STOCK SCION INTERACTION IN *Piper nigrum* L. GRAFTS

By

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(2017-12-006)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"STOCK SCION INTERACTION IN *Piper nigrum* L. GRAFTS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 11-10-2019

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CERTIFICATE

Certified that this thesis, entitled "**STOCK SCION INTERACTION IN *Piper nigrum* L. GRAFTS**" is a record of research work done independently by **Ms. Sarga George (2017-12-006)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Sarga George (2017-12-006),** a candidate for the degree of **Master of Science in Horticulture** with major in **Plantation Crops and Spices**, agree that the thesis entitled **"STOCK SCION INTERACTION IN *Piper nigrum* L. GRAFTS"** may be submitted by **Ms. Sarga George (2017-12-006),** in partial fulfillment of the requirement for the degree.

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Sarga George

Dedicated to my dear
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Introduction

1. INTRODUCTION

Black Pepper (*Piper nigrum* L.) also called 'King of spices' and 'Black Gold', is one of the most widely used spices in the world, occupying a position that is supreme and unique. The black pepper of commerce is the dried, mature fruits of the tropical, perennial climbing plant *Piper nigrum* L., which belongs to the family Piperaceae.

India is one among the countries where black pepper is being widely cultivated. In India, black pepper is cultivated to a large extent in Kerala, Karnataka, Tamil Nadu and to a certain extent in Maharashtra, North Eastern states and Andaman and Nicobar Islands. However our productivity is low compared to other pepper producing countries in the world. Among the various factors limiting the productivity of Indian pepper, pests, diseases and drought have prime importance. *Piper nigrum* is highly susceptible to foot rot disease caused by *Phytophthora capsici.*

Phytophthora capsici is a soil borne fungus which infects all parts of the plant and cause severe economic damage. *Phyotophthora* infection has both aerial and soil phases. Infection from soil affecting root and collar region is more fatal compared to aerial infection (Sarma and Anandaraj, 1997).

Some South American species of *Piper* are reported to be immune to *Phytophthora*. Turner (1971) proved that, *Piper colubrinum* and *Piper obliquum* are resistant to *Phytophthora* while, *P. scabrum*, *P. auduncum* and *P. treleasanum* showed partial resistance (Ruppel and Almeyda, 1965).

Grafting *Phytophthora* susceptible *P. nigrum* on resistant rootstocks is an effective method to bring foot rot under control. Grafting is the art of inserting a part of the plant into another plant in such a way that the two will unite and continue their growth. Plant part expected to produce the top of the new plant is deprived of its own root system and unites with another plant that supplies this part. (Adriance and

Brison, 2010). There were some reports from Sarawak, Brazil and India suggesting that, interspecific grafting is possible in *P. nigrum*. Many of the investors used *P. colubrinum* as rootstock to transfer diseases resistance to *P. nigrum*. But, it has been reported that, there is delayed incompatibity in the grafts involving *P. colubrinum.* The grafted plants showed good initial growth but deteriorated after some years (Alconero *et al.*, 1972).

Piper species are reported to show an anomalous stem structure. There are two rings of vascular bundles, peripheral as well as medullary and the later ones are scattered over the stelar region as in monocotyledons (Ravindran *et al.*, 2000).

In this context the present study was planned to find out whether there is proper graft union developing in grafts involving *Piper nigrum* and Brazilian species which are immune to *Phytophthora* and selecting the best rootstock and time of grafting. Anatomical studies of different *Piper* species and graft union will throw light on the establishment of graft union and reasons for incompatibility developing if any. The study also aims to analyse whether there will be proper development of graft union when Panniyur1 variety of *Piper nigrum* is used both as rootstock and scion.

Review of Literature

2. REVIEW OF LITERATURE

Black Pepper (*Piper nigrum* L.) also called 'King of spices' and 'Black Gold', is the most important and most widely used spice in the world, occupying a position that is supreme and unique. The black pepper of commerce is the dried, mature fruits of the tropical, perennial climbing plant *Piper nigrum* L., which belongs to the family Piperaceae. Even though India has the highest area under pepper cultivation, productivity is low. Among the various reasons pertaining to the lowest productivity, crop losses due to pests and diseases have the prime importance (Sarma and Anandaraj 1997). *Phytophthora* foot rot is the major production constraint in all pepper growing countries. Grafting *Phytophthora* susceptible *P. nigrum* on resistant rootstocks is a better method to bring foot rot under control.

Literatures on grafting in *Piper nigrum*, other spice crops and horticultural crops, anatomical studies on *Piper* sp., graft union and graft incompatibility are reviewed here.

2.1 GRAFTING STUDIES IN BLACK PEPPER

One of the earliest studies on grafting in *Piper nigrum* was reported from Puerto Rico. Gregory *et al.* (1960) obtained grafts of *P. nigrum* on five different American *Piper* species. In another study Hasan (1960) could produce dwarf grafts by using plageotropic shoots of *P. nigrum* as scion and *P. hirsutum* and *P. ariifolium* as rootstocks, but the scions died in due course. In Sarawak only initial coalescence was noticed between rootstock and scion, when intraspecific grafting was carried out using the cv. Kuching. There was no permanent fusion between wood of different varieties of *P. nigrum* (Waard and Zeven, 1967).

P. nigrum has been successfully grafted on to a number of *Piper* species including *P. aduncum*, *P. scabrum*, *P. treleaseanum* in Puerto Rico (Gaskins and Almeyda, 1969) *P. cubeba* L. and *P. unguiculatum* Ruiz & Pav. under glasshouse conditions (Garner and Beakbane, 1968) and *P. colubrinum*, *P. cubeba* and *P. hispidum* Sw. in Sarawak. But field plantings of *P. nigrum* grafts on *P. colubrinum* only have survived (Gaskins and Almeyda, 1969).

Albuquerque (1969) used different *Piper* species, resistant to *Phytophthora* as rootstock and identified *P. colubrinum* as best rootstock with 95 percent graft success. Forket method of grafting was tried at third or fourth internode. Good initial growth was shown by the grafted plants. Performances of plants in later years were not known. However Alconero *et al*. (1972) reported that after fourth year of grafting, Singapura variety of *P. nigrum* grafted on *P. colubrinum* was deteriorated. Longitudinal cracks were developed on the grafted portion and it was eventually rotted.

Interspecific grafting using *P. colubrinum* as rootstock was tried by workers in Sarawak. Single and double stocked grafts were produced by grafting cv. Kuching (Ku) on pink as well as green genotype of *P. colubrinum* and stated that the grafts involving pink genotype performed better and yield was comparable with that of ungrafted *Kuching* plants. Comparing the performance of Kuching on green colubrinum and *P. cubeba* it was reported that break down of Kuching on *P. cubeba* was slower to green colubrinum. But the yield of *P. cubeba* grafts were low (Anon. 1977, 1978, 1981).

Another trial was carried out in Sarawak to study the compatibility of intraspecific grafts. Kuching was grafted on different Indian as well as Indonesian cultivars of *P. nigrum*, such as Balankotta, Kalluvally, Uthirankotta, Cheriyakaniyakadan, Belantung and Djambi and results showed that Indonesian cultivars were more compatible with cv. Kuching than Indian cultivars (Anon. 1979).

Mathew and Rema (2000) reported that *Piper colubrinum* was compatible with black pepper as rootstock and can be used to mitigate the problem of *Phytophthora* foot rot. On the other hand, Krishnamurthy *et al*. (2003) failed to get grafts with resistance to *Phytophthora* for which rootstocks used were *P. colubrinum*, *P. scabrum* and *P. treleaseanum*, though all the three species showed resistant reaction on inoculation.

Studies carried out at IISR, Calicut revealed that among the various methods of grafting 78 per cent success was recorded with double root stock method using the cultivar 'Subhakara' as scion when grafted on *P. colubrinum*. Grafting at 50 cm height was ideal which encouraged vigorous growth of vines. Scions with two or three nodes sprouted earlier than single node shoots. Among the cultivars evaluated as scions, Poonjaramunda was found to be good for grafting on *P. colubrinum*. (IISR, 2001).

Graft recovery of *Piper nigrum* runner shoots on *Piper colubrinum* rootstock was influenced by varieties and month of grafting. Vanaja *et al*. (2007) evaluated the influence of varieties and season on graft recovery and claimed that regardless of the months of graft production and varieties, runner shoots performed best with highest graft recovery. February and March months were identified as conducive periods for getting high graft success (> 90%).

Krishnamoorthy and Parthasarathy (2009) reported that *P. colubrinum* could be effectively utilized as a rootstock for grafting black pepper to control foot rot and nematodes in addition to adaptability to marshy situations. The plant was immune to *Phytophthora capsici* and resistant to root knot nematodes. Among the various grafting methods tried, best performance was recorded with double rootstock method. One kg dry pepper per graft was obtained by third year of grafting. Field evaluation of grafts indicated that they remained healthy even nine years after grafting.

Janani (2009) studied the use of *Piper colubrinum* and *Piper hymenophyllum* as rootstock with orthotropic shoots of Panniyur 1 and Subhakara as scions and found 73.33 percent grafting success in the graft between five nodal cuttings of Subhakara as scion and *Piper colubrinum* as rootstock. 93.30 percent success was there on *Piper hymenophyllum*

However Arathi (2011) reported that among the two wild species, *Piper hymenophyllum* had more grafting success (42.78%) than *Piper attenuatum* (36.11%) and among the scion materials studied, runner shoots of Karimunda had the higher grafting success (44.44%) than Panniyur 1 (41.11%) on *Piper hymenophyllum.*

Another study of grafting *P. nigrum* on *Phytophthora capsici* resistant *P. colubrinum* suggested that, there were no abscission of the scion after five years of grafting but the grafted plants showed symptoms of drought stress during dry season and recorded fewer number of spikes (Ton, 2010).

Grafting Sreekara on *Piper hamiltoni*, a *Phytophthora* resistant species gave 50% success but the growth was poor because of the susceptibility of *Piper hamiltoni* to nematodes. *P. ornatum,* another species of *Piper* which was found to be resistant to major pathogens of *P. nigrum* was also used for grafting. Since *P. ornatum* was not compatible with *P. nigrum*, interstocks were used. The promising combination identified was Sreekara as scion grafted on *P. ornatum* with *P. hamiltoni* as interstock with a graft recovery of 80% (IISR, 2012).

Sourabha *et al.* (2017) studied the responses of varieties to grafting in black pepper in hill zone of Karnataka and reported that among twenty one varieties tried, Panniyur 1 and Panniyur 3 responded better to grfating on *Piper colubrinum.* There were significant difference in plant height, leaf length and leaf breadth and there were no incidence of foot rot also.

Some South American species of *Piper* were reported to be resistant to *Phytophthora*. In an experiment to investigate resistance in *Piper* species to infection by *Phytophthora palmivora* from *Piper nigrum*, Turner (1971) proved that South American species of *Piper* such as *Piper colubrinum* Link. and *Piper obliquum* Ruiz & Pav. were resistant to *Phytophthora*. Although necrosis was observed in another species called, *P. scabrum* Sw., sporangia of the pathogen could not be detected on the roots.

Ruppel and Almeyda (1965) reported partial resistance in wounded specimens of *P. auduncum* L., *P. scabrum* and *P. treleasanum* Britt. & Wils. from Puerto Rico.

2.2 GRAFTING STUDIES IN OTHER SPICE CROPS

Nutmeg was grafted on *Myristica fragrans*, *M. malabarica* and *M. beddomeii* during two seasons. Wedge grafting was found to be successful on all the rootstocks and highest success was recorded on *M. fragrans* (90%), followed by *M. malabarica* (76%) and *M. beddomeii* (68). Studies carried out at IISR, Calicut to understand the influence of root stock on productivity in nutmeg showed that the growth of the plant was more on wild rootstocks when compared to cultivated nutmeg (IISR, 2008).

A trial laid out to evaluate the growth and yield performance of orthotropic and plagiotropic grafts and seedlings of nutmeg indicated that the growth of the orthotropic grafts were much faster than the seedlings and plagiotropic grafts. Early flowering observed on grafts compared to seedlings and among the grafts, the plagiotropic grafts flowered earlier (IISR, 2008).

An investigation done at Regional Coconut Research Station, Bhatye, Maharashtra, on softwood grafting in nutmeg recorded a maximum of 80 percent success during the month of May (Haldankar *et al*., 1999).

When dwarf clove was grafted on to ordinary clove the resultant graft had the appearance of ordinary clove with increased internodal length of the scion shoot. The mean internodal length of the newly produced scion portion was increased to 1.66 cm showing the effect of rootstock on scion when new shoots/new leaves were formed as seen in an ordinary clove. But in contrast to that, when ordinary clove was approach grafted on dwarf clove the internodal length of the scion was reduced from 1.8 cm to 0.73 cm. The study indicated that with regard to the growth in clove, rootstock had a definite influence on the scion (Mathew *et al.*, 1999).

Stock scion studies on clove was carried out at IISR (2008) and they claimed that approach grafting of dwarf clove was successful in clove (Syzygium aromaticum) rootstock and the graft union was established in 6 – 8 months with a success of 64 percent and 100 percent field establishment. Among the various rootstocks used such *as S. aromaticum, S. heynianum , S. fruiticosum , S. cumini , S. zeylanium , S. lanceolatum* and *Eugenia uniflora*, clove was compatibile with *S. aromaticum* and *S. heynianum.*

Grafting of *Garcinia gummi - gutta*, *G. indica*, *G. xanthochymus* and *G. mangostana* were carried out on various related species. Soft wood grafting of *Garcinia xanthochymus* was standardized on 9 month old its own rootstock with 90 percent success. Grafting of *G. gummi - gutta* on *G. gummi - gutta , G. hombroniana* and *G. cowa* was successful with 89 percent, 80 percent and 24 percent success respectively. It has been reported that, there were 59 percent, 48 percent and 56 percent success respectively when *G. indica* grafted on *G. gummigutta*, *G. indica* and *G. cowa* and 70 percent success was recorded on grafting of *G. mangostana* on its own rootstock (IISR, 2008).

Experiments undertaken to explore the possibilities of patch budding and softwood grafting in tamarind at Rahuri reported that mid April to mid June and mid October were the best time for both softwood wedge grafting and patch budding under semi-arid conditions of Rahuri. Patch budding was found to be superior to soft wood wedge grafting. The scion sticks with 8 to 10 cm length involving 3 to 4 buds have been found suitable for wedge grafting in tamarind (Lalaji, 2001)

2.3 STUDIES ON GRAFTING IN OTHER HORTICULTURAL CROPS INVOLVING WILD AND OTHER SPECIES AS ROOTSTOCKS

Soft-wood grafting of sapota (*Achras sapota* syn. *Calocarpum sapota*) on local Khirni (*Manikhera hexandra*) was studied by Kulwal *et al.* (1985) and they claimed that soft-wood grafts of sapota could be prepared from mid July to mid August with more than 80% success. Fifty percent success was obtained from grafts produced during September to October under Akola condition. However Pampanna and Sulikeri (2000) reported that highest graft success (63.33%) was obtained for grafting done in May. In contrast, graft-take was very low (0-6.67%) for grafts produced during September to February.

Another study conducted in West Bengal by Ghosh *et al.* (2016) pointed out that, highest softwood grafting success in sapota was obtained when grafting was carried out during July (72%) followed by August (70%).

An investigation carried out on softwood grafting in jamun (*Syzygium cumini*) on two different rootstocks such as *S. cumini* and *S. operculatum* stated that 94 percent success was found with the rootstock *S. cumini* and 92 percent success was recorded with *S. operculatum* during the month of June (Gowda *et al.*, 2011).

Bacterial wilt resistance in eggplants can be imparted through grafting. It has been reported that two wild *Solanum* species such as *S. torvum* and *S. sisymbriifolium* can be utilized in grafting (Rahman *et al.*, 2002).

Wang *et al.* (2002) found a significant increase in survival and yield of cucumber when grafted onto *L. siceraria*, *C. moschata*, and *Benincasa hispida* (Thunb.) Cogn.

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2.4 STUDIES ON GRAFT UNION FORMATION IN DICOTYLEDONOUS PLANTS

Grafting is the art of connecting two pieces of living tissue together in such a manner that they will unite and subsequently grow and develop as one composite plant. Various events were taking place in the formation of a graft union. Adhesion of rootstock and scion, formation of callus tissue from uninjured, rapidly dividing parenchyma cells and callus bridge, differentiation of vascular cambium across the callus bridge and production of secondary xylem and phloem from the new vascular cambium are the stages of development of a graft union. Callus formation is vital in the healing process which influenced by plant type, physiological condition, environmental (temperature and relative humidity) and craftsmanship (Hartmann and Kester, 2002). Union formation is a continuous process and there is no definite time limit for the completion of each stage.

Singh (1960) reported that, in mango 2 -3 months were needed for the complete union formation. Charkrabarty and Sadhu (1988) also observed healing of graft joint in mango by 4 months after grafting.

Copes (1969) proposed that initiation of shoot growth by the scion was a good indication that cambium was present and tracheids had differentiated. Cambium maintained vascular connection in the callus bridge.

Union was influenced by anatomical, physiological and genetic variables (Andrews and Marquez, 1993; Edelstein *et al.*, 2004). Anatomical studies carried out in grafts of different fruit species showed that processes involved in union formation were similar. Adhesion of cells of rootstock and scion occured due to the deposition and polymerization of cell wall materials produced as a result of wound response (Errea *et al.*, 2001). Callus formation was a common response to wounding that occurred both in compatible as well as incompatible grafts (Moore and Walker, 1981;

Espen *et al.*, 2005). Pina *et al.* (2012) found that callus proliferation occurred both in compatible and incompatible grafts of *Prunus* spp. just one week after grafting.

It has been reported that in pea grafts, production of secondary xylem and phloem by reconstituted cambium in the callus bridge took place 12 days after grafting (McCully, 1983). However formation of xylem and phloem vessels in tomato graft occured 8 days after grafting (Fernandez *et al.*, 2004). Serdar and Soyla (2004) reported that, in chestnut grafts, new cambium, xylem and phloem tissues were formed two months after grafting. It was also observed that at least twelve months were needed for continuous cambial merging.

Lima *et al.* (2017) observed that, *Passiflora* grafts required 60 days for the completion of graft union.

2.5 GRAFT INCOMPATIBILITY

Graft incompatibility could occur due to various reasons *viz*, adverse physiological responses between graft partners, virus or phytoplasma transmission and anatomical abnormalities of vascular tissue in the callus bridge. There was no clear-cut distinction between a compatible and incompatible graft union. Incompatible graft partners failed to unite completely or unite initially and later showed symptoms of incompatibility. External symptoms of graft incompatibility included, lack of lignification, yellowing of foliage, decline in vegetative growth and vigor, braking of graft components and anatomical abnormalities (Hartmann and Kester, 2002).

Dogra *et al.* (2018) also reported that graft incompatibility may occurred due to structural or anatomical reasons, physiological and biochemical reasons, nutritional deficiency and presence of viruses at an early phase in response to grafting. All these abnormalities could cause mechanical weakening of the union which might occur in the first year after grafting or appeared several years later. Factors affecting graft-incompatibility were multiple but categorized into adverse physiological response between scion and rootstock and/or anatomical abnormalities of vascular system with the latter being the major cause (Mudge *et al.*, 2009).

Poorly aligned graft components resulted in slow cambial formation but severe misalignment may result in complete failure of graft (Asante and Barnett, 1997)

Graft incompatibility was common in pear cultivars grafted onto quince. The histological studies on graft union identified three typical symptoms of graft incompatibility in pear/pear and pear/ quince grafts such as bark discontinuity, cambial dysfunction and accumulation of starch in the scion. Little cell necrosis was observed at the interface of five month old incompatible grafts (Ermel *et al.*, 1999).

Machado *et al.* (2017) observed that, an eight-year-old pear graft on quince showed the symptoms of delayed incompatibility. Xylem (wood) bridged the gap between rootstock and scion during the early periods but there were no formation of bridging xylem after a few years.

However anatomical investigation on the formation of graft union in citrus grafts combinations proved that there was no difference in the healing processes of graft union both in compatible as well as incompatible grafts (He *et al.*, 2018).

It was observed that, the number of well differentiated sieve tubes was much lower in *Prunus* grafts with poor compatibility (Schmid and Feucht, 1981). Discontinuity in the vascular bundles at the graft union resulted in graft incompatibility in pepper/ tomato, tomato/pepper grafts. Because vascular discontinuity prevented the translocation of assimilates, mineral nutrients, and water between scions and rootstocks (Kawaguchi *et al.*, 2008).

Observations on the internal symptoms of graft incompatibility in unions of 6-month to 8-year-old ponderosa and western white pine grafts showed that common incompatibility symptoms were phloem and cortex necrosis, suberization, internal periderm formation, and invaginated xylem areas (Copes and Forest,1980).

By studying the stock scion interaction in *Prunus* sp. Pina *et al.* (2012) concluded that insufficient plasmodesmatal coupling at an early stage of development within one of the graft partners might have resulted in graft incompatibility.

Callus was formed as a reaction to wounding at the graft union. Callus differentiates into cambium and vascular tissues. A study of apricot grafted onto plum rootstock demonstrated that only some callus differentiated into cambium and vascular tissues, while a large portion of the callus never differentiated which interrupted the vascular connections (Pina and Errea, 2005).

In spite of lacking scion/stock vascular connections, Arabidopsis scions grafted on tomato rootstock flowered and produced seeds. This indicated some non vascular functional connections between the two plants, probably of parenchyma cells (Flaishman *et al.*, 2008). In the grafts of a monocot, *Vanilla* orchid, it was observed that scion survived and grew for two years with the union of parenchyma. But they could not survive when subjected to transpirational stress (Muzik, 1958).

Abundance of periderm rings was observed by Ermel *et al.* (1999) in a five month old pear/quince graft and concluded as one of the reasons for incompatibility. In some pine grafts, signs of incompatibility have been observed a few years after grafting and were explained by differences in radial growth between scion and stock that breaks tissue connectivity at the graft union (Savva *et al.*, 2004).

2.6 STEM ANATOMY OF *PIPER* SPECIES

Vascular anatomy of *Piper* species showed that there was a transition from the monocotyledonous to the dicotyledonous type of bundles in *Piperaceae*.

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The most distinguishing anatomical character of the family *Piperaceae* is the nature of vascular bundles in the stem. In all the species of genus *Piper*, vascular bundles were organized in two or more concentric rings (Yunker and Gray 1934; Trueba *et al.*, 2015) and secondary growth in most of the *Piper* sp. was limited to the outer vascular bundles only (Isnard *et al.*, 2012).

The inner series of vascular bundles in *Piper* was separated from an outer series by a sclerenchymatous ring. This pattern has been observed in *P. nigrum* L. and *P. colubrinum* Link (Ravindran and Remashree 1998); *P. hispidum* Sw. (Albiero *et al.*, 2006); *P. arboreum* (Souza *et al.*, 2009); and *P. mikanianum* (Kunth) Steud. (Duarte and Siebenrock, 2010).

Ravindran *et al.* (2000) identified outer, cortical or peripheral vascular bundles and inner, central or medullary bundles. Small and large bundles were arranged alternately in the outer ring. There were about 35- 40 such bundles. Medullary bundles were smaller than cortical bundles. Eight to ten such bundles were arranged in the parenchymatous pith region. Basic stem anatomy of orthotropic as well as plageotropic shoot remained same.

Ravindran and Remashree (1998) studied the anatomy of *Piper colubrinum* and reported that vascular structure of *Piper colubrinum* stem was basically similar to *P. nigrum*. There were 5-10 medullary bundles and 39-49 peripheral bundles in a 4-5 mm thick stem. Secondary thickening was restricted to the peripheral bundles only. Mucilage canal was present in the outer cortex region while there was no central mucilage canal as in *P. nigrum.* There was formation of cambial ring during secondary growth. The interfascicular cambium produced medullary rays and axial parenchyma towards inside and parenchymatous tissue towards outside.

Vascular anatomy of *Piper methysticum* was described by Hoffstadt (1916). Two systems of vascular bundles were identified in the stem of *Piper methysticum,* peripheral as well as pith vascular bundles. Vascular bundles in the pith region were arranged in two rows and peripheral bundles were of two types, larger primary and smaller secondary bundles. The stem was enlarged by cambial activity and divisions in the pith region.

The main cross sectional stem area of *Peperomia* consisted of parenchymatous ground tissue in which vascular bundles were embedded (Yuncker and Gray, 1934). The mechanical tissue or hypodermal layer in *Piper* sp. was present as an entire ring of thick walled cells towards the outside of the cross section (Isnard *et al*., 2012).

A study conducted on the stem anatomy of *Piper* species called *Piper sarmentosum* describes that, the epidermis consisted of an outer epidermal layer and an inner hypodermal layer. Thick cuticle covered the cells of epidermal layer externally. Cells of hypodermal layer were smaller and squarish. Outer cortex was collenchymatous and formed a continuous ring beneath the hypodermis, cells were angular and with heavily thickened walls. Inner cortex was parenchymatous, cells were circular. Vascular tissue was represented by two whorls of cortical and medullary bundles. There were 24 cortical bundles and one-third of them were larger than the others, all embedded in a wavy ring of 3 - 6 cell wide lacunar collenchyma tissue. Vascular bundles were collateral with phloem above and xylem below and a cambium in between. Medullary vascular bundles were about 5, similar to cortical bundles but larger in size, arranged in a ring in the middle region of pith (Raman *et al*., 2012).

Tepe *et al*. (2007) studied the stem anatomy of *Piper macrostachys* and claimed that there was a typical arrangement of tissues with a parenchymatous pith, medullary vascular bundles, a sclerenchymatous cylinder, peripheral vascular bundles, and a vascular cambium.

The stem anatomy of *Piper betle* L. was also studied. Like *Piper nigrum,* stem of *Piper betle* also had an inner irregular circle of primary vascular bundles interspersed with a large mucilage canal in the pith, more mucilage canals in the inner cortex, an undulating wall of sclerenchyma, and an outer ring of smaller cortical bundles and the pith was occupied with primary vascular (medullary) bundles (Beck 2011). But in another study Raman *et al.* (2012) identified the presence of a ring of mucilage canals in between the cortical and medullary rings of primary vascular bundles in the stem of *P. betle.* Central mucilage canal in the pith, secretory cells in the cortex, cortical fibers, and an endodermis with a casparian strip were also present in the stem.

Beck (2011) again showed that the stem of *P. excelsum* G. Forst. had two medullary bundles, occupied at the very center of the pith. Among the two vascular bundles, the central ones were irregularly grouped and enclosed by a thick cylinder of secondary xylem capped by phloem that was separated by wider medullary rays of primary parenchyma.

Saraswathy *et al.* (2013) indicated that the stem of *P. retrofractum* Vahl. was characterized by the presence of a large central mucilage canal surrounded by individual bundles scattered in a parenchymatous cortex. Central vascular bundles were encircled by a wavy ring of sclerenchyma followed by a ring of vascular bundles and medullary rays and the outer cortex was formed by pericycle and collenchymas.

Carlquist (1990) observed that woody Piperales were characterized by the presence of tall, wide, multiseriate rays and fascicular areas were separated by them.

General anatomical features of order piperales included reduced cambial activity, peculiar type of woodiness with large rays which resulted in the potential inability of interfascicular cambium to produce fusiform initials, lack of cambial activity in species of *Peperomia* and polycyclic distribution of vascular bundles (Isnard *et al.*, 2012).

2.7 EFFECTS OF ANATOMICAL FEATURES ON SUCCESS OF GRAFTING

Schubert (1913) attempted grafting in monocotyledons and concluded that a true union with functional vascular tissue could not be formed between plants that do not possess a cambium. In 1954, however, Muzik and Rue grafted several monocotyledonous lianes in addition to number of grasses. Intergeneric and interspecific grafts of lianes lasted for more than 8 months. The union of the bundles occurred in a curve rather than in a straight line. Similarly Garner and Beakbane (1968) observed a similar union formation in very young stems of *Piper* before a cambial sheath had formed. During secondary thickening normal cambial connection occurred as in dicotyledons. They also reported that, later in the development of the stem, the vascular strands of *P. nigrum* were separated by fibre flanges projecting from the outer fibrous sheath and disrupting the continuity of the cambium and this might account for the poor survival and field establishment of grafts.

Bond (1931) reported the presences of endodermis in the members of Piperaceae which was continuous over rays and vascular strands and it provided a sheath separating the phloem from the cortex. Trueba *et al.*, (2015) also identified an endodermis in the members of Piperaceae which occupied as a layer of cells of procambial activity at the limits of vascular system and the cortical area.

Empari and Sim (1986) proposed that, in grafts of *P. nigrum* on *P. colubrinum*, matching of all the vascular bundles of stock and scion were virtually impossible because of the scattered arrangement of the same. However, the adhesion of stock and scion apparently took place three days after grafting. There was no permanent union of the functional vascular tissues at the graft interface due to the tissue incompatibility between the graft partners.

Huang *et al.* (1994) observed that in chestnut, mismatch of phloem fiber bundles had an important effect on graft success. Stratified pattern of phloem bands formed fiber bundles in four areas of the circumference of the stem which developed towards the cambium and made the periphery of the xylem fluted or grooved. When grafting was performed by placing the scion on the phloem fiber bundle section of rootstock, a mass of parenchymatous tissue was found to be interrupted the normal vascular connection between graft partners. Such grafts survived for a few months and then died.

Materials and Methods

3. MATERIALS AND METHODS

The present study on stock scion interaction in *Piper nigrum* L. grafts was carried out at the Pepper Research unit of Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2017 December – 2019 March. The materials required for the experiment and the methods of experiment are described in this chapter.

3.1 EXPERIMENTAL MATERIALS

South American species of *Piper* which were reported to be resistant to *Phytophthora viz. Piper colubrinum, Piper auduncum* and *Piper arboreum* and also *Piper nigrum* were used as the rootstocks (Plate.1). Scion materials used in the study were orthotropic as well as plageotropic shoots of Panniyur 1 variety of *Piper nigrum* (Plate 2).

3.2 EXPERIMENTAL METHODS

The study was conducted as three experiments.

1. Grafting studies of *Piper nigrum* (orthotropes and plageotropes) on different rootstocks
2. Anatomical studies on *Piper* species
3. Anatomical studies on graft union

3.2.1 Grafting Studies of *Piper nigrum* (Orthotropes and Plageotropes) on Different Rootstocks

Grafting was done once in three months in order to standardize the best time of grafting. The seasons selected for grafting were December, March, June and September.

3.2.1.1 Raising of Rootstocks

Two nod semi hardwood cuttings of each rootstock were collected four months before grafting and planted in polythene bag (15 cm x 20 cm, 300 gauge) containing sand, soil and FYM in equal proportion. Cuttings were placed in a shade house for rooting and they were irrigated twice in a day. During summer mulching was given for the runner cuttings of *Piper nigrum*.

3.2.1.2 Method of Grafting

Grafting was performed under a shade house. Scion materials such as orthotropic and plageotropic shoots of *Piper nigrum* variety, Panniyur 1 were collected in the morning hours on the same day of grafting and they were kept moistened. Method of grafting followed was wedge grafting. The procedure is demonstrated in plate 3.

3.2.1.2.1 Preparation of Rootstock

Four month old healthy rooted cuttings of rootstocks were selected for grafting (Plate 3A). They were decapitated at a height of 15-20 cm from the base (Plate 3B). A cleft of 3-4 cm deep was given on the decapitated semi hardwood portion of the stem using a sharp grafting knife (Plate 3C).

3.2.1.2.2 Preparation of Scion

Lower portion of the scion was given a wedge shaped cut, 3-4 cm long by chopping off bark and a little wood from the two opposite sides (Plate 3D).

3.2.1.2.3 Grafting Procedure

The wedge of the scion was carefully inserted into the cleft of rootstock and secured the joint firmly with polythene strip (30 cm long, 2 cm broad, 150 gauge) (Plate 3E and F). Immediately after grafting, plants were kept in a mist chamber. High relative humidity was maintained by intermittent misting. During summer months special care was taken to maintain high humidity inside the mist chamber. Observations on sprouting were taken. Grafted plants were maintained in the mist chamber until they showed sprouting and later transferred to a polyhouse (Plate 5). Some of the one year old grafted plants were transferred to pot and main field and the growth was observed.

3.2.2 Design of Grafting Experiments

The grafting experiment was laid out in completely randomized design (CRD).

Number of grafts/ treatments – 30

3.2.2.1 Anatomical Studies

3.2.2.1.1 Hand Sectioning

Hand sections of stem of different *Piper* species such as *Piper nigrum, Piper colubrinum, Piper auduncum and Piper arboreum and* different graft unions (3 months and 6 months old) were taken using a microtome blade. Samples were fixed in formalin- acetic acid- alcohol mixture (Ethyl alcohol 95% - 50 ml, Glacial acetic acid – 5ml, Formalin – 10ml and water- 35ml).

Thin sections were selected and placed on a clean slide. Sections were flooded with an aqueous solution of 0.5 % saffranin O stain for less than one minute. Then gently removed the stain using a filter paper and the sections were washed by

Plate 1. Rootstocks

Piper colubrinum

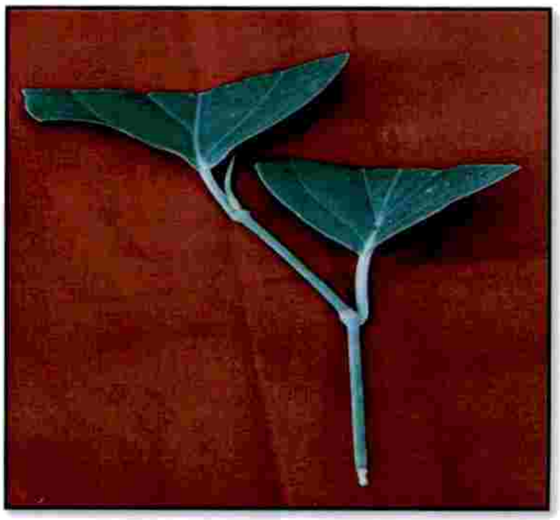
Piper auduncum

Piper arboreum

Piper nigrum

Plate 2. Scion

Orthotropic shoot of *P. nigrum* variety Panniuyr 1



Plageotropic shoot of *P. nigrum* variety Panniuyr 1

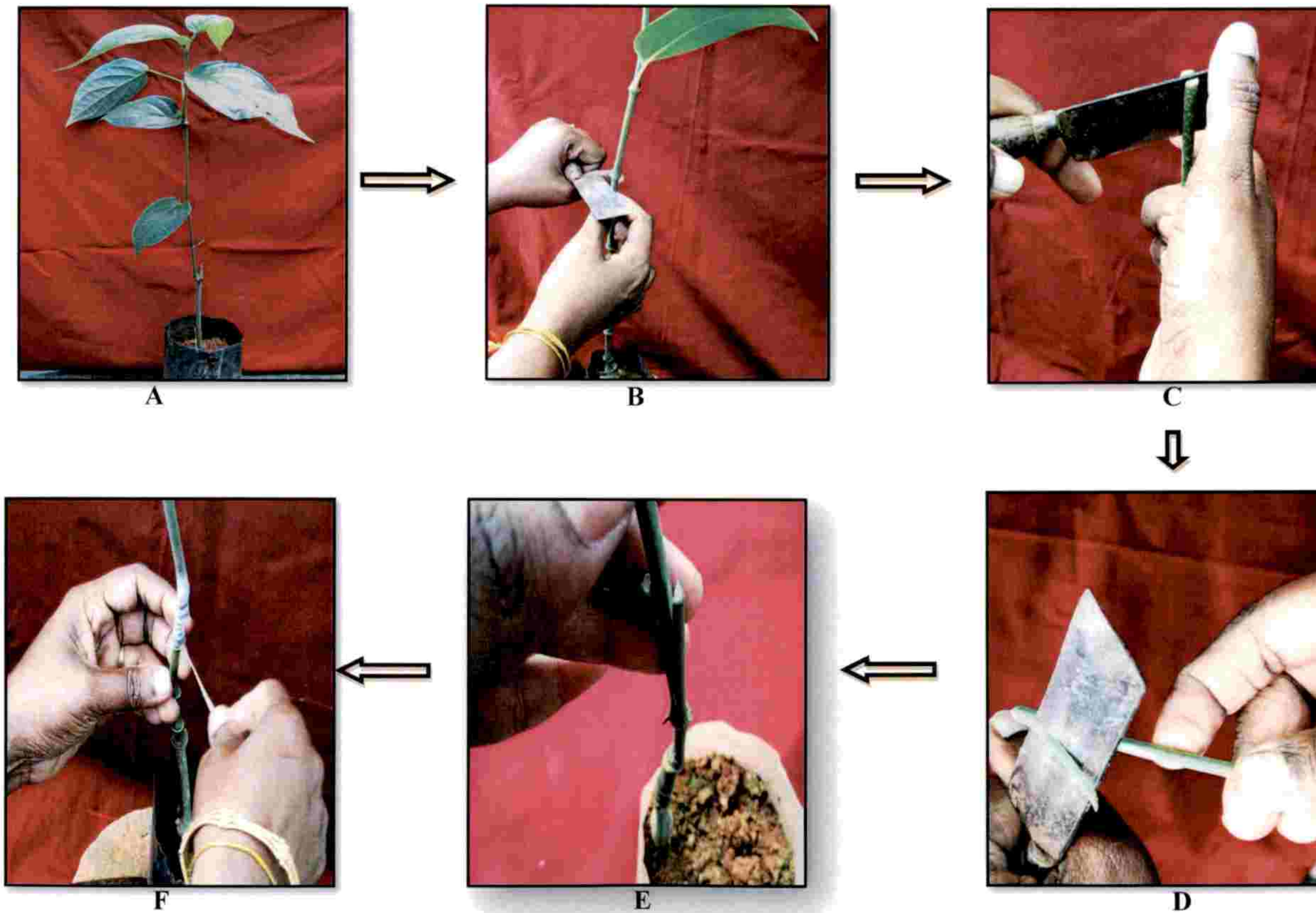


Plate 3. Steps in grafting (wedge and cleft grafting)

Plate 4. Newly grafted plants

Plate 5. Grafts in poly house

flooding with water followed by its removal. The process was repeated until there was no excess water around the sections. Later a drop of water was added over the sections and cover slip was placed over it.

3.2.2.1.2 Microtomy

Thin sections of representative samples of one year old graft union were taken using a sliding microtome. Sliding microtome is a device in which the block remains stationary and the knife moves across it (Prasad and Prasad, 1968).

Samples were cut into small pieces and fixed in the holder. Sections were taken by gently sliding the knife over the samples. Thin sections were selected and stained using aqueous solution of 0.5 % saffranin O stain.

Stained sections were observed under stereomicroscope (MZ8, Leica 10X). Image analysis was carried out using the software Catymage.

3.2.2.1.3 Preparation of Permanent Slide

Permanent slides of the sections were prepared by following the procedure given by Johansen (1940). For this, after staining the sections with saffranin, they were passed through a series of alcohol solution at different concentrations (70%, 90% and 95%) for complete dehydration. Dehydrated sections were subsequently dipped in acetone followed by xylene and finally mounted in DPX mountant and permanent slides (size 75mm x 25mm, thickness 1mm) were prepared.

3.2.2.1.4 Image Analysis

Stained sections were observed under stereomicroscope (MZ8, Leica 10X). Image analysis was done using an image analyzer which consisted of a microscope, digital camera and a PC (personal computer). Digitalized images were captured by digital camera and analyzed using a computer software (Catymage).

3.3 MAIN ITEMS OF OBSERVATION

Following observations were recorded to study the stock scion interaction in *Piper nigrum* L. grafts.

3.3.1 Number of Grafts

3.3.2 Number of Successful Grafts

Number of grafts survived after 6 months was counted and percentage of success in grafting was calculated.

$$\text{Percentage success (\%)} = \frac{\text{Number of successful grafts}}{\text{Total number of grafts}} \times 100$$

3.3.3 Number of Graft Failure

Number of graft failure was calculated by subtracting the number of successful grafts from total number of grafts.

3.3.4 Time Taken for Graft Union

Number of days taken for the sprouting of successful grafts was counted and expressed as the time taken for graft union.

3.3.5 Plant Height Six Months After Grafting

Height of the plant after six months of grafting was measured from the graft union to the tip of the vine using a tape and expressed in cm.

3.3.6 Number of Nodes Six Months After Grafting

Number of nodes of vine after six months of grafting was recorded.

3.3.7 Internodal Length Six Months After Grafting

Length between first ten nodes of six month old grafted plants was measured using a twine and scale and average worked out.

3.3.8 Number of Branches Six Months After Grafting

For grafts in which plageotropic shoots were used as the scion, number of branches six months after grafting was recorded.

3.3.9 Number of Spikes (if any) Six Months After Grafting

Grafts in which plageotropic shoots were used as scion, number of spikes were counted six months after grafting.

3.3.10 Incidence of Pests and Diseases

Incidence of pests and diseases was recorded throughout growth period of grafts.

3.4 ANATOMICAL OBSERVATIONS

Anatomical details of different *Piper* species and graft union at different stages such as 3 months, 6 months and 1 year after grafting were observed.

3.5 STATISTICAL ANALYSIS

Statistical analysis of data was done using WASP 2.0 software.

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Results

4. RESULTS

The present study entitled 'Stock scion interaction in *Piper nigrum* L. grafts' was carried out at Pepper research unit of Department of Plantation crops and spices, College of Horticulture, Vellanikkara during the period 2017 to 2019 so as to study the stock scion interaction in grafts of *Piper nigrum* L. on different South American species of *Piper* immune to *Phytophthora* foot rot and to standardize the best time of grafting. Grafting of *Piper nigrum* on its own rootstock was also done to study the feasibility of such grafting. The results of the study are presented here under.

4.1 GRAFTING STUDIES IN *Piper nigrum* L.

Grafting of *Piper nigrum* on four different rootstocks was carried out at four different months in order to determine the best rootstock and ideal month for grafting. The selected rootstocks were *P. colubrinum*, *P. auduncum*, *P. arboreum* and *P. nigrum.* Two node cuttings of rootstocks were raised in poly bags four to five months before grafting. Orthotropic and plageotropic shoots of *Piper nigrum* were used as scion. Grafting was carried out at four different months representing four different seasons such as March (summer), June (Monsoon), September (post monsoon) and December (winter). The design of the experiment was CRD and total number of grafts per rootstock per season was thirty. A season wise analysis of grafting success of *P. nigrum* on different rootstocks and the effect of rootstocks on growth of scion was carried out.

4.1.1 Orthotropic Shoot of *P. nigrum* as scion

4.1.1.1 *Evaluation of Grafting done in December*

4.1.1.1.1 *Success of Grafting*

For determining the success of grafting, sprouting of scion was observed at weekly intervals. Irrespective of rootstocks and seasons, sprouting of scion commenced between 8 and 12 days after grafting (DAG) and continued up to 30 DAG. Since maximum sprouting was observed at 30 DAG, number of successful grafts was recorded at that time.

The number of grafts prepared, number succeeded, per cent success and number of grafts failed to sprout are given in Table 1. As can be seen from the table, rootstocks had a profound effect on success of grafting when it was carried out during December. Among the four rootstocks, highest success in grafting was recorded with *P. nigrum*. Out of the thirty grafts of *P. nigrum*, twenty six got sprouted at 30 DAG giving a per cent success of 86.66 which was even greater than that of grafts on *P. colubrinum* (80%). But, the success of grafting of *P. nigrum* on other South American species of *Piper* such as *P. auduncum* and *P. arboreum* was low compared to *P. colubrinum*. About 60 per cent of grafts showed sprouting in grafts with *P. auduncum* as rootstock, while it was only 20 per cent with *P. arboreum*.

Two types of graft failure were noticed in these grafts, they were, shrinking and drying of scion within six to nine days after grafting. In the second type, scion remained green without sprouting even after sixty days of grafting and later dried. In case of grafts with *P. arboreum* as rootstock which recorded the lowest per cent success in grafting, the main symptom of graft failure observed was lack of sprouting of scion even after sixty days of grafting which remained green. Such grafts got dried after seventy days of grafting. Shrinking and drying of scion within six to nine days after grafting was prominently observed in grafts with *P. auduncum* as rootstock. Out of eighteen grafts failed, eleven grafts showed such type of graft failure. Minimum graft failure was observed in grafts of *P. nigrum* on its own rootstock (4 out of 30).

4.1.1.1.2 *Graft Survival*

Once the grafts sprouted (1 MAG) the successful grafts were carefully taken from the mist chamber to poly house and survival was observed.

Graft survival was indicated by grafts with actively growing scion. The number and percentage of such grafts were recorded at 3 MAG (months after grafting), 6 MAG and 12 MAG (Table 2). Although six grafts of *P. arboreum* could sprout within one month of grafting, they could not survive up to three months. At single leaf stage itself (within two months of grafting) scions of four grafts got dried and in two grafts rotting of rootstock was first observed immediately followed by drying up of scion. In case of all the other rootstocks, gradual decrease in the number of survived grafts and in the per cent graft survival was observed.

The survival percentage of grafts on *P. nigrum* gradually reduced from 86.66 to 73.33 within one year after grafting. Similarly in case of grafts on *P. colubrinum*, the gradual decrease in the per cent survival was from 80 (1 MAG) to 63.33 (12 MAG). But in case of grafts on *P. auduncum*, only 46.66 (3 MAG) was the per cent survival recorded which was further reduced and became half (23.33 %) by 12 MAG. Displacement of stock and scion due to improper joining and breakage of stock at the base (in case of six months to one year old grafts) due to the heaviness of scion were observed as the hindrance to graft survival in *P. colubrinum* as well as self grafts. Apart from these observations, rotting of stock and drying of scion were also noticed in grafts on *P. auduncum*.

Table 1. Effect of rootstocks on success of grafting done during December

Rootstock	Number of grafts prepared	Success of grafting		Graft failure		
		Number	Percentage	Grafts with dried scion	Grafts failed to sprout but remained green	Total no. of graft failure
P. colubrinum	30	24	80.00	4	2	6
P. auduncum	30	18	60.00	10	2	12
P. arboreum	30	6	20.00	4	20	24
P. nigrum	30	26	86.66	3	1	4

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Table 2. Effect of rootstocks on survival of graft (Grafting season – December)

Rootstock	Number of grafts prepared	Success		Survival					
		1 MAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	24	80.00	23	76.66	20	66.66	19	63.33
P. auduncum	30	18	60.00	14	46.66	10	33.33	7	23.33
P. arboreum	30	6	20.00	0	-	0	-	0	-
P. nigrum	30	26	86.66	24	80.00	23	76.66	22	73.33

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4.1.1.1.3 *Effect of Rootstocks on Growth of Scion*

The growth of established grafts (6 MAG) was observed by taking into account the growth charecterestics *viz.*, plant height, number of nodes and internodal length of scion. Mean values of these growth parameters of 23, 10 and 20 grafts on *P. nigrum*, *P. auduncum* and *P. colubrinum* respectively are furnished in table 3. Since none of the grafts with *P. arboreum* as rootstock could survive up to 6 MAG, observations on such grafts could not be taken. Observations on growth parameters of one year old grafts were also excluded since the attempt resulted in destruction of grafts via., braking of roots and disturbance of graft union.

It can be seen from the table that, when grafting of *P. nigrum* was carried out on South American species of *Piper* such as *P. colubrinum* and *P. auduncum*, growth of scion was much better compared to its own rootstock. Grafts on *P. colubrinum* excelled in growth with highest plant height (78.20 cm), number of nodes (13.04) and internodal length (6.14 cm). All these growth parameters of grafts with *P. auduncum* as rootstock were statistically on par with that of *P. colubrinum*. Scion of *P. auduncum* grafts were extended to a length of 66.35 cm by six months after grafting with 11.3 nodes and internodal length of 5.96 cm. Though the plant height and number of nodes of grafts on *P. nigrum* were statistically on par with that of *P. auduncum*, internodal length was significantly low (5.06 cm).

Table 3. Effect of rootstock on growth of scion (Grafting season- December)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)
P. colubrinum	78.20[a]	13.04[a]	6.14[a]
P. auduncum	66.35[ab]	11.30[ab]	5.96[a]
P. nigrum	48.03[b]	9.42[b]	5.06[b]
CD (0.05)	24.28	2.74	0.81

4.1.1.2 *Evaluation of Grafting done in March*

4.1.1.2.1 *Success of Grafting*

Grafting was carried out during March 2018 and observations on graft success, survival and growth of grafts were analyzed.

As stated earlier, success of grafting was indicated by the sprouting of scion. Within first month of grafting itself, maximum sprouting was observed during March also. So success percentage was calculated 1 MAG. Data regarding the number of grafts prepared, succeeded, per cent success and graft failure is given in table 4. As can be seen from the table, after one month of grafting 100 per cent sprouting was observed in self grafts. This was closely followed by grafts of *P. colubrinum* (96.66 %). Above fifty per cent success was recorded in *P. auduncum* grafts (63.33%). Using *P. arboreum* as rootstock 46.66 per cent sprouting was observed within first month of grafting.

Observations on graft failure indicated that, unsuccessful grafts on *P. arboreum* (16 out of 30) remained as such without sprouting for about three months and thereafter dried. In case of grafts with *P. auduncum* as rootstock, which confronted less graft failure compared to *P. arboreum*, prominently observed symptom of failure was shrinking and drying up of scion. Only one graft of *P. colubrinum* had remained without sprouting.

4.1.1.2.2 *Graft Survival*

As per the data given in table 5, gradual reduction in the number of successful grafts was noticed only in case of *P. auduncum* grafts (66.66 per cent to 33.33 per cent). Rotting of rootstock and gradual wilting and drying of scion was observed in all the grafts of *P. auduncum* which failed to survive after 6 months of grafting. The one graft of *P. colubrinum* which remained unsprouted 1 MAG, sprouted after two months giving 100 per cent survival at 3 MAG. But, at single leaf stage itself it got wilted and dried so could not grow further. Within 6 to 12 MAG, the per cent survival of *P. colubrinum* grafts was reduced from 96.66 to 83.33. During this period stunting was observed in two grafted plants and in other two, week growth of scion was observed even with a green and healthy rootstock.

Out of the fourteen succeeded grafts of *P. arboreum*, nine remained as such with a single small sprout for three months and later dried. Rests of the five grafts were observed with rotting of rootstock first and later complete drying of graft. Compared to South American species of *Piper*, *P. nigrum* on its own rootstock, performed better during March. Only 10 per cent reduction was recorded in self grafts within a period of three to twelve months after grafting. Reduction in the per cent survival of self grafts was attributed to the rotting of the stock from the base and subsequent yellowing and drying of scion.

Table 4. Effect of rootstocks on success of grafting done during March

Rootstock	Number of grafts prepared	Success of grafting		Graft failure		
		Number	Percentage	Grafts with dried scion	Grafts failed to sprout but remained green	Total no. of graft failure
P. colubrinum	30	29	96.66	0	1	1
P. auduncum	30	20	66.66	9	1	10
P. arboreum	30	14	46.66	0	16	16
P. nigrum	30	30	100.00	0	0	0

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Table 5. Effect of rootstocks on survival of graft (Grafting season – March)

Rootstock	Number of grafts prepared	Success		Survival					
		1 MAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	29	96.66	30	100.00	29	96.66	25	83.33
P. auduncum	30	20	66.66	15	50.00	14	46.66	10	33.33
P. arboreum	30	14	46.66	0	-	0	-	0	-
P. nigrum	30	30	100.00	29	96.66	29	96.66	27	90.00

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4.1.1.2.3 *Effect of Rootstocks on Growth of Scion*

In order to determine the influence of different rootstocks on growth of scion, growth characteristics such as plant height, number of nodes and internodal length of scion were recorded after six months of grafting. The mean of growth parameters of survived grafts after six months of grafting *ie.,* 29 grafts each of *P. nigrum* and *P. colubrinum* and 14 grafts of *P. auduncum* is given in table 6. Since complete graft failure was noticed in *P. arboreum* grafts by 6 MAG, data regarding the growth characteristics of such grafts are not included.

It may be seen from the table that, plant height and number of nodes differed significantly among the three rootstocks but not the internodal length. Highest growth vigour was shown by the grafts on *P. colubrinum* which was indicated by highest plant height (107.34 cm), number of nodes (16.06) and internodal length (6.50 cm). After *P. colubrinum*, plant height was highest in *P. nigrum* (69.74 cm) followed by *P. auduncum* (57.10 cm). Number of nodes in grafts with *P. auduncum* as rootstock was statistically on par with that of *P. nigrum*.

Table 6. Effect of rootstocks on growth of scion (Grafting season – March)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)
P. colubrinum	107.34^{a}	16.06^{a}	6.50
P. auduncum	57.10^{c}	9.35^{b}	5.96
P. nigrum	69.74^{b}	11.24^{b}	6.13
CD (0.05)	16.95	1.94	NS

4.1.1.3 *Evaluation of Grafting done in June*

4.1.1.3.1 *Success of Grafting*

The number of grafts prepared during June, number of successful grafts, per cent success and observations on graft failure are furnished in table 7. Since graft success was indicated by sprouting of scion, number of sprouted grafts and thereby per cent success was recorded at 1 MAG.

Out of thirty, twenty five grafts on *P. colubrinum*, sprouted giving the highest per cent success of 83.33 which was even greater than that of self grafts (66.66 %). A closely related per cent success of grafting was obtained by *P. auduncum* (46.66 %) and *P. arboreum* (43.33 %) in June. Among the five unsuccessful grafts of *P. colubrinum*, three remained green but without sprouting. Shrinking and drying of scion was observed in one graft within the first week of grafting itself and in another graft breaking of stock at the basal node was observed after two weeks of grafting.

The main symptoms of graft failure observed in *P. auduncum* grafts within the first month of grafting were, rotting of rootstock and subsequent drying of scion, shrinking and drying of scion, grafts without sprouts on the scion and breaking of the stock at the basal node. Among these, most widely observed symptom was rotting of rootstock. Out of the sixteen unsuccessful grafts, rotting of stock followed by complete drying of the graft was noticed in seven grafts and four grafts were observed with the symptom of shrinking and drying of scion. Along with these observations, grafts without sprouting and breaking of stock at the basal node were also observed in 3 and 2 grafts respectively.

As in the case of *P. auduncum*, rotting of stock was predominantly observed in *P. arboreum* grafts. Out of the seventeen unsuccessful grafts of *P. arboreum*, ten grafts were noticed with rotten stock and subsequent drying of scion. The general symptoms like shrinking and wilting of scion and grafts with unsprouted scion were observed in five and two grafts respectively. Along with the general symptoms of graft failure, two unsuccessful grafts of *P. nigrum* were observed with rotting at the graft union and which started to develop both upward and downward. In another two unsuccessful grafts, rotting of stock alone was observed.

4.1.1.3.2 *Graft Survival*

Number of grafts survived and the per cent survival indicated by actively growing scion were recorded at 3 MAG, 6 MAG and 12 MAG (Table. 8). Highest per cent survival was recorded by grafts on *P. colubrinum*. The twenty five grafts on *P. colubrinum* sprouted by 1 MAG, survived for more than three months. But after four months, scions of two grafts at 8 and 10 leaf stages respectively started wilting and later resulted in complete failure of the grafts.

Compared to grafts on *P. colubrinum*, per cent survival of grafts on other three rootstocks was low. After *P. colubrinum*, highest per cent survival was recorded in case of self grafts. In case of self grafts, within first three months itself, per cent survival of grafts reduced from 66.66 per cent to 50 per cent. Observations on the failed grafts indicated that, all the grafts which failed to survive was because of rotting of rootstock and subsequent wilting and drying of scion. A similar symptom was observed on the four grafts which failed to survive after four months of grafting so as the per cent survival was reduced from 50 per cent (3 MAG) to 36.66 per cent at 6 MAG. Breaking at the graft union was observed in a graft on *P. nigrum* at 11 leaf stage so as the survival percentage further reduced to 33.33 at 12 MAG.

Even though per cent success of *P. auduncum* as well as *P. arboreum* were similar, per cent survival recorded a wide variation. About half of the successful grafts of *P. auduncum* could not survive up to three months. Severe rotting was observed on the stock followed by the wilting and drying of scion. Thereafter not much reduction in the per cent survival was observed in *P. auduncum* grafts. At the sixth month of grafting, the one graft which showed week growth of scion got dried giving a per cent survival of twenty (6 MAG). Later on there no graft failure was observed in *P. auduncum* grafts.

The sprouted grafts on *P. arboreum* did not show normal growth. Out of the thirteen sprouted grafts, nine got dried after one month of sprouting. Four grafts remained at 2 to 3 leaf stage for one more month and then dried out.

4.1.1.3.4 *Effect of Rootstocks on Growth of Scion*

Overall growth of grafted plants was analyzed by recording the plant height, number of nodes and internodal length at six months after grafting. Mean values of these growth characteristics of successful grafts on *P. colubrinum*, *P. auduncum* and *P. nigrum* are furnished in table 9. Sine complete graft failure was recorded in *P. arboreum* grafts, data regarding the scion growth in such grafts are excluded.

As can be seen from the table, except internodal length other growth characteristics *viz.,* plant height and number of nodes showed significant difference among rootstocks. Grafts on *P. colubrinum* showed highest plant height (120.55 cm) and number of nodes (18.91). Internodal length did not differ significantly among rootstocks. Plant height and number of nodes of *P. auduncum* and self grafts were statistically on par.

Table 7. Effect of rootstocks on success of grafting done during June

Rootstock	Number of grafts prepared	Success of grafting		Graft failure		
		Number	Percentage	Grafts with dried scion	Grafts failed to sprout but remained green	Total no. of graft failure
P. colubrinum	30	25	83.33	1	3	5
P. auduncum	30	14	46.66	4	3	16
P. arboreum	30	13	43.33	5	2	17
P. nigrum	30	20	66.66	2	3	10

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Table 8. Effect of rootstocks on survival of graft (Season – June)

Rootstock	Number of grafts prepared	Success		Survival					
		1 MAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	25	83.33	25	83.33	23	76.66	22	73.33
P. auduncum	30	14	46.66	7	23.33	6	20.00	6	20.00
P. arboreum	30	13	43.33	4	13.33	0	-	0	-
P. nigrum	30	20	66.66	15	50.00	11	36.66	10	33.33

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Table 9. Effect of rootstocks on growth of scion (Season - June)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)
P. colubrinum	120.55[a]	18.91[a]	6.32
P. auduncum	69.25[b]	11.20[b]	5.94
P. nigrum	89.28[b]	13.66[b]	6.24
CD (0.05)	22.60	3.29	NS

4.1.1.4 *Evaluation of Grafting done in September*

4.1.1.4.1 *Success of Grafting*

The last season of grafting was September (2018). Number of grafts prepared, number succeeded, per cent success and observations on graft failure are given in table 10. As the success of grafting was indicated by the sprouting of scion, grafts were observed for sprouting and maximum sprouting was noticed 1 MAG. Number of successful grafts and the per cent success was recorded at 1 MAG.

As can be seen from the table, per cent success of grafting when it was done during September was ranging from 13.33 to 83.33 per cent. Highest per cent success of grafting was recorded by self grafts (83.33 %) followed by grafts on *P. colubrinum* (70%). Compared to these grafts, *P. auduncum* and *P. arboreum* recorded very low per cent success of grafting. When grafts on *P. auduncum* showed 26.66 per cent success, only 13.33 per cent success was given by *P. arboreum* rootstock.

Observations on graft failure indicated that, among the grafts of *P. arboreum* which recorded least success, the prominently observed symptom of graft failure was shrinking and drying of scion within first month of grafting. When twelve grafts were observed with such symptom, sprouting was lacking in eight grafts. Apart from these general symptoms of graft failure, rotting of stock was also observed in five grafts. In case of *P. auduncum* grafts, out of twenty two unsuccessful grafts, fifteen remained green without sprouting. Shrinking and drying of scion, rotting of rootstock and subsequent drying of scion were also noticed in these grafts. All the unsuccessful grafts of *P. nigrum* remained as such without sprouting one month after grafting.

4.1.1.4.2 *Graft Survival*

Graft survival indicated by the active growth of scion was recorded at one, six and ten months after grafting. Data regarding the survival of grafts are given in table 11.

When grafting was done during September, among the four rootstocks, highest success as well as survival was recorded by self grafts. Out of the 25 sprouted grafts, scion of only one graft could not produce more than three leaves and which got wilted at the second month of grafting. There was no reduction in per cent survival observed within a period of three to six months after grafting. But after six months, three grafts could not survive as the stock became rotten and scions became yellow and dried.

Grafts on South American *Piper* species *viz., P. colubrinum* and *P. auduncum* recorded a gradual reduction in the per cent survival from first to 10^{th} month after grafting. In both these grafts, there was 16.67 per cent reduction in survived grafts. General symptoms of graft failure observed in grafts on *P. colubrinum* were, yellowing and wilting of scion, breaking of stock at the basal node and detachment of scion from stock due to improper securing. In grafts on *P. auduncum*, in addition to the symptoms of failure in *P. colubrinum* grafts, rotting of stock and subsequent drying of scion was noticed. All the four sprouted grafts of *P. arboreum* showed very slow growth and the grafts dried up within three months after grafting (at two to three leaf stage).

4.1.1.4.3 *Effect of Rootstocks on Growth of Scion*

In order to determine the effect(s) of rootstocks on growth of scion, growth characteristics such as, plant height, number of nodes and internodal length were recorded at 6 MAG. Since grafts on *P. arboreum* could not survive up to six months, observations on the growth parameters of such grafts could not be recorded. The mean values of growth characteristics of survived grafts on *P. colubrinum*, *P. auduncum* and *P. nigrum* are furnished in table 12.

Grafts on *P. colubrinum* performed best as it recorded highest plant height (67.63 cm), number of nodes (10) and internodal length (6.54 cm). All the growth parameters of *P. auduncum* as well as self grafts were statistically on par.

Table 10. Effect of rootstocks on success of grafting done during September

Rootstock	Number of grafts prepared	Success of grafting		Graft failure		
		Number	Percentage	Grafts with dried scion	Grafts failed to sprout but remained green	No. of graft failure
P. colubrinum	30	21	70.00	2	4	9
P. auduncum	30	8	26.66	4	15	22
P. arboreum	30	4	13.33	12	8	26
P. nigrum	30	25	83.33	-	5	5

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Table 11. Effect of rootstocks on survival of graft (Season – September)

Rootstock	Number of grafts prepared	Success		Survival					
		1 MAG		3 MAG		6 MAG		10 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	21	70.00	20	66.66	17	56.66	16	53.33
P. auduncum	30	10	33.33	9	30.00	8	26.66	5	16.66
P. arboreum	30	4	13.33	0	-	0	-	0	-
P. nigrum	30	25	83.33	24	80.00	24	80.00	21	70.00

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Table 12. Effect of rootstocks on growth of scion (Season- September)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)
P. colubrinum	67.63[a]	10.00[a]	6.54[a]
P. auduncum	35.87[b]	7.25[b]	5.00[b]
P. nigrum	41.29[b]	7.91[b]	5.15[b]
CD (0.05)	9.81	1.22	0.69

4.1.2 Overall Effect of Rootstocks on Graft Success and Survival

The total number (and percentage) of successful as well as survived grafts obtained from each rootstock during the entire grafting period (December 2017 to September 2018) is analyzed in table 13.

Comparing the overall performance of rootstocks, grafts of *P. nigrum* on its own rootstock showed highest per cent success (84.16%) which was slightly greater than that of grafts on *P. colubrinum* (82.5 %). Compared to *P. colubrinum*, grafts on other South American *Piper* species *viz., P. auduncum* and *P. arboreum* recorded less per cent success in grafting. Using *P. auduncum* as rootstock, about fifty per cent success was obtained. Grafting on *P. arboreum* was least successful as it recorded the lowest per cent success (30.83 %).

A gradual failure of survived grafts was observed for all the rootstocks. When the percentage survival of grafts on *P. colubrinum* was 81.66 and 74.16 at three and six months after grafting respectively the survival percentage of self grafts was reduced from 75.83 to 72.50 per cent from three to six months after grafting. In case of *P. auduncum* grafts, per cent survival at three and six months after grafting was 37.5 and 31.66 per cent respectively. None of the grafts of *P. arboreum* could survive up to three months after grafting.

Table 13. Overall response of rootstocks to grafting (December 2017 to September 2018)

Rootstock	Total number of grafts prepared	Success		Survival			
				3 MAG		6 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	120	99	82.50	98	81.66	89	74.16
P. auduncum	120	62	51.66	45	37.50	38	31.66
P. arboreum	120	37	30.83	0	-	0	-
P. nigrum	120	101	84.16	92	75.83	87	72.50

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4.1.3 Effect of Month of Grafting on Graft Success and Growth of Scion

4.1.3.1 *Effect of Month of Grafting on Graft Success*

Per cent success of grafting (of each rootstock) when carried out during different months are given in table 14. When *P. colubrinum* was used as rootstock, success of grafting was ranging from 70 to 96.66 per cent. The highest grafting success was observed during March, while the lowest was recorded during September. A per cent success of 83.33 was noticed when grafting was performed during June which was closely followed by December (80 %).

Success of grafting using *P. auduncum* as rootstock was in the range of 26.66 to 66.66 per cent. Grafts using *P. auduncum* as rootstock in March recorded highest per cent success followed by December (60 %) and June (46.66 %). Comparatively low per cent success was observed in September (26.66 %). In the case of grafts using *P. arboreum* as rootstock, success of grafting ranged from 13.33 to 46.66. Highest grafting success was recorded when it was done during March. But, only 13.33 per cent success was obtained during September. When 43.33 was the success percentage noticed in June, it was only 20 per cent during December. In self grafts, the grafting success was in the range of 66.66 to 100 per cent. Hundred per cent grafting success was recorded during March. But, the lowest success was observed in the subsequent month *ie.*, June. Per cent success of grafting recorded during December and September were 86.66 and 83.33 respectively.

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Table 14. Effect of month of grafting on graft success

Month of grafting	Percent success of grafting			
	P. colubrinum	*P. nigrum*	*P. auduncum*	*P. arboreum*
December 2017	80.00	86.66	60.00	20.00
March 2018	96.66	100.00	66.66	46.66
June 2018	83.33	66.66	46.66	43.33
September 2018	70.00	83.33	26.66	13.33

4.1.3.2 *Effect of Month of Grafting on Growth of Scion*

Rootstock – *P. colubrinum*

Growth characteristics *viz*., plant height, number of nodes and internodal length of *P. colubrinum* grafts (six months old) obtained during different months are analyzed in table 15. Month of grafting significantly influenced plant height and number of nodes but not the internodal length. Plant height of *P. colubrinum* grafts varied from 67.63 cm to 120.55 cm. Highest plant height was recorded in the grafts of June (120.55 cm) which was statistically on par with that of March (107.34 cm). Height of grafted plants of December as well as September was also statistically on par. Month of grafting significantly influenced the number of nodes in scion. Grafts of June were recorded with highest number of nodes (18.91). Number of nodes in grafts of March, December and September were statistically on par.

Table 15. Effect of month of grafting on growth of scion

(Rootstock – *P. colubrinum*)

Season of grafting	Plant height	No. of nodes	Internodal length
December 2017	78.200^{b}	13.04^{bc}	6.14
March 2018	107.34^{a}	16.06^{ab}	6.50
June 2018	120.55^{a}	18.91^{a}	6.32
September 2018	67.63^{b}	10.00^{c}	6.54
CD (0.05)	24.50	3.07	NS

Rootstock – *P. auduncum*

In case of *P. auduncum* grafts, month of grafting influenced the number of nodes only (Table 16). Highest number of nodes was recorded in the grafts of June (11.2) which were statistically on par with that of March and December. Grafts of September were observed with lowest number of nodes (7.25). Other growth characteristics *viz.,* plant height and internodal length did not differ significantly.

Table 16. Effect of month of grafting on growth of scion

(Rootstock – *P. auduncum*)

Season of grafting	Plant height	No. of nodes	Internodal length
December 2017	66.35	11.30^{a}	5.96
March 2018	57.10	9.35^{ab}	5.96
June 2018	69.25	11.20^{a}	5.94
September 2018	35.87	7.25^{b}	5.00
CD (0.05)	NS	3.38	NS

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Rootstock – *P. nigrum*

There was significant difference in all the growth characters as far as *P. nigrum* grafts on its own rootstock were concerned (Table 17). Plant height of six month old *P. nigrum* grafts were found to be in the range of 41.29 cm to 89.28 cm. Grafts of June recorded highest plant height (89.28 cm) followed by March (69.74 cm). Plant heights of grafts were statistically on par when grafted during December and September.

Number of nodes in *P. nigrum* grafts varied between 7.91 and 13.66. When grafts of June were observed with highest number of nodes (13.66), the lowest was recorded with the grafts of September (7.91). Number of nodes in grafts of December and September were statistically at par. Internodal length in self grafts was in the range of 5.06 cm to 6.24 cm. Internodal length in grafts of March and June as well as December and September were statistically on par.

Table 17. Effect of month of grafting on growth of scion

(Rootstock – *P. nigrum*)

Season of grafting	Plant height	No. of nodes	Internodal length
December 2017	48.02^{c}	9.42^{bc}	5.06^{b}
March 2018	69.74^{b}	11.24^{ab}	6.13^{a}
June 2018	89.28^{a}	13.66^{a}	6.24^{a}
September 2018	41.29^{c}	7.91^{c}	5.15^{b}
CD (0.05)	14.75	2.43	0.78

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4.1.4 Using Plageotropic Shoot of *P. nigrum* as Scion

Grafting was carried out using plageotropic shoot of *P. nigrum* as scion. Evaluations on grafting done at four different months are given here under.

4.1.4.1 *Evaluation of Grafting done in December*

4.1.4.1.1 *Success of Grafting*

After grafting lateral shoots of *P. nigrum* on different rootstocks, grafts were observed frequently for sprouting. Commencement of sprouting was observed after shedding of leaves from scion which were retained during precuring. Maximum sprouting was noticed at 45 DAG. Since success of grafting was indicated by sprouting, number of successful grafts and per cent success was recorded at 45 DAG.

Total number of grafts prepared, number succeeded, per cent success and total number of graft failure are given in table 18. As can be seen from the table, highest per cent success in grafting was exhibited by self grafts (63.33%) closely followed by the grafts on *P. colubrinum* (60%). Other South American species of *Piper viz., P. auduncum* and *P. arboreum* recorded 33.33 and 23.33 per cent success respectively.

Generally observed symptoms of graft failure were shrinking and drying of scion within 14 DAG and grafts remained green but without sprouting even after two months of grafting. Twelve unsuccessful grafts of *P. colubrinum* were observed with wilted scion followed by complete drying of grafts. Failure of scion to sprout was prominantly observed in grafts on *P. auduncum* and *P. arboreum*. Out of the twenty unsuccessful grafts on *P. auduncum*, sixteen failed to sprout. Similar observation was recorded in grafts on *P. arboreum* also. Remaining grafts of both *P. auduncum* as well as *P. arboreum* showed wilting of scion and subsequent drying of the graft. Lack of sprouting, shrinking and drying of scion and detachment of scion from stock due to improper joining were observed in *P. nigrum* grafts.

Table 18. Effect of rootstocks on success of grafting done during December

Rootstock	Number of grafts prepared	Success of grafting		Total no. of graft failure
		Number	Percentage	
P. colubrinum	30	18	60.00	12
P. auduncum	30	10	33.33	20
P. arboreum	30	7	23.33	23
P. nigrum	30	19	63.33	11

4.1.4.1.2 *Graft Survival*

Number of grafts survived and percentage survival were recorded at three, six and twelve months after grafting. As shown in table 19, once the grafts got established, not much reduction in the per cent survival was observed thereafter. Lowest percentage reduction was observed in self grafts. These grafts exhibited 63.33 per cent graft success at 45 DAG. But, even after sprouting, scion showed wilting and subsequently dried out. Within one year period of grafting, the graft survival was reduced to 56.66 per cent.

Even though success of grafting with *P. colubrinum* rootstock was close to that of *P. nigrum*, per cent reduction in the graft survival was much more in *P. colubrinum*. Within one year period of grafting, percentage survival recorded a reduction from 60 to 40 per cent. Highest reduction was noticed from a period of 45 DAG to three MAG. Thereafter not much reduction in the survived grafts was observed. Out of the six grafts on *P. colubrinum*, which failed to survive during different months, three were observed with very slow growth of scion and later dried at two to three leaf stages. Rotting at the graft union was noticed in rests of the grafts. A ten per cent reduction in the number of survived grafts on *P. auduncum* was recorded within a period of one year after grafting. During the initial months, wilting and drying of scion was prominently observed but later rotting of stock was frequently noticed in these grafts.

Highest failure in grafting was observed when *P. arboreum* was used as rootstock. Even though 23.33 per cent grafts showed sprouting within 45 DAG, none of them could survive even up to three months after grafting as they showed wilting of scion and rotting of stock.

4.1.4.1.3 *Effect of Rootstocks on Growth of Scion*

Different growth parameters of grafted plants were recorded in order to determine the influence of rootstocks on growth of scion. Since plageotropic shoot was used as scion, along with plant height, number of nodes and internodal length, growth parameters *viz.*, number of branches and number of spikes (if any) were also recorded at 180 DAG. As none of the grafts on *P. arboreum* could survive up to 180 DAG, observations on growth of such grafts are excluded.

Data pertaining to the growth characteristics of scion grafted on different rootstocks during December is given in table 20. As can be seen from the table, grafts on different rootstocks did not differ significantly as far as growth of scion was concerned.

Table 19. Effect of rootstocks on survival of graft (Season – December)

Rootstock	Number of grafts prepared	Success		Survival					
		45 DAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	18	60.00	15	50.00	13	43.33	12	40.00
P. auduncum	30	10	33.33	8	26.66	7	23.33	7	23.33
P. arboreum	30	7	23.33	0	-	0	-	0	-
P. nigrum	30	19	63.33	18	60.00	17	56.66	17	56.66

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Table 20. Effect of rootstocks on growth of scion (Season – December)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)	No. of branches	No. of spikes
P. colubrinum	36.85	7.57	5.53	1.14	0.00
P. auduncum	31.26	4.60	7.11	1.10	0.00
P. nigrum	36.34	6.00	6.03	1.15	0.10
CD (0.05)	NS	NS	NS	NS	-

4.1.4.2 *Evaluation of Grafting done in March*

4.1.4.2.1 *Success of Grafting*

Number of grafts prepared during March, number succeeded, percentage success and number of graft failure are furnished in Table 21. Highest percent success in grafting was recorded when *P. nigrum* was grafted on its own rootstock (93.33 %). Comparatively low per cent success in grafting was recorded with *Piper* species other than *P. nigrum* when used as rootstock.

Among the South American *Piper* species, *P. colubrinum* exhibited highest per cent success (63.33 %) followed by *P. auduncum* (36.66 %) and *P. arboreum* (26.66 %). Predominantly observed symptom of graft failure in *P. colubrinum* grafts was shrinking and drying of scion within the first three weeks of grafting. Lack of sprouting was noticeably observed in grafts on *P. auduncum* as well as *P. arboreum*. But in self grafts, displacement of stock and scion due to improper joining was observed.

Table 21. Effect of rootstocks on success of grafting done during March

Rootstock	Number of grafts prepared	Success of grafting		Total no. of graft failure
		Number	Percentage	
P. colubrinum	30	19	63.33	11
P. auduncum	30	11	36.66	19
P. arboreum	30	8	26.66	22
P. nigrum	30	28	93.33	2

4.1.4.2.2 *Graft Survival*

Survival of grafted plants was monitored over a period of one year. A gradual reduction in the per cent survival of grafts was observed within a period of one year after grafting (Table 22). Highest reduction in the percentage survival was noticed in *P. arboreum* grafts. Twenty per cent of sprouted grafts failed within the first three MAG. A normal growth of sprouted scion was not observed in such grafts. Even before reaching at one full leaf stage, they were dried out. The grafts which showed good growth initially could not grow further because of rotting of stock and subsequent drying of the graft.

Compared to *P. arboreum*, success and survival of *P. auduncum* grafts were better. *P. auduncum* exhibited 36.66 per cent initial success in grafting but it was gradually reduced to 16.66 per cent within one year period of grafting. During the initial periods, shrinking and drying of scion were generally observed in these grafts but later rotting of stock was predominant. Among the South American *Piper* species, comparatively high per cent success and survival was observed in *P. colubrinum* grafts. Success in grafting using *P. colubrinum* as rootstock was recorded to be 63.33 per cent and it got gradually reduced to 30 per cent within one year of grafting. During the initial months of grafting, lack of sprouting was observed to be the cause of graft failure in grafts on *P. colubrinum*. However, rotting of stock was frequently observed after a period.

The least failure in grafting was shown by self grafts. Out of the twenty eight sprouted grafts, twenty could survive giving a per cent survival of 66.66 at 12 MAG. General observations on graft failure such as shrinking and drying of scion, lack of sprouting, rotting at the graft union were recorded in these grafts also.

4.1.4.2.3 *Effect of Rootstock on Growth of Scion (Season – March)*

Data pertaining to the growth characteristics of scion grafted on different rootstocks during March are furnished in table 23. Since none of the grafts on *P. arboreum* could survive up to six MAG, observations on such grafts were excluded.

As can be seen from the table, height of grafted plants varied from 33.91 cm to 45.36 cm. Self grafts recorded highest plant height (45.36 cm) while plant height of grafts on both *P. colubrinum* as well as *P. auduncum* were statistically on par. Similarly number of nodes also was recorded to be highest in self grafts (7.69) and there was no significant difference in the number of nodes of grafts on *P. colubrinum* and *P. auduncum*. However, a different trend was observed in case of internodal length. Grafts on *P. auduncum* recorded highest internodal length (7.33 cm) while that on *P. colubrinum* and *P. nigrum* were at par. Branching was not significantly different among the grafts on different rootstocks, however the highest number of branches recorded was two, in self grafts. Spiking was completely absent in all the grafts during a period of six months after grafting.

Table 22. Effect of rootstocks on survival of graft (Season – March)

Rootstock	Number of grafts prepared	Success		Survival					
		45 DAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	19	63.33	15	50.00	10	33.33	9	30.00
P. auduncum	30	11	36.66	8	26.66	6	20.00	5	16.66
P. arboreum	30	8	26.66	2	6.66	0	-	0	-
P. nigrum	30	28	93.33	25	83.33	21	70.00	20	66.66

Table 23. Effect of rootstocks on growth of scion (Season – March)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)	No. of branches*	No. of spikes
P. colubrinum	33.91[b]	4.66[b]	5.92[b]	1.91	0
P. auduncum	37.20[b]	5.08[b]	7.33[a]	1.91	0
P. nigrum	45.36[a]	7.69[a]	6.26[b]	2.00	0
CD (0.05)	7.50	2.14	0.79	NS	-

4.1.4.3 *Evaluation of Grafting done in June*

4.1.4.3.1 *Success of grafting*

Number of grafts prepared during June, percentage success and total number of graft failure are given in table 24. Success of grafting when carried out during June was in the range of 6.66 to 80 per cent. *P. nigrum* was recorded with highest per cent success (80 %). Among the other *Piper* species, grafts on *P. colubrinum* showed 66.66 per cent success while grafts on *P. auduncum* were recorded with 26.66 per cent success only. Very low success percentage was observed when *P. arboreum* was used as the rootstock (6.66 %).

Out of the twenty eight unsuccessful grafts of *P. arboreum*, twenty remained green for more than two months without sprouting and thereafter dried out. Rests of the grafts were observed with wilted scion. Similarly in *P. auduncum* grafts, lacking of sprouting was the predominantly observed symptom of graft failure. When eighteen grafts were recorded with such symptom, wilting of scion and rotting at the graft union was observed in other four grafts. On the other hand, shrinking and drying of scion was predominently observed in the unsuccessful grafts of *P. colubrinum* as well as *P. nigrum*.

Table 24. Effect of rootstocks on success of grafting done during June

Rootstock	Number of grafts prepared	Success of grafting		Total no. of graft failure
		Number	Percentage	
P. colubrinum	30	20	66.66	10
P. auduncum	30	8	26.66	22
P. arboreum	30	2	6.66	28
P. nigrum	30	24	80.00	6

4.1.4.3.2 *Graft Survival*

Data pertaining to the graft survival are furnished in table 25. It can be seen from the table that, within one year period of grafting, gradual reduction in the percentage of survived grafts was observed in case of self grafts only. In *P. colubrinum* as well as *P. auduncum*, reduction in the percentage survival was noticed only up to six MAG and thereafter no incidence of graft failure was observed.

The sprouted grafts of *P. arboreum* could not survive even up to three MAG as they were dried at the single leaf stage itself. Symptoms of graft failure observed in *P. colubrinum* as well as *P. auduncum* grafts were wilting and drying of scion at three to five leaf stages. Out of the twenty sprouted grafts of *P. colubrinum*, five grafts at their three to four leaf stages showed such symptom of graft failure. Five months after grafting, two more grafts showed yellowing and wilting of scion at four to six leaf stages. But, rotting of stock was noticed as the main reason of graft failure in self grafts. Out of the twenty four sprouted grafts of *P. nigrum*, seven failed to survive up to one year after grafting. Among these, rotten stocks were noticed in six grafts and the other one got dried because of breakage at the graft union.

4.1.4.3.3 *Effect of Rootstocks on Growth of Scion*

Data regarding the growth characteristics of scion grafted on different rootstocks during June are analyzed in table 26. It may be seen from the table that, height of grafted plants were in the range of 31.36 cm to 41.15 cm. Highest plant height was recorded by self grafts (41.15 cm) while plant height of grafts on *P. colubrinum* and *P. auduncum* were statistically on par. Similarly number of nodes also was observed to be highest in self grafts (7.33) and there was no significant difference in the number of nodes of *P colubrinum* as well as *P. auduncum* grafts.

Internodal length was also found to be highest in self grafts (6.27 cm). Internodal length of *P. colubrinum* as well as *P. auduncum* grafts were statistically on par. On the other hand, number of branches followed a different trend. There was no significant difference observed in the number of branches of *P. colubrinum* as well as *P. nigrum*. But lowest number of branches was recorded in *P. auduncum* (0.40). Spiking was very rarely observed in *P. colubrinum* and *P. auduncum* grafts but not in self grafts.

Table 25. Effect of rootstocks on survival of graft (Season – June)

Rootstock	Number of grafts prepared	Success		Survival					
		45 DAG		3 MAG		6 MAG		12 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	20	66.66	15	50.00	13	43.33	13	43.33
P. auduncum	30	8	26.66	5	16.66	4	13.33	4	13.33
P. arboreum	30	2	6.66	0	-	0	-	0	-
P. nigrum	30	24	80.00	20	66.66	18	60.00	17	56.66

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Table 26. Effect of rootstocks on growth of scion (Season – June)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)	No. of branches	No. of spikes
P. colubrinum	31.36^{b}	5.40^{b}	4.78^{b}	2.06^{a}	0.18
P. auduncum	34.00^{b}	5.80^{b}	5.44^{b}	0.40^{b}	0.20
P. nigrum	41.15^{a}	7.33^{a}	6.27^{a}	1.53^{a}	0.00
CD (0.05)	5.18	1.46	0.81	0.56	NS

4.1.4.4 *Evaluation of Grafting done in September*

4.1.4.4.1 *Success of Grafting*

Data regarding the number of grafts prepared during September, number succeeded, per cent success and graft failure are given in table 27. Comparatively low rate of sprouting was observed in September. The highest per cent success recorded was 53.33 only (self grafts). Among the South American *Piper* species, highest success in grafting was obtained when *P. colubrinum* was used as rootstock (40%) followed by *P. auduncum* (23.33 %). However, grafting was not a success with *P. arboreum* rootstock.

Observations on graft failure indicated that, about half of the unsuccessful grafts of *P. arboreum* remained as such without sprouting for about three months and thereafter dried out. Shrinking and subsequent drying was observed in rest of the grafts. In case of grafts with *P. auduncum* as rootstock, where there was less graft failure compared to *P. arboreum*, prominently observed symptom of failure was shrinking and drying up of scion within first month of grafting itself. In both *P. colubrinum* as well as *P. nigrum* grafts, unsuccessful grafts showed complete drying of scion alone, immediately after shedding of older leaves.

Table 27. Effect of rootstocks on success of grafting done during September

Rootstock	Number of grafts prepared	Success of grafting		Total no. of graft failure
		Number	Percentage	
P. colubrinum	30	15	40.00	15
P. auduncum	30	7	23.33	23
P. arboreum	30	0	0.00	30
P. nigrum	30	18	53.33	12

4.1.4.4.2 *Graft Survival*

Observations on per cent survival of grafts indicated that (Table 28), ten to thirteen per cent reduction in the survived grafts were recorded within a period of one year after grafting. Thirteen per cent reduction was noticed in *P. auduncum* as well as self grafts while it was only ten per cent in *P. colubrinum* grafts. Even after sprouting (45 DAG) five grafts of *P. colubrinum* were observed with wilted and dried scion. No further reduction was observed in survived grafts within a period of six MAG to ten MAG.

Gradual reduction in the percentage survival was observed in *P. auduncum* as well as self grafts. Out of the seven sprouted grafts of *P. auduncum* only three could survive up to ten months after grafting. Rests of the grafts periodically showed symptoms of failure. Wilting succeeded by drying of scion during the initial months and rotting of stock at the later stages were observed in the unsuccessful grafts. Apart from the general observations, rotting of stock was also observed as a hindrance for survival in self grafts.

4.1.4.4.3 *Effect of Rootstocks on Growth of Scion*

Different growth parameters of grafted plants were recorded in order to determine the influence of rootstocks on growth of scion. As none of the grafts of *P.*

arboreum could survive up to 180 DAG, observations on growth of scion in such grafts could not be recorded.

During September, grafts on different rootstocks did not differ significantly as far as growth of scion was concerned (Table 29). Spiking was very rarely observed in grafts on *P. colubrinum*.

4.1.5 Overall Effect of Rootstocks on Graft Success and Survival

4.1.5.1 *Plageotropic Shoot of P. nigrum as scion*

The total number (and percentage) of successful as well as survived grafts obtained from each rootstock during the entire grafting period (December 2017 to September 2018) is analyzed in table 30.

Comparing the overall performance of rootstocks, highest success in grafting of plageotropic shoot of *P. nigrum* was observed with its own rootstock (74.16 %). Among the South American *Piper* species used as rootstocks, *P. colubrinum* was recorded with highest per cent success (60%). Comparatively lower percentage of success was recorded with other *Piper* species *viz., P. auduncum* and *P. arboreum*. Grafting with the rootstock *P. arboreum* was least successful as it recorded the lowest per cent success (14.16 %).

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Table 28. Effect of rootstocks on survival of graft (Season – September)

Rootstock	Number of grafts prepared	Success		Survival					
		45 DAG		3 MAG		6 MAG		10 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	30	15	40.00	10	33.33	9	30.00	9	30.00
P. auduncum	30	7	23.33	6	20.00	5	16.66	3	10.00
P. nigrum	30	18	53.33	15	50.00	13	43.33	12	40.00

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Table 29. Effect of rootstocks on growth of scion (Season – September)

Rootstocks	Plant height (cm)	No. of nodes	Internodal length (cm)	No. of branches	No. of spikes
P. colubrinum	33.62	3.91	7.05	1.25	0.16
P. auduncum	37.25	4.06	7.55	1.31	0
P. nigrum	37.43	4.75	8.06	1.62	0
CD (0.05)	NS	NS	NS	NS	-

A gradual reduction in the percentage survival of grafts was observed for all the rootstocks. When it was 65 and 57.5 per cent at three and six MAG respectively for self grafts, the survival percentage of *P. colubrinum* grafts was reduced from 45.83 to 37.5 per cent from three to six months after grafting. In case of *P. auduncum* grafts, per cent survival at three and six MAG was 22.5 and 18.33 respectively. None of the grafts of *P. arboreum* could survive up to three months after grafting.

4.1.6 Effect of Month of Grafting on Graft Success and Growth of Scion

4.1.6.1 *Plageotropic Shoot of P. nigrum as Scion*

4.1.6.1.1 *Effect of Month of Grafting on Graft Success*

Per cent success of grafting (on each rootstock) when carried out during different months are given in table 31. Success of grafting using *P. colubrinum* as rootstock was ranging from 40 per cent to 66.66 per cent. Highest success was obtained when grafting was carried out during June (66.66 %) which was closely followed by March (63.33 %) and December (60%). But comparatively lower percentage success was recorded during September (40%).

As far as grafting success was concerned, among the different South American *Piper* species used as rootstocks, *P. colubrinum* was succeeded by *P.*

auduncum. Per cent success of grafting when *P. auduncum* was used as rootstock was in the range of 23.33 to 36.66. Highest per cent success was recorded when the grafting was carried out during March (36.66%) followed by December (33.33%). Compared to these two months, *P. auduncum* grafts obtained during June and September were recorded with lower per cent success (26.66 % and 23.33 % respectively).

Minimum graft success during all the months was observed with *P. arboreum* rootstock. It could obtain a maximum of 26.66 per cent (March) success only. Slightly lower per cent success was obtained during December also (23.33%). But much lower success was observed when grafting was performed during June and September. When 6.66 was the per cent success obtained during June, complete graft failure was observed when grafted during September.

Self grafts recorded highest per cent success during all the months. Wide variation in per cent success was observed in these grafts (53.33% to 93.33%). Self grafts obtained highest success when grafted during March (93.33) followed by June (80%). Comparatively lower per cent success was obtained when grafting was performed during December (63.33%) and September (53.33%).

Table 30. Overall response of rootstocks to grafting (December 2017 to September 2018)

Rootstock	Total number of grafts prepared	Success		Survival			
				3 MAG		6 MAG	
		Number	Percentage	Number	Percentage	Number	Percentage
P. colubrinum	120	72	60.00	55	45.83	45	37.50
P. auduncum	120	36	30.00	27	22.50	22	18.33
P. arboreum	120	17	14.16	2	1.66	0	-
P. nigrum	120	89	74.16	78	65.00	69	57.50

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Table 31. Effect of month of grafting on graft success

Month of grafting	Percent success of grafting			
	P. colubrinum	*P. auduncum*	*P. arboreum*	*P. nigrum*
December 2017	60.00	33.33	23.33	63.33
March 2018	63.33	36.66	26.66	93.33
June 2018	66.66	26.66	6.66	80.00
September 2018	40.00	23.33	0.00	53.33

4.1.6b Effect of month of grafting on growth of scion

Rootstock – *P. colubrinum*

Growth characteristics *viz.*, plant height, number of nodes and internodal length of *P. colubrinum* grafts (six months old) obtained during different months are analyzed in table 32. Month of grafting significantly influenced number of nodes, internodal length, and number of branches. But, in case of plant height and number of spikes, significant difference was not observed. Plant height of *P. colubrinum* grafts varied from 31.36 cm to 36.85 cm.

Number of nodes in six month old grafts of *P. colubrinum* was ranging from, 3.91 to 7.57. Highest number of nodes was recorded in the grafts of December (7.57). Number of nodes in the grafts obtained during March and June were statistically at par. The grafts of September were recorded with lowest number of nodes (3.91), however highest internodal length was observed in these grafts (7.05 cm). Internodal length in the grafts obtained during March, December and June were statistically on par. Branching was observed in grafts on *P. colubrinum*. Maximum number of branches recorded was 2.06 (June) and the minimum was 1.14 only (December). Spiking was rarely observed in these grafts (March, June and September).

Rootstock – *P. auduncum*

In case of *P. auduncum* grafts, month of grafting influenced the growth characteristics *viz.,* plant height, internodal length and number of branches. (Table 33). Plant height was ranging from 31.26 cm to 37.25 cm. Maximum plant height was recorded in the grafts of September which was statistically on par with that of March and June. Height of grafted plants of June (34) was on par with that of December (31.26) also. Number of nodes in grafts was not influenced by the month of grafting

Internodal length of grafts obtained during September, March and December were statistically at par. The lowest internodal length was recorded to be 5.44 cm (June). Maximum number of branches recorded in these grafts was 1.91 only (March). Number of branches in the grafts of December and September was statistically on par. While the lowest number of branches was recorded by the grafts of June (0.40). Spiking was observed only in the grafts of June (0.20).

Table 32. Effect of month of grafting on growth of scion

(Rootstock – *P. colubrinum*)

Month of grafting	Plant height	No. of nodes	Internodal length	No. of branches	No. of spikes
December 2017	36.85	7.57[a]	5.53[b]	1.14[c]	0.00
March 2018	33.91	4.66[bc]	5.92[ab]	1.91[ab]	0.25
June 2018	31.36	5.40[b]	4.78[b]	2.06[a]	0.18
September2018	33.62	3.91[c]	7.05[a]	1.25[bc]	0.16
CD (0.05)	NS	1.36	1.23	0.70	NS

Table 33. Effect of month of grafting on growth of scion

(Rootstock – *P. auduncum*)

Season of grafting	Plant height	No. of nodes	Internodal length	No. of branches	No. of spikes
December 2017	31.26^{b}	4.60	7.11^{a}	1.10^{b}	0.00
March 2018	37.20^{a}	5.08	7.33^{a}	1.91^{a}	0.00
June 2018	34.00^{ab}	5.80	5.44^{b}	0.40^{c}	0.20
September2018	37.25^{a}	4.06	7.55^{a}	1.31^{b}	0.00
CD (0.05)	5.33	NS	1.36	0.59	-

Rootstock – *P. nigrum*

Influence of month of grafting on growth of scion in self grafts is analyzed in table 34. As can be seen from the table, except internodal length, no other growth parameters of *P. nigrum* grafts were significantly influenced by the month of grafting. Grafts obtained during September secured highest internodal length (8.06 cm) whereas internodal length in grafts of other three months (December, March and June) were statistically on par. Even though there was no effect of grafting month on plant height, maximum plant height recorded was 45.36 cm (March) and the minimum was 36.64 cm (December). Similarly, number of nodes and number of branches in the self grafts were in the range of 4.75 to 7.69 and 1.15 to 2.00 respectively. Spiking was observed in the grafts of December (0.10) only.

Table 34. Effect of month of grafting on growth of scion (Rootstock – *P. nigrum*)

Season of grafting	Plant height	No. of nodes	Internodal length	No. of branches	No. of spikes
December 2017	36.34	6.00	6.03[b]	1.15	0.10
March 2018	45.36	7.69	6.26[b]	2.00	0.00
June 2018	41.15	7.33	6.27[b]	1.53	0.00
September2018	37.43	4.75	8.06[a]	1.62	0.00
CD (0.05)	NS	NS	1.14	NS	-

4.1.7 Incidence of Pests and Diseases

During the period of study, mealy bug infestation and grass hopper attack was observed in the grafted plants. However, this did not affect the survival of grafts. While, rotting of stock severely observed in the grafts involving *P. nigrum*, *P. auduncum* and *P. arboreum* as rootstocks, acted as a hindrance for graft survival. Stunting disease was observed in two grafts of *P. nigrum* on *P. colubrinum*.

Plate 6. Grafted plants at different stages of development

Plate 7. Unsuccessful grafts

4.2 ANATOMICAL STUDIES ON *PIPER* SPECIES

4.2.1 Stem Anatomy of *Piper nigrum*

Stem anatomy of *Piper nigrum* is described in plate 8. Transverse section of stem of *P. nigrum* showed the following features.

The epidermis consisted of a single layer of rectangular cells which were closely arranged. A well defined cuticle extended outer to the epidermis. Below the epidermis there were 2 – 3 layers of collenchymatous hypodermis. Sclereids were distributed in between the collenchymatous cells. Inner to the hypodermis there was a discontinuous band of sclerenchyma below which present 4 - 6 rows of parenchymatous cells. Two rings of vascular bundles were seen in *P. nigrum*, they were, cortical or peripheral and central or medullary bundles (Plate 8A).

There were 20 - 30 cortical bundles in a young stem but, as the plant get mature, number of cortical bundles increased to 25- 40. Small and large bundles arranged alternatively. Between two large bundles 2 -3 small bundles were observed. Cortical vascular bundles were collateral and open. A small sclerenchymatous cap was visible at the phloem end. Phloem was succeeded by cambium and xylem. Among the three xylem elements *viz*., vessels, tracheids and fibres were prominently observed in the cortical bundles. Below the xylem observed a wavy band of sclerenchyma (4 – 6 rows) (Plate 8C).

Pith lied inside the sclerenchymatous band. Closely arranged parenchyma cells were observed in this region. The pith region consisted of another ring of vascular bundles called medullary or central bundles which was larger than cortical bundles. These bundles were also collateral and open. There were 6 – 10 such bundles. Each bundle was observed with a sclerechymatous cap on either end (Plate 8E). Xylem vessels were more prominently observed in these bundles. A mucilage canal was present at the centre of the pith which was lined by secretary cells (Plate 8B).

Cambial connection between the cortical bundles was initiated in the observed stem section. Interfascicular cambium consisted of 3 - 4 rows of narrow elongated cells and towards the inner side which produced lignified parenchyma cells (Plate 8C). However cambial connection was absent between the medullary bundles. Periderm was also visible in this stem section (Plate 8D).

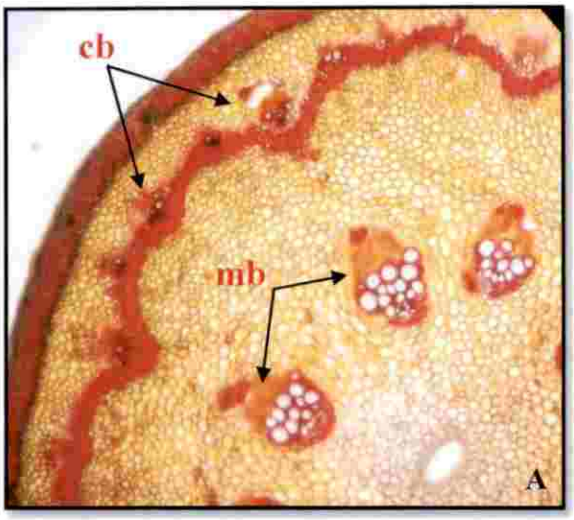
4.2.2 Stem Anatomy of *Piper colubrinum*

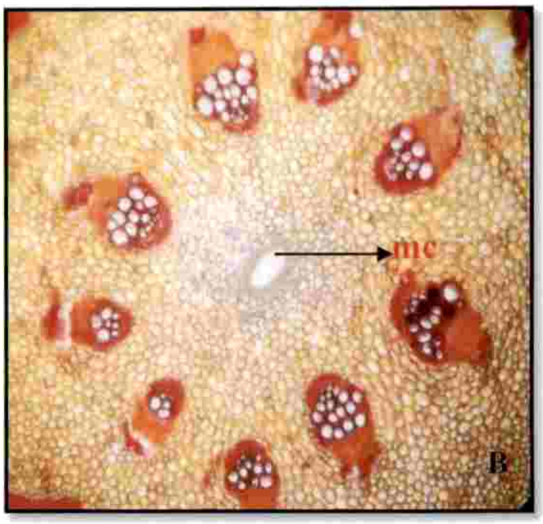
Stem anatomy of *P. colubrinum* is described in plate 9.

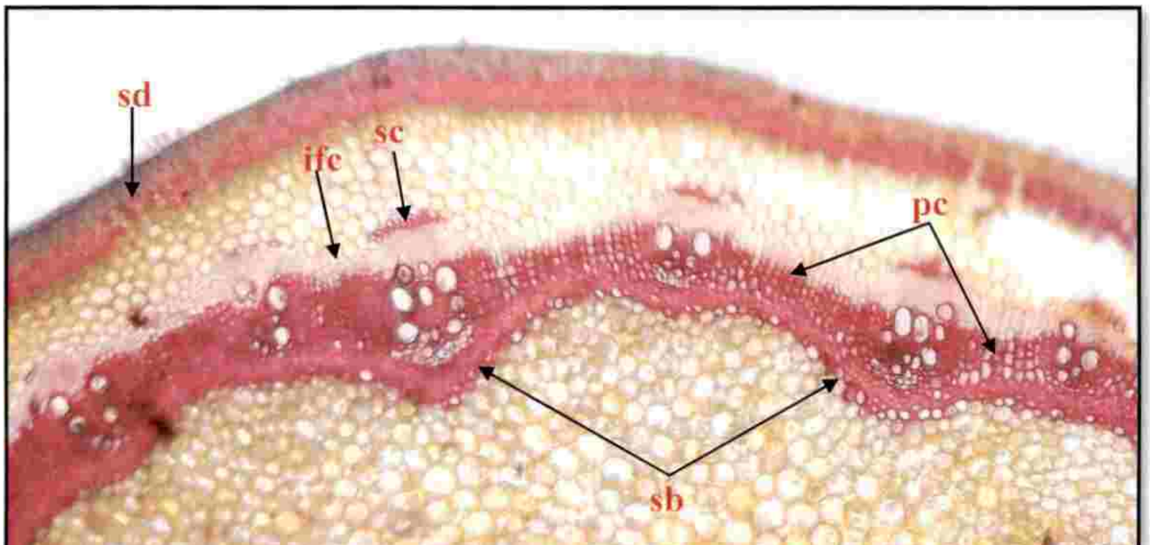
Basic stem anatomy of *P. colubrinum* and *P. nigrum* were similar. In the transverse section, *P. colubrinum* stem showed the following features. Epidermis was constituted by a single layer of rectangular cells covered externally by a cuticle. Inner to the epidermis collenchymatous hypodermis was visible which was constituted by 4 – 5 rows of cells. Small patches of collenchyma were observed in hypodermis separated by the extension of inner cortex (Plate 9A). Aerenchymatous cells were abundantly observed in the pith as well as cortex region (Plate 9E).

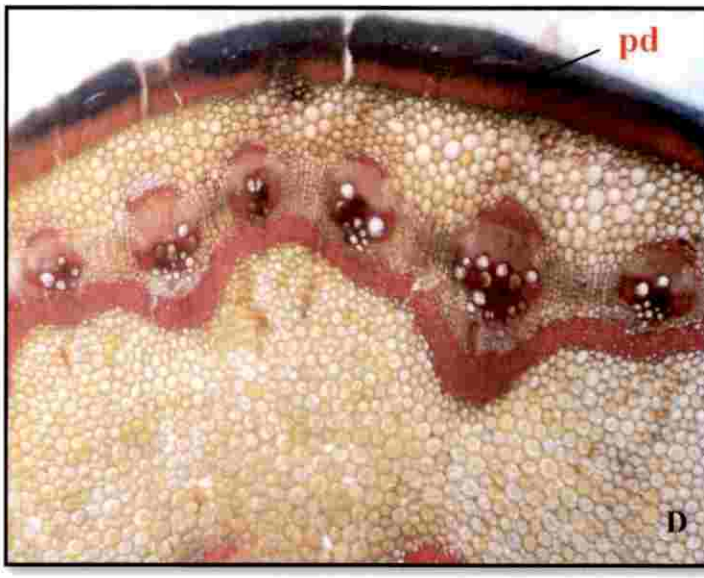
Vascular tissue was represented by polycyclic rings of cortical and medullary bundles (Plate 9B, 9C). There were 38 – 52 peripheral bundles and 7 – 12 medullary bundles in a 4- 6 mm thick stem. Instead of mucilage canal 1 - 3 medullary vascular bundles were observed at the centre of the pith. Very small mucilage canals were observed to be scattered over the stelar region (Plate 9C). All the vascular bundles were collateral and open. A clear cambial connection between the peripheral bundles was observed during secondary thickening (Plate 9F). But, it was restricted to the peripheral bundles only. Interfascicular cambium produced lignified parenchyma cells towards the inner side (Plate 9F). A distinct sclerenchymatous cap was visible above the peripheral bundles. Moreover a wavy band of sclerenchyma

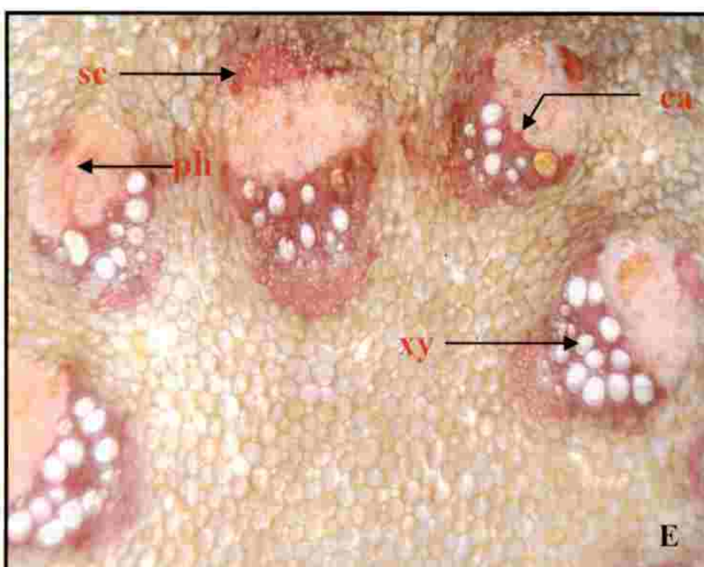
Plate 8: Transverse section of stem of *Piper nigrum*





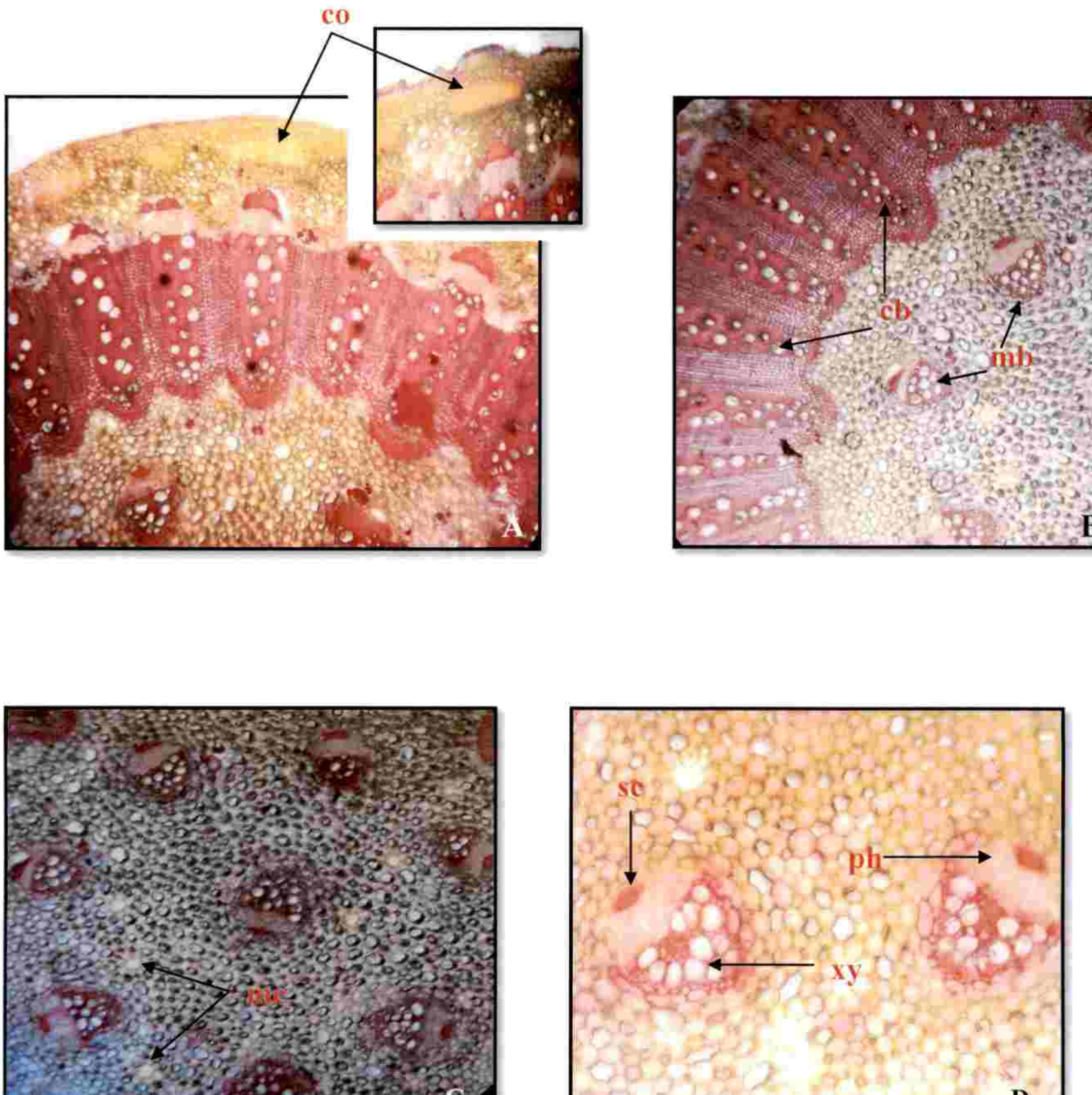


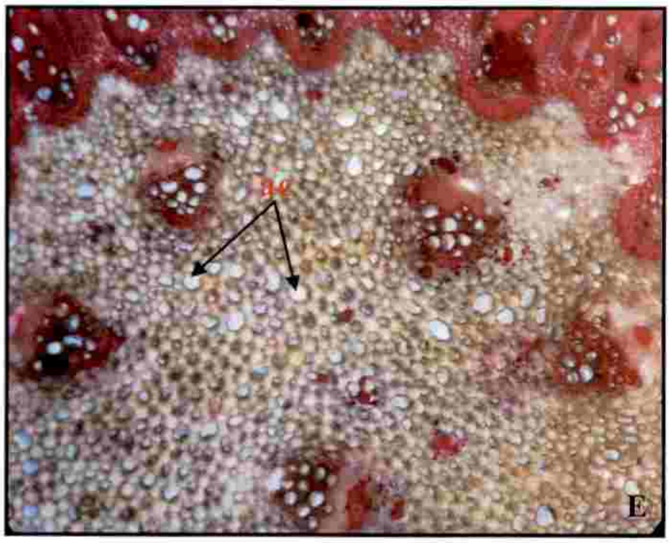


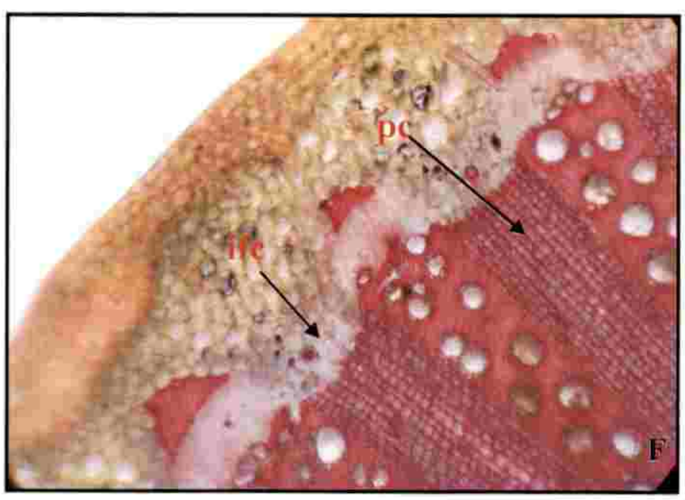


A. Polycyclic arrangement of vascular bundles (cb- cortical bundle; mb – medullary bundle) **B.** Stelar region showing medullary vascular bundles and central mucilage canal (mc – mucilage canal) **C.** Discontinuous band of sclerenchyma along with sclereides (sd) (ifc – interfascicular cambium; pc – lignified parenchyma; sb- sclerechymatous band; sc- sclerenchymatous cap) **D.** Stem showing secondary thickening (pd - Periderm) **E.** Medullary vascular bundles during secondary thickening (xy – xylem; ca - Intrafascicular cambium; ph-phloem; sc-sclerenchymatous cap). Scale bars: A, B, D = 500 μm; C, E = 200 μm.

Plate 9: Transverse section of stem of *Piper colubrinum*







A. Patches of collenchyma (co) in the hypodermis **B.** Polycyclic arrangement of vascular bundles (cb- cortical bundles; mb- medullary bundles **C.** Single vascular bundle at the centre (mc- mucilage canal) **D.** Enlarged view of medullary vascular bundles (xy- xylem; ph- phloem; sc- sclerenchymatous cap) **E.** Stelar region with abundance of aerenchyma (ac) **F.** Stem showing secondary thickening (ifc- interfascicular cambium; pc- lignified parenchyma) Scale bars: A, B, C, E = 500 μm; D, F = 200 μm.

was present just below the peripheral bundles. Many of the xylem vessels in the peripheral bundles were intermingled with these sclerenchyma cells.

A distinct pith region was absent. Medullary vascular bundles were scattered over the stelar region (Plate 9C). In the medullary bundles, sclerenchymatous cap was observed only at the phloem end (Plate 9D). Normal secondary thickening was absent in these bundles.

4.2.3 Stem Anatomy of *Piper auduncum*

Stem anatomy of *Piper auduncum* is described in plate 10.

Transverse section of the stem of *Piper auduncum* showed an epidermis with a single layer of cells. Epidermis was covered externally by a cuticle. Hypodermis was collenchymatous which possessed 4 – 6 rows of cells. Small patches of sclerenchyma were observed below the hypodermis (Plate 10A). Cortical and medullary bundles represented the vascular tissues (Plate 10B). They were collateral and open. Number of cortical vascular bundles was ranged from 46 – 53 in a young stem. Large and small bundles were arranged often alternately. Cortical and medullary bundles were closely placed but separated by a wavy band of sclerenchyma.

Secondary thickening was restricted to the cortical bundles only. Interfascicular cambium produced lignified parenchyma cells towards the innerside (Plate 10E). In a mature stem, cortical bundles existed as discrete strands separated by lignified parenchyma cells. Attached to the xylem vessels, tracheids were observed. Sclerides or xylem fibres were clearly visible in the cortical bundles (Plate 10F).

There were 12 – 15 medullary bundles at the stelar region (Plate 10C). Xylem vessels were abundantly observed in these bundles. Pith was large sized and constituted by closely arranged paranchymatous cells. Starch grains were abundantly observed in the cells of cortex as well as pith region. Oil glands were prominently observed in the stem sections of *P. auduncum* which were scattered over the stelar region. The pith was devoid of mucilage canal (Plate 10D).

4.2.4 Stem Anatomy of *Piper arboreum*

Stem anatomy of *Piper arboreum* is described in plate 11. Transverse section of the stem of *P. arboreum* showed the following characters.

Epidermis was constituted by a single layer of cells. Externally the epidermis was covered by a cuticle. Hypodermis was collenchymatous having 2 – 3 rows of cells. Very small patches of sclerenchyma cells (1- 2 rows) was observed below the hypodermis. Vascular tissues were represented by cortical or peripheral and medullary or central bundles which were collateral and open. Number of cortical bundles varied from 38 to 47 in a young stem. But as the stem got matured, number of bundles increased to 68 – 75. A sclerechymatous cap was clearly observed at the phloem end of the cortical bundles. Instead of forming into a wavy band, sclerenchyma cells were confined to a cap like structure at the xylem end (Plate 11A).

During secondary thickening, the cortical bundles were discretely separated by lignified parenchyma cells (Plate 11B). Medullary vascular bundles were distantly arranged from the central pith region. In a young stem, number of medullary vascular bundles varied from 12 – 16 and as it got matured, number increased to 39- 48. Secondary thickening was absent in these vascular bundles. Xylem elements *viz.,* vessels, trachieds and fibres were clearly observed in the vascular bundles. Intrafascicular cambium was constituted by 4 – 6 rows of cells (Plate 11E).

Pith was large sized with very closely arranged parenchymatous cells. Mucilage canal was absent in the pith region. Raphides (needle shaped crystal of calcium oxalate) were abundantly observed in the pith as well as cortex region (Plate 11D).

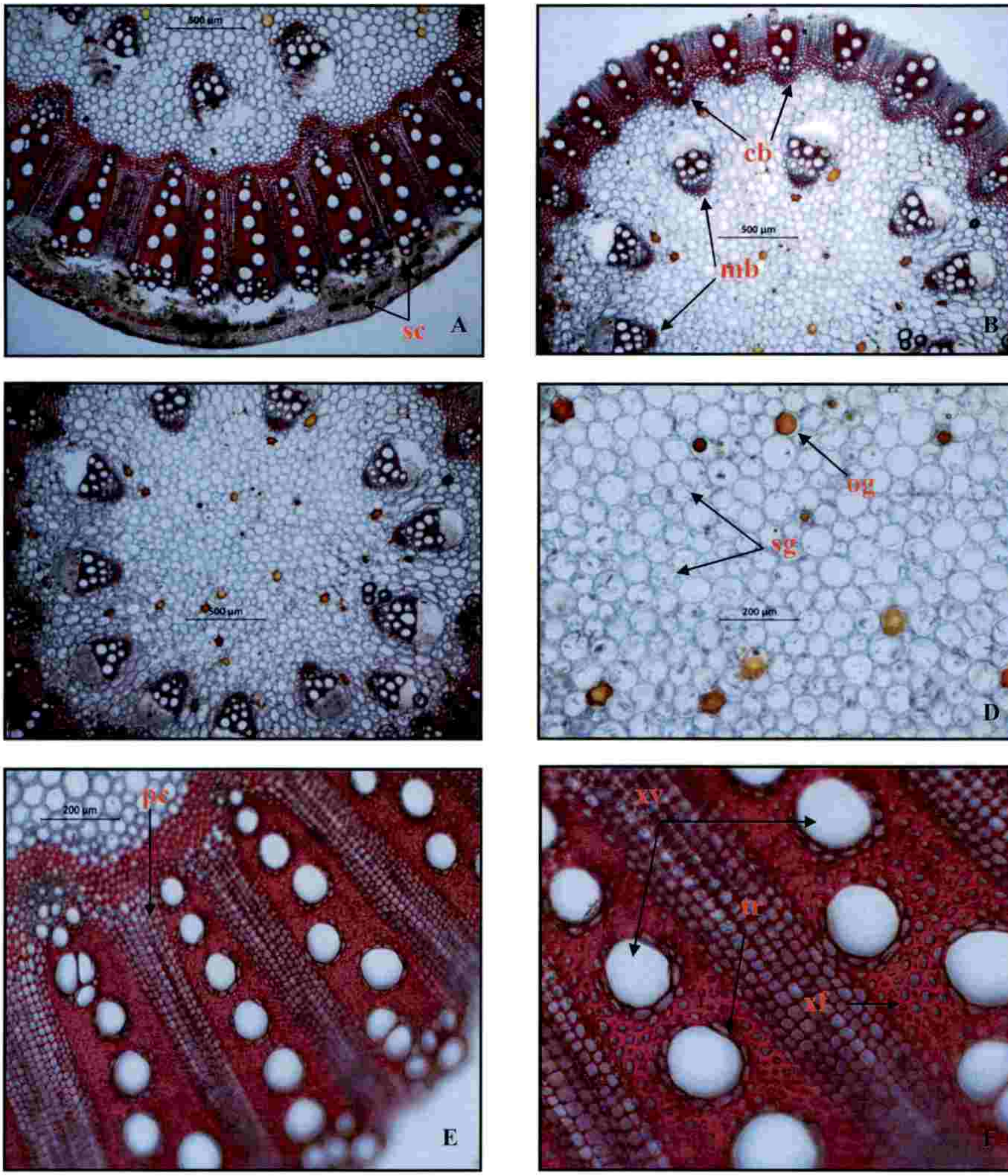


Plate 10: Transverse section of stem of *Piper auduncum*

A, B. Polycyclic arrangement of vascular bundles (sc- discontinuous sclerenchymatous patches; cb- cortical bundles; mb- medullary bundles) **C.** Parenchymatous pith **D.** Enlarged view of pith region containing oil glands (og) and starch grains (sg) **E.** Cortical bundles showing secondary thickening (pc- lignified parenchyma) **F.** Enlarged view of cortical xylem (xv- xylem; vessel tr- tracheids; xf- xylem fibres)

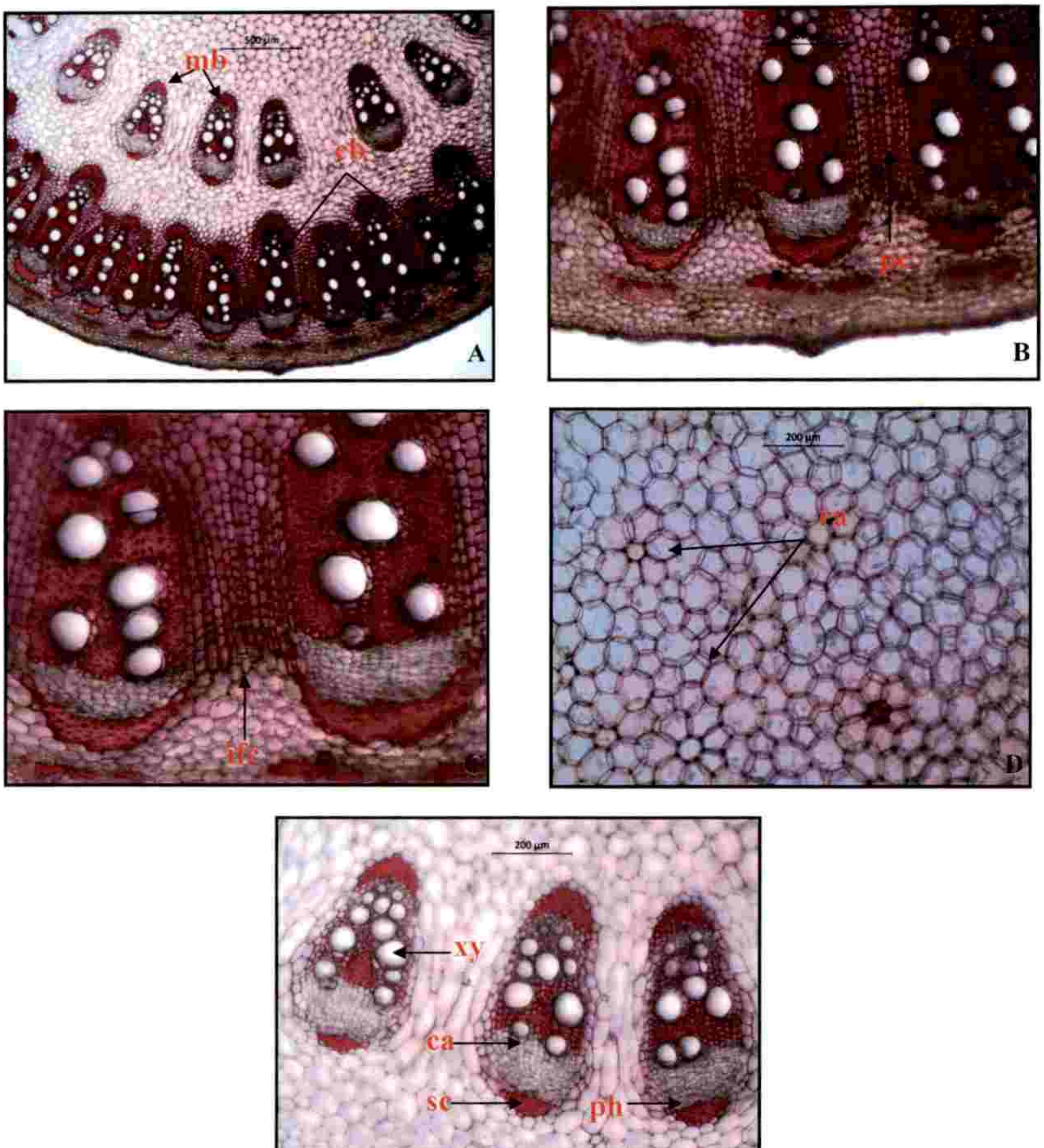


Plate 11: Transverse section of stem of *Piper arboreum*

A. Polycyclic arrangement of vascular bundles (cb- cortical bundles; mb- medullary bundles) B. Cortical bundles during secondary thickening (pc- lignified parenchyma) C. Enlarged view of cortical bundles (ifc- interfascicualr cambium) D. Pith region containing raphides (ra) E. Enlarged view of medullary bundles (xy- xylem; ca- intra fascicular cambium; ph- phloem; sc- sclerechymatous cap)

4.3 ANATOMICAL STUDIES OF GRAFT UNION

Anatomical structure of the graft union was examined in grafts involving *P. nigrum, P. colubrinum, P. auduncum* and *P. arboreum* as rootstocks and orthotropic and plageotropic shoots of *P. nigrum* variety Panniyur 1 as scion. Observations were made at three, six and twelve months after grafting in order to study the development of graft union at different stages and reason (s) for failure if any.

Preparation of hand sections with ordinary blade was difficult so also proper sections were not obtained using a microtome. The sections presented were obtained through hand section using microtome blades.

4.3.1 Anatomy of Graft Union Involving Orthotropic Shoot of *P. nigrum* as Scion

4.3.1.1 *Anatomy of P. nigrum Grafts on its own Rootstock*

The plates 12 - 14 show transverse sections of the grafts using *P. nigrum* as the rootstock at three, six and twelve months after grafting.

As can be seen from plates 12A - E, union of stock and scion was almost completed within three months after grafting. However some discrete strands of necrotic layer were observed at the stelar region (Plate 12A and B). Callus was visible on either side of these necrotic layers. Transverse sections of three month old grafts (Plate 12C) showed undefferentiation of phloem elements in the medullary bundles. Production of callus from the cambium of aforementioned medullary bundles was clearly observed in these sections. As plate 12D shows, there was no union of cortical cells of stock and scion observed in a three month old graft.

Anatomical structure of graft union at six month stage is described in Plate 13. As can be seen from plate 13A, in a six month old graft, there was marked reduction of necrotic layer at the stelar region. Differentiation of vascular bundles at the graft union was clearly observed in one of the sections (Plate 13B).

Cortical cells of stock and scion were also found to be united in a six month old graft. But individual cells in this region were not clearly differentiated (Plate 13C).

Anatomical structure of one year old graft union is described in plate 14. As can be seen from Plate 14A, stelar region of a one year old graft union was completely devoid of necrotic layers. In one of the sections, union was evident in the cortical region also (Plate 14B). But in some others, cortical cells of stock and scion were still separated (Plate 14C). One of the transverse sections of one year old graft showed completely differentiated medullary vascular bundles at the union (Plate 14D).

4.3.1.2 *Anatomy of P. nigrum Grafts on P. colubrinum*

Anatomical structure of graft union involving *P. nigrum* as scion and *P. colubrinum* as rootstock at different stages of development is described in plates 15–17.

The sections taken after three months of grafting showed that, at the stelar region, union of stock and scion was almost completed (Plate 15A). However some dark strands of necrotic layer were observed (Plate 15B). Rootstock and scion were still separated by the isolation or necrotic layer at the cortical and hypodermal region (Plate 15C and D). Towards this region callus production from both stock and scion was more. But, in one of the sections, union of cortical cells of stock and scion was observed. In this section, formation of wound repair xylem from the callus was also evident (Plate 15E).

Transverse sections of six month old grafts showed some broken strands of necrotic layer at the graft union (Plate 16A and B). However, healing was almost completed throughout the cut ends of stock and scion including at the cortical region (Plate 16C). But, in this region individual cells were not completely differentiated and a clear cambial connection between the cortical bundles of stock and scion was not been observed (Plate 16D). Abundant accumulation of starch grains was noticed alongside of rootstock near the union (Plate 16E).

Anatomical structure of one year old graft union was also examined. A dense necrotic area at the union was observed in all the sections taken (Plate 17). This necrotic area acted as a barrier between stock and scion. Comparatively large necrotic layer was developed on the scion side (Plate 17A). Necrotic layer developed on the rootstock was less thickened. The above mentioned necrotic area was found to be enlarging towards the stelar region (Plate 17B). Separation of stock and scion at the cortical region was also evident in one of the sections (Plate 17C). In another section, necrosis of cells belonging to the stock portion was observed along with the necrosis at the graft union (Plate 17D).

4.3.1.3 *Anatomy of P. nigrum Grafts on P. auduncum*

Anatomical structure of graft union of *P. nigrum* on *P. auduncum* at three month and one year stages are described in plates 18 - 19. Good transverse sections of six month old graft union were not obtained.

Transverse sections of three month old grafts showed the following features. Union of stock and scion was almost completed by three months after grafting. Some discrete strands of necrotic layer were visible at the union (Plate 18A). Callus was observed on either side of this necrotic layer (Plate 18B). More thickened necrotic layer was evident at the cortical region (Plate 18C). In some of the sections, union between cortical cells of stock and scion was not proper (Plate 18D and E).

One year after grafting, sections of graft union was taken and observed. Even after the completion of union between stock and scion, a dense necrotic area was found to be developed in the scion side at the stelar region (Plate 19A and B). Necrosis of cortical cells of scion was also observed (Plate 19C). At the cortical region, a proper union of stock and scion was not evident (Plate 19C).

4.3.1.4 *Anatomy of P. nigrum Grafts on P. arboreum*

During grafting, highest failure was confronted by the grafts involving *P. arboreum* as rootstock. Most of the grafts on *P. arboreum* remained green without sprouting even after two months of grafting. Transverse sections of such grafts were obtained.

Anatomical structure of a two month old graft involving *P. arboreum* as rootstock is described in plate 20. As can be seen from the plate, a wide gap between stock and scion was observed in an unsuccessful graft on *P. arboreum*. Callus formation was not evident beneath the isolation layers.

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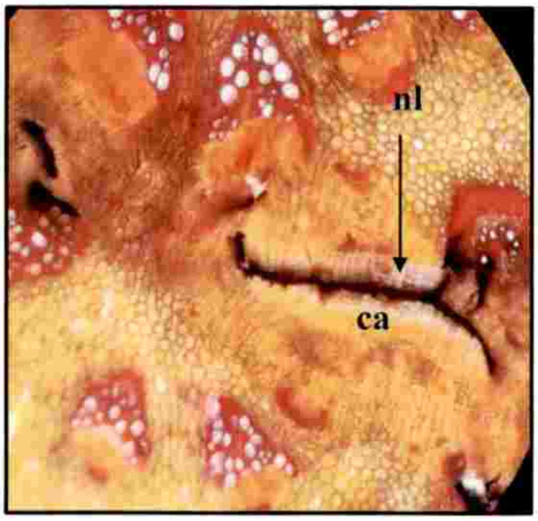
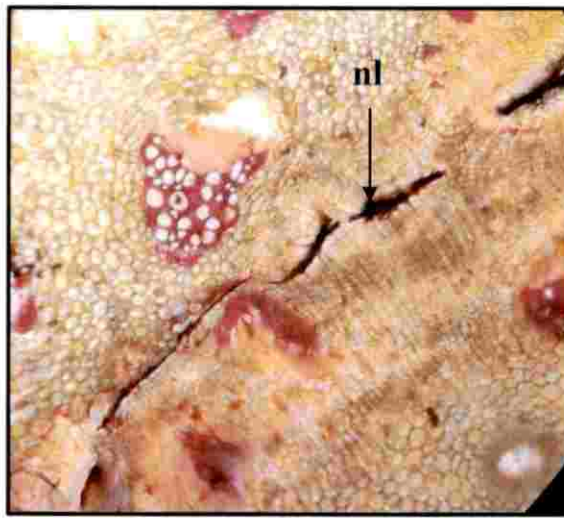
Plate 12: Three month old grafts of *P. nigrum* (orthotropic shoot) on its own rootstock

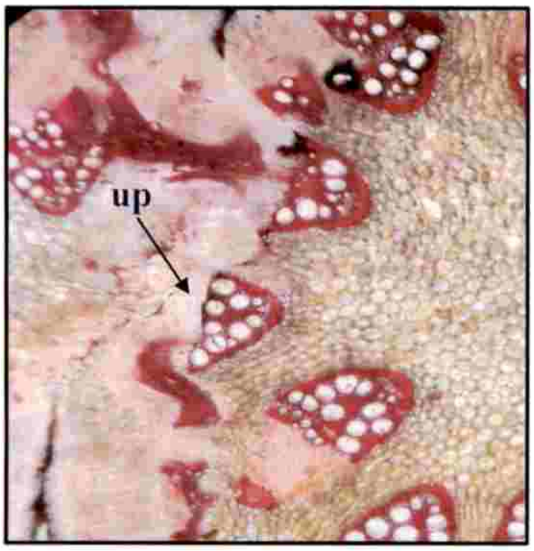


A



B

Plate 12A &B : Grafts showing discrete strands of necrotic layers at the union



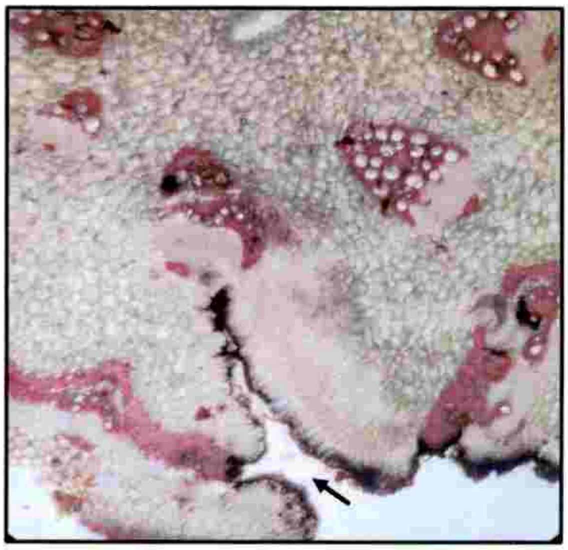


Plate 12C: Undifferentiated phloem elements (up)

Plate 12D: Incomplete union of grafting partners at the cortical region

Plate 13. Six month old grafts of *P. nigrum* (orthotropic shoot) on its own rootstock

Plate 13A: Complete union of stock and scion at the stelar region

Plate 13B: Transverse section of graft union showing newly differentiated vascular bundles (dvb)

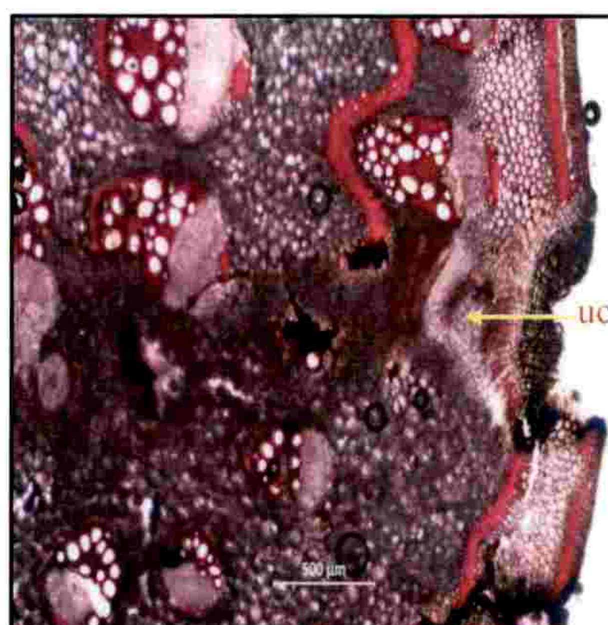


Plate 13C: Graft union showing undifferented cells (uc) at the cortical region

Plate 14. One year old grafts of *P. nigrum* (orthotropic shoot) on its own rootstock

Plate 14A: Completely united grafting partners at the stelar region

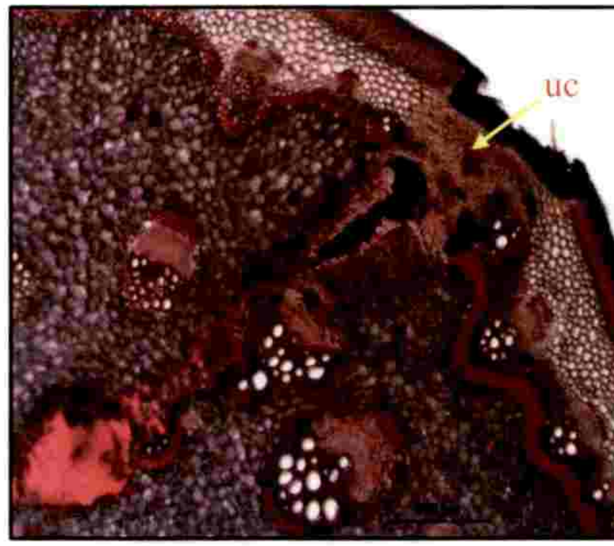


Plate 14B: Undifferentiated cells at the cortical region

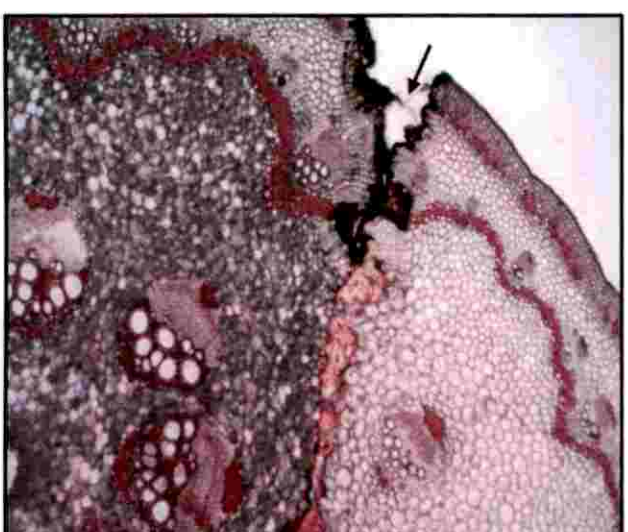


Plate 14C: Graft showing incomplete union at the cortical region

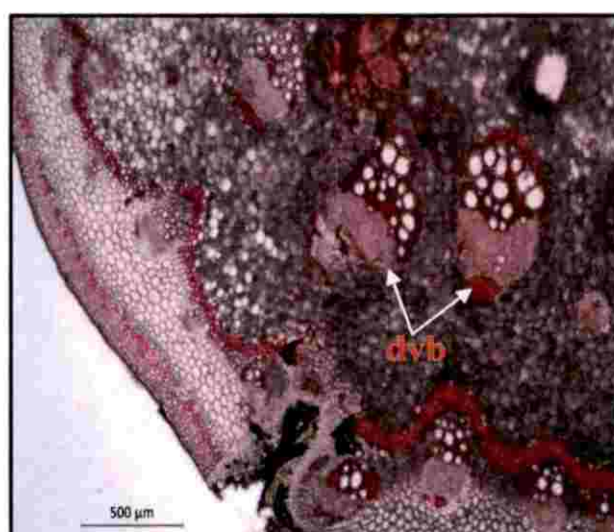
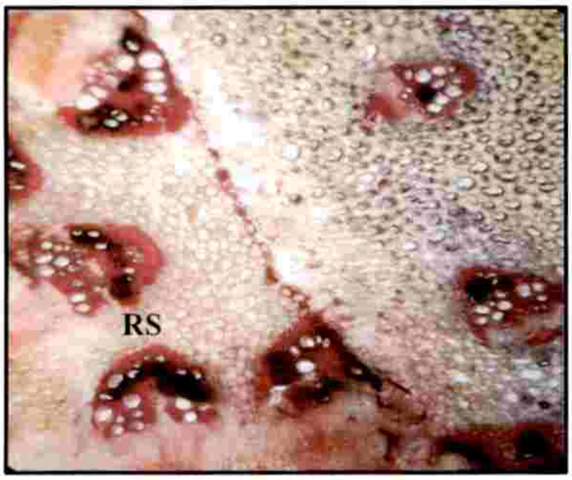


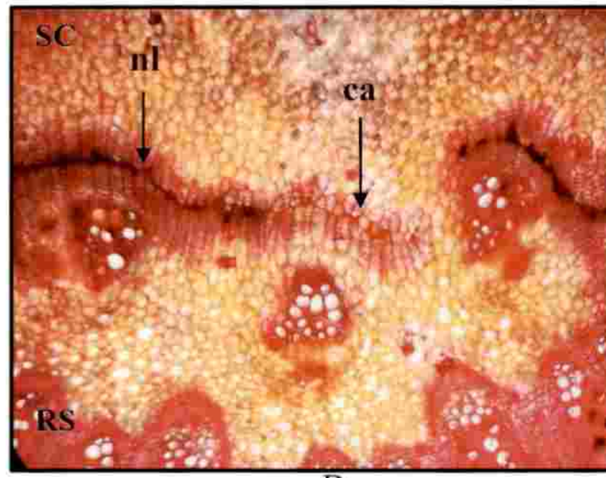
Plate 14D: Graft showing newly differentiated vascular bundles (dvb)

Plate 15. Three month old grafts of *P. nigrum* (orthotropic shoot) on *P. colubrinum*

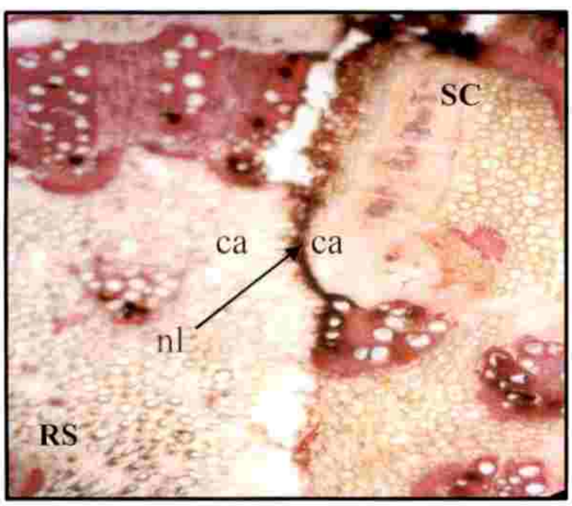
A



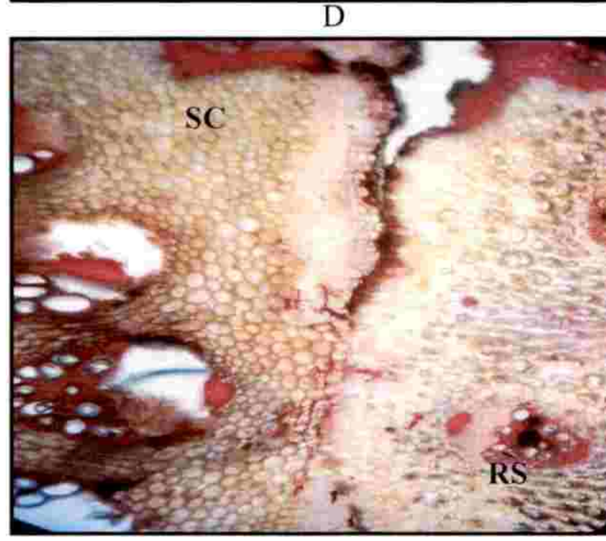
B



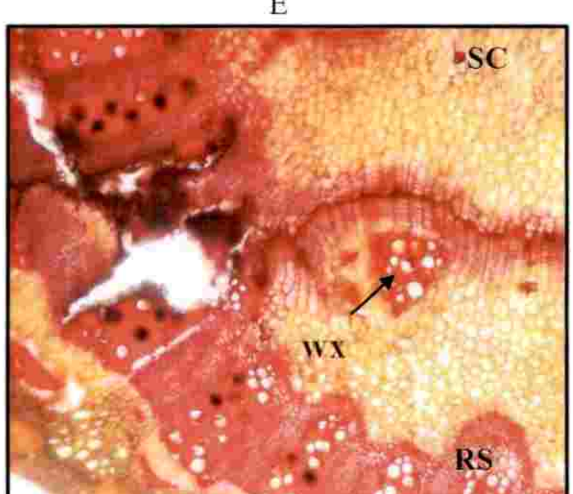
C



D

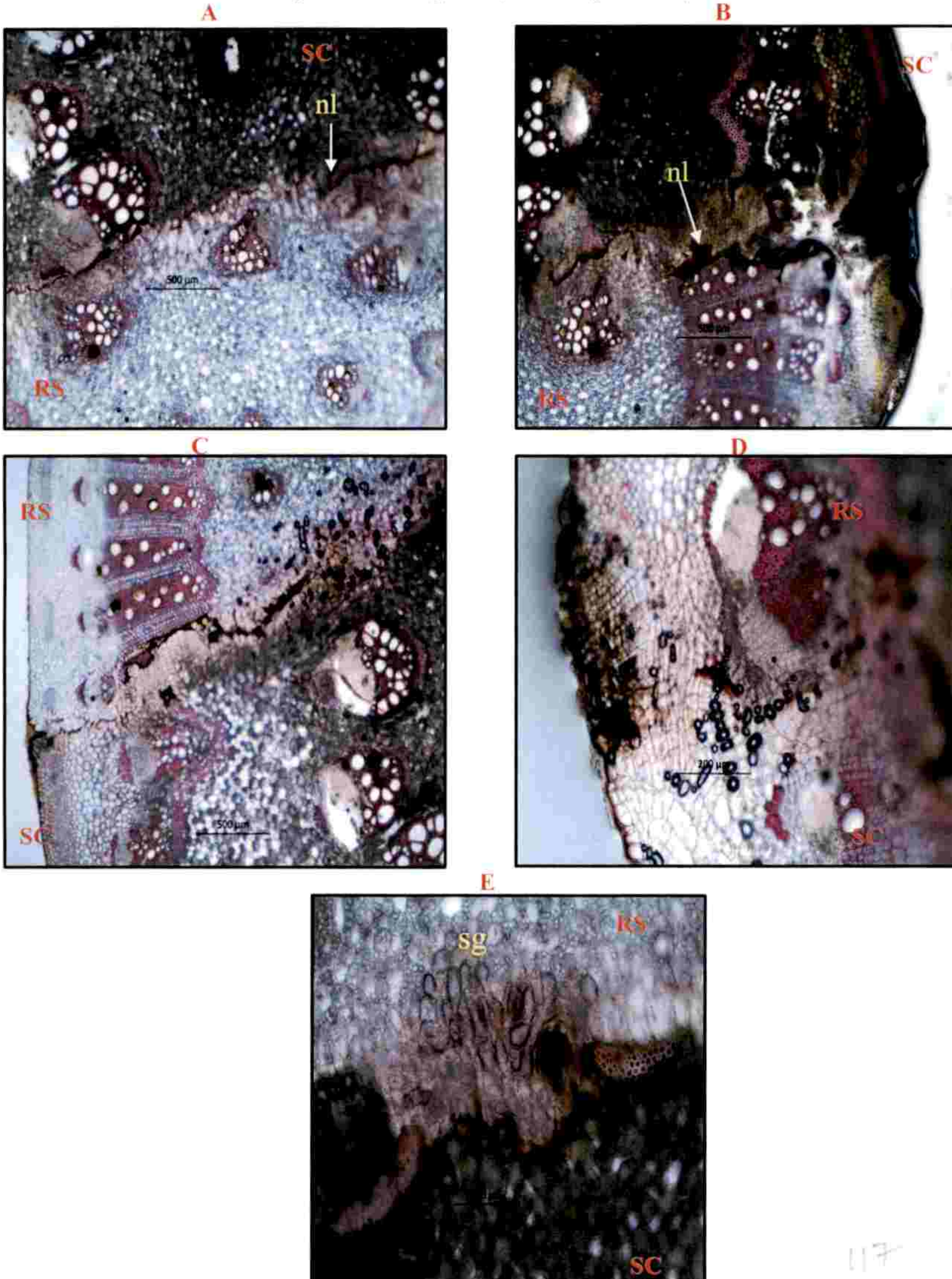


E



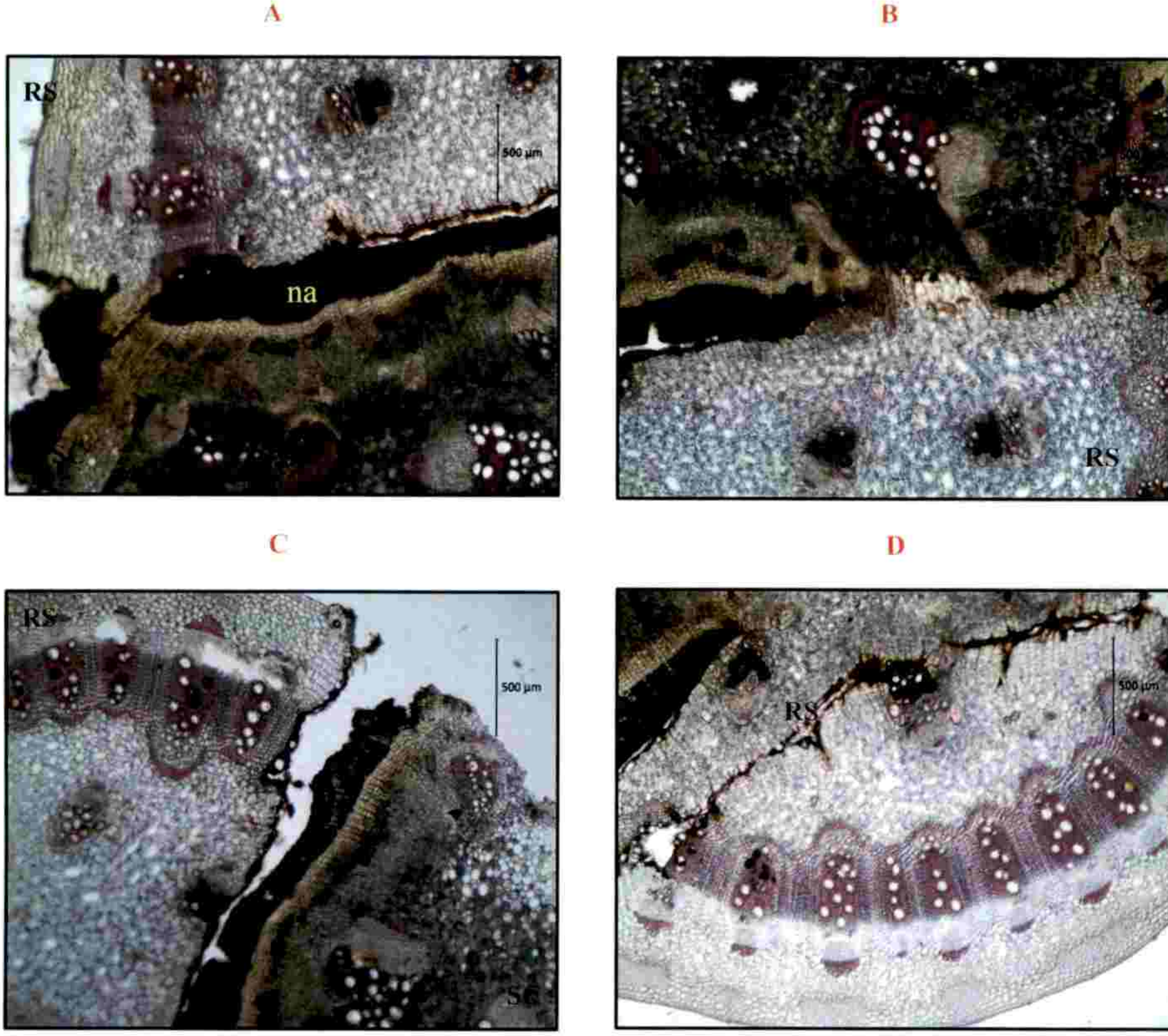
(RS- rootstock; SC- scion; nl- necrotic layer; ca- callus; wx- wound repair xylem)

Plate 16. Six month old grafts of *P. nigrum* (orthotropic shoot) on *P. colubrinum*



(RS- rootstock; SC- scion; nl- necrotic layer; sg- starch grain)

Plate 17. One year old grafts of *P. nigrum* (orthotropic shoot) on *P. colubrinum*



(RS- rootstock; SC- scion; na- necrotic area)

Plate 18. Three month old grafts of *P. nigrum* (orthotropic shoot) on *P. auduncum*

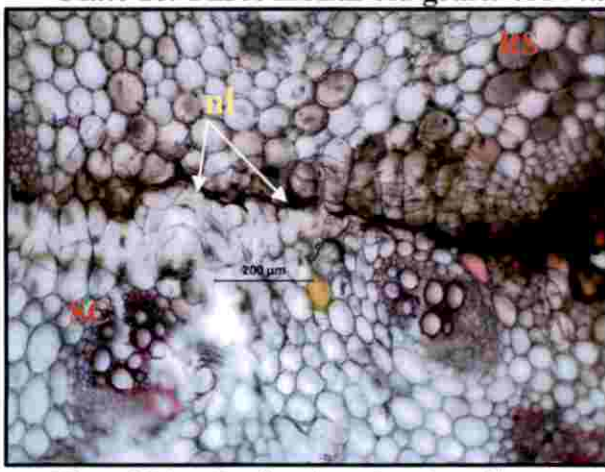


Plate 18A : Graft union showing discrete strands of necrotic layers

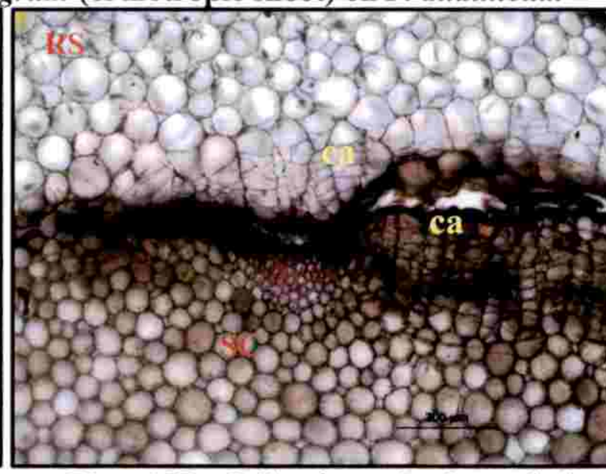


Plate 18B : Callus formation beneath the isolation layer

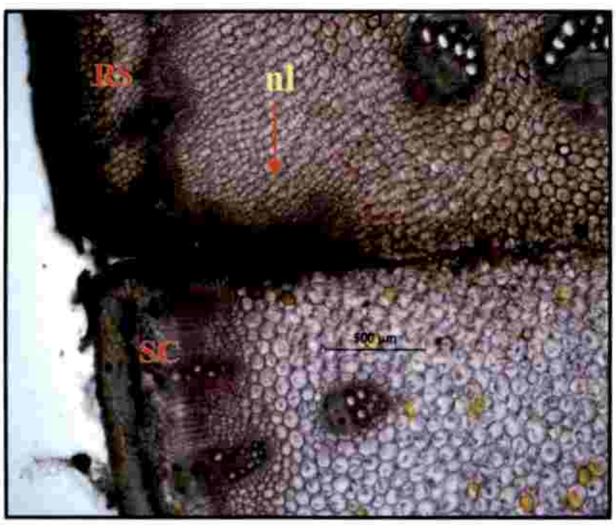


Plate 18C : Dense necrotic layer formed at the cortical region

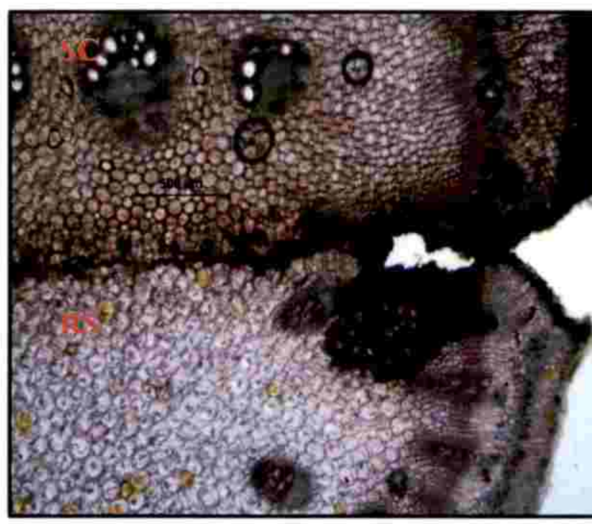


Plate 18D : Cortical region of graft without proper joining

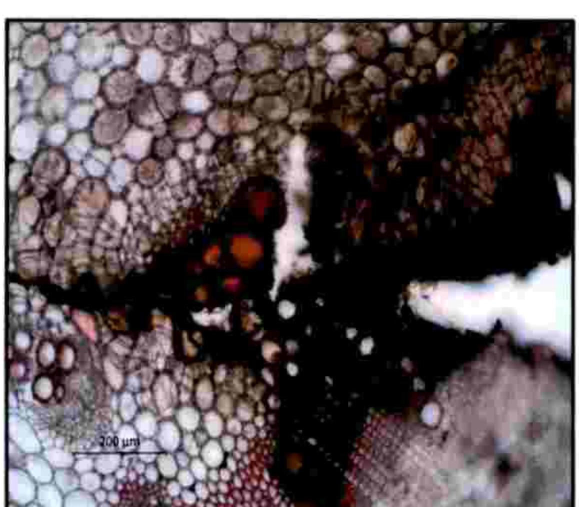
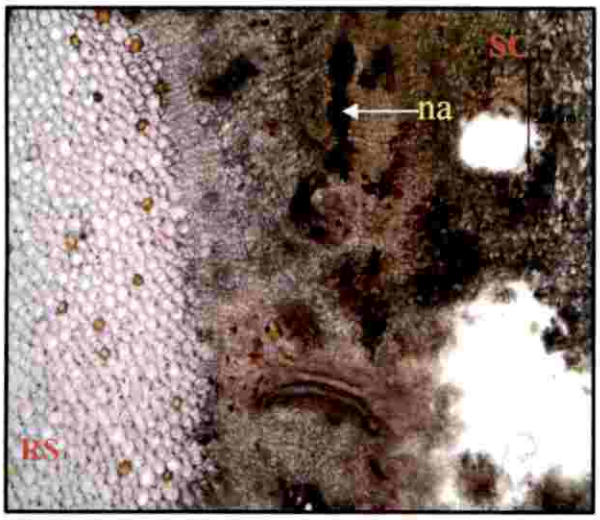


Plate 18E : Enlarged view of graft union at the cortical region with dense necrotic area

(nl- necrotic layer; ca- callus; na – necrotic area)

Plate 19. One year old grafts of *P. nigrum* (orthotropic shoot) on *P. auduncum*



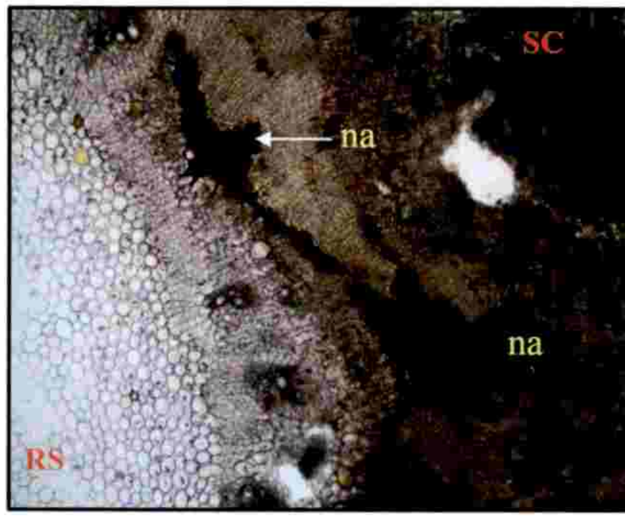


Plate 19A & B: Grafts showing necrotic area at the stelar region of scion

Plate 19C: Graft showing improper union at the cortical region and necrotic areas in scion

(Rs- rootstock; SC- scion; na – necrotic area)

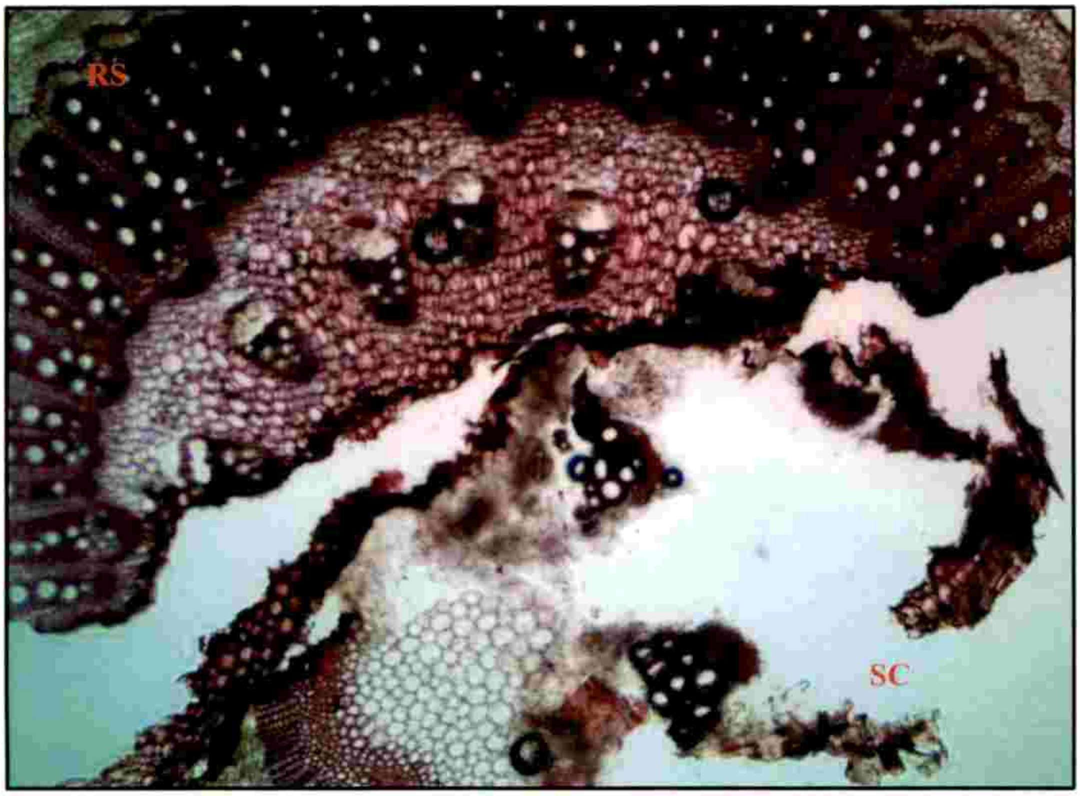


Plate 20. Two month old grafts of *P. nigrum* (orthotropic shoot) on *P. arboreum*

4.3.2 Anatomy of Graft Union Involving Plageotropic Shoot of *P. nigrum* as Scion

4.3.2.1 *Anatomy of P. nigrum Grafts on its own Rootstock*

Transverse sections of three month old graft involving plageotropic shoot of *P. nigrum* on its own rootstock were taken. Transverse sections of six months and one year old grafts are not included, since hand sectioning of grafts at advanced stages was difficult.

Anatomical structure of a three month old graft union is described in plate 21. Formation of a callus bridge between the isolation layers of stock and scion was clearly observed in one of the sections (Plate 21A). Union of stock and scion at the stelar region was almost completed by three months after grafting (Plate 21B). However, at the cortical region, they were still separated by isolation layers (Plate 21C). There was no evidence of callus bridging in this region.

4.3.2.2 *Anatomy of P. nigrum Grafts on P. colubrinum*

Transverse sections of three, six and twelve months old grafts involving plageotropic shoot of *P. nigrum* as scion and *P. colubrinum* as rootstock was taken. Anatomical structures of these grafts are described in plates 22- 24.

Plate 22 describes the anatomy of a three month old graft union. As can be seen from plate 22A, in a three month old graft, unbroken strand of necrotic layer was observed at the graft union. However, callus was observed beneath the isolation layers of stock and scion at the stelar region (Plate 22B). But, union was not evident at the cortical region (Plate 22C).

Anatomical structure of a six month old graft is described in plate 23. At the graft union necrotic layer appeared as discrete strands in these grafts (Plate 23A). In one of the sections, undifferentiated cells were observed at the cortical region of scion and this region was not in a proper contact with the cortical region of rootstock (Plate 23B and C). All the sections showed abundant accumulation of starch at the stock side.

Transverse sections of one year old graft union involving plageotropic shoot of *P. nigrum* as scion and *P. colubrinum* as rootstock are described in plate 24. As can be seen from the plates, a dense necrotic area was evident at the graft union. This was observed to be developed on the cut end of scion which acted as a barrier between stock and scion. Sectioning of one year old grafts was difficult, since individual cells of stock and scion were not clearly observed.

4.3.2.3 *Anatomy of P. nigrum Grafts on P. auduncum*

Transverse sections of graft union involving plageotropic shoot of *P. nigrum* as scion and *P. auduncum* as rootstock were taken at different stages of development. Aatomical structure of three and twelve month old grafts are described in plates 25 and 26. Anatomy of a six month old graft is not explained since good sections of such grafts were not obtained.

As can be seen from plate 25, in a three month old graft, union of stock and scion was almost completed. But individual cells were not differentiated. Remnants of necrotic layer remained at the stelar region (Plate 25A). While dense necrotic area was observed at the cortical region (Plate 25B).

Transverse sections of one year old graft showed dense necrotic area at the union (Plate 26A). Thickness of necrotic area was more at the cut end of scion. This dense necrotic area acted as a barrier between stock and scion. A proper union between grafting partners was not evident in one year old grafts of *P. nigrum* on *P. auduncum*. In one of the sections, necrosis of parenchyma cells of stock was observed (Plate 26B).

When grafting of plageotropic shoot of *P. nigrum* was carried out on different rootstocks, highest graft failure was recorded with *P. arboreum*. Hand sectioning was tried on the grafts on *P. arboreum* which remained green without sprouting. But, stock and scion got separated on taking transverse sections, indicating that there was no union between stock and scion.

Plate 21. Three month old grafts of *P. nigrum* (plageotropic shoot) on its own rootstock

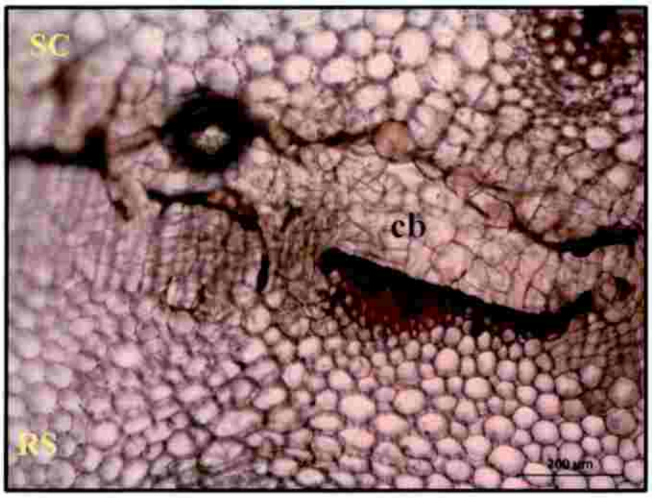


Plate 21A: Graft union showing formation of callus bridge (cb) between the isolation layers

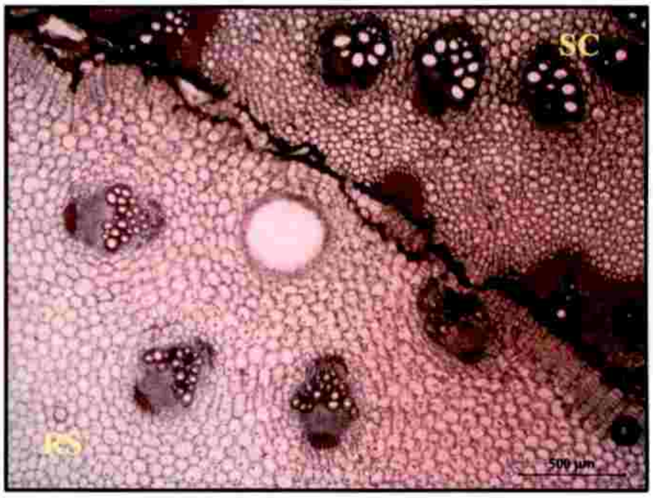


Plate 21B: Graft union without any gap at the stelar region

Plate 21C: Incomplete graft union

Plate 22. Three month old graft of *P. nigrum* (plageotropic shoot) on *P. colubrinum*

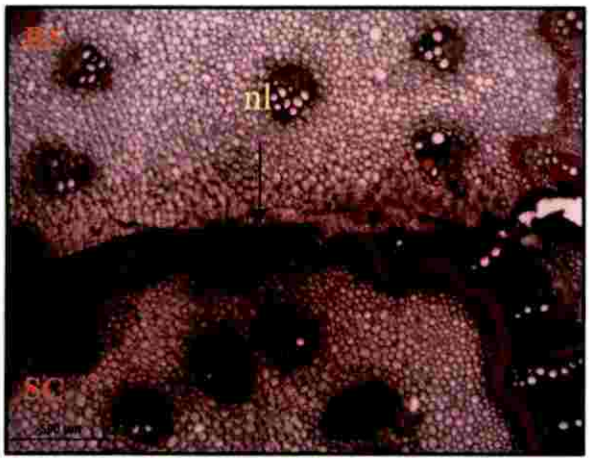


Plate 22A: Long strand of necrotic layer at the graft union

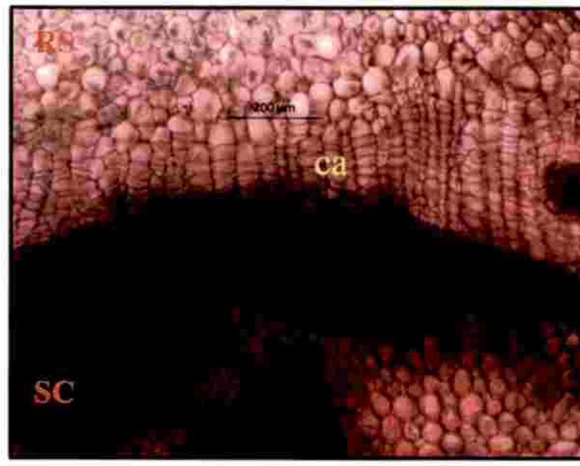


Plate 22B: Formation of callus beneath the isolation layers

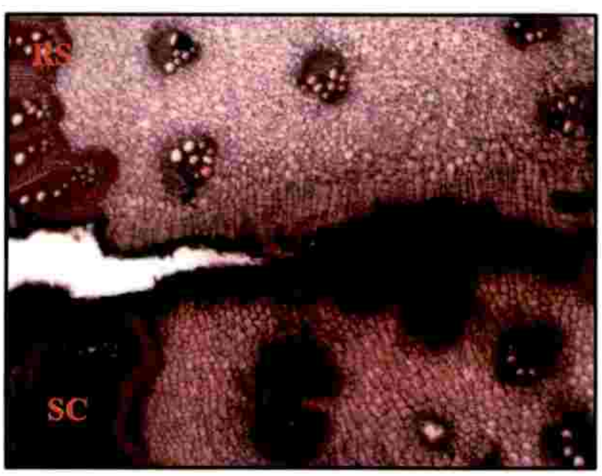
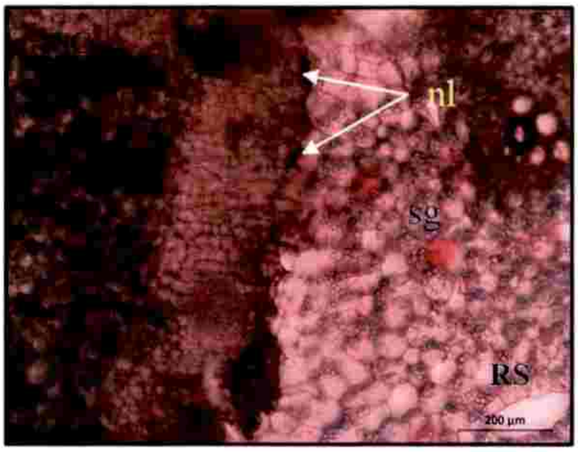


Plate 22C: Cortical region of stock and scion without proper joining

Plate 23. Six month old graft of *P. nigrum* (plageotropic shoot) on *P. colubrinum*



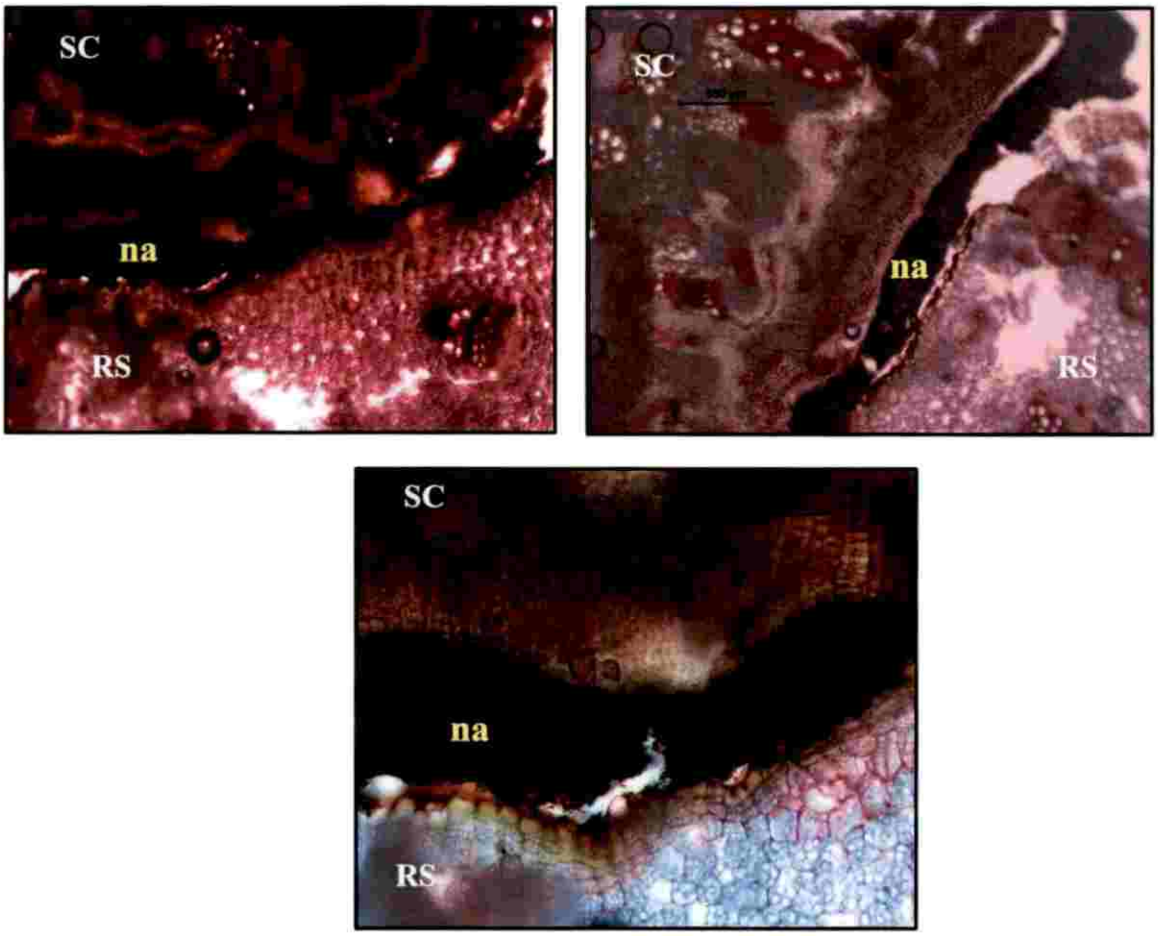
A

B

C

(RS- rootstock, SC- scion, ca- callus, nl- necrotic layer, sg- starch grain)

Plate 24. One year old graft of *P. nigrum* (plageotropic shoot) on *P. colubrinum*



na; necrotic area

Plate 25. Three month old grafts of *P. nigrum* (plageotropic shoot) on *P. auduncum*

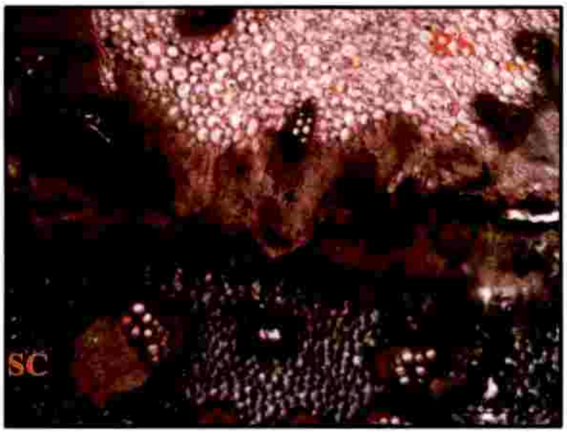


Plate 25A: Graft union with discrete strands of necrotic layer

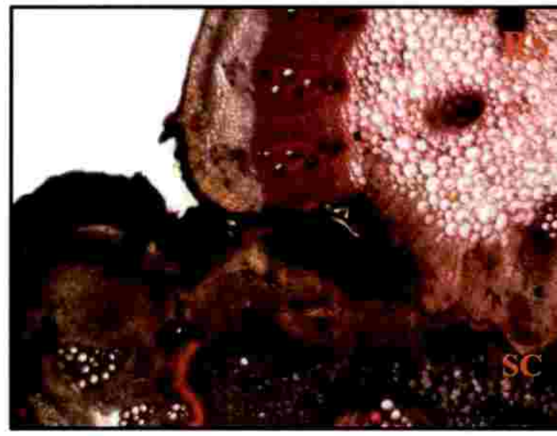


Plate 25B: Improper graft union at the cortical region

Plate 26. One year old grafts of *P. nigrum* (plageotropic shoot) on *P. auduncum*

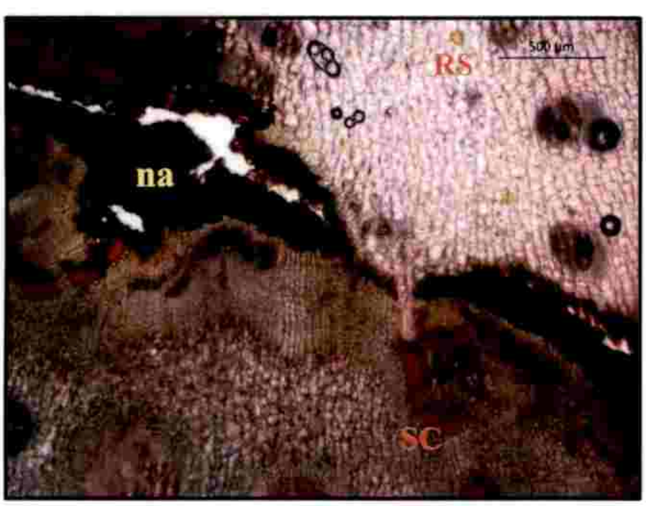


Plate 26A: Graft with necrotic area (na) at the union

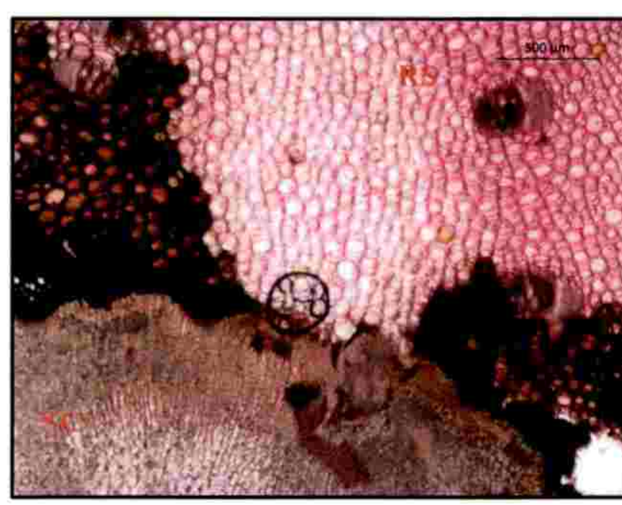


Plate 26B: Graft showing cell necrosis at the stock side

Discussion

5. Discussion

The present study entitled stock scion interaction in *Piper nigrum* L. grafts was carried out at Pepper Research unit of Department of Plantation crops and Spices, College of Horticulture, Vellanikkara during 2017 to 2019. The study was carried out as three experiments *viz.*, grafting studies, anatomical studies on *Piper* species and graft union. Results obtained from the study are discussed below.

5.1 GRAFTING STUDIES OF *P. nigrum* ON DIFFERENT ROOTSTOCKS

5.1.1 Scion – Orthotropic Shoot of *P. nigrum* variety Panniyur 1

5.1.1.1 Effect of Rootstocks on Graft Success

In the present study, grafting of *P. nigrum* was carried out on different South American *Piper* species *viz., P. colubrinum*, *P. auduncum* and *P. arboreum* which are immune to *Phytophthora* foot rot. Grafting of *P. nigrum* on its own rootstock was also done to study the feasibility of such grafting. Grafting was performed during four different months (December, March, June and September). Results obtained indicated that, highest per cent success was shown by the grafts of *P. nigrum* on its own rootstock followed by *P. colubrinum*, *P. auduncum* and *P. arboreum*. Among the four rootstocks, *P. nigrum* recorded highest per cent success in December (86.66%), March (100%) and September (83.33%) (Fig 1). Waard (1967) stated that intraspecific grafting is possible in *P. nigrum* by grafting *P. nigrum* variety, Kuching on its own rootstock.

When *P. colubrinum* was used as rootstock, highest percentage success recorded was 96.66 (March) (Fig 2). In confirmation with this observation, Vanaja *et al.* (2007) reported more than 90 per cent success in *P. nigrum* grafts on *P. colubrinum* when done during February- March.

In the present investigation, along with *P. colubrinum*, other two South American *Piper* species *viz., P. auduncum* and *P. arboreum* were also used as rootstocks. Highest percentage of success recorded by the grafts on *P. auduncum* was 66.66 (Fig 3), while that on *P. arboreum* was 46.66 (Fig 4). Attempts of grafting of *P. nigrum* on *P. auduncum* and *P. arboreum* were not reported earlier. However, Arathi (2011) attempted grafting in *P. nigrum* using two Western ghat species of *Piper* as rootstocks, *viz., P. hymenophyllum* and *P. attenuatum* with a grafting success of 42.78 and 36.11 per cent respectively.

Survival of *P. nigrum* grafts on *P. arboreum* was very low. None of these grafts could survive more than three months after grafting. This could be due to the anatomical mismatching of stock and scion, as *P. arboreum* possesses a large central pith region. Common symptoms of graft failure observed during the course of the present study were, shrinking and drying of scion and lack of sprouting in scion. Similar symptoms of graft failure were observed by Beena (1994) in nutmeg grafts.

5.1.1.2 Effect of Season on Graft Success

In the present study, irrespective of rootstocks, March was identified as the best month for grafting. When grafting was performed during March, grafts of *P. nigrum* on its own rootstocks showed 100 per cent success followed by grafts on *P. colubrinum* (96.66%), *P. auduncum* (66.66%) and *P. arboreum* (46.66%) (Fig 5). This was in accordance with the finding of Vanaja *et al.* (2007). They reported that, February and March were the best months for grafting *P. nigrum* on *P. colubrinum*.

5.1.1.3 Effect of Rootstocks on Growth of Scion

Among the various rootstocks tried, better growth of scion was observed in the grafts with *P. colubrinum* as rootstock. Six month old grafts on *P. colubrinum* obtained during June were recorded with highest plant height (120.55 cm) (Fig 1), number of nodes (18.91) (Fig 2) and internodal length (6.32) (Fig 3). Sourabha *et al.*

(2017) recorded only 39.7 cm plant height in a six month old graft of *P. nigrum* variety Panniyur 1 on *P. colubrinum* under Karantaka conditions. Number of nodes and internodal length of such grafts were reported as 7.7 and 6.7 cm respectively.

5.1.2 Scion – Plageotropic Shoot of *P. nigrum* variety Panniyur 1

Grafting of plageotropic or lateral shoot of *P. nigrum* on different rootstocks was also done in this study. Reports on grafting of lateral shoot of *P. nigrum* are very limited. Hence findings of this study are discussed with similar studies in other crops.

5.1.1.1 Effect of Rootstocks on Graft Success

In the present investigation, irrespective of month of grafting, highest per cent success was recorded by the grafts of *P. nigrum* on its own rootstock (Fig 9). This was followed by *P. colubrinum*, *P. auduncum* and *P. arboreum*. Highest per cent success exhibited by *P. nigrum* grafts on its own rootstock was 93.33 (March), while the grafts on *P. colubrinum* recorded 66.66 per cent (June) success (Fig 10). Grafts on *P. auduncum* and *P. arboreum* showed graft success of 36.66 per cent (March) (Fig 11) and 26.66 per cent (March) (Fig 12) respectively. Any of the grafts on *P. arboreum* could not survive more than three months after grafting. Similar studies on grafting using these rootstocks were not reported earlier. However, Hasan (1960) could produce dwarf grafts by using plageotropic shoots of *P. nigrum* as scion and *P. hirsutum* and *P. ariifolium* as rootstocks, but the scions died in due course.

5.1.1.2 Effect of Seasons on Graft Success

Seasonal effect on success of graft was also compared in this study. Except *P. colubrinum*, grafts on all other rootstocks showed highest success on grafting when grafting was performed during March. While, grafts on *P. colubrinum*, obtained during June recorded highest per cent success. This observation was similar to the findings of Rema *et al.* (2009) in nutmeg. They could obtain successful grafts in nutmeg during March. However, Das *et al.* (2011) reported highest grafting success in cashew during June.

5.1.1.3 Effect of Rootstocks on Growth of Scion

As far as growth of scion was concerned, there was no significant difference between grafts on different rootstocks obtained during September and December. At six month old stage, highest plant height was recorded by the grafts of *P. nigrum* on its own rootstock obtained during March (45.36 cm) (Fig 14). Grafts on *P. nigrum* recorded highest number of nodes (7.69) (Fig 15) and internodal length (8.06 cm) when grafting was carried out during March and September respectively. Branching and spiking was very limited in the observed grafts at six month old stage.

5.2 ANATOMY OF *PIPER* SPECIES

The stem structure of *Piper* species is anomalous among the dicotyledons, and bears some resemblance to monocotyledons also (Metcalfe and Chalk, 1950). The peculiar anatomical structure of *Piper* species might have affected the graft union. Anatomical investigation on *Piper* species was carried out to study the effect of its anomalous structure on success or failure of grafts.

The most unique feature of genus *Piper* is the polycyclic arrangement of vascular bundles. Number of rings of vascular bundles varied from species to species (Trueba *et al.*, 2015). In the present study, two rings of vascular bundles (cortical and medullary bundles) were observed in *P. nigrum*, *P. auduncum* and *P. arboreum*. Ravindran *et al.* (2000) reported the same feature in *P. nigrum*. Stem anatomy of *P. auduncum* and *P. arboreum* was not described earlier. However there are reports on the polycyclic arrangement of vascular bundles in other *Piper* species such as *Piper methysticum* (Hoffstadt, 1916), *Piper obtusilimbum* (Tepe *et al.*, 2007) and *Piper sarmentosum* (Raman *et al.*, 2012). The very centre of the pith was occupied by an additional medullary bundle in *P. colubrinum* as in *P. excelsum* (Beck, 2011).

Number of cortical as well as medullary bundles varied from species to species. In *Piper nigrum*, 25 – 40 cortical and 6 – 10 medullary bundles were observed. In the cortical region, small and large bundles were arranged often alternately. Medullary bundles were larger than cortical bundles. Intrafascicular cambium in the medullary bundle was 3- 4 celled. Similar observations were made by Ravindran *et al.* (2000) but the number of cortical and medullary bundles reported was 35 -40 and 8 – 10 respectively.

Number of cortical and medullary bundles recorded in the stem of *P. colubrinum* was 38-52 and 7-12 respectively. A cambial connection was clearly observed in the cortical bundles during secondary thickening. Ravindran and Remashree (1998) recorded 42- 46 and 11- 14 cortical and medullary bundles respectively in *P. colubrinum*. They also observed a clear cambial connection in the cortical bundles during secondary thickening. In a mature stem of *P. arboreum* 68 – 75 cortical bundles and 39 - 48 medullary bundles were observed. While in *P. auduncum*, the number of cortical and medullary bundles varied from 46 to 53 and 12 to 15 respectively.

The two rings of vascular bundles were separated by a wavy band of sclerenchma in all the *Piper* species under study except *P. arboreum*. Sclerechymatous separation of vascular bundles was reported by Yang and Chen (2017) in six *Piper* species distributed in the low mountainous forests of Taiwan *viz.*, *P. arborescens* Roxb., *P. kadsura* (Choisy) Ohwi, *P. kawakamii* Hayata, *P. kwashoense* Hayata, *P. sintenense* and *P. taiwanense*. Instead of forming into a wavy band, sclerenchyma remained as a cup like structure just below the xylem end of cortical bundle in *P. arboreum*. Yang and Chen (2017) reported a discontinuous band of sclerechyma in *P. betel* L.

In the present study, a central mucilage canal was noticed only in *Piper nigrum*. Similar mucilage canal was reported in the stem of *P.nigrum* (Garner and

Beakbane, 1968), *P. sarmentosum* (Raman *et al.*, 2012) and *P. retrofractum* Vahl. (Saraswathy *et al.*, 2013). However, Yang and Chen (2017) could not observe mucilage canal in *P. sintenense* and *P. taiwanense*. In *P. colubrinum*, instead of a large mucilage canal at the central pith region, the same was observed to be scattered in the stelar region, but reduced in size. Likewise in *P. betle* L, Khaing (2016) identified a ring of mucilage canals in the stelar region in addition to a central mucilage canal.

Large pith region was observed in *P. auduncum* and *P. arboreum*. Starch grains and oil glands were abundantly observed in *P. auduncum* while calcium oxalate crystals (raphides) were noticed in the pith and cortex region of *P. arboreum*. Consonantly, Dos *et al.* (2018) identified large parenchymatous pith region occupied by starch grains, secretory ideoblasts and calcium oxalate cryastals in *P. caldense*.

5.3 ANATOMY OF GRAFT UNION

Anatomical investigation on the graft union was carried out to know whether it is influenced by the anomalous stem structure of *Piper* species. Anatomical studies on grafts involving *Piper* species are limited, therefore, major observations derived from the present study are compared with the findings of similar studies conducted in other crops.

In the present study, transverse sections of three month old grafts on the rootstocks of *P. nigrum*, *P. colubrinum* and *P. auduncum*, showed a discontinuous isolation or necrotic layer at the graft union. Later, by six months after grafting, a gradual reduction of the same was observed. Reduction in the necrotic area was facilitated by callus. Callus formed beneath the isolation layer absorbed the necrotic layer. In confirmation with this observation, Selime and Ertan (2013) reported the development of necrotic area in a four month old chestnut – oak graft which was later destroyed by callus. They supposed that, development of necrotic area as a part of wound response is due to the oxidation of phenolic matters.

Formation of a callus bridge was clearly observed at the union of a graft involving plageotropic shoot of *P. nigrum* on its own rootstock. Hartmann and Kester (2002) reported that a callus bridging was necessary for the successful union of grafting components. In the present study, anatomical observations on *P. nigrum* grafts on its own rootstock revealed that, formation of graft union was a gradual process. Healing was almost completed at the stelar region by three months after grafting. At this stage, union occured between parenchymatous cells of stock and scion. A cambial connection was not evident at the stelar region. A similar union of respective parenchymatous cells of stock and scion was reported in vanilla grafts, which is a herbaceous monocotyledonous plant devoid of cambium (Muzik, 1958).

Cortical vascular bundles were not properly aligned in a six month old graft involving *P. nigrum* as scion and stock. But alignment of above mentioned cortical bundles was almost completed in a one year old graft union. But, a definite cambial connection between cortical bundles was not established within one year after grafting. Mahunu *et al.* (2013) reported that there is no definite time limit for the formation of a graft union. In Chestnut – Oak graft, Selime and Ertan (2013) could observe a clear cambial connection 7 months after grafting.

Three month old graft of *P. nigrum* on *P. colubinum*, showed the formation of a wound repair xylem from the callus. Hartmann and Kester (2002) reported that, there are evidences of differentiation of callus into vascular elements during graft union formation. In a six months old graft on *P. colubrinum*, abundant accumulation of starch grain was observed alongside of rootstock near the graft union. This was not observed in grafts on *P. nigrum* and *P. auduncum*. Even though starch grains were visible in the transverse sections of stem of *P. auduncum*, during grafting additional accumulation of starch grains was not observed. Evidence of such starch grain accumulation was reported by Simons and Chu (1985) in apple grafts. Starch accumulation in scion was observed as a reason for incompatibility by Ermel *et al.* (1999) in a five month old pear- quince graft.

A deviation from the normal anatomy of graft union was observed at one year stage. Excessive formation of dense necrotic area was observed in grafts of *P. nigrum* on *P. colubrinum* and *P. auduncum*. However this was not observed in grafts on *P. nigrum*. These necrotic tissues probably acted as a barrier between stock and scion. Similar findings on *P. nigrum* grafts were not reported earlier. However there are such reports on chestnut grafts which showed delayed incompatibility symptoms. In a six month old graft of chestnut on oak, Santamour (1988) observed dense necrotic area at the graft union acting as a barrier between grafting components. Similarly, Ermel *et al.* (1999) reported little cell necrosis in a five month old incompatible grafts of pear on quince.

A proper alignment of medullary bundles at the graft union was not observed in grafts of *P. nigrum* on *P. colubrinum* and *P. auduncum* at any stage of development. These were observed to be scattered at the graft union. In confirmation with this finding, Empari and Sim (1986) proposed that, in grafts of *P. nigrum* on *P. colubrinum*, matching of all the vascular bundles of stock and scion was virtually impossible because of the scattered arrangement of the same.

Anatomical structure of a two month old graft of *P. nigrum* on *P. arboreum*, which remained green without sprouting, showed wide gap between the grafting components. A similar observation was made by Mahunu *et al.* (2013) in an unsuccessful graft of cashew at two month stage.

Based on the findings of this study, it can be concluded that, best results were obtained in grafting of *P. nigrum* on its own rootstock. Interspecific grafting was possible in *P. nigrum* with *P. colubrinum* and *P. auduncum*. Among these immune species, *P. colubrinum* exhibited highest grafting success followed by *P. auduncum*. Similarly, when plageotropic shoot was used as scion, grafts on *P. nigrum* recorded highest grafting success followed by *P. colubrinum* and *P. auduncum* but the success rate was low. Irrespective of rootstocks, March was identified as the best month for grafting orthotropic shoot of *P. nigrum*. Grafts of plageotropic shoot of *P. nigrum* on *P. nigrum* and *P. auduncum* showed highest success during March, while grafts on *P. coubrinum* exhibited highest grafting success during June.

Piper species exhibited a peculiar arrangement of vascular bundles, hence the matching of all the vascular bundles of stock and scion was difficult. However, complete union of parenchymatous tissues of stock and scion was formed at the stelar region by three months after grafting. However, a complete cambial connection between the grafting partners at the cortical region was not evident even after six months of grafting. The peculiar observation made at the graft interface of one year old grafts on *P. colubrinum* and *P. auduncum* was the development of dense necrotic area. In spite of having dense necrotic area at the graft interface, grafts of *P. nigrum* on *P. colubrinum* and *P. auduncum* was showing vigorous growth, indicating compatibility between stock and scion. But the reasons for these phenomena are not clear. Further detailed studies are needed to throw more light on this aspect.

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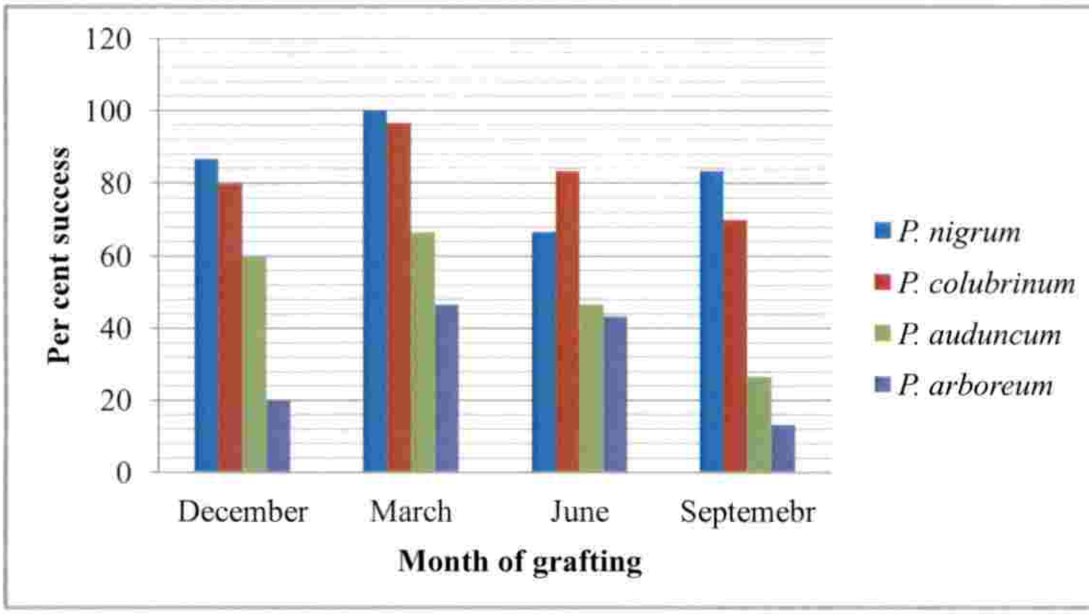

Figure 1. Effect of rootstocks on success of grafting (%)

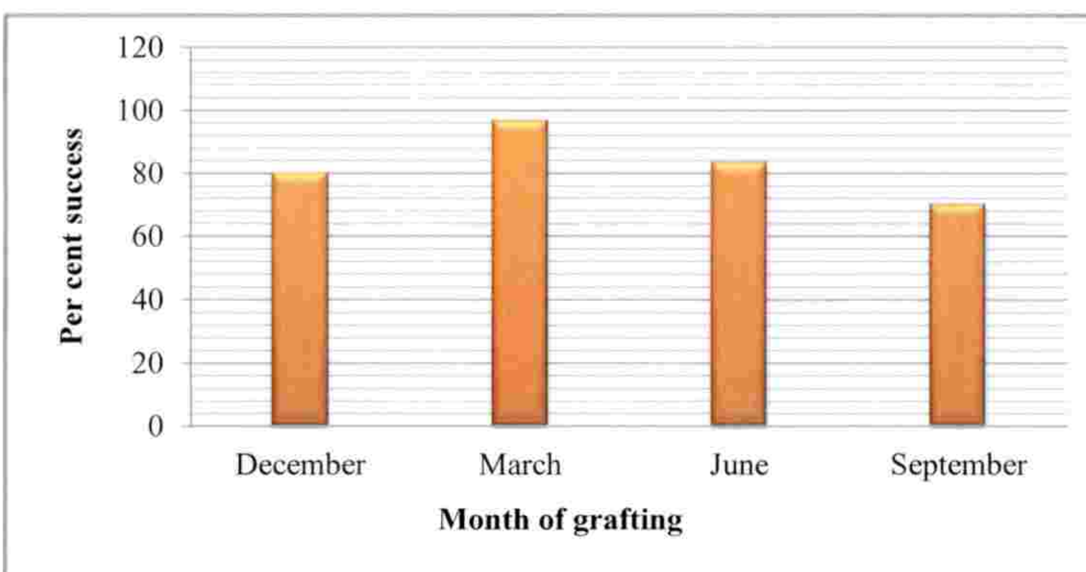

Figure 2. Success of grafting of *P. nigrum* on *P. colubrinum*

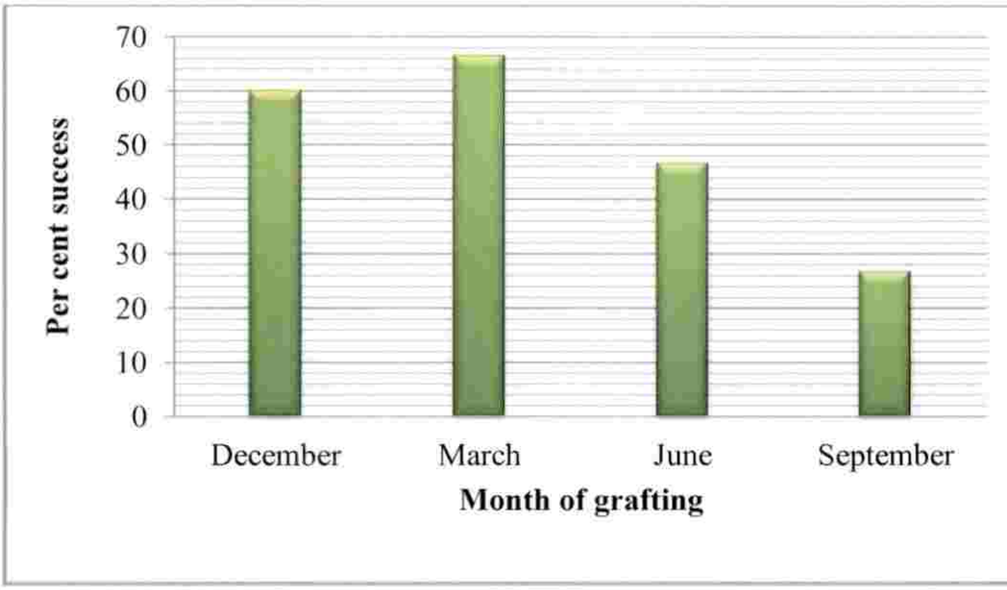

Figure 3. Success of grafting of *P. nigrum* on *P. auduncum*

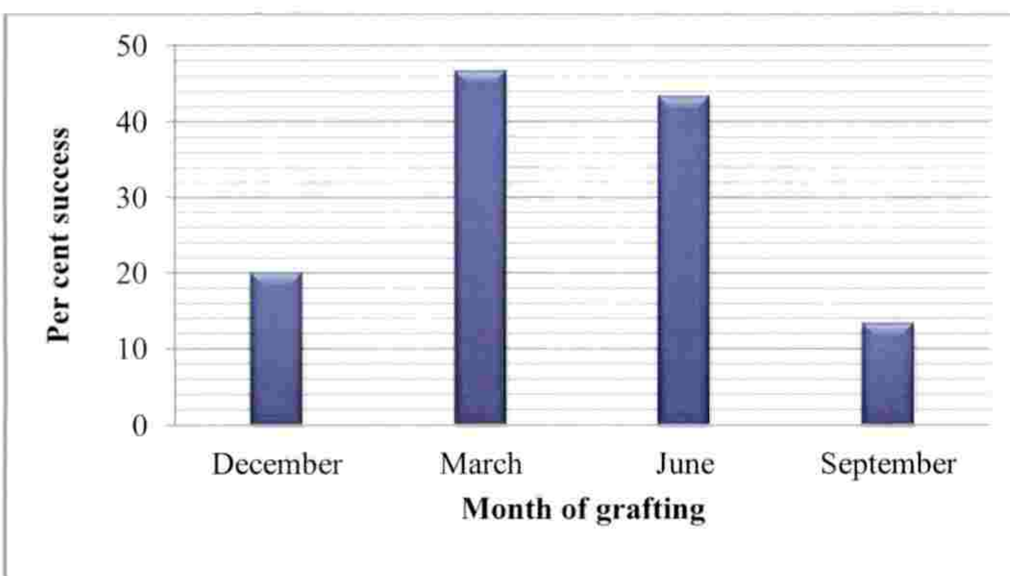

Figure 4. Success of grafting of *P. nigrum* on *P. arboreum*

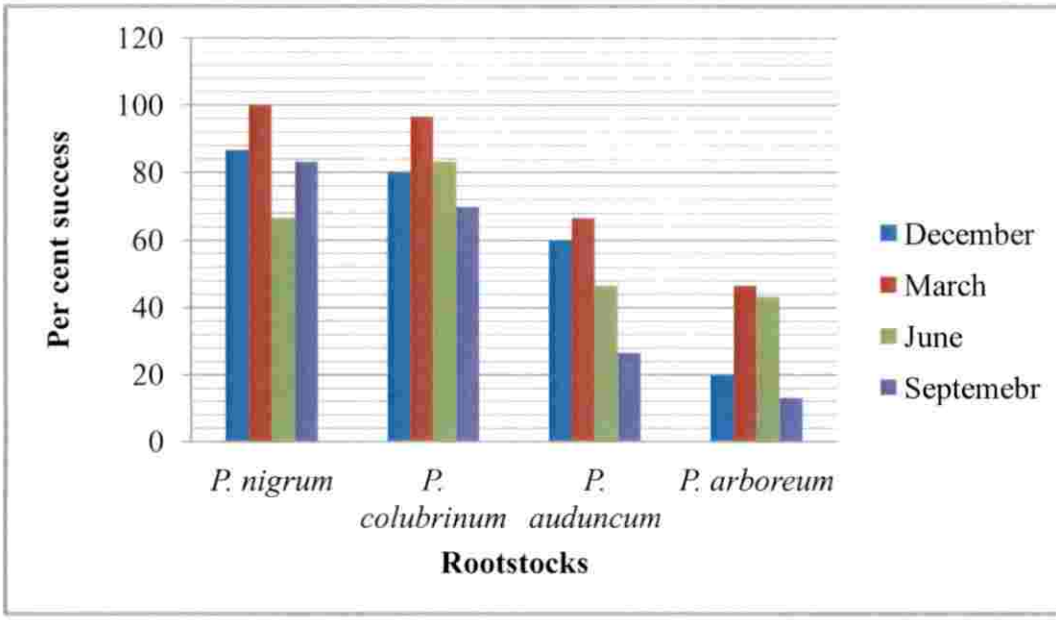

Figure 5. Effect of month of grafting on graft success (%)

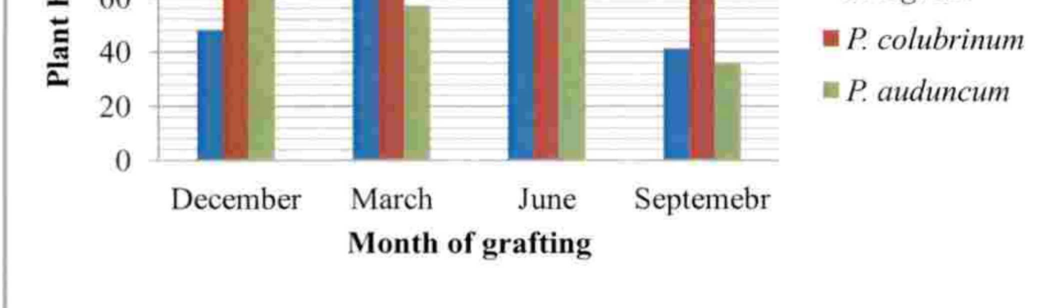

Figure 6. Effect of rootstocks on plant height (cm)

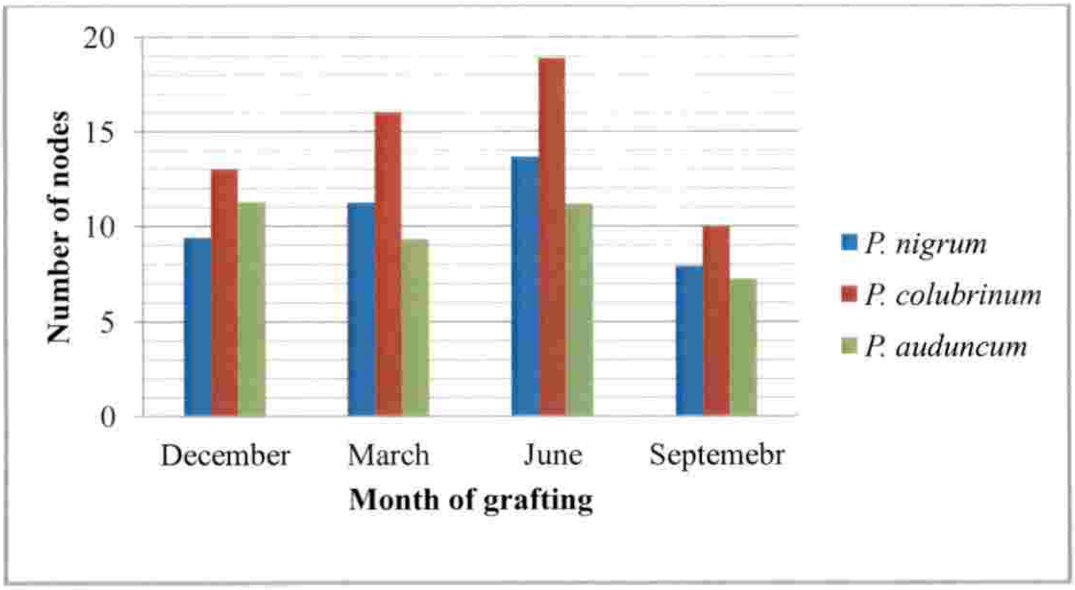

Figure 7. Effect of rootstocks on number of nodes

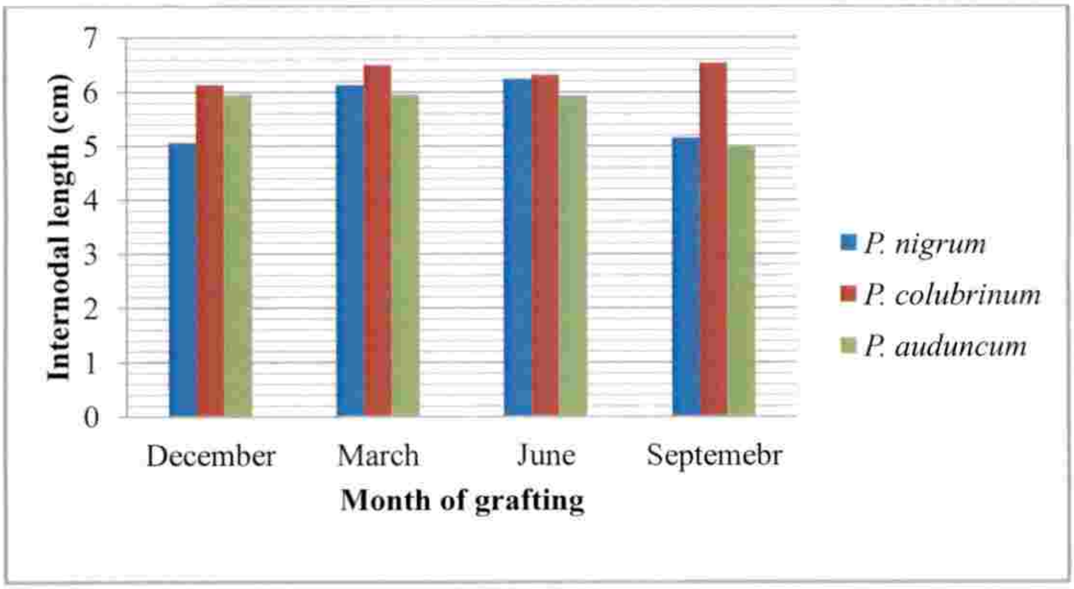

Figure 8. Effect of rootstocks on internodal length of scion (cm)

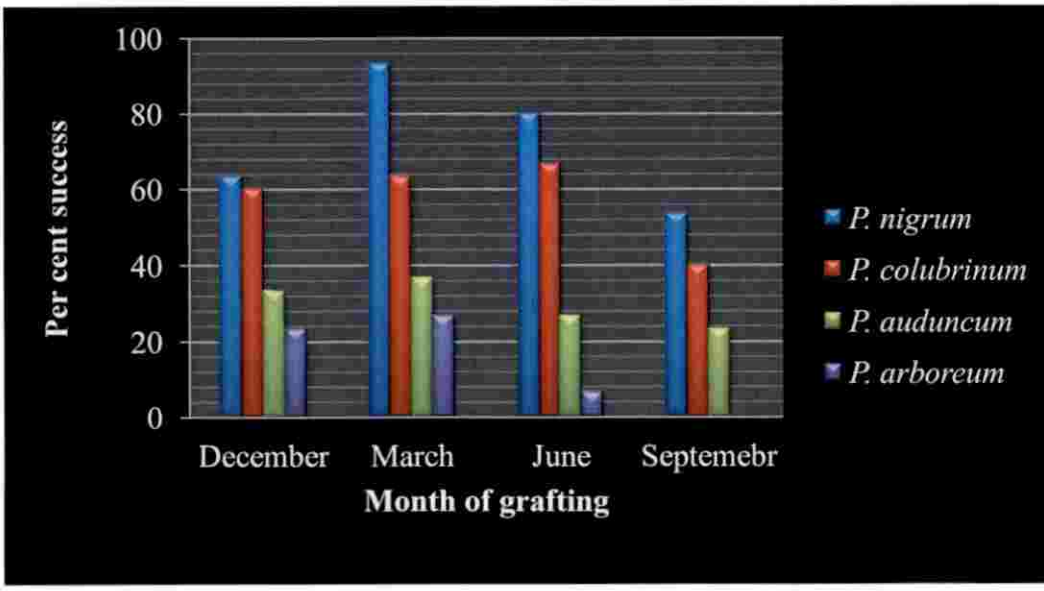

Figure 9. Effect of rootstocks on graft success (%) (Scion – plageotropic shoot of *P. nigrum*)

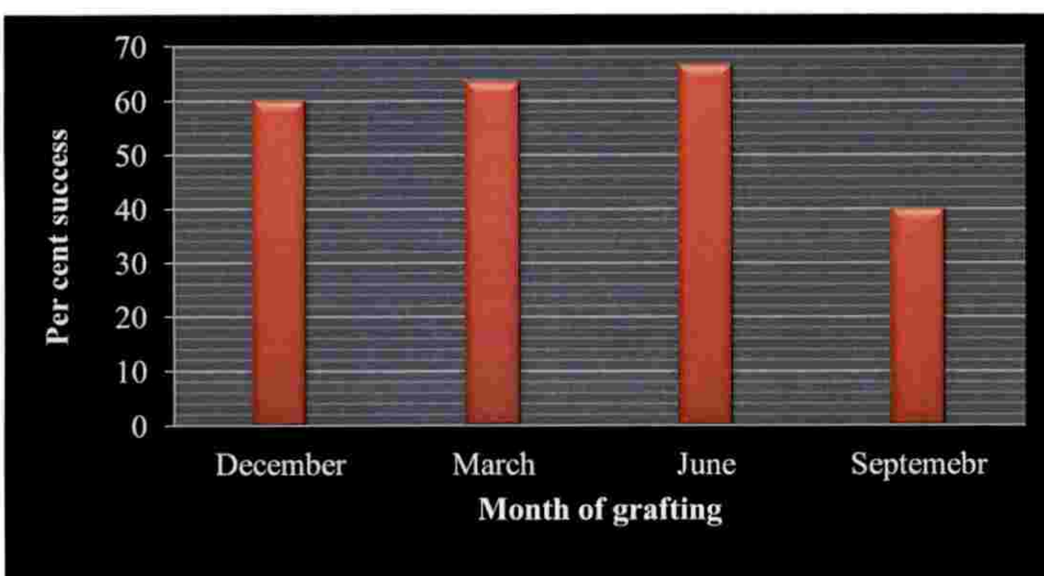

Figure 10. Success of grafting of *P. nigrum* (plageotropic shoots) on *P. colubrinum*

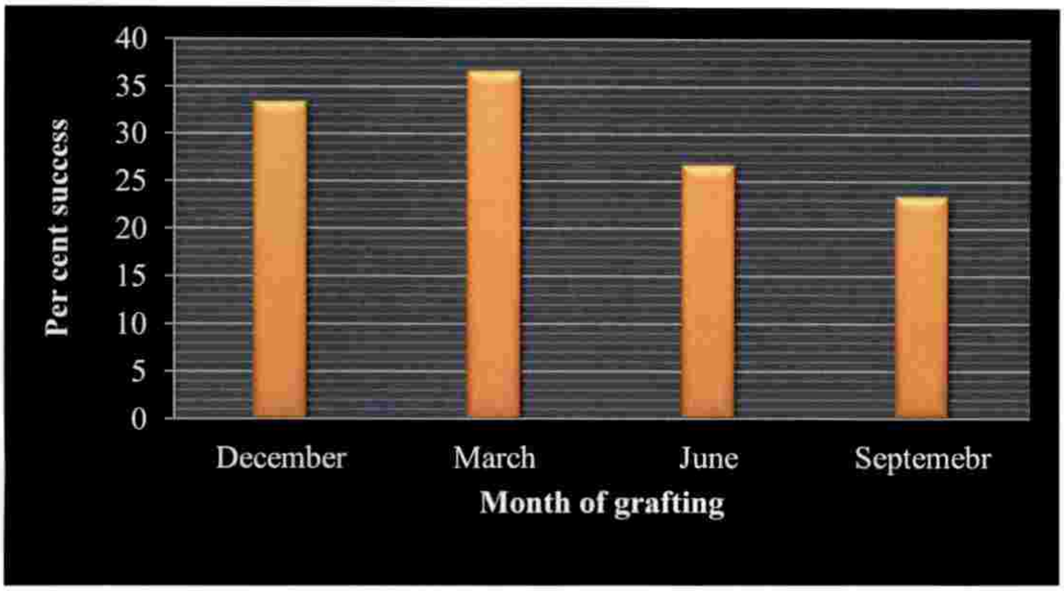

Figure 11. Success of grafting of *P. nigrum* (plageotropic shoots) on *P. auduncum*

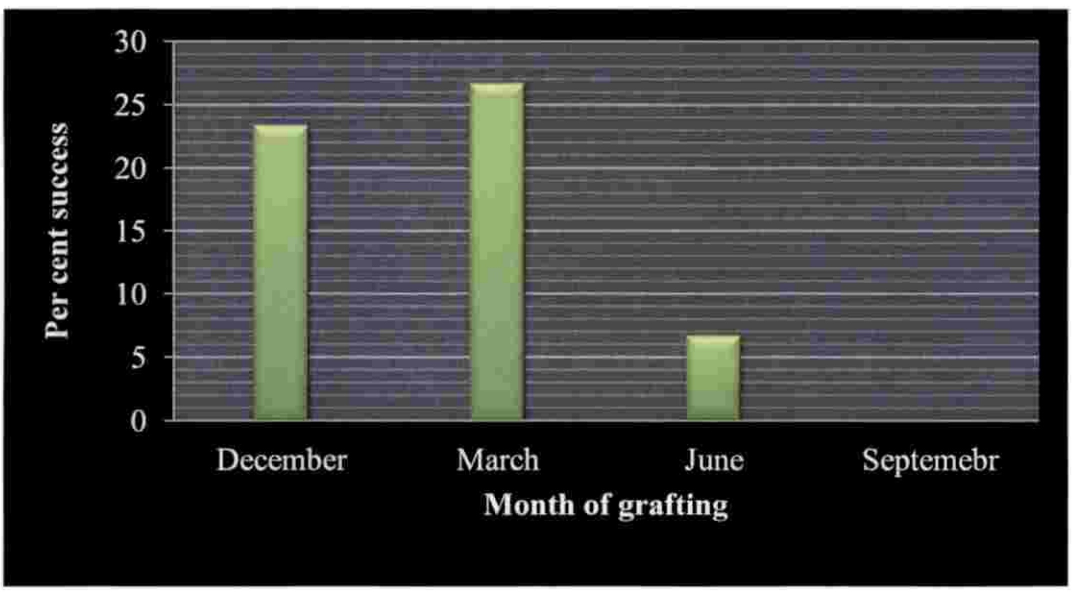

Figure 12. Success of grafting of *P. nigrum* (plageotropic shoots) on *P. arboreum*

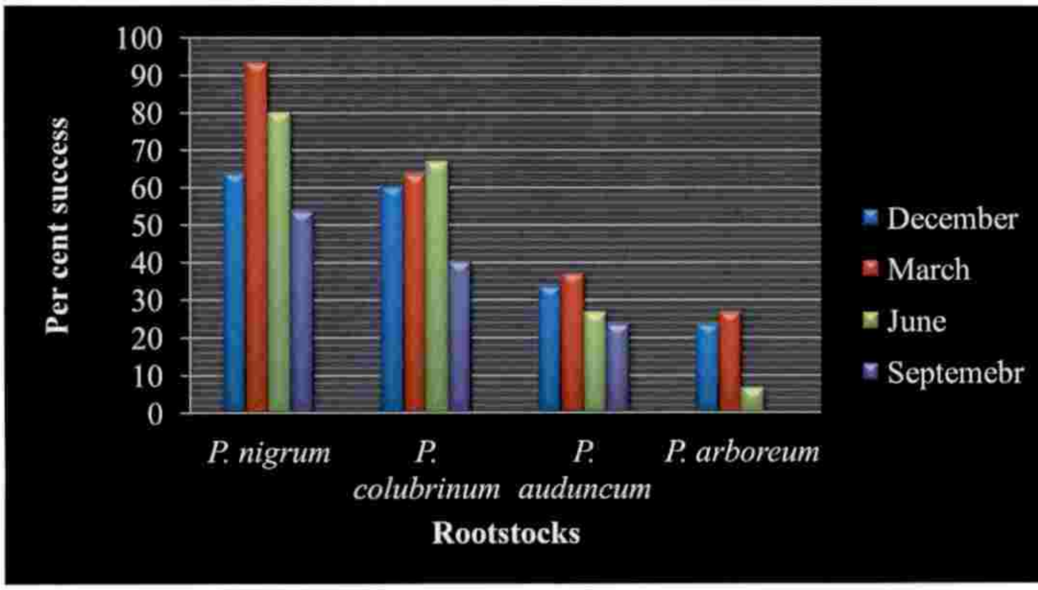

Figure 13. Effect of month of grafting on graft success (%)

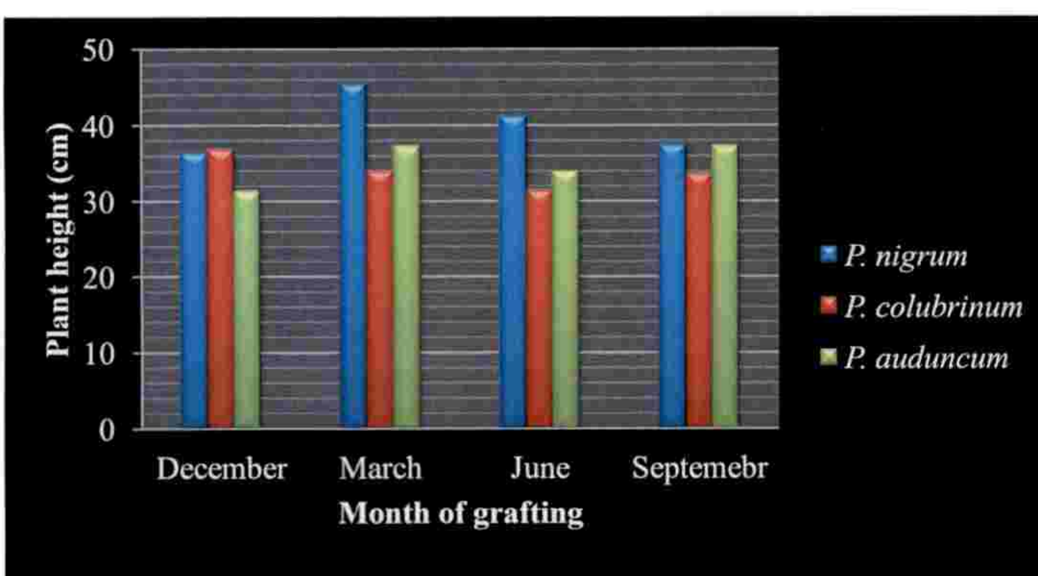

Figure 14. Effect of rootstock on plant height (cm)

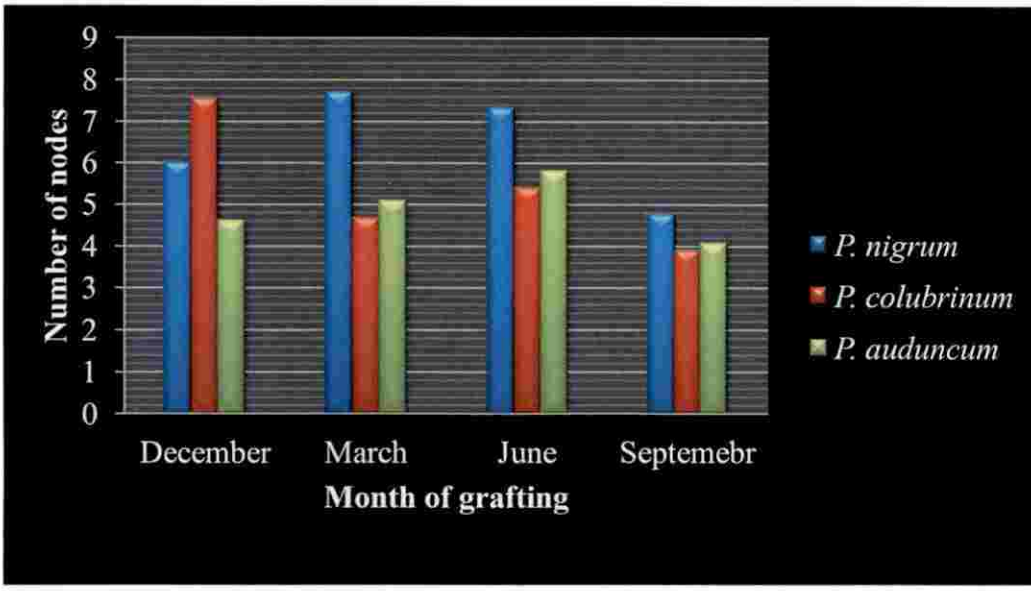

Figure 15. Effect of rootstocks on number of nodes

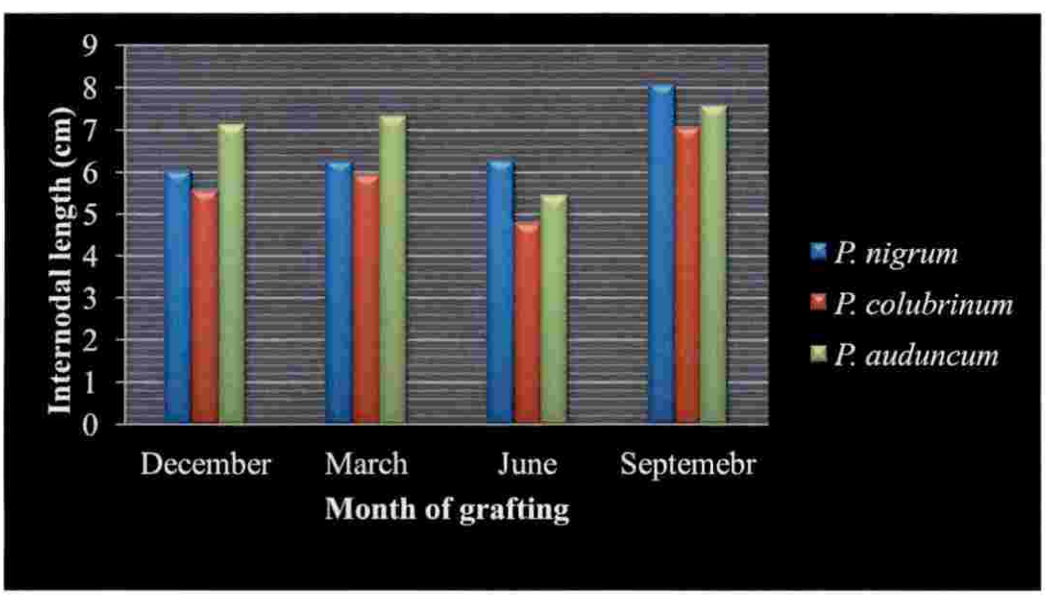

Figure 16. Effect of rootstocks on internodal length (cm)

Summary

6. SUMMARY

Black pepper (*Piper nigrum*) is an important spice crop. However, cultivation is affected by foot rot caused by *Phytophthora capsici*. South American *Piper* species are reported to be immune to *Phytophthora*. Since, South American species are not erasable with *P. nigrum*, grafting of *Phytophthora* susceptible *P. nigrum* on South American species of *Piper* is an effective way to bring foot rot under control. There are some earlier reports on grafting in *P. nigrum* using *P. colubrinum* as rootstock. But, a delayed incompatibility has been reported in such grafts. In this context, a study entitled, 'stock scion interaction in *Piper nigrum* L. grafts' was carried out at Pepper Research unit, Department of Plantation crops and Spices, College of Horticulture, Vellanikkara during 2017 December to 2019 March, so as to investigate the stock scion interaction in grafts of *P. nigrum* on different South American species of *Piper* and to standardize the best time of grafting.

Orthotropic as well as plageotropic shoot of *P. nigrum*, variety Panniyur 1 was grafted on three South American *Piper* species *viz.*, *P. colubrinum*, *P. auduncum* and *P. arboreum*. Grafting of *P. nigrum* on its own rootstock was also done to study the feasibility of such grafting. Grafting was performed during four different months *viz.*, December (2017), March (2018), June (2018) and September (2018). When orthotropic shoot of *P. nigrum* was used as scion, highest grafting success was recorded by the grafts of *P. nigrum* on its own rootstock followed by the grafts on *P. colubrinum*, *P. auduncum* and *P. arboreum*. *P. nigrum* grafts on its own rootstock showed 100 per cent success when the grafting was carried out during March. Highest per cent success recorded by the grafts on *P. colubrinum* was 96.66 (March). Grafts of *P. nigrum* on *P. auduncum* and *P. arboreum* showed a maximum graft success of 66.66 and 46.66 per cent respectively. Survival of *P. nigrum* grafts on *P. arboreum* was very limited. These grafts could not survive for more than three months after grafting. Irrespective of rootstocks, grafts of *P. nigrum* showed highest per cent success when the grafting was carried out during March. Hence the best time for grafting orthotropic shoot of *P. nigrum* was observed to be March. Although, grafting on *P. nigrum* showed highest per cent success, as far as growth of grafted plants (six month old) was concerned, grafts on *P. colubrinum* performed best with highest plant height (120.55 cm), number of nodes (18.91) and internodal length (6.32 cm).

When grafting of plageotropic shoot of *P. nigrum* was also carried out on different rootstocks, highest per cent success was recorded by the grafts of *P. nigrum* on its own rootstock (93.33%) followed by the grafts on *P. colubrinum* (66.66%), *P. auduncum* (36.66%) and *P. arboreum* (26.66%). When the seasonal effect on graft success was compared, it was observed that, grafts of plageotropic shoot of *P. nigrum* on *P. nigrum*, *P. auduncum* and *P. arboreum* recorded highest per cent success when grafting was performed during March. While, grafts on *P. colubrinum* showed the highest graft success when grafted during June.

As far as growth of grafts involving plageotropic shoot of *P. nigrum* on different rootstocks was concerned, at six month old stage, highest plant height was recorded by the grafts of *P. nigrum* on its own rootstock obtained during March (45.36 cm). Grafts on *P. nigrum* recorded highest number of nodes (7.69) and internodal length (8.06 cm) when grafted during March and September respectively. Branching and spiking was very limited in the grafts at six month old stage.

Anatomical investigations on different *Piper* species used as rootstocks were carried out to study the effect of anomalous structure of stem on success or failure of grafts. The most unique feature of genus *Piper* is the polycyclic arrangement of vascular bundles. In the present study, two rings of vascular bundles (cortical and medullary bundles) were observed in *P. nigrum*, *P. auduncum* and *P. arboreum*. While, in *P. colubrinum*, in addition to two rings of vascular bundles, a single medullary bundle was also observed at the centre. Number of vascular bundles varied from species to species. A central mucilage canal lined by secretary cells was visible only in case of *P. nigrum* stem. Compared to *P. nigrum* and *P. colubrinum*, a large pith region comprising of parenchyma cells was observed in *P. auduncum* and *P. arboreum*. In all the *Piper* species examined, during secondary thickening, cambial connection was limited to the cortical bundles only, while the medullary bundles remained as such without any cambial connection. However, they increased in size.

Transverse sections of graft union comprising of *P. nigrum* as scion and other *Piper* species as rootstocks was taken at different stages of development to understand the anatomical changes occurring during grafting and reasons for incompatibility developing if any. Transverse sections of three month old grafts on the rootstocks, *P. nigrum*, *P. colubrinum* and *P. auduncum*, showed a discontinuous isolation or necrotic layer at the graft union. Later, by six months after grafting, a gradual reduction of the same was observed. Reduction in the necrotic area was facilitated by callus. Complete union of wound portion including the cortical region was observed at six month old stage. Starch grain accumulation was abundantly observed in six month old grafts on *P. colubrinum*. A peculiar observation made in case of one year old grafts on *P. colubrinum* and *P. auduncum* was the development of a dense necrotic area at the graft interface alongside of scion. As earlier reports say, it acts as a barrier between stock and scion.

From the present study, it can be concluded that, both orthotropic and plageotropic shoots of *P. nigrum* can be successfully grafted on its own rootstock. Interspecific grafting is also possible in *P. nigrum* with *P. colubrinum* and *P. auduncum* as rootstocks. The best month identified for grafting both orthotropic as well plageotropic shoot of *P. nigrum* on *P. nigrum* and *P. auduncum* was March, while, June was the best month for grafting *P. nigrum* on *P. colubrinum*. Reasons for the development of dense necrotic area at the graft interface are not clear. More detailed studies on this aspect are needed to get a clear understanding.

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STOCK SCION INTERACTION IN *Piper nigrum* L. GRAFTS

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ABSTRACT OF THE THESIS

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ABSTRACT

Black Pepper (*Piper nigrum* L.) also called 'King of Spices' and 'Black Gold', is one of the most widely used spices in the world, occupying a position that is supreme and unique. India is one among the countries where black pepper is being widely cultivated. However our productivity is low compared to other pepper producing countries in the world. Among the various factors limiting the productivity of Indian pepper, a soil borne disease called foot rot caused by *Phytophthora* is of prime importance. *Piper nigrum* is highly susceptible to *Phytophthora* foot rot. It has been reported that some South American *Piper* spp. are immune to *Phytophthora*. Interspecific crossing between *Piper nigrum* and immune species has not been successful so far. Grafting on resistant rootstock is a method to escape soil borne inoculum.

The present study was conducted to assess the stock and scion interaction in grafts of *Piper nigrum* L. on different South American species of *Piper* immune to *Phytophthora* foot rot and to standardize the best time of grafting. The study was carried out as three experiments, *viz.,* grafting studies, anatomical studies on *Piper* species and anatomical studies on graft union at different stages of development.

Orthotropic as well as plageotropic shoots of *P. nigrum*, variety Panniyur 1 was grafted on three South American *Piper* species *viz.*, *P. colubrinum*, *P. auduncum* and *P. arboreum*. Grafting of *Piper nigrum* on the same species was also done to study the feasibility of such grafting. The trial was laid out in completely randomized design. When orthotropic shoot of *P. nigrum* was used as scion, highest grafting success was recorded by the grafts of *P. nigrum* on the same species (100%) followed by the grafts on *P. colubrinum* (96.66%), *P. auduncum* (66.66%) and *P. arboreum* (46.66%). Graft survival after one year of grafting was 90.00, 83.33 and 33.33 per cent for *P. nigrum/P. nigrum*, *P. nigrum/ P. colubrinum* and *P. nigrum/ P. auduncum* grafts respectively. Survival of *P. nigrum* grafts on *P. arboreum* was very limited. None of these grafts survived for more than three months after grafting. Irrespective of rootstocks, the best month

identified for grafting orthotropic shoot of *P. nigrum* was March. Although, self grafts showed highest per cent success in grafting, based on the growth of grafted plants (six month old), grafts on *P. colubrinum* was found to be the best with highest plant height (120.55 cm), number of nodes (18.91) and internodal length (6.32 cm).

The graft recovery was less when plageotropic shoot of *P. nigrum* was used as scion. The highest per cent success was recorded by the grafts of *P. nigrum* on the same species (93.33%) followed by the grafts on *P. colubrinum* (66.66%), *P. auduncum* (36.66%) and *P. arboreum* (26.66%). March was identified as the best month for grafting plageotropic shoot of *P. nigrum* on *P. nigrum*, *P. auduncum* and *P. arboreum*. However, grafts on *P. colubrinum* performed best when grafted during June. Graft survival after one year of grafting was 66.66, 30 and 16.66 per cent for *P. nigrum/P. nigrum*, *P. nigrum/ P. colubrinum* and *P. nigrum/ P. auduncum* grafts respectively. Survival of *P. nigrum* grafts on *P. arboreum* was very limited. As far as the growth of grafted plants was concerned, highest growth was shown by the grafts of *P. nigrum* on the same species.

In the anatomical studies of *Piper* spp. it was observed that, *Piper* species exhibited a peculiar arrangement of vascular bundles. Two rings of vascular bundles comprising of an outer, cortical and an inner, medullary bundles were observed in all the four *Piper* species examined. Mucilage canal was present only in *P. nigrum* while an additional medullary bundle was observed in *P. colubrinum*. Large pith region was the characteristic feature of *P. auduncum* and *P. arboreum*.

Examination of graft union at three, six and twelve months after grafting revealed that, graft union formation was completed by six months after grafting. One year old *P. nigrum/ P. colubrinum* grafts and *P. nigrum/ P. auduncum* grafts exhibited the formation of a dense necrotic area at the graft interface.

Based on the observations, it can be concluded that, due to the peculiar arrangement of vascular bundles in *Piper* species, matching of all the vascular bundles of stock and scion is difficult. However, complete union of parenchymatous tissues of stock and scion was formed within six months after grafting. But, a proper cambial connection between the grafting partners was not evident. Further studies need to be conducted to find out the reasons for the development of dense necrotic area at the graft interface.