8)	32.5 (34.9)	62.5 (52.2)	67.5 (55.2)	60.0 (50.8)
30	35.0 (36.3)	62.5 (52.2)	70.0 (56.8)	67.5 (55.2)	32.5 (34.8)	65.0 (53.7)	72.5 (58.4)	67.5 (55.2)
40	29.0 (32.6)	54.0 (47.3)	61.0 (51.3)	57.5 (49.3)	27.5 (31.6)	57.5 (49.3)	65.0 (53.7)	55.0 (47.9)
P_5 Forced + Ringed + Wounding								
20	38.0 (38.1)	65.0 (53.7)	75.0 (60.0)	67.5 (55.2)	35.0 (36.3)	70.0 (56.8)	77.5 (61.7)	65.0 (53.7)
30	42.5 (40.7)	72.5 (58.4)	80.0 (63.4)	75.0 (60.0)	40.0 (39.2)	75.0 (60.0)	85.0 (67.2)	72.5 (58.4)
40	32.0 (34.4)	62.5 (52.2)	70.0 (56.8)	67.5 (55.2)	37.5 (37.8)	65.0 (53.7)	72.5 (58.4)	62.5 (52.2)
Means of treatments								
a) Preconditionings : P_1			39.5(38.7)				40.6(39.3)	
P_2			44.0(41.4)				45.4(42.2)	
P_3			47.5(43.4)				47.4(43.4)	
P_4			53.7(47.1)				55.4(48.1)	
P_5			62.3(52.3)				63.1(52.9)	
C.D. (P=0.05)			6.38				5.70	
b) Size of cuttings : 20 cm			48.6(44.1)				49.1(44.4)	
30 cm			54.3(44.6)				55.0(48.0)	
40 cm			45.2(42.0)				47.0(43.2)	
C.D. (P=0.05)			4.90				4.42	
c) IBA Concentrations: 0 ppm			27.7(31.6)				29.1(32.5)	
2000 ppm			52.6(46.5)				54.0(47.4)	
3000 ppm			61.3(51.6)				64.0(53.3)	
4000 ppm			56.0(48.5)				54.4(47.6)	
C.D. (P=0.05)			5.20				5.10	

Figures in parentheses are angular transformed values.

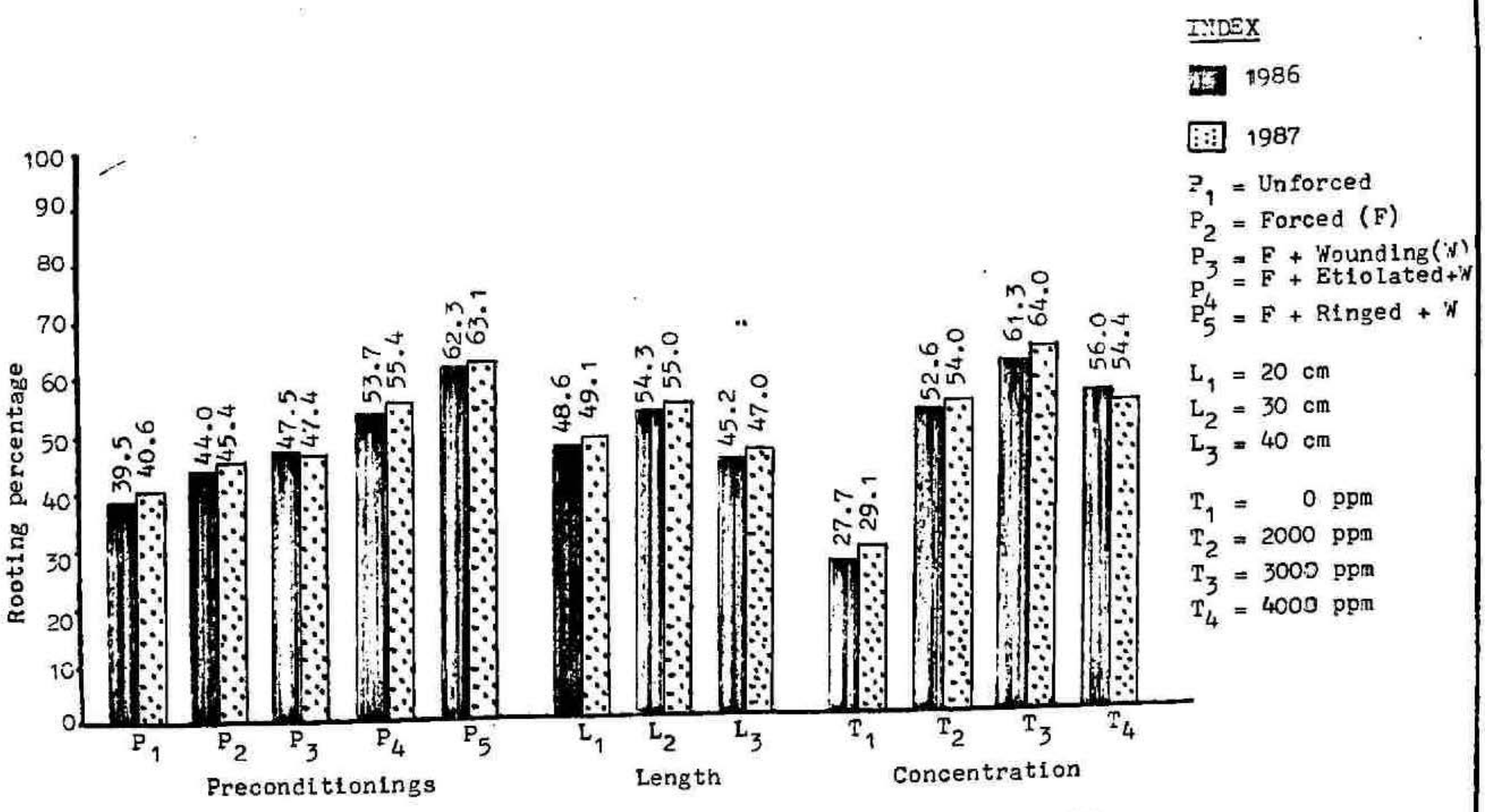


Fig. 12. Effect of preconditioning treatments, size of cuttings and IBA concentrations on rooting of cuttings of plum cv. Santa Rosa.

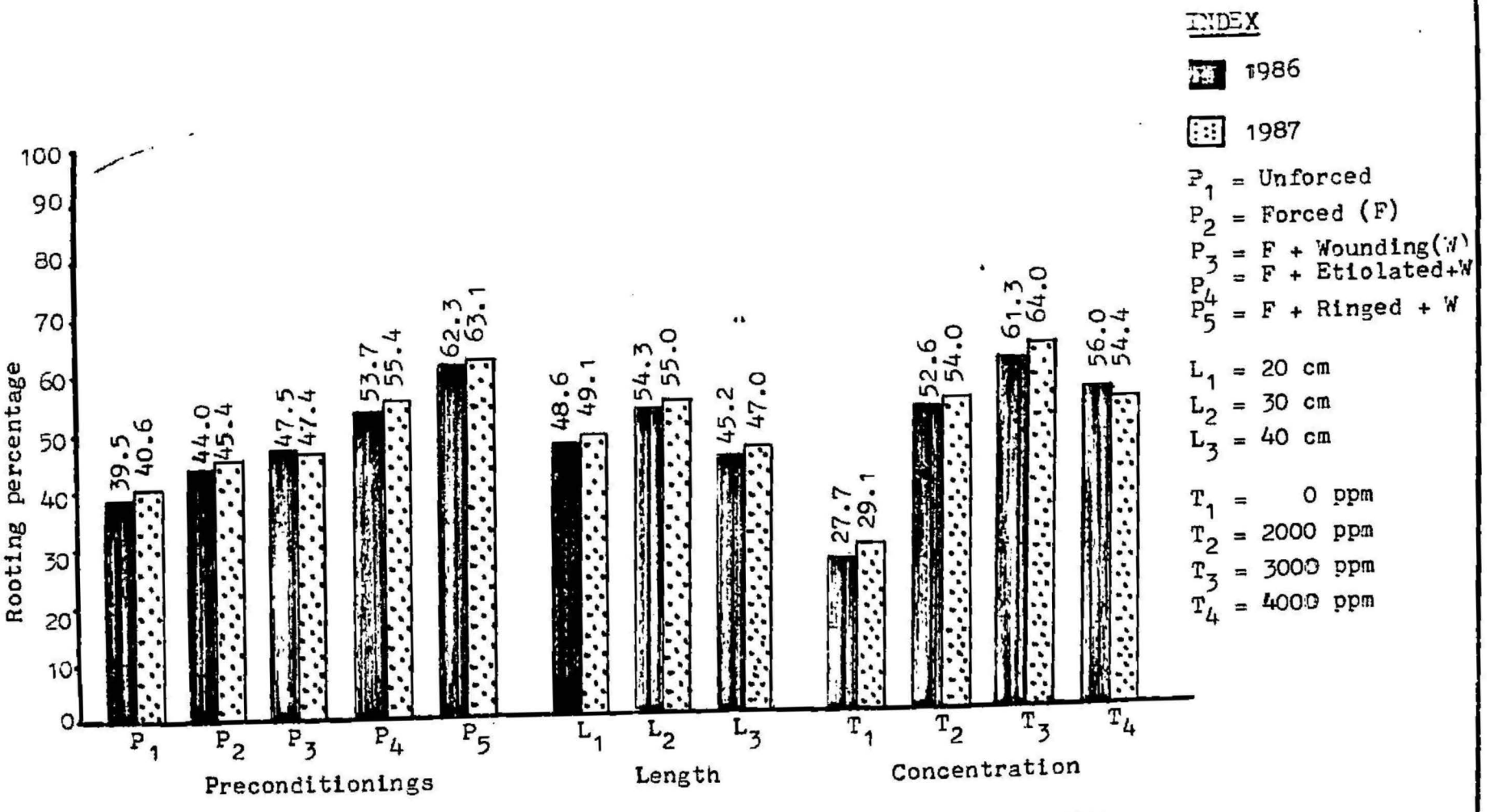
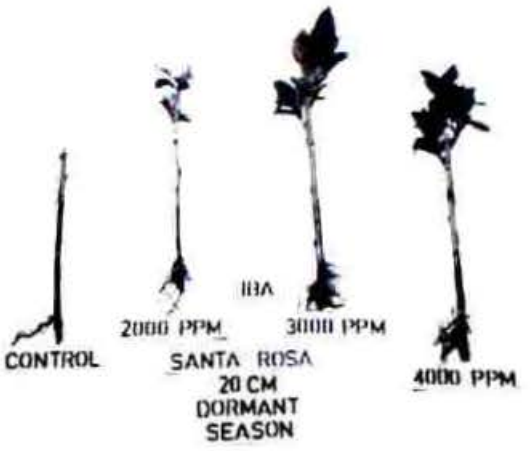


Fig. 12. Effect of preconditioning treatments, size of cuttings and IBA concentrations on rooting of cuttings of plum cv. Santa Rosa.

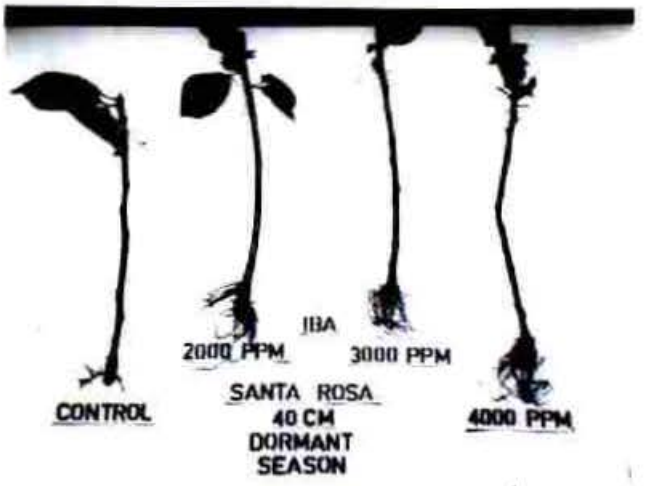
PLATE 3



A



B



C

Plate 3.A-C. Rooting behaviour in dormant planted hardwood cuttings of Santa Rosa as influenced by cutting sizes and IBA treatments.

2000 ppm IBA. Statistical analysis of the data showed that treatments of cuttings with 3000 ppm IBA solution was significantly better over 4000 ppm and 2000 ppm IBA. The extent of rooting in IBA 2000 ppm and IBA 4000 ppm did not differ significantly from each other. However, minimum rooting was recorded in control in both the years.

The interaction between preconditioning treatments, cutting sizes and IBA treatments was found to be non-significant during both the years, which implies that rooting of cutting was affected by either of these factors but they were independent of each other in this respect.

4.3. Shoot length

The data on the length of the newly emerged shoots of Santa Rosa plum as affected by various preconditioning treatments, cutting sizes and IBA concentrations are presented in Table 17.

It is evident from Table 17 that during 1986, treatment P_5 produced maximum shoot length (13.9 cm) followed by P_4, P_3, P_2 and P_1, which was statistically at par with each other except with P_1 which produced minimum (8.8 cm) shoot length. During the year 1987, more or less similar trend was observed. P_5 treatment produced maximum shoot length (14.6 cm) which was statistically at par with P_4 but significantly better than P_3, P_2 and P_1.

The size of cuttings gave significant effect on the average length of shoots in both the years. Maximum average

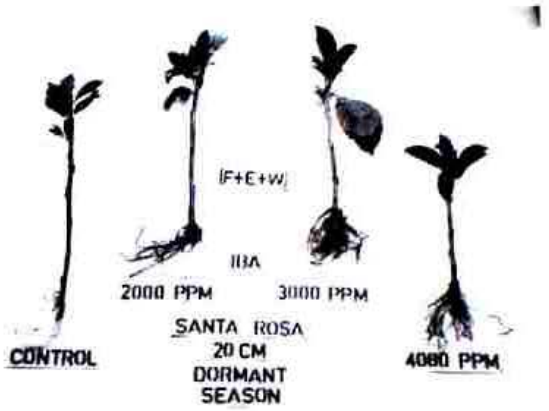
Table 17. Effect of preconditioning treatments, size of cuttings and IBA concentrations on main shoot length (cm) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	5.8	7.9	9.6	8.3	6.4	8.0	10.1	8.8
30	7.3	9.3	11.5	10.1	7.6	10.0	11.8	10.7
40	6.5	8.8	10.7	9.4	6.3	8.9	11.1	9.5
P_2 Forced								
20	7.0	9.0	11.6	10.1	7.8	9.8	12.3	11.0
30	8.2	10.9	13.7	11.5	8.9	11.8	14.6	12.3
40	7.8	10.2	13.0	11.3	7.4	10.9	13.3	12.0
P_3 Forced + Wounding								
20	7.7	10.5	13.5	11.5	8.4	11.4	14.1	12.3
30	9.0	11.8	16.0	13.5	10.0	12.9	15.8	13.1
40	8.3	11.1	14.9	12.6	8.6	12.1	15.5	13.4
P_4 Forced + Etiolated + Wounding								
20	8.5	12.0	15.0	13.1	9.0	13.0	15.4	13.6
30	9.8	12.9	17.0	14.6	10.8	14.4	16.3	14.8
40	9.2	12.5	16.0	13.6	10.1	13.4	16.5	15.0
P_5 Forced + Ringed + Wounding								
20	9.2	13.4	16.0	14.4	9.8	14.1	16.8	14.9
30	10.3	14.5	17.8	15.7	11.0	15.3	18.6	16.1
40	9.8	14.0	16.9	15.6	10.1	14.5	17.6	16.0

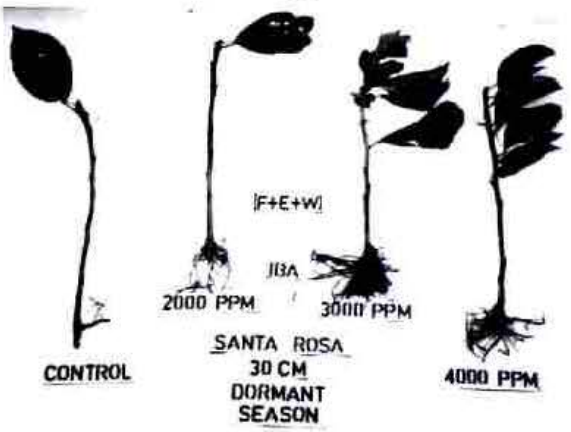
Means of treatments

a) Preconditionings :	P_1	8.8	9.1
	P_2	10.4	11.0
	P_3	11.7	12.3
	P_4	12.8	13.5
	P_5	13.9	14.6
C.D. (P=0.05)		1.22	1.21
b) Size of cuttings :	20 cm	10.7	11.3
	30 cm	12.3	12.8
	40 cm	11.6	12.1
C.D. (P=0.05)		0.95	0.93
c) IBA Concentrations:	0 ppm	8.3	8.8
	2000 ppm	11.2	12.0
	3000 ppm	14.2	14.6
	4000 ppm	12.4	12.9
C.D. (P=0.05)		1.09	1.08

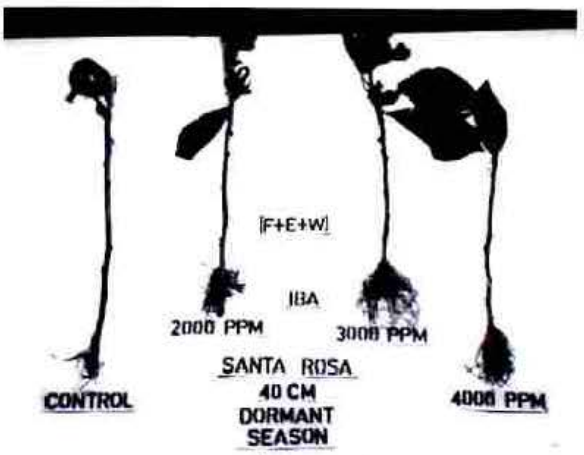
PLATE 4



A



B



C

Plate 4. A-C. Rooting behaviour of Santa Rosa hardwood cuttings involving forcing, etiolation, basal wounding and IBA treatments

shoot length of 12.3 cm and 12.8 cm were recorded from 30 cm long Santa Rosa cuttings during 1986 and 1987, respectively which was significantly higher than 20 cm and 40 cm long cuttings in both the years under study.

Cuttings treated with 3000 ppm IBA were observed to record maximum average length of the shoots (14.2 cm) followed by 4000 ppm and 2000 ppm IBA treatments during 1986. Shoots produced from the cuttings under control treatment showed least growth (8.3 cm). During 1987, 3000 ppm IBA treated cuttings produced maximum average shoot length of 14.6 cm which was significantly superior over other IBA treatments and the control.

The results in respect of interaction among different factors under study were not consistent and were found to be non-significant during both the years with respect to this character.

4.4. Diameter of shoots

The effect of preconditioning treatments, cutting sizes and IBA concentrations on the diameter of shoots produced by cuttings is given in Table 18.

The data in Table 18 show that during 1986 there was no significant differences among P_5, P_4, P_3, P_2 and P_1 treatments in respect of average diameter of shoots. However, these treatments were significantly better than treatment P_1 which had the minimum (0.45 cm) average shoot diameter.

Table 18. Effect of preconditioning treatments size of cuttings and IBA concentrations on main shoot diameter (in cm) from the cuttings of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings(cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	0.36	0.44	0.49	0.45	0.39	0.46	0.50	0.47
30	0.41	0.48	0.53	0.49	0.45	0.50	0.55	0.49
40	0.38	0.45	0.50	0.47	0.42	0.44	0.53	0.46
P_2 Forced								
20	0.40	0.46	0.51	0.49	0.44	0.48	0.52	0.47
30	0.46	0.51	0.56	0.54	0.49	0.53	0.58	0.54
40	0.45	0.49	0.54	0.51	0.45	0.49	0.56	0.55
P_3 Forced + Wounding								
20	0.43	0.46	0.53	0.50	0.45	0.50	0.54	0.51
30	0.49	0.54	0.59	0.54	0.48	0.58	0.57	0.55
40	0.46	0.50	0.56	0.53	0.45	0.52	0.56	0.51
P_4 Forced + Etiolated + Wounding								
20	0.45	0.50	0.56	0.53	0.48	0.54	0.58	0.55
30	0.48	0.55	0.61	0.57	0.51	0.60	0.61	0.58
40	0.47	0.54	0.58	0.53	0.49	0.53	0.56	0.54
P_5 Forced + Ringed + Wounding								
20	0.49	0.52	0.58	0.55	0.51	0.54	0.59	0.56
30	0.53	0.56	0.61	0.58	0.54	0.58	0.65	0.61
40	0.50	0.54	0.58	0.56	0.53	0.55	0.61	0.55
Means of treatments								
a) Preconditionings : P_1				0.45				0.47
P_2				0.49				0.50
P_3				0.51				0.52
P_4				0.53				0.55
P_5				0.55				0.57
C.D. (P=0.05)				0.027				0.027
b) Size of cuttings : 20 cm				0.49				0.51
30 cm				0.53				0.55
40 cm				0.51				0.52
C.D. (P=0.05)				0.021				0.022
c) IBA Concentrations: 0 ppm				0.45				0.47
2000 ppm				0.50				0.52
3000 ppm				0.55				0.57
4000 ppm				0.52				0.53
C.D. (P=0.05)				0.024				0.024

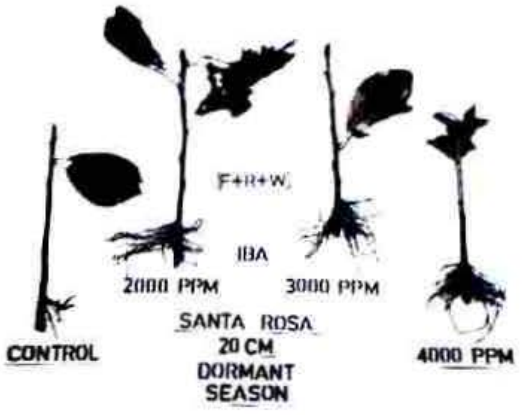
However, during the year 1987, maximum average shoot diameter was recorded in the cuttings of Santa Rosa plum under P_5 treatment which was significantly superior to those of P_3, P_2 and P_1 treatments but it was statistically at par with P_4 treatment. The treatment under P_1 registered relatively minimum shoot diameter with 0.47 cm.

During the year 1986, maximum (0.53 cm) average diameter of shoots was obtained in 30 cm long Santa Rosa plum cuttings which was significantly superior to 20 cm and 40 cm long cuttings. During the year 1987, maximum average daimeter of shoots with 0.55 cm was observed from 30 cm long cuttings which was statistically significant over 20 cm and 40 cm long cuttings. However, the shoot diameter did not show any significant difference between 20 cm and 40 cm long cuttings during both the years.

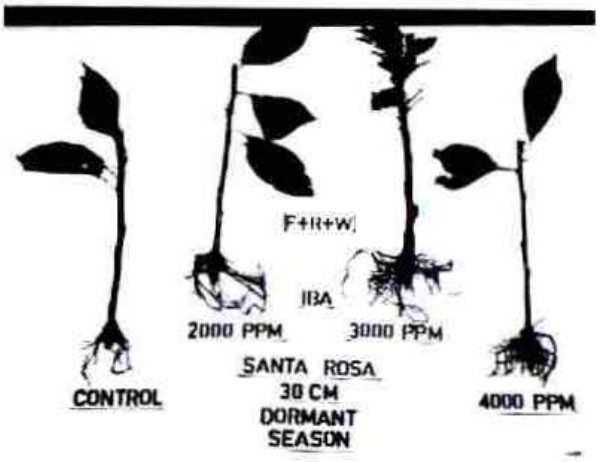
The different IBA treatments had also shown significant effect on diameter of shoots in both the years under study. Maximum shoot diameter of 0.55 cm and 0.57 cm were recorded in the 3000 ppm IBA treated cuttings which were significantly superior over 4000 ppm and 2000 ppm IBA treated cuttings in both the years. The concentrations of 4000 ppm and 2000 ppm IBA showed an identical effect on the average diameter of shoots in both the years. These were, however, significantly superibr to the treatment under control which produced the least diameter of shoots (0.45 cm in 1986 and 0.47 cm in 1987).

The interaction effects among different factors were not consistent and were found to be non-significant during

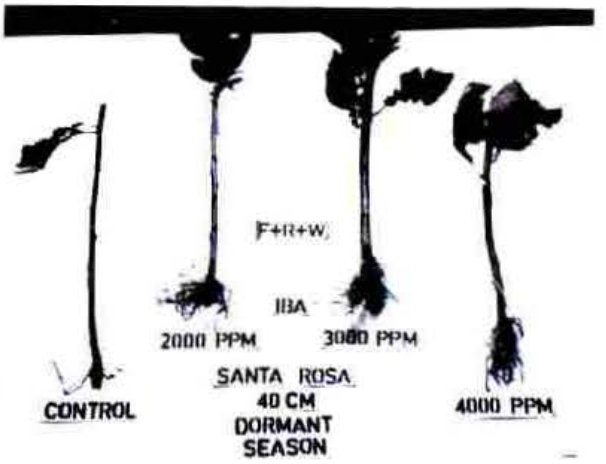
PLATE 5



A



B



C

Plate 5. A-C. Rooting behaviour in Santa Rosa hardwood cuttings involving forcing, ringing, basal wounding and IBA treatments

the years in respect of the diameter of shoots.

4.5. Number of primary roots per cutting

The data on the effect of different preconditioning treatments, cutting sizes and IBA concentrations on the average number of primary roots per cutting are presented in Table 19 and depicted in Fig. 13.

A perusal of the data reveals that maximum number of primary roots (19.7 and 21.1) were observed in P_5 treatment in both the years of study whereas the minimum number of primary roots (14.0 and 14.7) were observed under P_1 treatment.

The size of cuttings was observed to have no significant effect on the number of primary roots. However, the maximum number of primary roots (18.1 and 19.0) was observed in 30 cm long cuttings which was closely followed by 20 cm and 40 cm long cutting during both the years.

The different concentrations of IBA were found to have a significant effect on the number of primary roots per cutting. During 1986, maximum number of primary roots was observed under 3000 ppm IBA treatment (21.8) which was significantly superior over 4000 ppm (19.4) and 2000 ppm (17.2) IBA treatments. Similarly, during 1987, 3000 ppm IBA treated cuttings produced maximum number of primary roots per cutting which was significantly superior over other treatments. The minimum number of primary roots per cutting were observed during both the years under control.

Table 19. Effect of preconditioning treatments, size of cuttings and IBA concentrations on number of primary roots per cutting of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm) 0	2000	3000	4000	1987 IBA (ppm) 0	2000	3000	4000
P_1 Unforced								
20	7.8	14.3	18.0	16.0	8.5	15.0	18.5	16.2
30	8.7	15.2	19.7	17.0	9.5	16.0	20.0	17.5
40	6.8	12.8	16.7	15.5	7.5	14.3	17.8	16.0
P_2 Forced								
20	8.3	15.7	19.0	17.5	8.7	16.5	19.8	18.3
30	9.2	16.5	20.8	18.5	9.5	17.0	21.0	19.2
40	7.5	15.0	18.3	16.5	8.2	15.2	18.7	17.0
P_3 Forced + Wounding								
20	9.3	17.8	22.2	19.7	9.8	18.7	23.0	20.8
30	10.2	19.0	23.0	21.3	11.0	19.5	24.0	22.5
40	8.3	16.0	20.8	18.5	9.2	17.3	21.5	20.0
P_4 Forced + Etiolated + Wounding								
20	9.5	18.5	23.3	21.5	10.0	19.5	24.8	22.3
30	10.7	20.5	24.8	23.0	10.5	21.8	26.0	24.0
40	9.2	17.3	21.5	19.3	9.7	18.0	23.3	21.0
P_5 Forced + Ringed + Wounding								
20	10.5	19.8	26.0	22.3	11.2	21.5	26.8	23.7
30	11.3	21.5	27.8	24.0	12.3	23.0	29.5	26.3
40	10.3	18.5	24.0	20.8	11.0	20.9	25.7	22.2
Means of treatments								
a) Preconditionings : P_1				14.0				14.7
P_2				15.2				15.8
P_3				17.2				18.1
P_4				18.3				19.2
P_5				19.7				21.1
C.D. (P=0.05)				1.82				1.74
b) Size of cuttings : 20 cm				16.9				17.7
30 cm				18.1				19.0
40 cm				15.7				16.7
C.D. (P=0.05)				1.41				1.35
c) IBA Concentrations: 0 ppm				9.2				9.8
2000 ppm				17.2				18.2
3000 ppm				21.8				22.7
4000 ppm				19.4				20.5
C.D. (P=0.05)				1.63				1.55

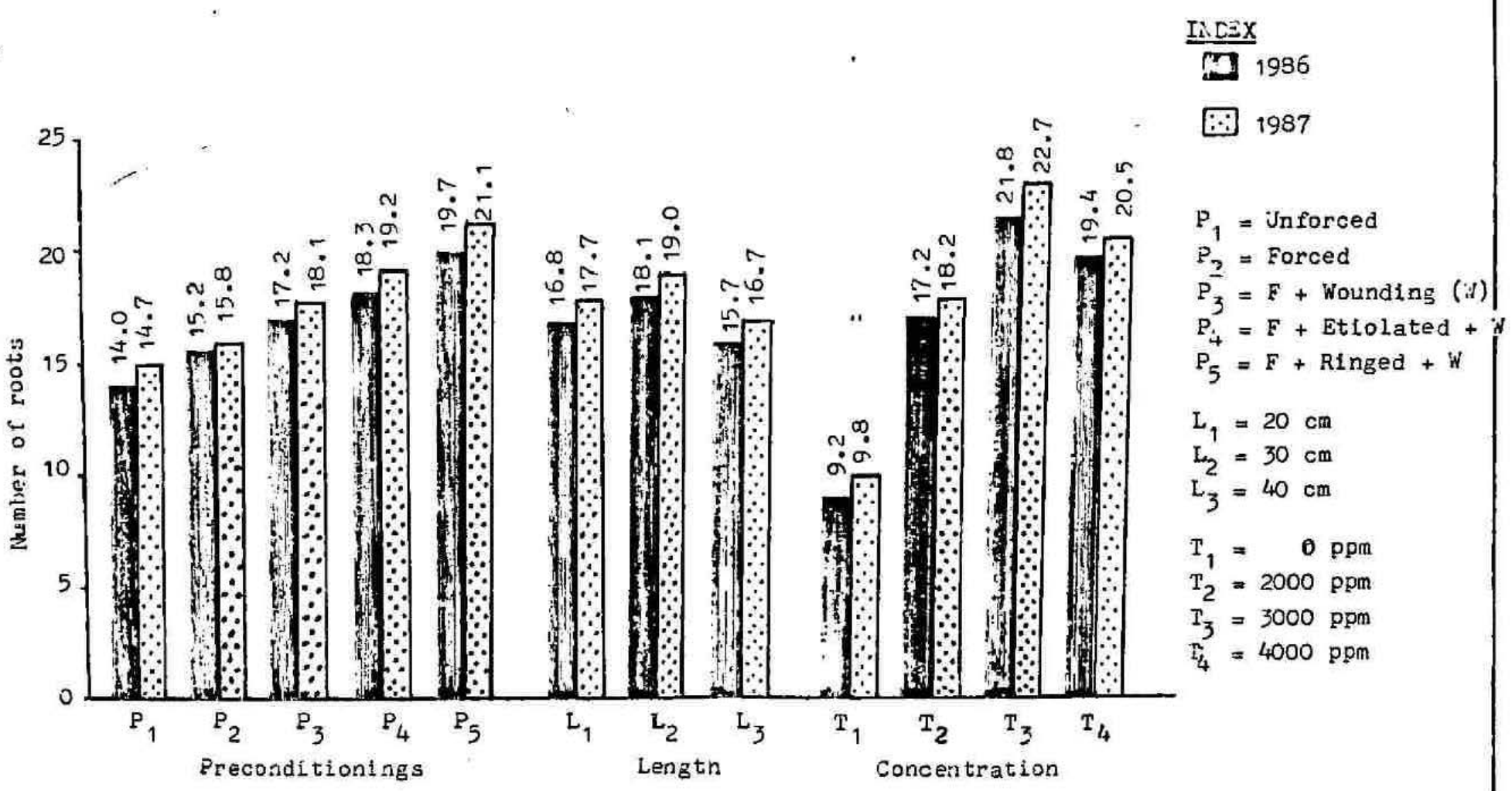


Fig. 13. Effect of preconditioning treatments, size of cuttings and IBA concentrations on number of primary roots per cuttings of plum cv.Santa Rosa

The interaction effects amongst different factors were found to be statistically non-significant in this regard.

4.6. Length of primary roots

The data recorded on the average length of roots in the cuttings of Santa Rosa plum cultivar as affected by preconditioning treatments, size of cuttings and IBA concentrations are presented in Table 20.

During 1986, the maximum root length measuring 13.1 cm was recorded in P_5 treatments and the minimum was recorded from P_1 treatment with 9.7 cm. Similarly, during 1987, the maximum root length (13.5 cm) was recorded from P_5 treatment and the minimum was obtained from P_1 treatment. However, in both the years, the differences between the preconditioning treatments were found to be non-significant.

Critical analysis of the Table 20 reflects that the size of cuttings had shown considerable effect on the length of the primary roots produced by the cuttings. The longest root was produced by 30 cm long cuttings (12.7 cm) which was significantly superior over 20 cm long cuttings (11.0 cm) and 40 cm long cuttings (10.5 cm) during 1986. Similarly, during the year 1987, maximum length of primary roots was obtained from 30 cm long cuttings (12.9 cm) which was significantly superior over 20 cm (11.3 cm) and 40 cm (11.0 cm) long cuttings. The differences between 20 cm and 40 cm long cuttings was found to be statistically non-significant.

Table 20. Effect of preconditioning treatment, size of cuttings and IBA concentrations on length of primary roots (in cm) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	5.5	11.4	11.1	9.3	6.0	11.8	10.9	10.0
30	7.0	13.4	12.6	10.9	7.4	14.0	12.4	11.0
40	5.3	11.4	9.8	8.3	6.2	11.0	10.3	9.2
P_2 Forced								
20	6.5	12.6	11.6	9.9	7.3	13.1	12.2	10.8
30	7.6	15.1	12.9	11.4	8.5	14.6	13.4	11.8
40	6.2	12.2	10.8	9.2	6.5	12.5	11.3	10.0
P_3 Forced + Wounding								
20	7.3	13.2	12.5	10.9	7.9	13.0	12.0	10.8
30	8.1	16.5	13.8	12.2	8.6	16.9	14.2	12.5
40	6.8	14.0	11.1	10.1	7.2	14.4	11.6	9.9
P_4 Forced + Etiolated + Wounding								
20	8.0	15.0	13.4	11.8	8.5	14.6	13.2	12.1
30	8.5	17.5	14.8	13.4	9.0	17.8	15.4	13.5
40	7.6	14.6	12.0	11.1	7.5	15.0	13.2	11.4
P_5 Forced + Ringed + Wounding								
20	8.6	15.9	13.9	12.4	8.9	16.1	14.6	12.8
30	9.1	18.5	16.1	14.0	9.5	18.0	16.5	13.7
40	8.2	15.6	13.2	11.9	9.0	16.3	13.8	12.2

Means of treatments

a) Preconditionings:	P_1	9.7	10.0
	P_2	10.5	11.0
	P_3	11.4	11.6
	P_4	12.3	12.6
	P_5	13.1	13.5
	C.D. (P=0.05)	1.05	0.94
b) Size of cuttings:	20 cm	11.0	11.3
	30 cm	12.7	12.8
	40 cm	10.5	11.0
	C.D. (P=0.05)	0.81	0.73
c) IBA Concentrations:	0 ppm	7.4	7.9
	2000 ppm	14.5	14.6
	3000 ppm	12.7	13.0
	4000 ppm	11.1	11.5
	C.D. (P=0.05)	0.94	0.84

Similarly, IBA treatments were found to have a significant effect on the length of the primary roots. During 1986, the maximum length of the primary roots measuring 14.5 cm was obtained in 2000 ppm IBA treated cuttings. The 3000 ppm and 4000 ppm IBA treated cuttings also gave equally good response producing 12.7 cm and 11.1 cm long primary roots respectively against 7.4 cm from untreated cuttings. IBA at 3000 ppm was found to be significantly different from 4000 ppm IBA treated cuttings. More or less similar effect of IBA treatments was observed on the mean length of the primary roots during the year 1987. All the cuttings treated with 2000 ppm IBA showed higher average length of the primary roots, which was significantly better than 3000 ppm, 4000 ppm and control.

The results in respect of interaction were not consistent and found to be non-significant during both the years under study.

4.7. Diameter of primary roots

The diameter of the primary roots in Santa Rosa plum cuttings were presented in Table 21.

It may be seen from the data presented in Table 21 that during 1986, the maximum diameter of primary roots (0.26 cm) was recorded under P_5 treatments. However, this treatment did not differ significantly with other preconditioning treatments except from P_1, which produced significantly smaller root diameter (0.21 cm). During the year 1987, the cuttings from P_5 treatment produced maximum

Table 21. Effect of preconditioning treatments, size of cuttings and IBA concentrations on diameter of primary roots (in cm) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	0.16	0.24	0.23	0.21	0.19	0.25	0.24	0.22
30	0.19	0.25	0.24	0.23	0.18	0.26	0.25	0.23
40	0.17	0.24	0.22	0.20	0.17	0.24	0.23	0.20
P_2 Forced								
20	0.18	0.26	0.25	0.24	0.20	0.27	0.25	0.24
30	0.20	0.27	0.25	0.25	0.22	0.28	0.25	0.25
40	0.17	0.25	0.24	0.24	0.19	0.26	0.24	0.23
P_3 Forced + Wounding								
20	0.21	0.28	0.27	0.25	0.19	0.26	0.25	0.24
30	0.21	0.29	0.26	0.24	0.21	0.28	0.26	0.25
40	0.18	0.26	0.25	0.24	0.18	0.25	0.24	0.23
P_4 Forced + Etiolated + Wounding								
20	0.20	0.28	0.27	0.26	0.22	0.27	0.26	0.25
30	0.21	0.29	0.28	0.27	0.23	0.29	0.27	0.26
40	0.20	0.26	0.25	0.24	0.21	0.26	0.25	0.24
P_5 Forced + Ringed + Wounding								
20	0.22	0.29	0.28	0.28	0.23	0.30	0.29	0.26
30	0.23	0.30	0.29	0.28	0.24	0.31	0.30	0.27
40	0.21	0.28	0.27	0.26	0.22	0.29	0.28	0.26

Means of treatments

a) Preconditionings :	P_1	0.21	0.22
	P_2	0.24	0.25
	P_3	0.25	0.24
	P_4	0.25	0.25
	P_5	0.26	0.27
C.D. (P=0.05)		0.015	0.014
b) Size of cuttings :	20 cm	0.24	0.25
	30 cm	0.25	0.26
	40 cm	0.23	0.24
C.D. (P=0.05)		0.012	0.011
c) IBA concentrations:	0 ppm	0.20	0.21
	2000 ppm	0.27	0.27
	3000 ppm	0.26	0.25
	4000 ppm	0.25	0.24
C.D. (P=0.05)		0.014	0.013

average diameter of primary roots with 0.27 cm which was significantly better than other preconditioning treatments. The P_1 treatment produced minimum diameter of roots, which was significantly lower than that produced under P_4, P_3 and P_2 which were at par with each other.

Data presented in Table 21 further reveals that the size of the cuttings had significant effect on the diameter of roots in both the years. During both the years, maximum diameter of roots were produced by 30 cm long Santa Rosa plum cuttings, which was significantly superior over 20 cm and 40 cm long cuttings. However, the differences between 20 cm and 40 cm long cuttings was found to be non-significant.

During the year 1986, the effect of different IBA concentrations on the diameter of primary roots was found to be significantly better than control. The maximum diameter of root (0.27 cm) was exhibited by the cuttings treated with 2000 ppm IBA which was significantly superior over 4000 ppm IBA treatments but was at par with 3000 ppm IBA treatment. During the year 1987 almost similar trend was observed with the maximum diameter of primary roots (0.27 cm) recorded in 2000 ppm IBA treated cuttings. Statistically, 2000 ppm IBA was significantly better than 3000 ppm, 4000 ppm IBA treatments and control. However, the 3000 and 4000 ppm treatments were at par with each other, but significantly better than control.

The differences due to interaction effects among different factors were found to be non-significant in

respect of the diameter of the primary roots.

4.8. Number of secondary roots per cutting

The observations on the development of secondary roots as affected by the preconditioning treatments, size of cuttings and different IBA treatments are given in Table 22.

During the year 1986, maximum number of secondary roots (52.0) were recorded in the cuttings under P_5 treatment, which was significantly better than P_3, P_2 and P_1 treatments but was at par with P_4. Almost similar effect was observed during 1987 in this character in which the maximum number of secondary roots (55.0) was obtained from P_5 treatment and the minimum (42.0) was recorded under P_1 treatment. The P_5 treatment was significantly superior over all the other preconditioning treatments, except P_4 treatment with which it was at par.

It was also observed that the size of cuttings had significant effect on the number of secondary roots per cutting. During 1986, the maximum average number of secondary roots per cutting (48.4) was observed in 30 cm long cuttings which was significantly superior over 40 cm long cuttings producing 42.6 secondary roots per cutting but it was statistically at par with 20 cm long cuttings (45.8). Similarly, during 1987, 30 cm long cuttings showed highest number of secondary roots per cutting with 50.8 roots which was significantly better than 40 cm long cuttings

(46.5) but found to be at par with 20 cm long cuttings with 48.6 roots.

A perusal of the data in Table 22 indicates that all the cuttings treated with different IBA concentrations gave significantly better results as compared to control but the treatment with 3000 ppm IBA proved to be the best in both the years under study producing 62.0 and 65.0 secondary roots per cutting during 1986 and 1987, respectively. Statistically, 3000 ppm IBA treatment was significantly superior over 4000 ppm IBA (52.0 and 54.7) and 2000 ppm IBA (46.6 and 51.4) during both the years under study. Cuttings under control produced minimum number of secondary roots per cutting in both the years of study with 21.2 and 23.5 secondary roots per cutting respectively.

The interaction effects between IBA concentrations and other preconditioning treatments of shoots as well as different size of cuttings in respect of the development of secondary roots was found to be non-significant in both the years under study.

4.9. Survival percentage of the rooted cuttings

The data recorded on survival percentage as affected by preconditioning treatments, cutting sizes and IBA concentrations is presented in Table 23.

During both the years, the maximum field survival of the rooted cuttings (66.7 and 68.9 per cent) were recorded

Table 22. Effect of preconditioning treatments, size of cuttings and IBA concentrations on number of secondary roots per cutting of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	18.5	40.0	54.5	46.5	20.5	45.0	57.5	48.3
30	20.2	43.5	57.3	48.0	21.8	42.3	58.0	49.8
40	17.5	37.0	50.3	42.2	18.7	41.0	52.3	45.0
P_2 Forced								
20	20.7	43.0	57.5	49.5	22.5	47.5	60.8	51.7
30	21.8	46.3	63.0	51.0	24.3	49.5	62.0	53.3
40	16.7	40.8	54.0	45.0	19.0	44.8	58.5	49.0
P_3 Forced + Wounding								
20	23.0	46.5	60.5	51.5	24.3	49.8	63.5	54.2
30	24.0	48.3	66.5	53.3	25.8	52.0	70.3	56.9
40	18.3	44.0	58.2	49.0	20.5	62.8	63.7	51.0
P_4 Forced + Etiolated + Wounding								
20	24.5	50.2	65.3	56.0	25.5	53.8	68.0	58.3
30	26.0	52.3	70.2	57.3	26.8	55.3	74.5	61.0
40	20.5	46.8	62.3	53.0	22.8	51.0	65.8	55.2
P_5 Forced + Ringed + Wounding								
20	25.8	53.5	69.5	59.3	27.0	58.7	73.0	62.5
30	28.3	55.5	74.5	61.3	28.5	60.2	77.5	64.0
40	22.7	50.8	66.7	56.5	24.8	54.0	70.3	59.3

Means of treatments

a) Preconditionings :	P_1	39.7	42.0
	P_2	42.4	45.2
	P_3	45.3	49.6
	P_4	48.7	51.5
	P_5	52.0	55.0
C.D. (P=0.05)		4.89	3.72
b) Size of cuttings :	20 cm	45.9	48.6
	30 cm	48.4	50.9
	40 cm	42.6	46.5
C.D. (P=0.05)		3.79	2.88
c) IBA concentrations :	0 ppm	21.2	23.5
	2000 ppm	46.6	51.4
	3000 ppm	62.0	65.0
	4000 ppm	52.0	54.7
C.D. (P=0.05)		4.37	3.32

under P_5 treatment closely followed by P_4, P_3 and P_2 treatments. Statistically, the treatments P_5, P_4 and P_3 were at par with each other though all of them were significantly better than P_1 treatments. P_1 treatment recorded the minimum (55.9 and 56.3 per cent) field survival during 1986 and 1987, respectively. However, P_1 treatment was at par with P_2 during 1986.

It is evident from the data given in Table 23 that the size of cuttings had shown significant effect on field survival in both the years under study. During the year 1986, maximum survival of 64.6 per cent was noted with the planting of 30 cm long cuttings which was significantly superior over 20 cm and 40 cm long cuttings. The minimum field survival (59.4 per cent) was recorded from the 40 cm long cuttings. In 1987, maximum field survival of 66.2 per cent was recorded from 30 cm long cuttings which was statistically at par with 20 cm long cuttings but significantly superior over 40 cm long cuttings with 62.5 per cent field establishment.

The effect of IBA at different concentrations on the survival of cuttings was found to be high during 1986. The maximum field survival of cuttings (71.2 per cent) was resulted from 3000 ppm IBA treated cuttings followed by 4000 ppm and 2000 ppm IBA. The lowest survival percentage of 43.0 per cent was observed in control treatment. Statistically, 3000 ppm IBA treatment was significantly superior over 4000 ppm and 2000 ppm IBA treatments and the control.

Table 23. Effect of preconditioning treatments, size of cuttings and IBA concentrations on field survival of rooted cuttings (in per cent) of plum cv. Santa Rosa

Preconditioning treatments/size of cuttings (cm)	1986 IBA (ppm)				1987 IBA (ppm)			
	0	2000	3000	4000	0	2000	3000	4000
P_1 Unforced								
20	45.8	56.7	61.9	57.4	45.9	56.3	63.0	62.0
30	41.5	62.0	67.7	62.2	35.4	60.9	68.9	61.3
40	40.0	54.9	60.4	50.0	43.8	55.8	64.0	56.7
P_2 Forced								
20	41.7	62.2	66.4	62.6	45.8	65.6	70.0	68.3
30	42.5	65.7	73.7	68.3	39.6	69.1	74.4	69.8
40	44.8	61.2	66.7	62.1	45.8	64.2	62.6	61.6
P_3 Forced + Wounding								
20	39.6	65.8	70.8	67.9	37.9	69.5	72.3	68.0
30	43.8	68.1	76.6	73.1	46.3	70.0	76.8	74.0
40	41.7	64.6	68.9	66.8	39.8	66.7	70.2	62.7
P_4 Forced + Etiolated + Wounding								
20	39.2	68.3	73.2	70.9	43.8	71.0	75.1	72.2
30	46.2	70.5	77.8	73.3	50.4	72.9	79.9	75.7
40	39.8	67.3	72.5	65.9	43.8	68.5	74.6	71.0
P_5 Forced + Ringed + Wounding								
20	43.8	68.3	76.9	71.2	46.3	71.8	77.8	67.9
30	48.3	75.9	80.6	75.6	52.5	78.8	85.4	79.4
40	44.2	69.9	73.3	71.9	45.8	71.0	76.8	73.0
Means of treatments								
a) Preconditionings : P_1				55.9				56.3
P_2				59.8				61.7
P_3				62.3				62.8
P_4				63.7				66.5
P_5				66.7				68.9
C.D. (P=0.05)				4.91				5.23
b) Size of cuttings : 20 cm				60.5				62.5
30 cm				64.6				66.2
40 cm				59.4				61.0
C.D. (P=0.05)				3.80				4.06
c) IBA concentrations: 0 ppm				43.0				44.4
2000 ppm				65.4				67.3
3000 ppm				71.2				72.9
4000 ppm				66.6				68.3
C.D. (P=0.05)				4.39				4.69

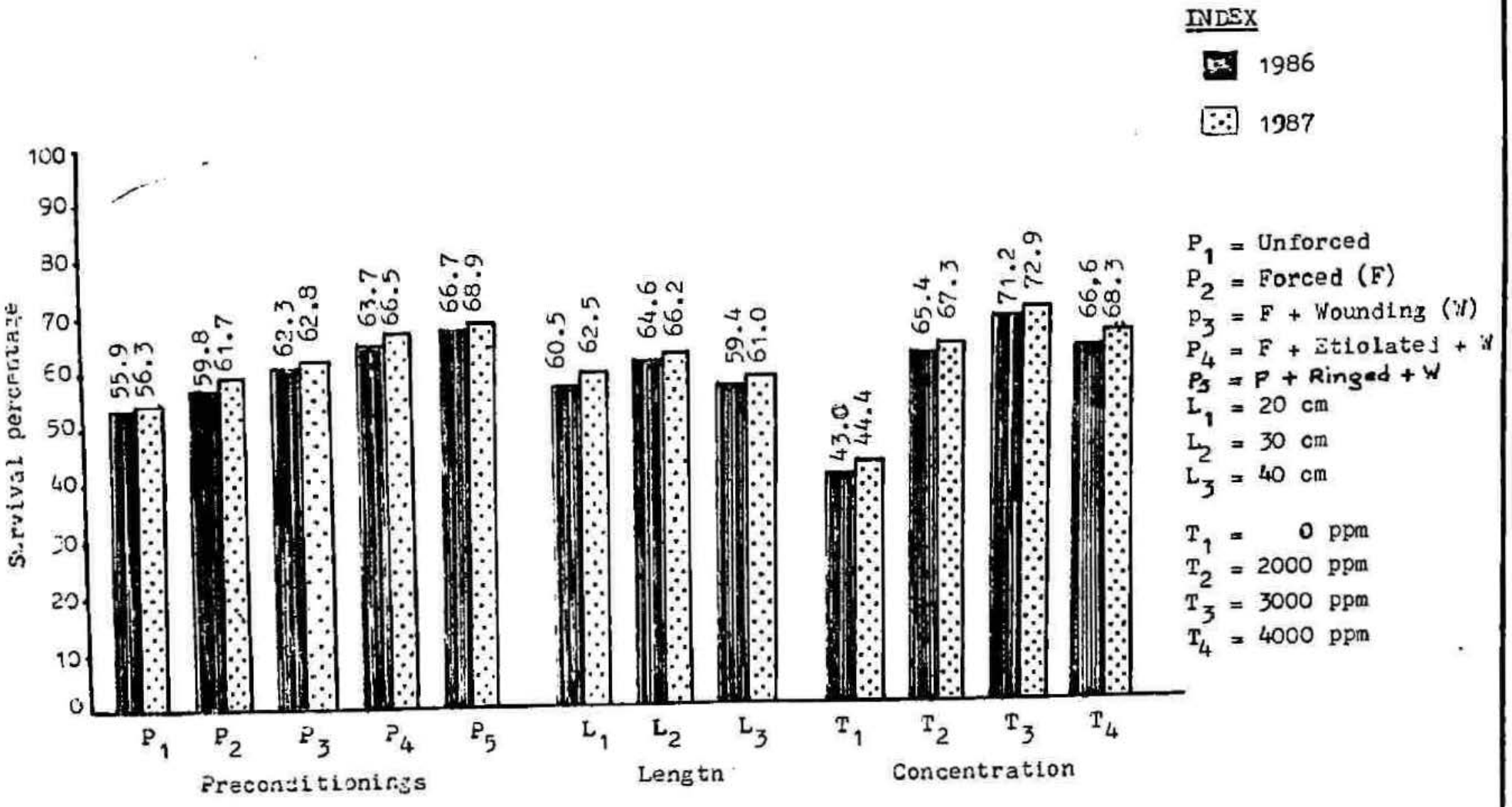


Fig. 14. Effect of preconditioning treatments, size of cuttings and IBA concentrations on field survival of rooted cuttings of plum cv.Santa Rosa

During the year 1987, the maximum field survival (72.9 per cent) was recorded in 3000 ppm IBA treatment which was statistically at par with 4000 ppm IBA treated cuttings but significantly better than 2000 ppm IBA treated cuttings and cuttings under control. The minimum field survival of 44.4 per cent was obtained from those cuttings under control.

The interaction effects among the preconditioning treatments, cutting sizes and IBA concentrations was found to be non-significant and not consistent in respect of this character.

5. Biochemical status of Santa Rosa plum cuttings as affected by various preconditioning treatments of shoots

The data on the various biochemical constituents of Santa Rosa plum cuttings as affected by various preconditioning treatments of shoots were recorded and are presented in Table 24.

5.1. Total nitrogen

It may be seen from the data given in Table 23 that during the year 1986, hardwood cuttings of Santa Rosa plum under NPT recorded maximum (0.80 per cent) nitrogen content followed by F+R treatment with 0.69 per cent. The minimum nitrogen content (0.64 per cent) was recorded under F+E treatment. During the year 1987, the maximum nitrogen content of 0.75 per cent was observed from cuttings under NPT while the minimum (0.61 per cent) was obtained from F+E treatment. The nitrogen content in the cuttings showed

Table 24. Mean values or some biochemical constituents (on dry weight basis) as affected by various preconditioning treatments and their relationship with rooting of hardwood cuttings of Santa Rosa plum

Year and Preconditioning treatments	Total rooting (%)	Total nitrogen (%)	Total carbohydrates (%)	C/N ratio	Total phenols (mg/g)	O.D. phenols (mg/g)
1986						
F+E	55.0	0.64	22.1	34.5	36.1	10.3
F+R	61.0	0.69	24.6	35.6	34.7	9.2
NPT	47.1	0.80	21.8	27.3	32.6	8.7
(r)	-	-0.73**	+0.47	+0.87**	+0.66*	+0.38
1987						
F+E	56.0	0.61	21.1	34.6	34.8	9.9
F+R	63.0	0.63	25.6	40.6	32.3	8.5
NPT	46.5	0.75	22.8	30.4	28.4	8.0
(r)	-	-0.84**	+0.54	+0.90**	+0.51	+0.34

F = Forcing; E = Etiolation; R = Ringing; and NPT = No preconditioning treatment

*Significant at 5 per cent
**Significant at 1 per cent

significant negative correlation with the rooting ability of the cuttings in both the years under study.

5.2. Total carbohydrates

Highest percentage of carbohydrates (24.6 per cent) was found in the cuttings under F+R preconditioning treatment while the lowest content (21.8 per cent) was recorded in NPT during 1986. More or less similar trend was observed in carbohydrate content ranging between 21.1 to 25.6 per cent with maximum occurring in F+R treatment while the minimum content was recorded under NPT during the year 1987. The response of carbohydrate content with respect to the rooting ability of the cuttings was found to be positively correlated during both the years under study.

5.3. Carbohydrates/Nitrogen ratio (C/N ratio)

The data presented in Table 24 revealed that C/N ratio ranged from 27.3 to 35.6 during the year 1986 and 30.4 to 40.6 during 1987, with maximum level of C/N ratio under F+R treatment and the minimum under NPT in both the years of study. The C/N ratio was positively correlated to the rooting percentage of Santa Rosa plum cuttings as was shown by highly significant correlation coefficient in both the years under study.

5.4. Total phenols

It is evident from Table 24 that the maximum content (36.1 and 34.8 mg/g) of total phenols were found in cuttings

under F+E treatment and the minimum contents (32.6 and 30.4 mg/g) were obtained in NPT during 1986 and 1987. The correlation between total phenols content and the rooting ability of the cuttings was found to be significant during 1986 but non-significant during 1987.

5.5. Ortho dihydric phenols (O.D. phenols)

The highest amount of O.D. phenols (10.3 and 9.9 mg/g) were observed under F+E treatment and the minimum contents of O.D. phenols (8.7 and 8.0 mg/g) were observed under NPT during 1986. The contents of O.D. phenols were positively correlated with the rooting of the cuttings but were found to be non-significant in both the years.

6. Study on the response of tissue culture in respect of shoot proliferation and root initiation

6.1. Development of callus in apical meristem

The initiation of callus was observed from all the cultivars during the third week of cutting of apical meristems. Callus initiation of Santa Rosa plum cultivar appeared first during the earlier part of the third week of the trial. In the beginning compact, whitish-green granular structures appeared near the interface of the tissue and the medium, which gradually spread all over the apical meristem, resulting into an apparent increase of the tissue mass. The gradual growth of callus in the other three cultivars was also observed in the latter part of the third week. The calli grew gradually but significantly over

the duration of the experiment in all the cultivars. These results are presented in Table 25. The rate of growth was quite fast in the beginning. The tissue grew almost five times its weight during the first four weeks and the mass of calli obtained at the end of 8th and 12th week of the

Table 25. Periodic callus growth*(in gram) from apical meristem of four plum cultivars

Cultivars	4 weeks	8 weeks	12 weeks	Mean
Santa Rosa	5.4	35.6	65.5	35.5
Beauty	4.6	27.2	62.7	31.5
Greengage	3.0	16.8	37.5	19.1
Early Transparent Gage	3.4	21.5	39.8	21.6
Mean	4.1	25.3	51.4	-
C.D. (P=0.05)	2.7	4.7	8.2	

*Periodic callus growth results are the means of six separate experiments

experiment were still significantly higher than the precedding observations.

The calli of Santa Rosa and Beauty cultivars grew faster than the calli in Greengage and Early Transparent Gage from the beginning of the experiment. The gap became significantly wider in the later part of the trial. The total callus growth obtained in Santa Rosa and Beauty was significantly higher than that of Early Transparent Gage end Greengage at the end of the 4th, 8th and 12th week. Similarly,



Plate 6. Development of callus in apical meristem explant of some plum cultivars.
SR = Santa Rosa BE = Beauty ET = Early Transparent Gage GG = Greengage



Plate 7. Shoot proliferation in explants of plum <u>in vitro</u>

there was no significant differences between Early Transparent Gage and Greengage except during the second transfer.

6.2. Proliferation rate of explants from apical meristems and shoot

There were no signs of growth in the calli from apical meristems, even upto the first month of planting of the explants. However, the explants were observed to be green and a number of shoots emerged out from the callus after six weeks of planting. Similarly, explants of shoot tips with lateral bud had also exhibited the signs of shoot proliferation after six weeks. The data on shoot proliferation rate of apical meristem and shoot tip explants are presented in Table 26.

Table 26. Proliferation rate* of apical meristem and shoot tips with lateral bud as explants in four plum cultivars under in vitro

Cultivars	Average number of shoots per culture		Mean
	Apical meristem as explant	Shoot tip with lateral bud as explant	
Santa Rosa	13.2	9.5	11.4
Beauty	11.7	6.8	9.3
Greengage	7.3	4.8	6.1
Early Transparent Gage	8.2	5.6	6.9
Mean	10.1	6.7	-

C.D. (P=0.05) for

Explant	= 0.71
Cultivar	= 1.01
Interaction (Explant x Cultivar)	= 1.42

*Shoot proliferation results are the means of six separate experiments

The data given in Table 26 revealed that cultivars, type of explants and their interaction were found to be significant at 5 per cent level of significance.

Significant difference was observed due to the effect of cultivars on the average number of shoots per explant. It is evident from the data presented in Table 26 that explants from Santa Rosa plum cultivar produced the maximum number of shoots (11.4) per explant which were significantly better than Beauty, Early Transparent Gage and Greengage producing 9.3, 6.9 and 6.1 shoots per explant in descending order.

It was also found that the type of explant had also shown significant effect on the production of average number of shoots per explant. When the apical meristem was used as explant it produced the maximum number of shoots (10.1) per explant which was significantly superior over shoot tip with lateral bud as explant.

The interaction effects between cultivars and type of explants gave the highest number of shoots (13.2) per culture when apical meristems from Santa Rosa were used as explants while the minimum number of shoots (4.8) per explant was recorded when shoot tips with a lateral bud were used as explants obtained from Greengage plum.

6.3. Plant characteristics of in vitro derived plantlets of plum

6.3.1. Shoot length

A perusal of data in Table 27 showed that explants

Table 27. Mean characteristics of *in vitro* derived plantlets of plum*

Cultivar	Average shoot number per explant	Average shoot length (cm)	Average shoot diameter (cm)	Rotting per cent	Average root number/plant	Average root length (cm)
Santa Rosa	11.4	2.1	0.25	37.8	9.6	3.5
Beauty	9.3	1.9	0.22	33.0	7.5	3.0
Greengage	6.1	1.4	0.20	27.9	5.2	2.5
Early Transparent Gage	6.9	1.7	0.19	28.8	5.4	2.8
C.D. (P=0.05)	1.01	0.46	N.S.	2.38	0.93	0.26

*Shoots and root characters are the means of six separate experiments

obtained from Santa Rosa produced the maximum shoot length (2.1 cm). There was however, no significant difference in shoot length among Santa Rosa, Beauty and Early Transparent Gage. However, Santa Rosa was found to give better shoot length which was significantly superior to Greengage in this character.

6.3.2. Diameter of shoot

The data on the effect of different cultivars on the diameter of *in vitro* derived shoots is presented in Table 27.

A critical analysis of the data showed that the maximum diameter of 0.25 cm was recorded *in vitro* derived shoot obtained from Santa Rosa plum. However, the differences in shoot diameter in the plantlets derived from different cultivars were not statistically significant.

6.3.3. Percentage of rooting

The data in Table 27 revealed that the highest rooting percentage was recorded in Santa Rosa plum cultivar which was significantly superior to all the other cultivars. The percentage of rooting in the *in vitro* derived shoots of Early Transparent Gage and Greengage was statistically at par with each other.

6.3.4. Number of roots per plant

It is evident from Table 27 that the *in vitro* derived shoots of Santa Rosa plum produced the maximum number of

roots per plant which was significantly superior to all the four cultivars. However, the number of shoots for Early Transparent Gage and Greengage were statistically at par with each other in this respect.

6.3.5. Root length

The perusal of data presented in Table 27 indicated that the plantlets of Santa Rosa had significantly maximum root length per plantlet. There was, however, no significant difference between the plantlets of Early Transparent Gage and Greengage whereas Beauty was significantly over the last two cultivars in this respect. The minimum root length was obtained from Greengage plum cultivar.

6.4. Effect of IBA and NAA concentrations in the rooting media on rooting behaviour of *in vitro* derived shoots of plum

6.4.1. Percentage of rooting

The data in respect of mean rooting percentage of *in vitro* derived shoots of plum cultivars are presented in Table 28. The data given in Table 28 revealed that the effect of cultivars and growth regulator treatments on rooting percentage were found to be significant whereas their interaction was not significant with each other.

The highest rooting percentage (37.8 per cent) was recorded in Santa Rosa plum which was significantly superior to all the other cultivars under study. The lowest rooting per cent was recorded in Greengage with 27.9 per cent, which was at par with that of Early Transparent Gage but significantly

Table 28. Effect of IBA and NAA supplements in MS medium on rooting percentage from in vitro derived shoots of four plum cultivars*

Treatment	Concentration (mg/l)	Cultivars				Mean
		Santa Rosa	Beauty	Greengage	Early Transparent Gage	
Control	-	22.8	19.4	12.0	15.2	17.4
IBA	0.5	33.4	31.0	27.0	29.2	30.2
IBA	1.0	53.6	44.2	35.4	38.6	42.9
IBA	2.0	41.8	37.0	32.2	27.8	34.7
NAA	0.5	44.8	39.2	33.4	35.2	38.2
NAA	1.0	37.2	32.3	28.5	30.0	32.0
NAA	2.0	31.0	28.2	26.8	25.2	27.8
Mean	-	37.8	33.0	27.9	28.8	-

C.D. ($P=0.05$)

Treatment	= 3.14
Cultivar	= 2.38
Interaction (Treatment x Cultivar)	= N.S.

*Rooting results are the means of six separate experiments



Plate 8. Proliferation of shoots in shoot tip explants of plum.



Plate 9. Rooting of plantlets of plum in MS rooting medium *in vitro*.

less as compared to Beauty.

The effect of different growth regulator treatments on the percentage of rooting of in vitro derived shoots were also significantly different from each other. The proliferated shoots planted in the MS medium containing 1.0 mg/l IBA under aseptic conditions gave significantly higher percentage of rooting than those treated with different concentrations of IBA and NAA. The in vitro derived shoots planted in MS medium devoid of any IBA and NAA showed the least percentage of rooting in this respect.

The results in respect of interaction effect between cultivars and treatments were not consistent and were found to be non-significant with respect to rooting.

6.4.2. Number of roots per plantlet in vitro

The results recorded on the average number of roots are presented in Table 29.

Table 29 showed that the maximum number of roots (9.6) was obtained in the proliferated shoots of Santa Rosa plum cultivar which was significantly superior to the other three plum cultivars. The minimum average number of roots per plant was recorded in Greengage cultivar with 5.2 roots. The in vitro derived shoots of Early Transparent Gage and Greengage were statistically at par with each other but were statistically inferior to Beauty in this respect.

Table 29. Effect of IBA and NAA supplements in MS medium on average number of roots per plantlet from *in vitro* derived shoots of four plum cultivars*

Treatment	Concentration (mg/l)	Cultivars				Mean
		Santa Rosa	Beauty	Greengage	Early Transparent Gage	
Control	-	4.6	3.4	1.8	2.4	3.1
IBA	0.5	8.2	6.4	5.6	5.2	6.4
IBA	1.0	14.6	13.3	7.5	8.6	11.0
IBA	2.0	11.2	8.5	6.1	5.8	7.9
NAA	0.5	12.4	10.2	6.8	7.8	9.3
NAA	1.0	9.2	5.4	4.6	4.0	5.8
NAA	2.0	7.0	5.2	3.8	4.2	5.1
Mean	-	9.6	7.5	5.2	5.4	-

C.D. ($P=0.05$)

Treatment = 1.23
Cultivar = 0.93
Interaction (Treatment x Cultivar) = N.S.

*Average number of roots per plantlet is the means from six separate experiments

The effect of different concentrations of IBA and NAA on number of roots per plantlet were also significantly different from one another. It is evident from Table 29 that the rooting medium containing IBA at 1.0 mg/l produced maximum number of roots (11.0) which was significantly superior to other IBA and NAA treated rooting media. The MS rooting media containing 1.0 mg/l and 2.0 mg/l NAA were statistically at par with each other in this respect and the latter showed the minimum average number of roots per plantlet producing 5.1 roots.

The interaction between cultivars and treatments was not consistent and found to be non-significant. However, it was observed that *in vitro* derived shoots of Santa Rosa plum when planted in the MS rooting medium containing 1.0 mg/l of IBA produced the maximum number of roots per plantlet.

6.4.3. Root length

The data in respect of average root length per proliferated shoot which were planted in rooting media under aseptic condition are presented in Table 30.

It may be seen from the data presented in Table 30 that the maximum root length (3.5 cm) was recorded with *in vitro* proliferated shoots of Santa Rosa plum cultivar which was significantly better than those of Beauty, Early Transparent Gage and Greengage. The minimum average length of root per plantlet was produced by Greengage plum cultivar

Table 30. Effect of IBA and NAA supplements in MS medium on average root length (cm) from *in vitro* derived shoots of four plum cultivars*

Treatment	Concentration (mg/l)	Cultivars				Mean
		Santa Rosa	Beauty	Greengage	Early Transparent Gage	
Control	-	1.7	1.2	0.60	0.9	1.1
IBA	0.5	3.2	2.8	2.5	2.6	2.8
IBA	1.0	4.6	4.1	3.5	3.7	4.0
IBA	2.0	4.0	3.4	3.0	3.3	3.4
NAA	0.5	4.3	3.9	3.3	3.5	3.8
NAA	1.0	3.7	3.2	2.7	2.9	3.1
NAA	2.0	3.0	2.4	2.0	2.2	2.4
Mean	-	3.5	3.0	2.5	2.8	-

C.D. (P=0.05)

Treatment = 0.35
Cultivar = 0.26
Interaction (Treatment x Cultivar) = N.S.

*The average root length is the mean of six separate experiments



Plate 10. In vitro rooted plantlets of plum.



Plate 11. Weaning of in vitro rooted plantlet of plum under light and temperature controlled room.

with 2.5 cm long roots.

It is evident from Table 30 that the rooting medium containing 1.0 mg/l IBA was statistically at par with the rooting medium containing 0.5 mg/l NAA but was significantly better than the rest of the treatments with respect to the production of maximum root length (4.0 cm). However, the minimum root length was produced by MS rooting medium containing only the basal medium.

The results in respect of interaction were not consistent in between cultivars and treatments. However, the maximum root length of 4.6 cm per plantlet was recorded from those *in vitro* derived shoots which were planted in the MS rooting medium containing 1.0 mg/l IBA concentration.

6.4.4. Field survivability of rooted plant

There was no success in field establishment of the rooted plants due to non-availability of temperature and moisture controlled glass house (phytotron) which is a must for *in vitro* derived rooted plants as those plants cannot be planted directly in the field just after the weaning treatment in the temperature and moisture controlled laboratory.

C H A P T E R - V

D I S C U S S I O N

DISCUSSION

The experiments on the propagation of four plum cultivars, two each from Prunus salicina Lindl. and P. domestica L. through hardwood and semi-hardwood cuttings and by tissue culture were conducted during 1986-88. The results obtained from the present investigations are discussed below under different headings:

1. Varietal variations in rooting, growth development and field establishment

In the present studies, maximum sprouting and shoot development were recorded in Santa Rosa cuttings. However, there were no significant variations in sprouting behaviour amongst different cultivars. Similarly, there was no correlationship between sprouting and rooting behaviour as well as with the field establishment of the rooted cuttings.

While comparing with overall performance of cultivars under the present investigations, the maximum rooting percentage was recorded in Santa Rosa followed by Beauty which belong to Japanese plum species whereas poor rooting was exhibited by European plum cultivars, Greengage and Early Transparent Gage. The development of primary and secondary roots in respect of their numbers, length and diameter were also found to be better in Japanese plum cultivars. This shows that Japanese plum species could be stimulated to

induce rooting relatively more easily than European plum species. This also indicates that the European plum species belong to the difficult-to-root category. The poor rooting behaviours in cuttings of European plums in the present studies might be attributed to their genetic constitutions besides their physiological conditions and anatomical characteristics. These findings are in accordance with the observations of Bartolini and Roselli (1979) and Bartolini et al. (1982) who had found that eight Japanese plum cultivars rooted better than nine others of European group with or without IBA treatments.

The performance of rooted cuttings of different cultivars in respect of their field establishment was more or less in line with the observations recorded in the rooting behaviour. In the present studies, highest field establishment was recorded in Santa Rosa in both the years while the lowest was recorded in Greengage during 1986 and Early Transparent Gage during 1987.

Differences among the cuttings of different plum species in respect of field survival might be explained in view of variations in characters like the number, length and diameter of roots as well as the size of the shoots. This may also be due to the variations in the physiological maturity of rooted cuttings, internal food reserves and retention of adequate number of leaves at the time of transplanting of rooted cuttings. The usefulness of leaves

on rooting and field establishment has already been emphasized by various workers (Went, 1938 and Faustov, 1969).

The poor establishment of the rooted cuttings of European plum species can also be explained on the probable grounds that its cuttings took longer time for the initiation of rooting and this in turn might have depleted maximum internal food reserves in the cuttings by the time they become ready for transplanting. Such cuttings also produced poor root systems as compared to the Japanese plum species in the present study, which is in conformity with the earlier findings of Bartolini and Roselli (1979).

2. Seasonal variations in rooting, growth development and field establishment

In the present studies, although the maximum sprouting was obtained during summer in both the years there were only little variations on sprouting with respect to different seasons and sprouting had no significant association with rooting and field survivability. In both the years, the development of shoot length and diameter was also better in the summer planted cuttings. The possible explanation of the marked seasonal contrast in the manifestation of the vegetative growth and development in the cuttings in different plum cultivars may possibly be due to the morphological status of the mother plants from which the cuttings were collected as stated by Wells (1963). This might also be due to differences in environmental conditions such as temperatures and humidity prevailing during the

experimental periods. The cuttings collected during summer season were leafy semi-hardwood, the buds were active in growth and the cuttings were planted under mist where better growth conditions such as temperature and humidity could be maintained at optimum levels during rooting and vegetative growth. On the other hand, cuttings collected during dormant and autumn seasons were fully matured hardwood cuttings which were planted under adverse field conditions. The observations of Hartmann and Kester (1983) regarding seasonal variations on vegetative growth in stem cuttings also support the present findings.

In the present studies, maximum rooting was recorded from summer planted cuttings. The development of higher primary and secondary roots as well as superior length and diameter of roots were also recorded during this season in both the years under study. These findings on seasonal effect on the development of root systems are in agreement with the findings of Hartmann and Brooks (1959), Hartmann and Loreti (1965) and Brainerd and Evert (1980) who have also reported that in deciduous plant species, leafy semi-hardwood cuttings root better during summer season while hardwood cuttings root better during dormant season. The presence of leaves in the cuttings at the time of planting not only supply nutrients but also specific root forming substance called rhizocaline to the site of rhizogenesis which increases rooting (Went, 1929, 1938). Similarly, Faustov (1969) reported that the cuttings of several species which

retained their leaves rooted better than those which shed their leaves during the rooting period. Hartmann and Kester (1983) also reported that if hardwood cuttings were taken when the buds were in the rest period, they had no stimulating effect on rooting, but when the buds were active, they showed a strong root promoting effect. Further more, the stem cuttings taken from juvenile growth phase, root much more readily than those taken from the adult growth phase (Gardner, 1929). Since the summer planted cuttings were obtained from the current season growth which are still immature in age, the maximum rooting was also obtained from these cuttings during summer season. The presence of more sclerenchymatous ring in the hardwood cuttings and increased inhibitory substances during dormancy as reported by Fadl and Hartmann (1967) might be the reasons for poor rooting in autumn and dormant seasons.

3. Effect of IBA concentrations on rooting, shoot growth and field establishment

In the present experiments, treatment of cuttings with IBA resulted in higher rooting performance, better shoot growth and maximum field survival. However, the sprouting percentage of the cuttings obtained under different IBA treatments were inconsistent and had no impact on either rooting behaviour or field establishment. The IBA application at 2000 ppm concentration resulted in maximum rooting and field survival of semi-hardwood cuttings of all the four cultivars whereas in dormant and autumn seasons

maximum rooting were recorded with 3000 ppm IBA irrespective of type of cultivars. These observations regarding the effect of 2000 ppm IBA during summer under mist and 3000 ppm IBA during dormant and autumn seasons under field conditions on the rooting of cuttings of plums are more or less in conformity with the findings of several other research workers (Nicotra, 1971; Kapetanovic et al., 1972; Ivanicka and Pastyrik, 1978; Fontanazza and Rugini, 1980 and Rathore, 1983). The lower rooting with IBA at 4000 ppm was considered to be due to a toxic effect of this growth regulator on root initiation. On the other hand, lower concentrations of 2000 or 3000 ppm IBA showed better exploitation of regenerative capacity of the cuttings.

The variation in the rooting percentage in different cultivars of plum with IBA treatments might be due to their inherent genetic constitution as reported by Zatyko (1959). The differential response in the rooting of cuttings among different plum species with IBA treatments can also be due to variable response of species to different concentrations. According to Hartmann and Kester (1983) the purpose of treating cuttings with growth regulators is to increase percentage of rooted cuttings, to hasten root initiation, to increase the number and quality of roots and to increase uniformity of rooting.

The response of cuttings to different IBA treatments in respect of average number, length and diameter of roots per cutting, was almost similar in both the years. It was

also observed in the present studies, that lower concentration of IBA at 2000 ppm increased root length significantly but the number of roots was less as compared to 3000 ppm and 4000 ppm IBA concentrations. These observations are in conformity of the earlier findings of Nahlawi and Howard (1972) and Devlin (1978) who reported that higher concentrations of IBA increased the number of roots per cuttings whereas low concentration increased the root length and diameter. Similar findings on the clonal propagation of plum and peach were reported by Howard and Nahlawi (1973), Chauhan and Reddy (1974) and Dwivedi et al. (1979). Nanda (1975) also reported that IBA treatments increased the initiation of cell division and elongation in meristematic tissues, differentiation of cambial initials into root primordia and in the mobilization of reserve food materials by enhancing the activity of hydrolyzing enzymes. Thus, it appears that IBA treatments had a marked effect on rooting as well as on rapidity of root and shoot development than control.

In the present studies, the number and size of the roots had shown an important role on the field establishment of the rooted cuttings. The size of the shoots at the time of transplanting in turn promoted the vegetative growth of the shoots. This relationship was quite discernable in the present study. The present observations on the relationship between rooting and field survival is in the conformity with the findings of various research workers (Grzyb, 1975;

Hartmann, 1982 and Sinha et al., 1983). The better survival of rooted cuttings under IBA treatments as compared to control in the present studies are in conformity with the findings of Bartolini and Roselli (1976), Pathak et al. (1975) and Hartmann (1982) who reported that rooting of cuttings showed definite and significant survival responses to the IBA concentrations and basal wounding in the cuttings of different plum cultivars.

The higher percentage of rooting in semi-hardwood cuttings of different plum species, especially European plum species when planted under mist during summer as compared to hardwood cuttings planted during dormant and autumn might be due to the beneficial effects of mist on rooting. The misting showed beneficial effect on cuttings because of maintenance of high humidity around the cuttings which reduced the rate of respiration and transpiration thereby keeping the cuttings alive until rhizogenesis. These findings are in accordance with the observations of Merganov (1978) and Rathore (1983) who had reported good success in rooting and field survivability in different plum species when treated with IBA and raised under intermittent mist conditions.

5. Effect of preconditioning treatments on rooting, growth development and field establishment

In the present studies, it was observed that in Santa Rosa, forced cuttings produced higher rooting, more number of roots and better root and shoot characteristics. It also

gave higher field survival percentage. Further improvement in rooting of cuttings was noticed when they were given either ringing or etiolation treatments. Thirty centimeter long cuttings from forced and ringed shoots when given a wounding-cut in the base of the cuttings and treated with 3000 ppm IBA prior to planting gave the best results followed by preconditioning with etiolation of the shoots in the mother plants. This results clearly indicated the importance of forcing, etiolation or ringing, wounding-cuts, size of cuttings and IBA treatments in the induction of rooting, shoot growth and the field establishment of Santa Rosa plum cuttings. The present observations support the findings of Fontanazza (1969), Nicotra (1971) and Nanda (1975).

It has been observed that cuttings from plants in the juvenile stage can be induced to root more easily as reported by Gardner (1929) and Sax (1962). In the present experiment the newly sprouted shoots from the cut ends of thick branches have been termed forced shoots as suggested by Mukherjee and Chatterjee (1979) and these have given significantly higher rooting than unforced shoots due to their juvenile nature. This observation was also supported by the findings of Gautam (1986).

In the present studies, etiolation of shoots before cuttings were made was observed to promote better rooting, better characteristics of roots, shoot growth and field survival, as has been reported by Knight and Wilt (1927),

Teaotea and Pandey (1961), Hartmann and Hess (1963) and Hartmann and Kester (1968). The better performance of root characteristics in etiolated shoots might be due to decrease in starch content, cell wall thickness and cell wall deposits. This finding is also in accordance with Nanda et al. (1979) who had reported that etiolated stems had more parenchyma cells and more tissues in a less differentiated condition thereby making them to react strongly to applied auxins for root formation and acting synergistically as a rooting cofactors.

Ringing of shoots in the mother plants before the cuttings are made to induce maximum rooting and growth characteristics in the present study. This treatment was found to be significantly better than all the preconditioning treatments in the present study in both the years. The present observation is in conformity of the findings with that of Stoltz and Hess (1966), Fontanazza and Rugini (1980) and Hartmann and Kester (1983). These findings also get support from the work done by Purushotham et al. (1982) who indicated that ringing followed by etiolation improved rooting percentage in coffee cuttings. Similar findings was also reported by Gautam (1986) in the cuttings of some temperate fruit plants. The better response of ringing might be due to the reason that ringing blocks the downward movement of carbohydrates, hormones and other possible root promoting factors which induced root initiation and resulted in increased root formation. Sen and Bose (1959) suggested that changes in physiological status of the

regenerating tissue consequent upon ringing is an important condition in the formation of roots in relatively difficult-to-root cuttings. The present observations also supported these findings.

In the present study it was observed that wounding-cuts given in the basal ends of the cuttings also induced more rooting and plant growth in the preconditioned cuttings. Maximum rooting, more number of primary and secondary roots, better root length and diameter, better shoot size and higher field survival were observed when wounding-cut was given to forced shoots with ringing. This might be due to greater absorption of applied growth regulator by disrupting cambium tissues at the base of the wounded cuttings as was reported by Hartmann and Kester (1983). Wounding of cuttings also facilitated the outward penetration of the newly emerged roots and their subsequent development.

In the present studies, it was observed that 30 cm long preconditioned hardwood cuttings produced maximum rooting and field establishment. This observation was in conformity with that of Fontanazza (1969) and Chauhan and Reddy (1974) who obtained better rooting and field survival with 30 cm long cuttings of some plum cultivars. However, the present finding is not in line with the observations made by Nahlawi (1973) and Pathak et al. (1975) who had reported better rooting with 60 cm long hardwood cuttings of plum.

Treatment of the preconditioned cuttings with IBA at 3000 ppm was found to be optimum for rooting, shoot growth and field survival in the present studies. This finding is in conformity with that of Fontanazza (1969) who reported better rooting with 3000 ppm IBA treated cuttings with longitudinal incisions at the bases. Nahlawi and Howard (1970, 1971) also observed rooting improvement in hardwood cuttings by ringing the shoots and wounding the cuttings at the base prior to IBA treatment between 500-5000 ppm.

It was quite encouraging to note that the technique of combining forced and ringed or forced and etiolated shoots with IBA and wounding-cuts has been successful in rooting of cuttings of Santa Rosa plum. This method of inducing forced shoots combined with preconditioning treatments may also give good results in rooting of cuttings in other plum cultivars which have not rooted satisfactorily so far.

6. Biochemical status of cuttings in relation to rooting behaviour

A marked difference was observed in the levels of nitrogen and carbohydrates in the cuttings of different plum species in both the years. The rooting percentage of cuttings was positively correlated with carbohydrate contents but negatively correlated with the nitrogen content. This correlation indicated that an increase in carbohydrate contents resulted in proportionate increase

in the rooting percentage, but increase in nitrogen content had a retarding effect on the rooting percentage of different plum species in the present study. These findings are well supported by the observations of Starring (1924) and Calma and Richey (1930). These workers have also advocated that the cuttings with sufficient amount of carbohydrates rooted more easily.

In the present studies, plum cuttings collected during summer season had less nitrogen and relatively moderate amount of carbohydrates as compared to the cuttings taken during dormant and autumn seasons in both the years. As a result of this, these cuttings exhibited higher C/N ratio as compared to dormant and autumn planted cuttings. The C/N ratio was also relatively higher in Santa Rosa plum cuttings and was minimum in Greengage in both the years. However, the C/N ratio of the cuttings indicated only a slight variations during the two years, which ranged from 21.1 to 32.8 in 1986 and 22.8 to 35.4 in 1987 in different cultivars. This slightly higher C/N ratios might be due to the favourable environmental conditions prevailing during 1987 which was found conducive for increase in C/N ratios and hence better growth performances. A positive correlation was observed between the percentage of rooting of cuttings and the level of C/N ratio in both the years. These observations are in agreement with those of Starring (1924), Pearse (1943), Sen and Basu (1960) and Hartmann and Kester (1983) who reported that the

cuttings high in carbohydrates and low in nitrogen contents generally produced good rooting, cuttings having intermediate proportions of carbohydrates to nitrogen produced both roots and shoots whereas highly vegetative cuttings produced neither roots nor shoots.

The levels of total phenols and O.D. phenols were found to be maximum in hardwood cuttings planted during January in both the years. However, the minimum total phenols were observed in October planted cuttings in case of Japanese plums whereas in European plum cultivars the minimum total phenols contents were observed during summer season in both the years. The minimum O.D. phenols contents were observed in October planted cuttings in all the four cultivars in both the years of study. The highest levels of total phenols were exhibited by Beauty in both the years while the lowest total and O.D. phenols were recorded in Santa Rosa in 1986 and Early Transparent Gage in 1987.

In the present studies, positive correlation coefficients between total and O.D. phenols and the rooting ability of the cuttings were observed in Japanese plum cultivars whereas European plums showed negative correlations between total phenols and rooting and a positive response of O.D. phenols in respect of rooting ability of the cuttings. The present findings on the positive response of the total phenols with the seasonal rooting percentage of Japanese plums are in conformity

with the observations of Gautam (1986) who reported a positive correlation of total phenols and O.D. phenols with the rooting of cuttings in some temperate fruit plants. Rana (1987) also reported similar positive correlation of total phenols and O.D. phenols with rooting ability of cuttings in some Prunus cultivars and rootstocks. The present findings is also supported by Nanda and Kochhar (1985) who had reported a positive correlation between phenolic compounds and rooting of cuttings in some easy-to-root plants. Similarly, Hess (1965) reported phenolic compounds as stimulators of root initiation.

According to Nanda and Kochhar (1985) easy-to-root plant species contain more quantity of free and bound acid fractions (Rfs 0.4-0.6) of the total phenols such as ferulic, p-hydroxybenzoic and p-coumaric acids which caused significant promotion on rooting. The positive correlation-ship between phenolic compounds and the rooting of cuttings in Santa Rosa and Beauty in the present studies may be due to the presence of such quantity of free and bound acid fractions of total phenols in these cultivars.

Basu et al. (1969) also reported that the exogenous application of phenol catechol exhibits synergistic effect with IAA in root formation on stem cuttings. The synergism of phenols with IAA is probably because phenols prevent the destruction of endogenous auxin as they exhibit the enzyme system that oxidizes IAA or stimulates auxin synthesis from

tryptophan and frees the auxin and makes it available at a site which only IAA can satisfy (Hess, 1967, 1968; Basu et al., 1969; Basu, 1969, 1970 and Bose et al., 1972). On the other hand, the negative response with the seasonal rooting per cent of European plums may be due to the presence of more level of monophenols which act as cofactors of IAA oxidase which can nullify the effect of phenolic synergists as reported by Schneider and Wightman (1974).

Although, hardwood cuttings of plum cultivars were quite rich in the carbohydrates and O.D. phenols, yet less rooting was recorded. Hence, some other factors seem to be present which are responsible poor root initiation process of plum.

7. Status of endogenous promoters in the cuttings

In the present experiment, the Mung bean bioassay test was adopted to study the effect of growth promoters in the fresh (zero day) and 60 days after planting of cuttings in four plum cultivars.

In fresh semi-hardwood cuttings of Japanese plum, the areas of promotion were observed at Rfs 0.2-0.4 and 1.0, whereas after 60 days in the rooted cuttings strong promoters were seen to exist at Rfs 0.2-0.5 and 0.9-1.0. In the fresh hardwood cuttings the promoting activity were found at Rfs 0.1-0.4 and 0.8-1.0 but after 60 days of planting, the activities of rooting promoters were decreased at Rfs 0.2-0.4 and 0.9-1.0. However, Santa Rosa cuttings

showed the presence of more rooting promoters as compared to Beauty. These two areas at Rfs 0.2-0.4 and 0.9-1.0, according to Hess (1964) correspond to cofactors 2 and 4, respectively. Taylor and Odom (1969) have also reported the similar promoter activities regardless of the type of cuttings and pre-treatments.

In semi-hardwood cuttings of European plums, the zones of promotion were at Rfs 0.1, 0.3-0.5 and 1.0 in case of Greengage whereas in Early Transparent Gage the promoter activity was observed at Rfs 0.1-0.6 and 0.9-1.0. After 60 days in the rooted cuttings these root promoting activities were markedly reduced at Rfs 0.1-0.5 and 1.0 in both the cultivars. In hardwood cuttings more root promoting activities were observed in fresh basal segments at Rfs 0.2-0.5 and 1.0 and after 60 days in rooted cuttings these root promoting activities were markedly reduced at Rfs 0.3-0.4 and 0.9-1.0, particularly in Early Transparent Gage.

Gasto et al. (1967) and Mandez et al. (1968) have also observed that although both inhibitor and promoter zones were present in easy as well as difficult-to-root species but the presence of inhibitor was much stronger in the later case.

Rooting potentiality of cuttings of different plants have been attributed to the variation in endogenous level of cofactors (Hess, 1961 and Herman and Hess, 1963). Hess

(1962-1968) identified these factors and concluded that synergistic effect of phenolics was responsible for protection of IAA from oxidase activity. Hess (1959) stated that in cuttings which are difficult-to-root, only neutral cofactors can stimulate the root differentiation. In European plum cuttings, the level of these promoters/ cofactors was very less, therefore, it might be due to that reason the cuttings regenerated with more difficulty. These observations are in conformity with the findings of Masuda and Yamamato (1970) and Davis (1973).

8. Status of endogenous inhibitors in the cuttings

The bioactivity of the inhibitors which was noticed in the basal extracts of fresh plum cuttings in summer was observed to be reduced markedly in the rooted cuttings after 60 days as seen by cress seed germination bioassay. Similarly, there was marked activity of inhibitory substance in the basal extracts of fresh plum cuttings but the zones of inhibition increases with slight reduction in their activities wnen the basal ends of the hardwood rooted cuttings were tested 60 days after planting of cuttings. Besides, the appearance of peak zones of inhibition at Rfs. 0.5-0.7 at which ABA standards are reported to move by Abdel Renman et al. (1975), there were also strong inhibitory activity at Rf.0.4 and 0.8-0.9 which might be the phenolic compounds as reported by Nanda and Kochhar (1985). This appearance of peak zones of inhibition at Rfs.0.4-0.9 indicated that IAA action was stimulated by some phenolic inhibitors by forming a complex with auxin. These findings

get an active support from the studies made by various workers (Basu et al., 1970 and Lipecki et al., 1975) and elaborated by Hartmann and Kester (1972).

Spiegel (1954, 1955) in grape vine and Fadl and Hartmann (1967) in pear found that cuttings at some period of year are difficult to root because of natural inhibitors localized respectively in buds, in leaves or in stem. Possibly the intact leaves of European plums continued the synthesis of the inhibitors noticed in the present studies and hence may be due to the increased inhibitor level that these cuttings gave poor root initiations.

Thus, in the present studies, besides other factors one of the reasons of poor rooting in cuttings of European plum species may be the strong inhibitory activity of ABA-like substances which appears to have counteracted the effects of all endogenous promoters and exogenous application of indole butyric acid. The decreasing rooting response in hardwood stem cuttings as compared to semi-hardwood cuttings may be due to the advancing maturity of the wood used as cuttings which is also ascribed to an increase in the content of inhibitory substances and decrease in the content of promotory substances (Nanda and Kochhar, 1985). They also reported that the inhibitory substances isolated from the cuttings of difficult-to-root species inhibit root formation even on cuttings of easy-to-root plant species. The present observations on better rooting during summer under mist condition is supported by the findings of Nanda (1975) who had reported that the inhibitory substances decrease if

the cuttings are exposed to longer photoperiods.

9. Biochemical status in preconditioned cuttings

With the etiolation and ringing of forced shoots there was a marked increase in the levels of total carbohydrates, C/N ratios and phenolic compounds. However, the level of nitrogen was slightly reduced in the present studies in both the years. The present observations support the findings of Allan et al. (1980). Stoltz and Hess (1966) who reported that the amount of carbohydrates per cutting is higher in ringed cuttings due to its accumulation above the ring and etiolated parts of the shoot as compared to non-ringed cuttings.

A positive correlation was found between the rooting of cuttings from preconditioned shoots and the levels of carbohydrates, C/N ratio and phenolic compounds. Smith et al. (1975) also observed the similar findings. They mentioned that although hardwood cuttings of different lengths differed significantly in their rooting potential but no clear relationship with initial food reserve status was found. However, numerous investigators including Kelley (1966), Stoltz and Hess (1966), Taylor and Odom (1970), Gautam (1986) and Rana (1987) have concluded that factors leading to high carbohydrate content of stock plants favour the rooting of cuttings. The significance of high C/N ratio for rooting, shoot growth and survival has also been reported earlier by Mokashi et al. (1982) in grapes.

Mukherjee and Chatterjee (1979) suggested that the ringing effects can sometimes be attributed to interruption of supplies of root produced hormones especially cytokinins and gibberellins. On the other hand, Allan et al. (1980) opined that the translocation of growth and rooting substances is reduced from the terminal shoot portions with ringing and hence results in better rooting of cuttings and plant development. Thus, in the present investigations the higher amount of carbohydrates in the ringed and etiolated Santa Rosa plum shoots and the possible reduction in translocation of growth and rooting substances from these terminal shoot portions could explain the good rooting and field survival obtained in the etiolated and ringed cuttings as compared to the non-preconditioning treatments.

10. Varietal effect on callus formation in vitro

Callus formation from the apical meristem occurred within three weeks in the present studies. As the results indicate, the increase in callus mass was influenced markedly by the type of cultivar planted in the MS culture medium. The rate of callus formation was faster and their tissues grew almost more than twelve times its weight in the 12th week as compared to its weight in the 4th week in Santa Rosa plum cultivar. Similar trend was also observed in case of Beauty which along with the Santa Rosa belongs to Japanese plum species, Prunus salicina. However, the rate of growth was quite slow in case of European plum (Prunus domestica) cultivars Greengage and Early Transparent Gage

which may be due to the presence of endogenous auxin and other growth regulators in the plant tissues which causes a loss of polarity and makes the cell division irregular, thus resulting into the proliferating mass of unorganized cells.

According to Gautheret (1966), the results fulfilled by the presence of growth stimulants in the nutrient medium suggest that the factors responsible for de-differentiation are none other than those that stimulate cell multiplication. The physiological conditions of the explant materials were also ascribed to varietal variation by Whiteley and Abbott (1976). The best plant characteristics was obtained from the Japanese plum species in the present studies which is in conformity with the findings of Rosati et al. (1980) but not with that of Bassi (1984) who obtained better results in European plum cv. Prune d' Agen clone 707.

11. Proliferation rate of explants from apical meristem and shoot tips with lateral bud

In the present investigations it was observed that the apical meristems produced the highest number of in vitro proliferated shoots per explant as compared to the average number of shoots proliferated from the shoot tip with a lateral bud. This may be due to the rapid multiplication of cells in the apical tip of a shoot

and also due to the highest probability of producing lacking detectable levels of viruses and other pathogens as reported by Hammerschlag (1982) in his earlier studies. Jones et al. (1977) also reported higher potential of shoot proliferation in apical meristem whereas the shoot tips having one or two buds had a lower rate of shoot proliferation but had a higher survival rate.

12. Effect of growth regulators in rooting

Out of various auxins tried, in the present studies the best rooting behaviours was obtained on IBA supplemented MS rooting medium at a rate of 1.0 mg/l. In the same experiment, good rooting characteristics were also obtained with NAA at 0.5 mg/l and IBA at 2.0 mg/l. It was also observed that with the increase in NAA concentrations in the rooting medium which is made up of with the addition of 0.1 mg/l IBA and 0.1 mg/l GA_3 in the basal medium, there was a decreasing trend in their rooting behaviours which indicates the adverse effect this hormone can have when present in higher concentrations on their rooting behaviours. But the present finding is not in conformity with that of Ruzic and Cerovic (1985) who had obtained better rooting when the culture medium was supplemented with 1.0 mg/l NAA followed by 0.5 mg/l NAA and 0.5 mg/l IBA, respectively. Pietropaolo and Reisch (1984), however obtained similar results as in the present studies with plum cv. Pozegaca.

C H A P T E R - VI

S U M M A R Y

SUMMARY

The present studies on the propagation of some plum species by cuttings and tissue culture were conducted in the Department of Pomology and Fruit Technology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan during 1986-88 to study the rooting and other growth behaviours of the cuttings as well as of the explants. The results thus obtained in the present investigations have been summarized below:-

1. Maximum percentage of rooting was recorded in Santa Rosa plum cuttings whereas minimum rooting was recorded in the cuttings of Early Transparent Gage.

2. Semi-hardwood cuttings planted during summer under mist gave better success in rooting as compared to hardwood cuttings planted during dormant and autumn seasons in all the four cultivars.

3. IBA at 3000 ppm was found to be the optimum concentration for rooting of hardwood cuttings planted during dormant and autumn seasons whereas IBA at 2000 ppm gave higher success in rooting of semi- hardwood cuttings planted during summer under mist.

4. Maximum development of primary and secondary roots occurred in the cuttings of Santa Rosa treated with 3000 ppm IBA in all the seasons.

5. The cuttings treated with 2000 ppm IBA attained the maximum root length and diameter as compared to those cuttings treated with IBA at 3000 and 4000 ppm and control.

6. The cuttings of the different cultivars which produced the highest number of primary and secondary roots showed better shoot growth.

7. Maximum sprouting and shoot growth were observed in Santa Rosa plum cuttings in all the seasons as compared to other cultivars.

8. Maximum field survival percentage was recorded in Santa Rosa whereas the minimum was recorded in Early Transparent Gage.

9. The semi-hardwood cuttings planted during summer under mist gave relatively higher field survival as compared to autumn and dormant planted hardwood cuttings.

10. IBA at 3000 ppm was found to give higher percentage of field survival as compared to other treatments.

11. Preconditioning treatments involving forcing and ringing gave relatively higher rooting and field establishment of the rooted cuttings.

12. Basal wounding of preconditioned stem cuttings followed by the application of 3000 ppm IBA to such cuttings resulted in increased rooting and higher field survival.

13. Treatment comprising of forcing, ringing, woundfng and IBA at 3000 ppm resulted in increased rooting, better root and shoot development and increased field survivability.

14. Better rooting and growth characteristics of the cuttings and higher field survivability of the cuttings were recorded in 30 cm long preconditioned cuttings.

15. Relatively higher total nitrogen contents were recorded in Beauty as compared to other cultivars.

16. Maximum total nitrogen contents were recorded in cuttings planted during January while the minimum was recorded from July planted cuttings.

17. Cuttings of Santa Rosa recorded maximum total carbohydrates while the cuttings of Greengage had recorded minimum content of total carbohydrates.

18. Maximum C/N ratio was observed in Santa Rosa plum cuttings while the minimum was observed in Greengage.

19. Summer season cuttings recorded maximum C/N ratio while the autumn season cuttings recorded minimum C/N ratio.

20. Maximum total phenols and O.D. phenols were obtained in Beauty whereas the minimum values were recorded in Santa Rosa.

21. Relatively higher total nitrogen, carbohydrates, total phenols and O.D. phenols contents were recorded in dormant (January) cuttings as compared to summer and autumn planted cuttings.

22. A negative association was obtained between total nitrogen contents and the rooting percentage of the cuttings.

23. A positive correlation was obtained between the levels of carbohydrates, C/N ratio, O.D. phenols and the rooting ability of the cuttings.

24. A positive correlation was recorded between the levels of total phenols and the rooting percentage of Santa Rosa and Beauty whereas a negative correlation was recorded between total phenols and the rooting ability of cuttings in Greengage and Early Transparent Gage.

25. In Mung bean bioassays, relatively higher bioactivity of promoters at Rfs. 0.2-0.4 was observed in the basal extracts of semi-hardwood cuttings collected during summer as compared to the bioactivity of promoters in the basal extracts of hardwood cuttings obtained during dormant and autumn seasons.

26. In Mung bean bioassays, the promotive activities of IAA-like substances in the basal extracts of the cuttings at Rfs. 0.2-0.4 were more in the fresh basal extracts at zero day as compared to the basal extracts in the rooted cuttings after 60 days. However, slightly increased bioactivity of promoters was observed at Rf. 1.0 in case of rooted cuttings.

27. In cress seed germination innibition bioassays, maximum level of endogenous inhibitory substances was observed in the basal extracts of Early Transparent Gage.

28. The ABA-like inhibitory substances observed at Rfs 0.5-0.8 were more stronger in the basal extracts of fresh cuttings as compared to rooted cuttings after 60 days.

29. Apical meristem explants gave more shoot proliferations as compared to shoot tip explants with a lateral bud under tissue culture studies.

30. Santa Rosa gave better rooting and shoot growth in vitro as compared to other cultivars.

31. Addition of 1.0 mg/l IBA in MS rooting medium gave higher rooting percentage with better root quality.

32. Explants from Greengage exudated more quantity of phenolic compounds into the culture medium.

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A P P E N D I C E S

APPENDIX-I

Monthly meterological data of the University Campus for 1986 and 1987 as per weather bulletin maintained by the Department of Soil Sciences and Water Management, College of Forestry, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Nauni at 1135.5 metre (a.m.s.l.).

Month	Atmospheric Temperature (°C) Maximum		Minimum		Average relative humidity (%)		Rainfall (mm)	
	1986	1987	1986	1987	1986	1987	1986	1987
January	16.3	15.4	-1.0	4.8	67.3	62.9	-	61.0
February	16.1	19.9	3.3	7.9	72.2	69.5	61.0	40.0
March	22.7	22.8	13.5	11.3	74.2	63.7	159.4	17.0
April	29.1	27.7	17.2	14.5	72.2	66.4	15.0	27.0
May	30.1	26.2	19.5	14.1	70.4	74.9	17.2	307.0
June	33.4	32.1	23.0	18.0	73.1	79.2	249.0	77.0
July	30.3	31.3	22.3	20.1	85.3	81.1	270.6	109.0
August	31.0	28.7	22.5	19.0	86.1	85.6	181.0	164.0
September	29.6	29.9	19.3	18.4	80.9	79.6	71.4	100.0
October	27.6	27.8	14.0	14.1	72.3	57.0	79.8	81.0
November	20.0	23.1	9.3	4.5	72.5	53.1	21.0	-
December	13.9	20.5	4.0	1.5	48.6	54.9	31.0	8.0

APPENDIX-II

Composition of Murashige and Skoog medium*

		Concentration (mg/l)
Major elements		
Ammonium nitrate	NH_4NO_3	1650
Potassium nitrate	KNO_3	1900
Calcium chloride	$CaCl_2.2H_2O$	440
Magnesium sulphate	$MgSO_4.7H_2O$	370
Potassium dihydrogen orthophosphate	KH_2PO_4	170
Ferric sodium EDTA		36.70
Minor elements		
Boric acid	H_3BO_3	6.2
Manganese sulphate	$MnSO_4.4H_2O$	22.3
Zinc sulphate	$ZnSO_4.7H_2O$	8.6
Potassium iodide	KI	0.83
Sodium molybdate	$NaMoO_4.2H_2O$	0.25
Cupric sulphate	$CuSO_4.5H_2O$	0.025
Cobaltous chloride	$CoCl_2.6H_2O$	0.025
Micro-elements		
Thiamine HCl		0.5
Inositol		100.0
Nicotinic acid		0.5
Pyridoxine HCl		0.5
Glycine		2.0
Organic constituents		
Sucrose		2-30000
Agar		6-7000
Hormones		
Benzylaminopurine	BAP	(Concentrations vary with different subjects and at different stages of propagation)
Indole-3-butyric acid	IBA	
Napthalene acetic acid	NAA	
Gibberellic acid	GA_3	

* Murashige and Skoog (1962).

APPENDIX-III

Mean value of total phenolic contents*(mg/g) exuded by explants into the culture medium.

Cultivars	Apical meristem as explant	Shoot tip with lateral bud as explant	Means
Santa Rosa	25.3	33.5	29.4
Beauty	28.9	39.6	34.2
Early Transparent Gage	30.1	37.2	33.7
Greengage	33.8	40.2	37.1
Means	29.5	37.6	-
C.D. (P=0.05)	1.12	1.28	-

*Results are the means of six separate experiments.

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