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Structural and Biochemical Study of Apple (*Malus pumilo* L.) Bark Splitting Disorder in Balochistan

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Abstract: The structural and biochemical study of apple bark splitting (ABS) disorder was conducted in Balochistan. Anatomical results revealed that the cells of the phloem were crushed and large lysigenous air cavities were formed. The cells of the vascular cambium were also crushed there was no contact between the cells of the vascular cambium and functional phloem or young xylem cells. The air cavities were present throughout the bark extended until cambial layers. The exude which was oozed out of the bark was phloem sap and rich in sugar and also contained little amount of proteins. The chemical analysis of the exude had high contents of Na, K, Mg, Fe, Ni and Co in ABS infected trees as compared to the healthy trees. However, the Cu content was same in both trees and Zn content was higher in exudates of the healthy tree as compared to ABS infected trees.

Key words: *Malus pumilo* L., apple, apple bark splitting disorder (ABS), exude

INTRODUCTION

The apple (*Malus pumilo* L.) is the major fruit crop of Balochistan. Majority of the area under apple production in Pakistan (40,000 ha) is in Balochistan (33,000 ha). Apple bark splitting (ABS) is a common problem of apple trees in Balochistan particularly in Quetta, Pishin and Ziarat. This problem is a growing threat to and constraint on apple production in Balochistan[1-3].

Based on the field observation in Quetta, Mastung and Pishin by Gregly[2] it was concluded that ABS is a clearly defined syndrome, which is characterised by the following symptoms: An oily patch was appeared on the trunk and main branches. The sap causing the oily patch was clear and watery. No discernable deposit was left on the tree when the sap dried up. Oily patches were observed from June till November. No new patches were found from December till June. The splits were formed at the sites of oily patch and ranged from 25-75 mm in length and 4-8 mm in width. The splits were randomly distributed on the trunk and branches. They were longitudinally oriented, no horizontal split was found. The bark around the split was lifted from the underlying wood and the edges of the split were slightly ragged. The bark can be stripped off easily around the split and the wood underlying the split seemed to be normal but somewhat discoloured due to exposure to the open air. Root rot of both feeding and supporting roots was a common feature. The exude coming out of the bark is sweet. ABS causing leaf chlorosis, yellow vein clearing, radish brown necrosis, leading to defoliation and dieback of main branches. Restricted growth with small leaves and shortened internodes also occurred especially on dieback-affected branches. Affected trees may die suddenly during the hot season of July and August.

ABS is relatively new disease, which is lethal and is growing threat to the apple production in Quetta, Mastung, Pishin and Ziarat areas of Balochistan. There are two hypothesis proposed about ABS. First hypothesis was proposed by Geard[1] that, bark splitting was the result of disturbed cambial activity. According to him abnormal functioning of the cambium resulted in the lack of attachment of the bark to the underlying wood. The second hypothesis was also proposed by Geard[4]. According to this hypothesis aeration of the bark from the underlying wood was a result of extraordinary fluctuation in the degree of hydration of the xylem. This fluctuation was resulted from excessive water application; low transpiration and depleted root system reduced the water uptake capacity. Both hypotheses explained the ABS is a physical or physiological disorder not a pathological problem.

ABS infected samples of plant sap and tissue from the stem and root were analysed by electron microscopy, inoculation tests to virus, polymerase chain reaction, detection of plant prokaryotes, fungal and bacterial

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isolation did not show any evidence that ABS was caused by pathogens[3]. He suggested that ABS was caused by improper irrigation, lack of soil management, deep plantation, heavy clayey soil without proper aeration, water logging due to excessive irrigation during the period of fruit production and ripening. These practices lead to the low oxygen supply to roots, decrease in aerobic soil microorganism, nutrient deficiency and toxicity. A number of studies on the effect of over irrigation in trees has shown injuries to the plants[5-7]. Flooding also cause nutrients deficiency which results in an injury of the meristematic regions[5,8].

The aim of the present study was to find out the cause of ABS and find the possible control mechanism to this unique disorder of apple crop in Balochistan. In order to achieve the above objective the following study was performed: anatomical observation of healthy and infected bark and chemical analysis of the exudes of infected and healthy trees.

MATERIALS AND METHODS

The samples of the bark of apple (*Malus pumilo* L.) were collected from both healthy and ABS affected trees from Quetta, Pishin and Ziarat. The bark samples were collected from July to October 1998. The samples of the exudes were also collected from the same trees. For the exudes collection the oily bark and oily surface of the wood below the bark was removed and washed in distilled water. The samples of the exudes were concentrated and used for chemical analysis.

Anatomical studies: The samples of the healthy and affected bark were soften in HCL solutions of various concentrations. The soft samples were sectioned at the Forest Institute, University of Peshawar with the sliding microtome. The sections were stained with safranin and fast green and mounted in canada balsam. Stained sections were photographed using Olympus Polarizing microscope (Model BHSP) with Olympus photographic system (Model PM-10AD) attachment at the Centre of Excellence in Mineralogy, University of Balochistan, Quetta, Pakistan.

Protein assay of the exudes: The protein content of the exudes was estimated by using Biuret method. The 100 ml exude sample was extracted in 0.2 M Potassium phosphate buffer (pH 6.9), centrifuged at 3000 rpm for 15 min. and supernatant was used for protein assay. A routine assay contained 2 ml of protein extract, 2 ml of distilled water and 6 ml of biuret reagent (3 g of copper sulphate, 5 g of sodium potassium tartrate, 5 g of potassium iodide in 1,000 ml of 0.2 Sodium hydroxide solution). This mixture was incubated at 37°C for 10 min. Absorbance was measured at 520 nm. Bovine serum albumin (BSA) was used as protein standard.

Estimation of sugar content: For each assay 1 ml of the exude sample prepared for protein assay was mixed with 1 ml of 1% starch solution and incubated at 30°C for 3 min. Then 2 ml DNS reagent (1 g of 3,5-dinitrosalicylate, 6 g of sodium hydroxide in 1000 ml of aqueous solution) was added and incubated at 100°C for 5 minutes. Then 10 ml distilled water was added. The absorbance of the mixture was measured at 250 nm. Controls were prepared with the addition of starch solution. Two standards, containing 1 ml of 1% starch (substrate) and the other 1 ml of 0.1% maltose (product) were used.

Chemical analysis of the exude: Exude mixture (100 ml) was filtered through the filter paper (Whatman filter paper No. 1). Filtered samples were placed on hot plate for evaporation till the volume became 50 ml. These samples were placed in the oven for further drying then in furnace for complete drying. Dried samples were dissolved in 5 ml of HCl and made the volume 50 ml by several washing with HCl. Samples were processed for the analysis of various elements by Atomic Absorption Spectrophotometer model 2380 Perkinelmnmer U.S.A. Sodium and potassium were analysed by flame photometer PFP7 at PCSIR Labs, Quetta[9].

RESULTS AND DISCUSSION

Morphological studies: The apple bark splitting is a very common problem of apple crop in Balochistan. In the early stage of ABS disorder an oily patch appeared on the main trunk or branches (Fig. 1). This patch remains until the rainfalls. The number of oily patches increased with time. Then a vertical split in the bark was formed surrounded by

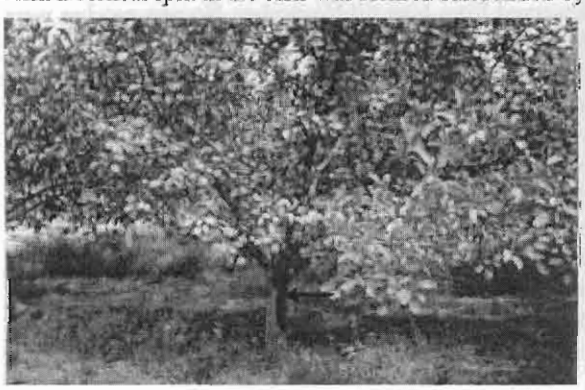

Fig. 1: An ABS infected apple tree showing oily patch

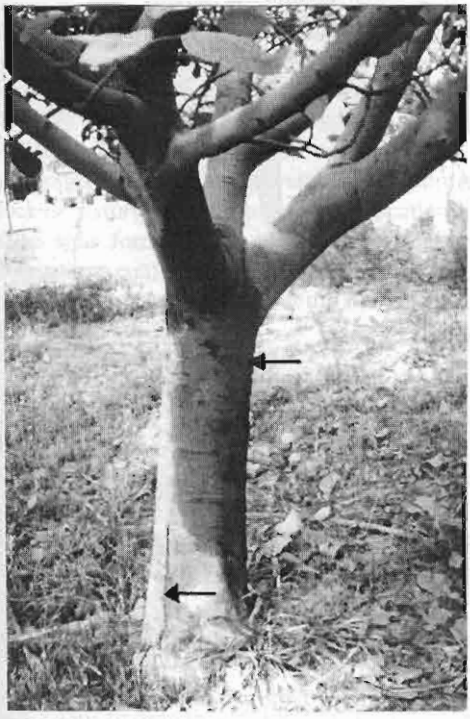

Fig. 2: An ABS infected tree showing oily patch and split in the bark

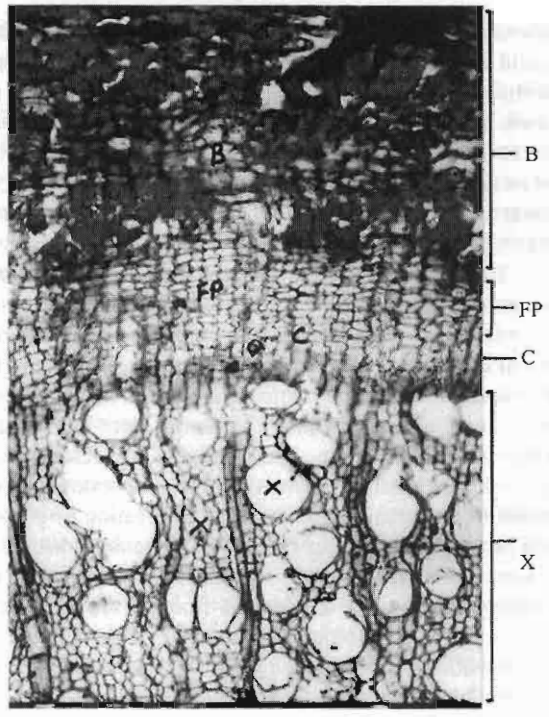

Fig. 4: Cross section of wood sample containing bark of healthy tree B: Bark, V: Vascular cambium, FP: Functional Phloem, X: xylem

Fig. 3: An ABS infected dead apple tree having no leaves and fruit

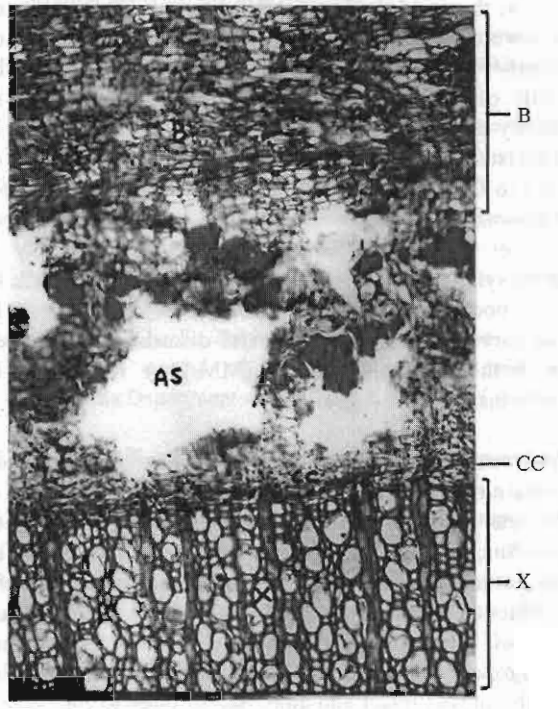

Fig. 5: Cross section of wood sample containing bark of ABS infected tree. AS: Air spaces, B: Bark, CC: Crushed cambium, X: xylem

oily exude (Fig. 2). The bark at the split became loose and could be removed easily. Further damage of the tree lead to the senescence and abscission of leaves and finally the death of the tree (Fig. 3). Roots of the affected tree were rotten and had poor structure. The morphological observations in our study were agreed with the observation of the earlier workers[1-3] who had reported that ABS is a lethal problem.

The oily substance was probably the phloem sap, which exuded out due to some physical or physiological disorder of the bark. The splits were more obvious during the month of July till October. The exude leaks out in the hot season when the temperature reaches or exceeds 40°C in Quetta and Pishin. The cracks were increased in number with the time. From August to October high diurnal temperature fluctuation may be exerted some physiological disturbance in the translocation of phloem sap from the leaves to the roots. Because during this season when fruits were set and near to ripened the photosynthates were transported from leaves to roots[10]. Due to this physiological state the large amount of photosynthates were in the phloem of branches and trunk and any blockage in the transporting stream can cause the bark to split and release the exude out. Along the bark splitting, at the later stage of ABS disorder the leaves were senesced and abscised. The infected tree died within 2 to 4 years from the beginning of ABS.

In the early stages of ABS the roots of the infected tree were normal but later on the roots were rotten which Gregly[2] and Dabek[3] observed. They suggested that the death of the roots was probably as a result of lake of supply of photosynthates from the leaves to the root. In the case of over irrigation of garden during the months of July to October also reduces the aeration in the soil for the roots. The stem borers attacked on the infected trees at later stage of ABS disorder. The lake of supply of photosynthates to roots poor soil aeration and attack of stem borers may cause the death of an infected tree. In our survey it was found that ABS disorder was observed in both local and clonel (MM.111) rootstocks in Balochistan.

Anatomical studies: The cross section of wood containing bark of the healthy tree is shown in Fig. 4. At the periphery bark was present and consisted of non-functional phloem cells having crushed sieve cells, ray parenchyma and pockets of sclerenchyma. The cells of functional phloem present next to the vascular cambium had few layers of cells. All the cells of this region were hexagonal in shape and arranged in rows. The vascular cambium was intact and consisted of three to four layers of cells. Inside the vascular cambium xylem was composed of vessels, xylem parenchyma and ray parenchyma cells. The type of the wood was diffused porous. The

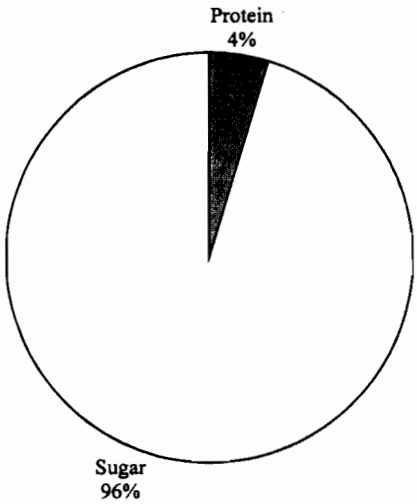

Fig. 6: Total sugar and protein contents in the exude of ABS infected apple tree

anatomical study was in consistent with the morphological observations.

The cross section of the wood from ABS affected area is shown in Fig. 5. The bark was consisted of functional and non-functional phloem cells. Functional phloem cells were not intact with the vascular cambium. Lysigenous air spaces were observed in the region of functional phloem. The cells of the vascular cambium and functional phloem were degraded. Ray cells were also degraded. The cells of the vascular cambium were also crushed. There were large air cavities present in the region of non-functional and functional phloem. These air cavities were formed by the lysis of cells of the phloem. These cells were crushed and finally the splits formed started from cells of the vascular cambium through the young phloem to the older phloem.

The fuller rose beetle problem was very common in the citrus fruits in California[11]. In order to block the Fuller Rose beetle movement from the ground to the fruits the oil based stickum mixed with various coloured paints was applied on the bark of trunk in which the beetles were captured and were unable to damage the fruit. Due to the over heating at the site of stickum application the bark was split on the sunny side of the trunk during summer[11]. In the cross section of the bark, crushed phloem cells were observed the cells of vascular cambium were also broken and the vessels of the wood were also compressed at the site of split[11]. The bark split of the citrus lead to the death of the severely infected trees. In this case the splitting was mainly due to over heating of the bark during summer on the sunny side of the trunk.

Similarly in present study, the oily exudes on the tree trunk and branches may heat up the surface of the bark during hot months of June, July and August. Due to the

temperature difference inside and outside the bark during summer may formed a split in the bark of tree. During this period the fruits were ripening and the new leaf formation was very little. Therefore, the bulk of the phloem sap containing photpsynthates was transported to the roots for storage. If there is any physiological disorder that will block or disturb the path of sap translocation may result in the split formation in the bark. Present anatomical observations confirmed the damage of functional phloem cells as well as cambium cells at the site of split in the bark of tree.

Sugar and protein estimation of the exude: The exude of the phloem was mainly rich in carbohydrates. The predominant sugar was sucrose as the transporting sugar in majority of plants[12]. In addition to sucrose many other metabolites were present in the phloem, such as sugar phosphates, organic acids, nucleotide phosphates, amino acids, enzymes, ions, hormones and exogenous chemicals[13,14].

The exude of the ABS infected tree was also rich in sugar content. The total sugar content was 96% and total protein content was 04%. These results were agreed with the previous finding that major translocates of the phloem were sugar and little amount of protein, the enzymes[13,15]. The exude of the ABS infected tree was the phloem sap.

Chemical analyses of exude: A difference was found in the concentration of various elements between the infected trees as compared to the normal trees. The contents of Ca, Mg, Fe, Mn, Ni, Na and K were higher in the exude of infected trees, while Cu content was same in both and Zn content was higher in the exude of healthy tree.

The zinc deficiency include little leaf and rosette of apples, peaches and pecans, resulting from growth reduction of young leaves and stem internodes[5] this retardation of growth might result partly from its requirements for the production of growth hormone, indol acetic acid (IAA) and partly its association with number of enzymes[16]. In the present results the lower amount of Zn in the exude of an infected tree might be the cause of reduction in leaf and shoot growth.

It has been reported that copper deficiency caused rolling of leaves "die back" and "exauthema" of fruit trees in which leaves wither fall and bark becomes rough and splits, exuding gummy substances[10]. We were unable to find the copper deficiency in the exudes obtained from ABS infected trees. Some elements readily move through the phloem from older leaves to younger ones and then to storage organs. These elements include N, P, K Mg and Cl. Others such as B, Fe and Ca are much less mobile and the mobility of S, Zn, Mn, Cu and Mo usually intermediate[5]. It was observed that the phloem exude were rich in most of the essential elements which were translocated from shoot to root.

In the present study it was unable to find that deficiency of any essential nutrient is the cause of ABS. However, over irrigation of orchard at the time of fruit set may cause poor aeration of roots, blocks the supply of photpsynthates to roots and diurnal temperature fluctuation may cause bark splitting and finally the death of infected tree.

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