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Mortality of air-layered plants of litchi (*Litchi chinensis*) and histopathology of affected roots in Bihar, India

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Litchi or lychee (*Litchi chinensis* Sonn.) is one of the most important subtropical evergreen fruit tree belonging to the family Sapindaceae. It is aptly known as "Queen of Fruits" owing to its attractive colour, taste and juicy aril. The top five litchi producing countries are China, India, Taiwan, Thailand, and Vietnam (FAO 2002). The acreage under litchi cultivation in India was 84,000 ha with a production of 585,000 tonnes during 2013-14 (NHB 2016). Major litchi producing states in India are Bihar, West Bengal, Assam and Jharkhand. Bihar contributes about 45% of total litchi production and has 40% of the acreage (Kumar *et al.* 2014a).

Litchi can be multiplied sexually but owing to disadvantages of seedling plants such as plants propagated from seed are not genetically the same as the parent plant, this is propagated through vegetative means. Of the various methods, air layering or marcotting is the most common and convenient method (Castro and Silveira 2003, Mitra 2004). Air layering involve induction of rooting of the branch, yet tied to the mother plants by means of a complete girdling involving removal of a strip of bark followed by wrapping the girdled region with moistened substrate. The girdling causes photo assimilates and hormones transported by the phloem to be retained in the region of the layers and shows increasing nutrient availability to new root formation (Hartmann *et al.* 2011). However, the major bottleneck associated with this method of propagation is the high mortality of layers after severing them from the mother plants and establishment in the nursery on their own root systems (Sharma *et al.* 1990, Syamal and Singh 1993). Hence, the objective of this study was to investigate the extent and aetiology of mortality of air-layered plants after its planting in polybags, and to examine histopathology of affected versus healthy roots of these plants.

The experiment was conducted during 2014-2015 at National Research Centre on Litchi (NRCL), Muzaffarpur (26° 05'87" N, 85°26'39" E, 47 m asl), Bihar, India. The air-layered samplings were detached from mother plants in the morning h in the 1st week of October and about 70% of their leaves were defoliated. After removal of polyethylene cover, these air-layers were dipped in Carbendazim (0.02%) solution for 2-3 min to avoid any pathogenic contamination. The potting mixture (media) for mass propagation of litchi identified at NRCL (Kumar *et al.* 2014b) was used for planting air layers in black nursery polybags (30 cm size, 200 µm thick). This consisted of riverbed soil + vermicompost in a ratio of 2:1 and vermiculite was added @ 50 g per 3 kg potting mixture. Sufficient quantity of potting mixture was prepared after thorough mixing (5-6 times) to fill about 750 numbers of polyethylene bags. Each polybag was filled with approximately 3 kg potting mixture. Half of the bags were filled with mixtures before planting and remaining half portion was filled after putting the litchi air-layers (cv. 'Shahi') in the middle portion and pressing from the sides. Plants were watered by garden water cans and kept in the net house having misting facility. These plants were arranged in six blocks each having 150 plants constituting three replications of 50 plants each. Misting was given 3-4 times daily for proper humidity inside the net house. Relative humidity inside net house was 70±5% with mean maximum temperature 28.5-32°C and the minimum 15.8-21.2°C during the growth period of potted plants. With the operation of misting facility inside net house a reduction in daily mean air temperature was about 1.0-1.5°C compared to outside conditions. After one month, NPK was added @5 g per plant in the form of diammonium phosphate, urea and muriate of potash in two split dosages (Kumar *et al.* 2014b). All due care was taken to raise healthy and vigorous saplings.Mortality of plants was recorded at 20 days interval up to 100 days.The data were analyzed using SAS® 9.2 statistical computing software and subjected to analysis of variance (ANOVA). The least significant differences (LSD) between means were computed at 5% significance level ($P < 0.05$).

Symptomatic plants were collected from nursery and isolation of fungal and bacterial plant pathogens were

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tried on potato dextrose agar (PDA) and nutrient agar (NA) medium, respectively from rhizosphere soil, roots and leaves. Isolations from symptomatic leaf and affected roots were made by surface-disinfesting small fragments of tissue in 0.5% NaOCl, double-rinsing in sterile water, and plating onto PDA or NA medium. Serial dilution technique was followed for isolation from rhizospheric soil. Dishes were incubated at 28±1°C for six days and axenic cultures were obtained. Pathogenicity test was carried out by soil inoculation (toothpick method by Keeling 1982) with the pure culture of isolated fungi. Plants were inoculated 7 days after planting by inserting toothpick tip overgrown with mycelia of *Fusarium solani.* Five toothpick per plant was inoculated near the rhizosphere of the plant.

To study histopathology of roots of air-layered plants, symptomatic and healthy plants of same age were brought to laboratory. The plastic of polybags were torn opened to get plants along with soil. Then they were dipped in a bucket filled with water to remove soil and obtain intact root system. Condition of roots were visually observed and photographed. Thereafter, root bits were taken and put between half-cut potatoes and thin slices were made by using a new stainless steel blade. These slices were dipped in water and tissue sections of roots were picked up by a fine hair brush onto a glass slide. A drop of lactophenol was put on the root section and a cover glass was placed over it. The slides were observed under brightfield of a Nikon Fluorescence Microscope (model Eclipse T*i*-5). Photomicrographs of anatomical structures were captured using a digital charge-coupled device (CCD) microscope camera (DS-Ri1).

Results of the studies on mortality of air layered plants revealed that the first conspicuous symptoms were mild drooping of young leaves followed by development of discoloured gray, water-soaked lesions. These lesions enlarged with progressive tissue necrosis and turned dark gray to black. Leaf lamina started folding from margins towards midrib. In advance stage, entire foliage wilted leaving behind blackened hanging leaves on stem that later dried off (Fig 1A). These symptoms were thus different from the typical symptoms of wilting caused by *Fusarium solani* in litchi that starts with yellowing of foliage and drooping of leaves leading to wilting of the plants (Kumar *et al.* 2011), without development of any water-soaked lesions. After planting of air-layers in polybags, initial roots developed were white, puffy or inflated having fine secondary roots with numerous root hairs, that in due course in healthy plants, degenerated after the development of fibrous root system. In the dying plants, however such fibrous root system did not develop (Fig. 1B and 1C) and plants might have starved of soil nutrients to support its growth.

The cumulative mean mortality of air-layered plants in six different nursery blocks varied significantly from 10.3–26.7%. The maximum mortality of plants was observed around 60 days of planting (Fig 2). A mean mortality of about 10% in transplanted air-layers of litchi was also observed by Chawla and Mehta (2015) in different growing media,viz. litchi orchard soil + FYM (1:1) + PGPR@ 50g/ kg, soil + FYM (1:1) + PGPR @50 g/kg and litchi orchard soil + coco peat + vermicompost.

Aetiology of mortality of air-layered plants revealed that though from rhizosphere soil samples of some of the dying plants, *Fusarium solani* was isolated but in pathogenicity test, typical symptoms of mortality were not reproduced. Also, there was no evidence of plugging of vascular bundle by fungal hyphae. No other pathogenic fungus or bacterium was isolated from roots and leaves of affected plant. Thus, primary reason for plant mortality, appeared to be the failure of plants to develop fibrous root system to mine soil nutrients. There might be exhaustion of reserve nutrients in air layered plants after 60 DAP in polybags. A detailed physiology of growth and establishment of air layers after planting in polybags need to be examined to get insight into the problem.

Studies of histopathology of roots showed that the

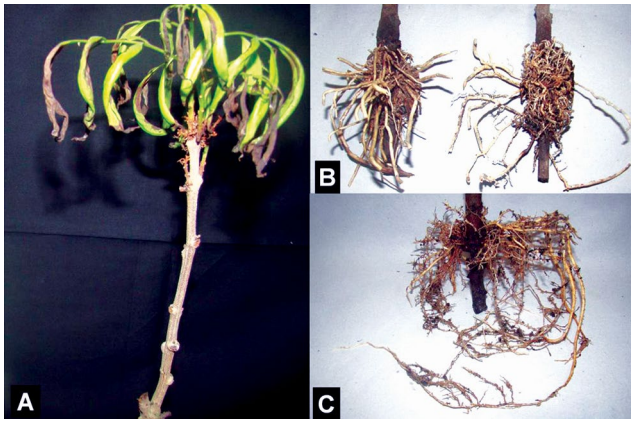

Fig 1 A. Symptoms on foliage prior to mortality of air-layered plants; B. Condition of initial roots developed after 60 days of planting in wilting/dying (Left) and healthy (Right) plants (note the initiation of fibrous root system in healthy plant); and C. Fibrous root system developed in healthy plant after 90 days of planting.

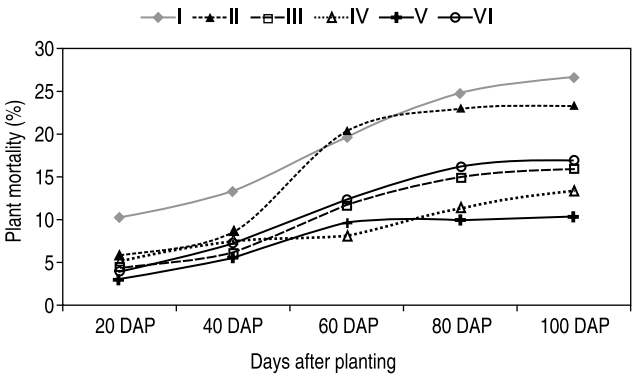

Fig 2 Mortality of litchi air-layered plants in different blocks (I-VI) of a nursery at NRCL, Muzaffarpur. The least significant difference (LSD)for mortality of air-layered plants ($P<0.05$) at 20, 40, 60, 80 and 100 days after planting (DAP) were 1.56, 0.96, 1.33, 1.24 and 1.47, respectively.

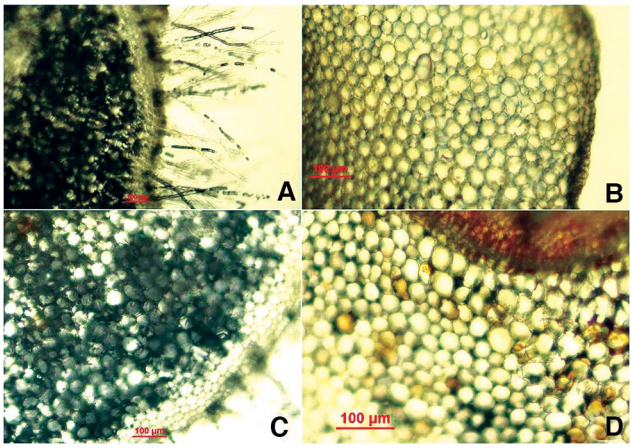

Fig 3 Transverse section of inflated root of diseased and healthy air-layered plants; A. Epidermis and root hairs of diseased root; B. Epidermis in healthy root lacking root hairs; C. Cortex of diseased root; D. Cortex in healthy roots.

initial roots of air-layered plants were inflated, puffy roots with fine root hairs. When transverse sections of the root from dying and healthy plants of same age were examined under microscope, marked differences were noticeable.The affected plant at the time of mortality (about 60DAP) had only inflated roots having root hairs that were bi-layered with rippled wall. At the verge of degeneration they were necrosed showing black discolouration. Surprisingly, no root hairs were found on inflated roots of healthy plants after 60 DAP (Fig 3). Difference in cortex region was also apparent. The cortical cells of healthy roots appeared as systematically arranged, white pebbles without any discolouration and were tightly packed in contrast to diseased roots which had black discolouration and cells were loosely packed. In healthy roots, endodermis were multi-layered with well-organized cells in vascular region while in diseased roots, endodermis was single layered with disorganized cells in vascular region.

SUMMARY

Mortality of layers after severing them from the mother plants and establishment in the nursery on their own root systems is a major constraint in producing large number of quality planting material. Studies were conducted to know the extent and aetiology of mortality of air-layered plants after its planting in polybags, and examined histopathology of affected versus healthy roots of these plants. The results showed that the maximum mortality of plants was around 60 DAP in polybags, the mean mortality being in the range of 10.3 to 26.7%. After 60 DAP, the inflated (puffy) roots failed to support growth of plants. Mortality of the plants occurred because there was no development of fibrous root system, coupled with possibly exhaustion of reserve nutrients in air-layered plants. Thus, it was conclusively proved that mortality of air-layers in initial stage was a growth related problem rather than of a pathogenic aetiology. Further physiological study of growth and establishment of air-layers after planting may be examined to have more insight into the problem that may improve survival rate of air-layers in nurseries.

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