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Studies on Micro Propagation of Jackfruit
2-A comparative Histological Studies on *in vitro* and *ex vitro* Plants of Jackfruit

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Abstract: An *in vitro* and *ex vitro* plants produced of the first part in this trial were used as a source of the histological samples, the histological studies were under taken for comparing between the anatomical structures of *in vitro* root and shoot and *in vivo* root and shoot; study the initial tissues of *in vitro* root and shoot as affected by the studied growth regulators (BA, Kinetin, NAA, IBA, coumarin and paclobutrazol) in MS and half strength of MS media; and study the relationship between these anatomical features and the successful micropropagation of jackfruit. The old roots of jackfruit seedlings were lacked of developed vascular system, which cause the snap of the root or the breakable roots and decrease of the root's nutrition supply and hence decrease the root growth and cause some problems and death of seedlings. Media contained the paclobutrazol increased an *in vitro* root diameter or thickness by increasing the cortex layers, also the *in vitro* roots seems to be initiated in the internal tissue (compact thick-walled parenchymatous cells) of the *in vitro* callus that originated from the middle thin-walled parenchymatous cells of the *in vitro* stem base cortex. The *in vitro* shoots (shootlets) initiated in the inner tissue of this basal callus as a results of culture on MS medium+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. adenine sulphate, the uniseriate trichomes originated by lateral extension of the outer walls of the epidermis cells. Chlorenchyma and collenchyma cells were noticed in the whole studied jackfruit plant tissues, as they act as a conjunctive tissues, besides the storage cells were found. The structures of an *in vitro* stem and leaf are lack of cuticle and trichomes, which have many functions in controlling of evapotranspiration, water absorption and temperature and hence decrease the successful plantlet's percentage of the jackfruit during the acclimatization and may be cause the vitrification beside some growth regulators (NAA). Buds initiated from the cortex of seedling, the *ex vitro* stem and leaf have a good cuticle, trichomes, conjunctive and flexible tissues (fibres) compared to an *in vitro* stem and leaf which make the plant tolerate the dryness and diseases.

Key words: Histological features . Stem and root jackfruit . *In vitro* and *ex vitro* . *Artocarpus heterophyllus*

INTRODUCTION

The jackfruit, *Artocarpus heterophyllus* lam, of the family Moraceae. It is found a lack knowledge about the jackfruit histological features in all studies. So, the goals of this study were compared between the anatomical structure of *in vitro* shoot (shootlet) and *in vivo* shoot (acclimatized shoot), *in vitro* root and *in vivo* root and study the original or initial tissues of *in vitro* root and shootlet, as all the previous studies lead to explain and illustrate the anatomical structure development of jackfruit's shoot and root during *in vitro* and acclimatization stages, which could be help to control and raise the success percentage of jackfruit's micro-propagation, specially in the acclimatization by practice some applied or practical procedures.

Also, explain the relationship between some anatomical features of an *in vivo* shoots and roots (acclimatized plantlets) and success of the acclimatization of micropropagated plantlets.

Ziv *et al.* [1] indicated that growth retardants increased rapid growth, shortened stems, inhibited leaf expansion and formation of clusters. Hazarika [2] reported that paclobutrazol (0.5-4 mg/L.) in the rooting medium reduced stomatal apertures and wilting after transfer to compost, increased epicuticular wax, shortened stems, chlorophyll content and thickened roots. Chen and Ziv [3] said that plant growth retardants affect cell division and cell enlargement, probably by interfering with gibberellin biosynthesis.

MATERIALS AND METHODS

The plant materials were an *in vitro* samples took from plantlets grew on MS media with BA, NAA and kinetin during the callus and multiplication

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stages, while MS or half-strength of MS media with NAA,IBA,coumarin and paclobutrazol were used in the rooting stage. Also,an *ex vitro* samples took from the plantlets grew into the growing media of sand and soil,sand and peat,and soil,burned rice hull and fibrous sheath of date palm (as mentioned in the first part of this trial). At the end of the rooting experiment, *in vitro* root segments, shootlet segments, *in vitro* root emergence area (original or initial tissue of the root in *in vitro* mass) and shootlet's emergence area (initial tissue of the shootlet in *in vitro* mass) have taken as *in vitro* samples for the anatomical studies, also *in vivo* or acclimatized shoots and roots segments have taken at end of the acclimatization experiment as *in vivo* or acclimatization samples for the anatomical studies, samples were killed and fixed in 70% FAA, stored in 70% ethanol, softened a minimum of 2 weeks in glycerol-alcohol solution. Transverse sections have taken at 25 and 50 μ using a hand-fed sliding microtome, the most clearness sections were double stained with safranine-fast green, mounted in Canada Balsam [4, 5], microscopically examined and photographed on Kodak colour film, investigation and discuss the comparison of *in vitro* roots with *in vivo* roots and shootlets with *in vivo* shoots, also explain and illustrate the original or initial tissue of an *in vitro* roots and shootlets.

Besides, explain the relationship between some anatomical features (trichomes) of an *in vivo* roots and shoots and success of the acclimatization of an *in vitro* plantlets.

RESULTS AND DISCUSSIONS

The shoot and stem structure: Figure 1 showed the following transverse sections

- *In vitro* stem comprised of 2 layers of compact rectangular cells formed the epidermis carried some feeble uniseriate trichomes; cortex consisted

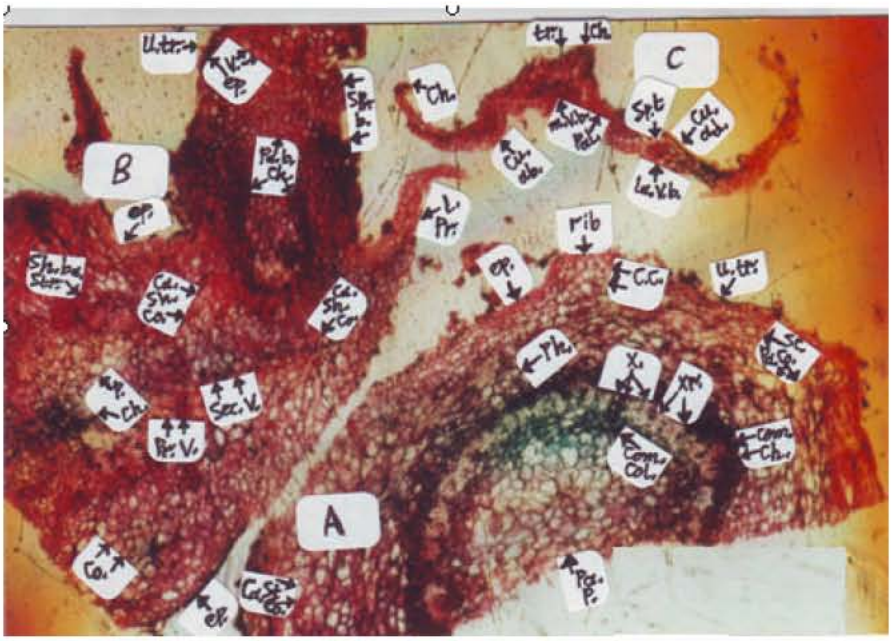

Fig. 1: Transverse sections of *in vitro* stem (A); new *in vitro* shoot initiated from the basal callus of *in vitro* stem cultured on MS+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. adenine sulphate (B); and an *in vitro* leaf (c) of *Artocarpus heterophyllus* Lam. (X 52). Details: section (A): rib., rib; ep., epidermis (2 layers); C.C., compact parenchymatous cells; u.tr., uniseriate trichome; S.C., secretory canal; Pa. Co., parenchyma of the cortex; ph., phloem; x., xylem; Xr., xylem rays; com. ch., companion chlorenchyma; com. col., companion collenchyma; Pa. P., parenchymatous cells of the pith; and Ca. St. Co., callus of the stem cortex (parenchyma). Section (B): U. tr., uniseriate trichome; V., vessels; ep., epidermis; Spr. B., sprouted bud; Pa. b., parenchyma of the bud; Ch. Chlorenchyma; Ca. sh. Co., callus of the shoot cortex ; l.pr., leaf primordium; Sec. V., secondary vessels; Pr. v., primary vessels; P. pith; Co., cortex; and Sh. Ba. Str., shoot base structure. Section (c): Cu. ad., cuticle of adaxial side of the leaf (one layer); la.v. b., lateral vascular bundle; Sp. t., spongy tissue; m. v. b., middle vascular bundle; pal., palisade (chlorophyll parenchyma); tr., trichome; Ch., chlorenchyma; and cu. ab., cuticle of abaxial side of the leaf (one layer)

of a parenchymatous cells that concentrated and thickened at the ribs of the stem and compacted under directly the epidermis layers, besides, the callus tissue originated from the middle thin -walled parenchymatous cells of the cortex; the vascular cylinders are arranged in a circle beneath the cortex and comprised of phloem adjacent to the cortex and xylem alternating with a similar number of xylem rays, the vascular cylinders externally accompanied by a circular compact chlorenchyma cells and internally accompanied by a circular compact collenchyma cells that externally limited the central pith comprised of an irregular parenchymatous cells with irregular intercellular spaces.

- Basal callus of *in vitro* stem showed a regenerated *in vitro* shoot initiated from this basal callus originated from an *in vitro* stem cortex, as a result of culture on medium of MS+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L Kinetin+25 mg/L. adenine sulphate. An *in vitro* shoot seems to be initiated from the inner tissue of the callus; and the new shoot structure comprised of epidermis with one layer and carried some small uniseriate trichomes originated by lateral extension of their outer walls, centric vascular bundles with a clear xylem vessels, the pith is absent at this stage, chlorenchyma bundles at the base of the new shoot, as they may be act a conjunctive tissues, some leaf primordia and the main parenchyma tissue. This section, also showed an *in vitro* shoot base structure, as previously described beside appearance of the secondary vessels in the section.
- An *in vitro* leaf comprised of one layer of cuticle on both adaxial and abaxial sides of the leaf, chlorenchyma cells occupied the ribs of the surface, little amount of feeble small trichomes, few of stomata, palisade tissue, spongy tissue and vascular bundles.

Figure 2 showed the transverse section of the stem rib of jackfruit seedlings. The section comprised of the leaf structure and the rib stem structure. Firstly, the structure of the leaf are the cuticle on the adaxial and abaxial sides, compact epidermis with 2 layers, spongy tissue of chlorenchyma loosely arranged cells, palisade tissue of a parenchymatous cells with chlorophyll and the leaf base with a conjunctive tissues of both chlorenchyma and collenchyma cells. Secondary, the rib stem structure comprised of cuticle; epidermis of 2

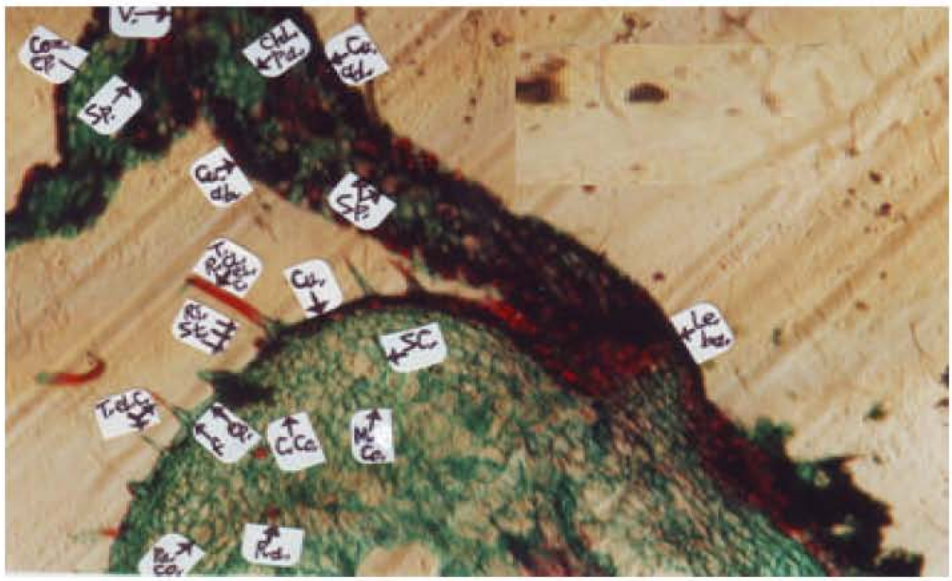

Fig. 2: Transverse section of the stem rib of *Artocarpus heterophyllus* lam. seedlings at the age of 2 years. Notice: the primary structure of the leave; uniseriate type of the trichomes at different growth stages and the structure of the stem rib (X144). Details: Com. ep., compact epidermis (2 layers); Sp., Spongy tissues (chlorenchymatous loosely arranged cells); V., vessels; Chl. Pa., chlorophyll parenchyma cells (palisade tissue); Cu, ad., Cuticle of adaxial side of the leaf; Cu.ab., cuticle of abaxial side of the leaf; le. ba., leaf base; Cu., cuticle; Ra., Racine; sc., secretory canal; Ri. st., Rib of the stem; Pa. Co., parenchyma cells of the cortex; M. co., Middle cortex (parenchymatous cells); C. co., compact cortex (collenchymatous cells); T. el. C., Terminal elongated cells of the trichomes; f., foot of the trichome (2 cells in thichness); ep., epidermis of the stem.; and T.R.cl.el.c., Terminal red colored elongated cells of the trichome

Fig. 3: Magnified portion of the transverse section of Fig. 1B (X 144): Notice: the new *in vitro* shoot initiated from the callus originated from cortex of *in vitro* shoot base. Details: Co., cortex; ep. epidermis; spr. bud, sprouted bud; Ch., chlorenchyma; Col., collenchyma; v., vessels; u. tr., uniseriate trichome; and Ca. sh. ba., callus of *in vitro* shoot base

layers; trichomes originated from the outer layer of the epidermis cells by lateral extension of their outer walls, the trichome comprised of a terminal long cell that turned into a red color and foot with two cells in thickness; the outer cortex layers lay beneath the epidermis and consists of a compact collenchyma cells, the middle and inner cortex layers with their parenchema cells, racine and secretory canals inside the cortex. This structure indicated that the stem and the leaf have a bundant amount of cuticle, trichomes and other tissues, which giving many benefits for the plant, i.e., resist or tolerate to the dryness, defect of the irrigation and against the diseases.

Figure 3 illustrated a magnified portion of the Fig. 1B, which showed the inner tissue of the callus, which consider the origin of the new shoot. Also, it showed the structure of new *in vitro* shoot; i.e., epidermis with one layer, cortex of a parenchyma cells, chlorenchyma bundles, vessels and collenchyma cells as a conjunctive tissues. An *in vitro* stem and shoot structures showed less amount of a conjunctive tissues, cuticle, vessels and trichomes; and increase in the cortex and pith percentages compared to an *ex vitro* structure, that reflected a feeble structure, less water and nutrients absorption and increase in the water loss, which lead to failure in their acclimatization. Also, the lack of cuticle may be cause an vitrification phenomenon of *in vitro* shootlets.

This structure of an *in vitro* stem and leaf seems to be lack of cuticle and trichomes, which decrease the evapotranspiration, increase the water absorption, modification of the temperature loss and protect the plantlets against the diseases [6,7], hence, these few feeble trichomes could not do the previous functions of the *ex vitro* trichomes and consequently causes an decrease in success percentage of an *in vitro* plantlets of jackfruit during the acclimatization stage.

Figure 4 illustrated that the transverse section of the bud primordium of jackfruit initiated from the cortex layers lay under the epidermis, the bud primordium consisted of some of the leaf primordia, external tunica and corpus tissues. Also, the transverse section of the main stem of jackfruit seedlings at 2 years old, showed the structure of the stem comprised of thick cuticular layer, epidermis of one layer; outer cortex of a compact collenchyma cells, middle cortex of a parenchymatous cells, inner cortex of a compact collenchyma cells; racine, secretory canals and tannins inside the cortex; vascular circles at the inner cortex and at near the centre, which comprised of secondary phloem and a secondary xylem, accompanied by a large amount of alternated fibres bundles and lignified cell

Fig. 4: Transverse section of the main stem of *Artocapus heterophllus* lam. seedlings at the age of 2 years. Notice: bud primordia; the vascular bundles arranged in a ring and the concentric bundles developed in the pith. (X 52)

Details:B. Pr., Bud primordia; L. Pr., leaf primordi; Tu., Tunica; Cor, Corpus; Cu., cuticle; EP., Epidermis; Gr., Groove; T., Tanins; O.C.Co., Outer compact cortex (collenchyma); M. P. Co., Medial parenchyma cortex (collenchyma); i.c.co., inner compact cortex; S. C., Secretory canal; Ra., Racine; F. b., Fibrous bundles; Se.ph., Secondary phloem; Li. ci. ce. r., Lignified circular cell rays; Se.x., Secondary xylem; St. R., Stele ringe; Con. b., concentric bundles and C. p., Compact pith (chlorenchyma)

rays; the pith consists of a compact chlorenchyma cells. From the previous structure, it was noticed that the bud primordium was lack of a vascular connection, which cause a failure of growth of many buds forming on the jackfruit stem, hence the little branches are characteristic of the jackfruit seedlings. Besides, the stem section filled with a numerous conjunctive and flexible tissues in a bundle forms, which giving a force and flexibility for the stem to resist the break. Also, the numerous vascular cylinders permit with a good absorption and movement of both water and nutrients, but this structure face to the structure of the roots that are lack of a bundant vascular tissues, as the root structure may be consider the most important problem that cause the death of jackfruit seedlings or at least, the weak growth of the seedlings.

These results were in parallel with those of Ramamurthy and Savithrama [8], which decided that Cassia meristemoids located at the sub epidermal level were only able to differentiate into shoots. Also, Tetsumura and Yukinaga [9] said that the origin of the adventitious shoots was the pericycle of persimmon explants. Besides, Zhu and Welander [10] reported that meristem-like structure, which develop into *in vitro* shoots arose from surface of callus of pear *in vitro* produced leaves. Also, Paiva-Neto *et al.* [11] said that Cytokinin Zeatin-induced meristemoids originated mainly from wounding tissues of Annatto and thidiazuron (TDZ) induced a high level of mitotic division resulting in several proliferation zones nearby the epidermis and outer cortical tissues, although calli occurred at the basal end of shoots; Brutti *et al.* [6] elucidated that poor development of trichomes on *in vitro* leaves considered to be one of the major causes for high rate of transpiration leading to low rate of survival micropropagated plants; Agren-Schemske [7] decided that the function of trichome in light piping, alter heat loss and aid in reducing water loss through transpiration, also, the trichomes protect against herbivory, pathogens and act in storage and secretion of secondary metabolites; Perez-Estrada *et al.* [12] reported that the trichomes play an important roles in management of water content of the micropropagated plants; and finally, Bandyopadhyay *et al.* [13] suggested that three types of trichomes, viz., slender uniseriate, slender multiseriate and stellate, were found in *in vivo* leaves only, while, the small, stalk, globose and glandular trichomes were in *in vitro* leaves, significant positive correlation was observed between water content and leaf area covered with trichomes in both *in vitro* and *in vivo* leaves.

Fig. 5: Transverse section of the growing root of *Artocarpus heterophyllus* lam. seedlings at the age of 2 years. Notice: a numerous root hairs; peripheral arrangement of the vessels and the storage cells. (X144). Details: rh., root hair; Li. Pa. r., lignified parenchymatous rays (conjunctive tissues); Pe., periderm; Per; L., pericyclic layers; x., xylem; C. com. Pa., concentric companion parenchymatous cells; Com. F., companion fibres; ph., phloem; St. C., storage cells; Com. Pa., companion paranchymatous cells and Ca., cambium

The root structure: The transverse section of the growing root of jackfruit seedlings with 2 years old (Fig. 5), shows the main tissue systems; i.e., a-the outer tissue comprised of the periderm that consists of a single layer of thick-walled cells with a prominent cuticle layer and having a potentiality to firm abundant small root hairs by a lateral extension of their outer walls, besides, these small root hairs may be also originated from the underlying layer of the pericycle followed by the periderm layer. This periderm layer seems to be consider the external layer of the pericyle layers, which consists of a small size cells having the potentiality to divide. b-The internal tissue comprised of a large amount of thin-walled parenchymatous cells that are limited externally by the pericyle layers.

These parenchymatous cells with a triangular intercellular spaces, seems to be initiated from the inner layers of the pericyle and constitutes the most outer layers of a little amount of the vascular cylinders, which compost of a phloem, little of secondary phloem, xylem, little of metaxylem and a little of 1-2 layer of cambium. The vascular cylinders accompanied by a large amount of thick-walled parenchymatous cell rays reached to the pith, that occupy by a compact thick-walled parenchymatous cells and some of the storage cells.

From the previous description, it could be noticed that the old root of jackfruit seedlings was lacked of developed vascular system, as illustrated in a little amount of secondary vascular tissues, which cause the snap of the roots or the breakable roots, in spite of the presence of the conjunctive tissues of a moderate amount of a thick-walled parenchymatous cells. These roots lacked of the vascular cylinders did not affected the water absorbion because of these roots is formed abundant small root hairs; but the lack of the vascular cylinders, especially, the phloem may be cause decrease of the root's nutritious supply, hence decrease the root growth.

This observation could be fortified by the presence of the storage cells in the root section. Also, the presence of unsuffient peridermal layer may be one of the causes of the root infected by some soil diseases, as it found in this trial that some of the jackfruit seedlings infected with root rot and fusarium, that may be cause the dieback. Also, the old root almostly free of the secratory canals.

Figure 6 illustrated that the transverse section of an *in vitro* callus at *in vitro* shoot base cultured on ½ MS+2 mg/L NAA+3 mg/L IBA+1 mg/L coumarin+1 mg/L pacloburazol. The section showed that the main tissue of an *in vitro* callus of jackfruit, comprised mainly of the compact thick-walled parenchymatous

Fig. 6: Transverse section through *in vitro* callus of *in vitro* shoot base cultured on ½ MS+2mg/L NAA+3 mg/L IBA+1mg/L. coumarin+1mg/L. paclobutrazol and through the regenerated root of jackfruit (X 144). Notice: an *in vitro* root primordium originated from the inner parenchyma of the callus and structure of the new root (right). Details: O.col. L., outer collenchyma layers (brown cover); I.Pa. tis., inner parenchyma tissue; r. pr., root primordium; St. ca. sh. ba., structure of callus of *in vitro* shoot base; Ne. r. st., new root structure; ep., epidermis; co, cortex, pe. L., pericyclic layers; pr. x., primary xylem; and Pr. ph., primary phloem

cells (internal tissues) surrounded by a circular tissue of collenchyma layers (external tissues). The root primordia seems to be originated from the internal tissues of the callus. The primary structure of *in vitro* roots consists of (Fig. 6) a ruptured epidermis with one layer of thick-walled parenchymatous cells; an developed cortex comprised of a small rectangular parenchymatous cells with thin walls and small intercellular spaces; 1-2 layer of pericycle cells, that seems to be produce the cortex towards the outside and produce a narrow vascular system towards the inside; the primary vascular system is more compact and the stele is composed two phloem strands, accompanied by a few amount of parenchyma cells, beside the xylem ridge comprised of some 2 vessels occupied the root centre forming a solid core and the pith is absent, hence, the stele is regarded as a proptostele. Also, the used treatment included paclobutrazol may be increase the root diameter or thickness, by increase the size of cortex layers as shown in this figure in an *in vitro* regenerated root structure.

These results were in agreement with those of Hazarika [2] who reported that paclobutrazol in the rooting medium increased epicuticular wax and thickened roots. Besides, Chen and Ziv [3] revealed that plant growth retardants affect cell division and cell enlargement, probably by interfering with gibberellin biosynthesis. In the parallel, El-Bahr *et al.* [14] decided that rootlets of *ex vitro* date palm appeared thicker in cross-sections than *in vitro* plantlets; such increase was allowed to the largest area occupied by exodermis, hypodermis and central vascular cylinder, which appeared thicker and more developed in *ex vitro* plantlets. Also, Jambor-Benczur *et al.* [15] illustrated that *in vitro* developed roots of *Prunus* had a broad cortex and narrow vascular cylinder with less developed xylem elements. In the same trends, the roots initiated in the cuttings from the pith parenchyma in mango [16] and in coffee from parenchyma of the xylem rays [17].

The old roots of jackfruit seedlings were lacked of developed vascular system, which cause the snap of the root or the breakable roots and decrease of the root's nutrition supply and hence decrease the root growth and cause some problems and death of seedlings. Media contained the paclobutrazol increased an *in vitro* root diameter or thickness by increase of the cortex layers, also the *in vitro* roots seems to be initiated in the internal tissue (compact thick-walled parenchymatous cells) of the *in vitro* callus that originated from the

middle thin-walled parenchymatous cells of the *in vitro* base stem cortex. The *in vitro* shoots (shootlets) initiated in the inner tissue of this basal callus as a result of culture on MS+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kin.+25 mg/L. Adenine sulphate, the uniseriate trichomes originated by lateral extension of the outer walls of the epidermis cells. Chlorenchyma and collenchyma cells were noticed in the whole studied jackfruit plant tissues, as they act as a conjunctive tissues, besides the storage cells were found. The structures of an *in vitro* stem and leaf are lack of cuticle and trichomes, which have many functions in controlling of evapotranspiration, water absorption and temperature and hence decrease the successful plantlet's percentage of the jackfruit during the acclimatization and may be cause the vitrification beside some growth regulators (NAA). Buds initiated from the cortex of seedling, the *ex vitro* stem and leaf have a good cuticle, trichomes, conjunctive and flexible tissues (fibers) compared to an *in vitro* stem and leaf which make the plant tolerate to the dryness and diseases.

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