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Studies on Micro Propagation of Jackfruit
1-Behaviour of the Jackfruit Plants Through the Micropropagation Stages

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Abstract: *In vitro* and *ex vitro* experiments were carried out during 2004/2005 and 2005/2006 seasons and took some observations until 2007 year on jackfruit plant (*Artocarpus heterophyllus* lam.) to detect the suitable methodology for micro propagating the jackfruit as a pronounced method for using different explants (apical growth, nodal segments, new leaves and cotyledons) of the available rare plant material found in Egypt for obtaining a good number of the plants diseases-free suffice for spreading this tree which have many benefits (fresh, canned or industrial food, therapic purposes, furnitures, …etc). The effects of sterilizants (ethanol at 70%, $HgCl_2$ at 0.1-0.3% and chlorox at 10-20%) with aid of antioxidants (ascorbic and citric mixture) and 0.1% Tween 20 on the survival and aseptic explants %, effect of MS medium alone or with BA at 2 and 5 mg/L., NAA at 0.5 mg/L., kinetin at 0.5 mg/L. and adenine sulphate at 25 mg/L. on the callus formation and differentiation, the multiplication and the vitrification (hyperhydricity); effect of MS, ½ MS alone or with NAA at 0.5-2 mg/L., IBA at 0.5-3 mg/L., coumarin at 0.5-1 mg/L. and paclobutrazol at 0.5-1 mg/L. on the rooting and the vitrification; possibilities of transferring the plantlets from *in vitro* to *in vivo* or *ex vitro* conditions using different growing media of sand : soil (1:1, v/v), sand : peat moss (1:1, v/v) and soil : burned rice hull : fibrous sheath of date palm (1:1:1, v/v/v) by studying the effect of the growing media on the survival plants %, leaf and shoot number/plant and height of the plant; and effect of the collection dates of the explants (June-July, December-January and from 3 months old seedlings on September-October), all effects were investigated. The studies revealed that 70% ethanol for 2 min.+0.2% $HgCl_2$ for 5 min.+15% "Clorox" for 15 min. with the antioxidants, was the most effective sterilization treatment, as it is recorded a good percentages of the survival and aseptic explants at all studied dates and for all explants types; besides using the explants of apical growth during Dec.-Jan. and for those of 3 month old seedlings and using the explants of the new leaves during June-July, were achieved the best significant aspectic and survival explants %. The variant response to the sterilizants between the explants may be due to the different anatomical structures, especially the surface tissues. MS medium+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kin.+25 mg/L. adenine sulphate and explants of apical growth and nodal segments, were recording significant values of a good callus formation and differentiation, multiplication and produced the lower significant vitrification %. NAA and kinetin seems to be necessary for callus formation but raised significantly the vitrification%. The explants collected during Dec.-Jan. and those of 3 months old seedlings gave the best callus formation and differentiation, while those of the seedlings gave significant values of a good multiplication and less significant vitrification %. Shootlets produced of either nodal segments or new leaf segments and media of ½ MS+3 mg/L. IBA and ½ MS+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. paclobultrazol+1mg/L. coumarin recorded the higher significant rooting and the lower significant vitrification %. Paclobutrazol seems to be inhibited the vitrificantion, while NAA, coumarin or IBA seems to be increased it. All growth estimations during the acclimatization were increased with increase of dates recording these estimations and reached to the maximum values after 3 month of the planting date, beside, the best growing mixture in the acclimatization was soil : burned rice hull : fibrous sheath of the date palm.

Key words: Micropropagation . jackfruit plants . *Artocarpus heterophyllus* . Hyperhydricity

INTRODUCTION

The jackfruit, *Artocarpus heterophyllus* lam is one of the family *Moraceae*, which an excellent example of a food prized in some countries and allowed to go to waste in others. The jacks are such large and interesting fruits and the trees so well-behaved that it is difficult to explain the general lack of knowledge concerning them. The tree is evergreen and monoecious. The compound or aggregate fruit is green to yellow when ripe with weight of 4.5-50 kg. The interior of the fruit consists of large "bulbs" of yellow banana-flavored flesh, each

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bulb encloses a smooth, oval, light-brown "seed". There may be 100-500 seeds in a single fruit. The jack fruit tree originated at the Western Ghats, spread and cultivated across the world; i.e., in South India, the jackfruit is a popular food ranking next to the mango and banana. Government horticulturists promote the planting of jackfruit tree along high ways, water ways and rail roads (as Egypt have much of these roads, specially at the upper Egypt, where the humic tropical climate and rich deep soils are fitting for cultivating the jackfruit) to add to the country's food supply.

In Egypt, the main crop of jackfruit (it bears fruits during the year) ripen from June to July as the first part of this period has lack of the fruit production, which permit with a good early market and extend during an ordinary fruit production period, as jackfruits are rare and attractive fruit; and thus, it get a high completion ability. The crop was about 25-40 large fruits per tree (in India, it may be reach to 150 large fruit per tree) and consequently, the tree give a good economic fruits. The ripe fruits could be cold storage for 3-6 weeks. The fruit has a highly food value; i.e., ripe-fresh pulp contains a good quantities (per 100 g of edible portion) of protein (1.9 g), fat (0.3 g), carbohydrates (25.4 g), Ca (22 mg), P (38 mg), Fe (0.5 mg), Na (2 mg), K (407 mg), Vitamin A (540 I.U.), Niacin (4 mg) and ascorbic acid (10 mg). Fresh seeds are considered to be high in starch and good sources of vitamin B_1 and B_2. Jackfruits have a wide uses as follows: the full grown and unripe fruits, including the seeds, cooked green and served as a vegetables; the flesh of the unripe fruit has been canned in brine or with curry, dried and kept in tins, pickled with spices; The ripe bulbs made into ice cream, chutney, jam, jelly and paste, jackfruit nectar, powder, dried and fried in oil and salted for eating like potato chips. The seed boiled or roasted and eaten or preserved in syrup like chestnuts, canned in brine, dried seeds are ground to make flour for baking. Tender leaves and young male flower clusters cooked or eaten by cattle. The latex serves as birdlime or household cement. Jack wood is a good timber and can be exported or is superior to take for furniture as it resembles mahogany. The seeds and bulbs consider tonic, cooling, nutritious and overcoming the influence of alcohol; the roots is a remedy for skin diseases. The bark has 3.3% tannins made into cloth and cordage. In Egypt, all of the previous uses could be practice or applied to obtain many benefits. "Safeda", "Khaja" and "Handia" are the most important varieties. In the tropical and sub-tropical areas, the jackfruit could be propagated by the seeds, budding, inarching, air layers, cutting of young wood and tissue culture [1]. Also, the jackfruit tree could be bearing fruits twice yearly [2] and hence give an economic crop.

All propagation methods needs aid of hormones (except the grafting) and take more time, efforts and more experience except the tissue culture; also, in Egypt, there are not any orchard of the jackfruit to investigate and test the previous methods, but we can find a rare single tree in the public garden, which served as ornamental tree; besides, very few nurseries obtained the seeds by the exportation or from the local few trees and try to propagate them but the traits were failed because of the seedlings needs more cares in their growth i.e., plant house, some specific horticultural procedures were not existed and may be unsuitable climate or soil conditions. So, many problems objected propagation and spread of the jackfruit in Egypt as clear previously.

Many investigators studied *in vitro* propagation (tissue culture) of the jackfruit, such as Adiga *et al*. [3] decided that sucrose was a good C source, as cultures supplemented with sucrose or sugar at 3 or 4% produced more shoots and GA3 (6 mg/L) promoted shoot length.

Singh *et al.,* [4] mentioned that jackfruit was successfully micro propagated by culturing nodal segments on modified MS containing 18 mg/L. BA (benzyladenine)+0.2 mg/L.IBA (indole-3-butyric acid), the highest number (4-5) of usable shoots was developed on nodal segments taken from *in vitro*-proliferated shoots by enhancement of auxiliary branches after 4 subcultures on MS with 2 mg/L. BA+0.2 mg/L. IBA, *in vitro* grown successful rooted (58.7%) in half-strength MS with 1 mg/L. IBA, rooted shootlets were hardening (80% success) in sterilized mixture of sand+soil and farmyard manure (1:1:1).

Rahman [5] assured that a low level or absence of nutrients especially N, in the substrates used for acclimatization of *in vitro* rooted plantlets of jackfruit, at least 20 days resulted in significantly more growth and survival of plantlets, while, a nutrient feeding after 20 days improved growth and survival of plantlets.

Rajmohan and MohanaKumaran [6] reported that plantlets of jackfruit were hardened by exposure to high light intensity for 1 week, then transferred to vermiculite medium under 90-100% RH and treated with ½ MS salts. The survival rate was 55.6% after 8 weeks.

Rajmohan and MohanaKumaran [7] decided that light regime of darkness in the establishment stage for 4 weeks only was necessary for the successful micropropagation of jackfruit.

Ziv *et al*. [8] indicated that growth retardants increased rapid growth, shortened stems, inhibited leaf expansion and formation of clusters. Hazarika [9] 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 [10] stated that plant growth retardants affect cell division and cell enlargement, probably by interfering with gibberellin biosynthesis, the growth retardants used in liquid cultures to overcome hyperhydric malformation or vitrification. This phenomenon made vegetative shoots soft, glassy, translucent water retention in organs during differentiation and proliferation stages and succulent appearance that can result in cultures deteriorate and fail to proliferate. It may be attributed to inhibition of lignin and cellulose synthesis, excess water uptake and effect of some growth regulators (NAA).

Yanes-Paz *et al*. [11] indicated that treatment with 5 mg/L. fertilizer combi II+GA_3 at 100 mg/L. provoked a homogeneous and significant increment of pineapple plantlet's growth.

This study aimed to investigate the tissue culture technique as a pronounced method for propagation of the jack fruit and reach to a good *in vitro* propagation methods recommended for the jackfruit's propagation which are producing a higher numbers and free diseases plantlets, that permit with spread of the jackfruit in Egypt to add many yield and resources to our country and open the door to import an important cultivars and micropropagation of them. Also, the tissue culture technique was more fitted for using of the lowest amount of the plant material-found in Egypt-for the propagation and it permit with use many parts of a rare single tree in Egypt (seed, …, leave and current shoots) during the year, hence, this technique could be reach to the highest number of the propagated plant on the opposite to the traditional methods (cutting, …etc.)

The ultimate goal of this study is to find out the best possibilities procedures of sterilization, establishment, multiplication, elongation, rooting and acclimatization of jackfruit under Egyptian conditions.

MATERIALS AND METHODS

This work was carried out at the Plant Tissue culture laboratory and the greenhouse of the Pomology Dept., Fac. of Agric., Cairo Univ., to detect the suitable methodology for micropropagating the jackfruit trees, during the period from 2004-2007.

Plant materials (source of explants), explant types and date of the culture: The plant materials used for obtaining the explants included the following:

- Individual jackfruit trees were growing at the Botanical (plant) garden at Aswan governorate.
- Individual jackfruit tree growing at El-Zohereia garden, Cairo governorate, as it believes that these trees belong or native to kapa type of jackfruit (the fruits have thick peel, firm to crisp flesh, high quality with less aroma and cut open with a knife).
- Seedlings at age of three months (recommended by Rajmohan and MohanaKumaran, [7]), where it noticed in this trial that the seedlings had old of more than 3 months had more woody tissues neared to the tissues of the adult trees. These Seedlings produced from the extracted seeds of the collected fruits of the previous trees and cultivated and growing at a special farm at Kerdasa region, Giza governorate.

The explants of the tree were collected on June-July and December-January of 2004 and 2005. The used explants were the cotyledons of the fresh seeds collected during June-to-July of 2004 and 2005 years; also, the apical growth without leaves (5-10 mm), nodal segments (10-15 mm) and new leaf's segments, which collected from the seedlings at age of 3 months during the period of September-to-October of 2004 and 2005 years and collected from the current shoots of the tree during both of the period of June-to-July and the period of December-to-January of 2004 and 2005 years. The used seedlings were produced by cultivating the fresh seeds collected during June-July of 2004 and 2005.

Nutrient medium: The basal nutrient medium was Murashige and Skoog [12] medium, pH was adjusted to 5.7±0.1 after preparation of the medium, adding 30 g. sucrose/L. and 100 mg/L. myo-inositol before the addition of agar (6.0 g/L.), cooking the medium, distributing into culture jars (200 ml), each jar contained 35 ml of the cooked medium, capping with polypropylene closure and autoclaved at 121°C and 15 Ib/in^{2} for 20 min. All autoclaved culture jars were incubated at 27±2°C. Growth regulators were supplemented with the previous basal medium before adjusting pH.

The sterilization experiment: To evaluate effect of some sterilizants on percentage of the explants survival (percentage of alive explants) and percentage of non-contaminated explants. The previous mentioned explants were washed by tap water followed by a solution of soap for 3 min and 5 min under distilled water, the half number of each type of the explants were soaked in antioxidants (100 mg/L. citric and 150 mg/L. ascorbic) solution for 2 hours, then soaked under aspectic conditions in the sterilizants solution with few

drops (0.1%) tween-20, the sterilization treatments included the following:

- 70% ethanol for 2 min., then 0.1% mercuric chloride ($HgCl_2$) for 10 min.
- 70% ethanol for 2 min., then 0.2% $HgCl_2$ for 5 min.
- 0.3% $HgCl_2$ for 3 min.,
- 0.3% $HgCl_2$ for 5 min.,
- 70% ethanol for 2 min., then 10% Clorox (5.25%NaOCl) for 10 min.
- 70% ethanol for 2 min, then 10% Clorox for 15 min.
- 70% ethanol for 2 min., then 15% Clorox for 10 min.
- 70% ethanol for 2 min., then 15% Clorox for 15 min.
- 20% Clorox for 5 min.
- 20% Clorox for 10 min.
- 70% ethanol for 2 min.
- 70% ethanol for 5 min.
- 70% ethanol for 2 min., then 0.2% $HgCl_2$ for 5 min. and 15% Clorox for 15 min.

After sterilization, explants were rinsed in a sterilized distilled water 3 times, cultured individually into culture jars containing MS basal medium and incubated for 2 weeks at 25±2°C and 16 hr illumination of 2000 lux (white fluorescent lamps). Data have taken at end of the experiment (10-15 days).

Callus formation and differentiation, multiplication, elongation and vitrification phenomenon as affected by the different media: The recovered explants of each explant's type (sterilized or aseptic and alive or survival explants) have been cultured individually into jars (150 ml.) containing 40 ml. of the following media:

- MS (control),
- MS supplemented with 5 mg/L. BA (Benzyl adenine).
- MS supplemented with 2 mg/L. BA.
- MS supplemented with 2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin (kin.)
- MS supplemeted with 5 mg/L. BA+0.5 mg/L. Kin.+0.5 mg/L. NAA.
- MS supplemeted with 5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kin.+25 mg/L. adenine sulphate.

Cultures were incubated under the same conditions previously described in the sterilization experiment, the explants were sub cultured onto the same media every 3 weeks and repeatedly sub cultured at least 2 times. Callused explants and multiplicated shootlets were repeatedly subcultured onto the same media for 2 times at least. Also, the explants of apical growth, nodal segments and leaf segments have taken from these shootlets and cultured onto the same media. Data in averages have taken for each estimation after 2 repeated subcultures at least (1.5 month) as data were photographed and recorded for each explant's type as follows: callused explants percentage, number of shootlets/callused explant, diameter of callus (cm) and number of shootlets/ explants of shootlet of the callused explants, shootlet's length (cm), number of leaves/shootlet and hyperhydric shootlet's percentage.

Rooting experiments: Firstly, hyperhydric shootlet's percentages were recorded in this experiment. The rooting experiments included the following two experiments:

Effect of IBA and NAA on the rooting stage: Multiplicated shootlets produced from the previous experiments were separated into individual shootlets groups, in according to the explants produced them and cultured onto the following rooting media:

- ½ MS.
- ½ MS+1 mg / L. IBA
- ½ MS+3 mg/L. IBA.
- ½ MS+0.5 mg / L. IBA.
- ½ MS+2 mg/L. NAA
- ½ MS+0.5 mg / L. NAA
- ½ MS+1 mg/L. NAA+1 mg/L. IBA
- ½ MS+0.5 mg/L. NAA+0.5 mg/L. IBA.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA
- ½ MS+1 mg/L. NAA+3 mg/L. IBA.
- MS.

Data of the rooted shootlet's percentage, root number/shootlet, hyperhydric shootlets percentage and root length (cm) were recorded after at least 2 repeated subscultures, as they repeatedly subcultured every 3 weeks and start the experiment of the second rooting experiment (coumarin and paclobutrazol).

Effect of coumarin and paclobutrazol on the rooting stage: According to the results of the first experiment, media of ½ MS+2 mg/L. NAA+3 mg/L. IBA and ½ MS+1 mg/L. NAA+1 mg/L. IBA, proved to be the best, so these media were chosen for the second rooting experiment, as these media were used as a basal media to study the effect of coumarin and paclobutrazol. Individual shootlets produced from the multiplication stage were also used in this

experiment; the shootlets were cultured onto the following media:

- ½ MS+1 mg/L. coumarin.
- ½ MS+0.5 mg/L. coumarin.
- ½ Ms+1 mg/L. paclobultrazol.
- ½ MS+0.5 mg/L. paclobutrazol.
- ½ MS+1 mg/L. NAA+1 mg/L. IBA+1 mg/L. coumarin.
- ½ MS+1 mg/L. NAA+1 mg/L. IBA+0.5 mg/L. coumarin.
- ½ MS+1 mg/L. NAA+1 mg/L. IBA+1 mg/L. paclobutrazol.
- ½ MS+1 mg/L. NAA+1 mg/L. IBA+0.5 mg/L. paclobutrazol.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. Coumarin.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+0.5 mg/L. Coumarin.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. paclobutrazol.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+0.5 mg/L paclobutrazol.
- ½MS+1mg/L. NAA+1mg/L. IBA+1mg/L. coumarin + 1mg/L. paclobutrazol.
- ½ MS+1 mg/L. NAA+1 mg/L. IBA+0.5 mg/L. coumarin+0.5 mg/L. paclo.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. coumarin+1 mg/L. paclo.
- ½ MS+2 mg/L. NAA+3 mg/L. IBA+0.5 mg/L. coumarin+0.5 mg/L. paclo.

The same previous data in the first rooting experiment were recorded at the end of this experiment (1.5 month) and after at least 2 repeated subcultured every 3 weeks.

Acclimatization experiment: Rooted plantlets (5-8 cm length with 3-4 leave produced *in vitro*) were washed thoroughly with tap water 3 times to remove all traces of agar, then immersed in a fungicide vitafax (0.1% for 3 min.) and cultured individually in a perforated black plastic pots (8 cm) filled with one of the following sterilized growing media (v/v): a-Sand : soil (1:1). b-Sand : peat (1:1). and c-Soil : burned rice hull : fibrous sheath of date palm (1:1:1); covered with transparent plastic sheets (which were punched up 3cm from two sides) and grown under the following greenhouse conditions : light intensity at about 1500 Lux for 16 hours per day were provided by white fluorescent lamps, the temperature of about 28±2°C, high relative humidity using white polyethylene bags for 3 week to maintain humidity over plantlets and were irrigated by saturation twice during the 3 weeks after transplanting. The transparent plastic sheets were completely removed at the end of these 3 weeks; after one month of recovering the plantlets, the acclimatized plants were transplanted into perforated black plastic bags (15x25 cm) filled with the previous growing media. Plants were irrigated at 3 days intervals with tap water containing 0.1% benlate and ½ MS salts; plants were sprayed at 3 weeks intervals with a solution containing "kirstalon" (NPK fertilizer at 19-19-19) at the rate of 0.5 g/L., also Fe and Mg were applied to the solution at the rate of 0.1 g/L. for each element and a wetting agent was added to the solution; plants were grown under the previous greenhouse conditions for 3 months. In all *ex vitro* (*in vivo*) experiments, each treatment consisted of 3 replicates with 10 plants for each replicate (one plant for pot).

At the end of this experiment (3 months after acclimatization), samples have taken for the anatomical studies and at 0, 1, 2 and 3 months of acclimatization, the following parameters were estimated on the acclimatized plants as follows: survival percentage of the acclimatized plants, leaves number/plant, shoot number/plant and height (cm) of the vegetative growth.

The experimental design and the statistical analysis: All experiments were conducted using a completely randomized design. Three replicates were used in each treatment; each replicate included 5 explants for *in vitro* experiments and included 10 plantlets for *in vivo* acclimatization experiments; also, each experiment was repeated at least twice. All data were averaged and differences among the means of the different treatments were compared using the L. S. D. test as described by Steel and Torrie [13]. In the case of percentages, the original data were firstly arcsine-transformed prior to the statistical analysis.

RESULTS AND DISCUSSIONS

Firstly, some observations have taken as the following:

- Indirect organogenesis seems to be dominant in this trial and the direct organogenesis have not observed in an *ex vitro* explants,on the opposite of an *in vitro* explants.
- An embryogenesis phenomenon have not observed at all during all *in vitro* stages.
- Vitrification phenomenon have found during all *in vitro* stages except the sterilization experiment.
- The explants devoided of the callus still alive, but without any growth.
- The formed callus was hard or compact type.

The acclimatized seedlings produced of *in vitro* culture seems to be sharply influenced by Giza governorate conditions (Kerdasa), as they are dried and

burned at the winter conditions, especially the current shoot tips; so, it is recommended by cultivating these seedlings under the protected or covered conditions in the winter for 2 years at least.

Sterilization experiment: The effect of sterilizants, type of explants and anti-oxidants on the survival and aseptic explant percentages for June-July cultures is shown in Table 1. It is obvious that the new leaves explants with anti-oxidant treatment had the superior value of aseptic explants (72.15%), while the nodal segments explants without anti-oxidants had the lowest significant value (43.65%). Concerning the survival explants percentages, the nodal segment explants with anti-oxidants had the highest significant value (80.92%), while the new leaves explants without anti-oxidants had the lowest significant value (33.72%). The others explants with or without anti-oxidants produced intermediate values with significant differences among them. Referring to the effect of sterilizants used, data cleared that the treatment of 0.3% $HgCl_2$ for 5 minutes achieved the higher significant values of the aseptic explants (100 and 95% for the first and the second seasons, respectively) and recorded the lower significant values of alive explants (27.62 and 20% for the first and the second season, respectively), whilst, the treatment of 70% ethanol for 2 minutes+10% Clorox for 10 minutes recorded the lower significant values of the aseptic explants (10% for the two seasons) and recorded the higher significant values of alive explants (92.37 and 88.25% for the first and the second season, respectively). The action of $HgCl_2$ may be due to the known effect of $HgCl_2$ in lysis of microbial cells or reaction with thiol groups in the microbial enzymes, as well as the chlorox have a known effect as a powerful antimicrobial agent [14]. There are significant differences between the explant used in response to the sterilization treatments, these differences may be due to that they have a variant anatomical structures, especially the surface tissues. So all treatments of $HgCl_2$ gave the higher significant values of the aseptic explants %, but they have injured the explant's tissues and consequently gave the lower significant values of the alive explants %. On the other hand, the treatments of a relatively higher concentrations of chlorox gave a good values due to the previous known effect of "Chlorox". In this respect, it could be recommended with the treatment of 70% ethanol for 2 minutes+0.2% $HgCl_2$ for 5 minutes+15% "Clorox" for 15 minutes, as a good treatment recorded a satisfied results of both the aseptic and the survival explants percentages as shown in Table 1. The interactions between the different

Table 1: Effect of the sterilizants, type of the explants, seasons and the anti - oxidants on percentages of the survival (alive) and non-contaminated (aseptic) explants of the jackfruit trees, collected on JuneJulyof 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Type of exps. (exps.)		Apical growth (5-10 mm) without leaves				Nodal segments (10-15 mm)				New leaves segments				Cotyledons segments					
Anti-oxidants treatment (A trs.)		+		-		+		-		+		-		+		-		AVs.	
Measurements																			
Sterilization treatments (S. trs.)		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	Asept.	Alive
70% ethanol for 2 min.+	S_1	66	100	60	73	53	100	47	73	73	87	66	73	53	80	60	60	59.75	80.75
0.1% $HgCl_2$ for 10 min.	S_2	60	100	53	66	53	93	47	66	73	93	60	66	60	80	66	60	59.00	78.00
70% ethanol for 2 min.+	S_1	73	93	80	66	73	87	73	60	93	80	87	60	66	60	73	47	77.25	69.12
0.2% $HgCl_2$ for 5 min.	S_2	80	93	73	60	73	93	66	60	93	80	80	60	66	66	66	40	74.62	69.00
0.3% $HgCl_2$ for 3 min.	S_1	100	53	100	33	100	60	93	40	100	40	100	20	100	47	100	40	99.12	41.62
	S_2	93	47	100	33	93	53	87	27	100	40	100	13	100	47	100	33	96.62	37.87
0.3% $HgCl_2$ for 5 min.	S_1	100	33	100	27	100	40	100	27	100	27	100	7	100	33	100	27	100.00	27.62
	S_2	93	27	100	20	87	33	80	20	100	20	100	0	100	27	100	13	95.00	20.00
70% ethanol for 2 min.+	S_1	13	100	13	93	20	100	7	100	20	87	7	66	0	100	0	93	10.00	92.37
10% Clorox for 10 min.	S_2	13	93	13	87	20	93	7	93	20	87	7	66	0	100	0	87	10.00	88.25
70% ethanol for 2 min.+	S_1	33	93	27	73	33	93	20	87	40	66	27	53	20	100	20	73	27.50	79.75
10 % Clorox for 15 min.	S_2	27	93	33	66	27	93	13	80	47	53	33	47	27	93	27	66	29.25	73.87
70% ethanol for 2 min.+	S_1	47	80	33	60	53	87	27	66	60	53	40	40	33	80	27	53	40.00	64.87
15 % Clorox for 10 min.	S_2	47	73	40	60	53	80	27	66	53	53	47	33	40	80	27	60	41.75	63.12
70% ethanol for 2 min.+	S_1	73	73	53	47	66	80	40	66	73	47	60	33	53	73	33	47	56.37	58.25
15 % Clorox for 15 min.	S_2	73	66	60	47	60	73	47	53	66	40	66	20	60	66	40	40	59.00	50.62
20 % Clorox for 5 min.	S_1	73	60	66	33	60	73	47	53	93	33	80	20	53	60	33	33	63.12	45.62
	S_2	66	53	60	40	60	73	47	53	93	33	87	13	53	60	27	40	61.62	45.62
20 % Clorox for 10 min.	S_1	93	47	80	27	73	53	53	40	100	20	93	7	73	33	53	20	77.25	30.87
	S_2	93	40	80	20	73	47	47	33	93	13	100	0	80	27	60	13	78.25	24.12
70% ethanol for 2 min.	S_1	27	93	13	100	0	100	0	100	33	53	40	40	13	13	0	0	15.75	62.37
	S_2	20	93	7	100	0	100	0	100	40	60	47	40	7	13	0	0	15.12	63.25
70% ethanol for 5 min.	S_1	33	80	20	93	0	100	0	100	60	40	47	27	20	0	0	0	22.50	55.00
	S_2	27	87	13	93	0	100	0	93	53	40	53	20	13	0	0	0	19.87	54.12
70% ethanol for 2 min.+	S_1	100	87	100	66	87	100	60	100	100	53	100	33	100	66	100	47	93.37	69.00
0.2 $HgCl_2$ for 5 min.	S_2	100	93	100	60	93	100	53	93	100	47	100	20	100	60	100	33	93.25	63.25
+15% Clorox for 15 min.																			
AVs.	S_1	63.92	76.31	57.31	60.85	55.23	82.54	43.61	70.15	72.69	52.77	65.15	36.84	52.61	57.31	46.08	41.54	57.07	59.78
	S_2	60.92	73.69	56.31	57.84	53.23	79.31	43.69	64.38	71.61	50.69	67.69	30.61	54.31	55.31	47.15	37.31	56.41	56.24
General Avs.		62.42	75.00	56.81	59.34	54.23	80.92	43.65	67.26	72.15	51.73	66.42	33.72	53.46	56.31	46.61	39.42	56.74	58.01
LSD (0.05)																			
Trs	S_1	18.667	28.731	23.531	25.471	18.806	24.110	18.660	25.136	25.901	19.166	35.148	19.816	18.461	25.281	19.386	24.190	27.943	29.617
	S_2	15.031	26.915	22.816	24.650	16.510	23.411	19.015	23.510	24.760	18.506	28.990	22.112	19.912	28.566	23.766	22.417	28.251	28.840
Seasons		4.610	3.823	2.391	4.893	2.795	3.795	0.160	8.196	2.658	3.462	2.956	7.676	2.959	3.164	1.890	5.603	1.408	4.005
Seasons x Trs.		19.658	29.530	23.882	26.719	20.158	24.907	19.715	25.819	26.382	20.159	32.463	22.510	20.864	30.185	24.361	25.174	29.218	30.390
Trs. x S. x Anti. Oxid. (Alive) =				28.363				26.318				22.864				28.226			-
Trs. X S. x Anti-oxid. (Asept.) =				22.916				21.581				31.164				23.568			-

Exps. X Anti-oxid. (Asept.) = 17.993 and Exps. X Anti-oxid. (Alive) = 19.268

1: Asept. %, 2: Alive %

Table 2: Effect of the sterilizants, type of the explants, seasons and the anti-oxidants on percentages of the survival (alive) and non-contaminated (aseptic) vegetative explants of the jackfruit trees, collected on December-Januaryof 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Type of exps. (exps.)		Apical growth (5-10 mm) without leaves				Nodal segments (10-15 mm)				New leaves segments					
Anti-oxidants treatment (A trs.)		+		-		+		-		+		-		AVs.	
Measurements															
Sterilization treatments (S. trs.)		1	2	1	2	1	2	1	2	1	2	1	2	Asept	Alive
70% ethanol for 2 min.+	S_1	73	100	66	87	53	93	40	66	80	33	73	13	64.17	65.33
0.1% $HgCl_2$ for 10 min.	S_2	66	100	60	73	47	87	33	66	87	27	73	13	61.08	61.00
70% ethanol for 2 min.+	S_1	80	100	80	66	66	80	60	53	100	27	93	13	79.83	56.50
0.2% $HgCl_2$ for 5 min.	S_2	80	93	80	60	66	87	66	60	93	20	87	7	78.67	54.50
0.3% $HgCl_2$ for 3 min.	S_1	100	53	100	47	93	66	93	47	100	7	100	0	97.67	36.67
	S_2	100	53	100	40	93	60	100	33	100	0	100	0	98.83	31.00
0.3% $HgCl_2$ for 5 min.	S_1	100	33	100	33	87	33	80	20	100	0	100	0	94.50	19.83
	S_2	100	33	100	27	93	27	93	20	100	0	100	0	97.67	17.83
70% ethanol for 2 min.+	S_1	27	100	20	100	13	80	0	93	27	27	13	13	16.67	68.83
10% Clorox for 10 min.	S_2	27	100	27	93	13	93	0	93	33	27	13	7	18.83	68.83
70% ethanol for 2 min.+	S_1	33	100	33	73	20	93	13	73	53	13	40	7	32.00	59.83
10 % Clorox for 15 min.	S_2	33	93	27	66	27	87	13	80	66	13	40	7	34.33	57.67
70% ethanol for 2 min.+	S_1	53	80	40	66	47	80	20	60	73	13	53	7	47.67	51.00
15 % Clorox for 10 min.	S_2	47	80	40	60	53	73	27	60	66	13	53	13	47.67	49.83
70% ethanol for 2 min.+	S_1	80	66	60	47	60	73	33	66	80	26	73	7	64.33	47.50
15 % Clorox for 15 min.	S_2	73	66	60	53	60	80	40	60	73	20	66	0	62.00	46.50
20 % Clorox for 5 min.	S_1	73	60	66	40	60	66	40	47	100	7	93	0	72.00	52.17
	S_2	73	66	66	40	53	73	40	53	100	7	93	0	70.83	39.83
20 % Clorox for 10 min.	S_1	100	53	93	33	66	47	47	33	100	7	100	0	84.33	28.83
	S_2	100	47	80	27	60	47	40	33	100	0	100	0	80.00	25.67
70% ethanol for 2 min.	S_1	27	100	13	100	0	93	0	93	47	7	53	7	23.33	66.67
	S_2	20	93	13	100	0	100	0	87	53	13	60	7	24.33	66.67
70% ethanol for 5 min.	S_1	33	87	20	100	0	93	0	87	73	7	53	7	29.83	63.50
	S_2	33	93	20	93	0	93	0	87	66	7	60	0	29.83	62.17
70% ethanol for 2 min.+	S_1	100	93	100	66	80	93	53	93	100	27	100	13	88.83	64.17
0.2 $HgCl_2$ for 5 min.+	S_2	100	100	100	73	87	100	47	87	100	20	100	13	89.00	65.50
15% Clorox for 15 min.															
AVs.	S_1	67.61	78.85	60.85	66.0	49.61	76.15	36.85	63.92	79.46	15.46	72.61	6.69	61.17	52.37
	S_2	65.54	78.23	59.46	61.92	50.15	77.46	38.38	63.00	79.77	12.85	72.69	5.15	61.00	49.77
General Avs.		66.57	78.54	60.15	63.96	49.88	76.80	37.61	63.46	79.61	14.15	72.65	5.92	61.08	51.07
LSD (0.05)															
Trs	S_1	24.626	29.718	25.816	24.518	19.396	29.416	18.015	25.213	30.419	28.619	38.126	6.614	27.861	30.485
	S_2	26.116	30.111	26.116	25.367	22.541	30.175	18.864	26.061	32.006	24.118	39.293	6.218	27.516	29.836
Seasons		3.015	1.001	2.182	7.631	2.010	1.884	2.458	1.229	0.815	5.316	0.369	1.637	28.011	30.935
Seasons x Trs.		26.415	30.861	26.314	26.358	31.390	30.195	18.985	26.917	31.519	26.780	39.009	6.792		
Trs. x S. x Anti. Oxid. (Alive) =				25.680				27.395				5.806			-
Trs. X S. x Anti-oxid. (Asept.) =				26.041				20.618				35.114			-

Exps. X Anti-oxid. (Asept.) = 10.035 and Exps. X Anti-oxid. (Alive) = 28.318

1: Asept. %, 2: Alive %

Table 3: Effect of the sterilizants, type of the explants, seasons and the anti-oxidants on percentages of the survival (alive) and non-contaminated (aseptic) vegetative explants of the jackfruit seedlings of 3 month old, collected on September-October of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Type of exps. (exps.)		Apical growth (5-10 mm) without leaves				Nodal segments (10-15 mm)				New leaves segments					
Anti-oxidants treatment (A trs.)		+		-		+		-		+		-		AVs.	
Measurements															
Sterilization treatments (S. trs.)		1	2	1	2	1	2	1	2	1	2	1	2	Asept.	Alive
70% ethanol for 2 min.+	S_1	73	100	66	80	60	100	53	66	80	93	60	73	65.33	85.33
0.1% $HgCl_2$ for 10 min.	S_2	66	100	66	73	53	100	47	60	73	93	66	73	61.83	83.17
70% ethanol for 2 min.+	S_1	80	100	87	73	80	93	73	53	100	87	87	66	84.50	78.67
0.2% $HgCl_2$ for 5 min.	S_2	80	93	80	66	73	100	66	60	93	80	93	60	80.83	76.50
0.3% $HgCl_2$ for 3 min.	S_1	100	53	100	40	100	53	93	33	100	47	100	20	98.83	41.00
	S_2	100	53	100	33	100	60	93	27	100	40	100	20	98.83	38.83
0.3% $HgCl_2$ for 5 min.	S_1	100	33	100	27	100	40	93	27	100	20	100	0	98.83	24.50
	S_2	100	40	100	27	93	40	87	33	100	20	100	0	96.67	26.67
70% ethanol for 2 min.+	S_1	20	100	20	93	27	100	7	100	20	80	0	60	15.67	88.83
10% Clorox for 10 min.	S_2	20	100	13	100	20	100	0	100	20	87	7	66	13.33	92.17
70% ethanol for 2 min.+	S_1	33	100	27	66	33	100	20	80	40	60	33	53	31.00	76.50
10 % Clorox for 15 min.	S_2	33	93	20	66	33	93	20	87	47	60	33	60	31.00	76.50
70% ethanol for 2 min.+	S_1	53	80	33	53	60	87	33	60	60	60	47	40	47.67	63.33
15 % Clorox for 10 min.	S_2	47	87	40	60	53	87	27	73	60	53	40	40	44.50	66.67
70% ethanol for 2 min.+	S_1	80	80	53	40	66	80	47	53	66	53	66	27	63.00	55.50
15 % Clorox for 15 min.	S_2	73	73	53	47	66	73	40	66	73	47	66	33	61.83	56.50
20 % Clorox for 5 min.	S_1	66	60	60	40	66	73	53	53	100	40	87	20	72.00	47.67
	S_2	73	60	66	40	60	80	47	60	93	33	80	20	69.83	48.83
20 % Clorox for 10 min.	S_1	100	53	80	20	80	53	53	33	100	13	100	0	85.50	28.67
	S_2	93	47	73	27	73	60	53	33	100	13	100	0	82.00	30.00
70% ethanol for 2 min.	S_1	27	100	7	100	0	100	0	100	40	60	47	40	20.17	83.33
	S_2	27	93	13	93	0	100	0	100	40	60	47	40	21.17	81.00
70% ethanol for 5 min.	S_1	27	87	13	87	0	100	0	100	60	40	53	33	25.50	74.50
	S_2	33	87	13	93	0	100	0	100	60	40	53	27	26.50	74.50
70% ethanol for 2 min.+	S_1	100	93	100	73	93	100	66	100	100	53	100	33	93.17	75.33
0.2 $HgCl_2$ for 5 min.+	S_2	100	93	100	66	100	100	60	100	100	53	100	33	93.33	74.17
15% Clorox for 15 min.															
AVs.	S_1	66.08	79.92	57.38	60.92	58.84	83.00	45.46	66.00	74.31	54.31	67.69	35.77	61.63	63.32
	S_2	65.00	78.38	56.69	60.84	55.69	84.08	41.54	69.15	73.77	52.23	68.08	36.31	60.13	63.50
General Avs.		65.54	79.15	57.03	60.88	57.26	83.54	43.50	67.57	74.04	53.27	67.88	36.04	60.88	63.41
LSD (0.05)															
Trs	S_1	30.618	33.361	18.018	18.256	26.301	32.008	21.181	28.511	18.812	23.906	31.550	18.706	25.106	24.641
	S_2	15.781	19.152	21.990	17.100	23.666	34.150	16.996	29.703	17.256	22.417	32.312	19.170	22.015	25.815
Seasons		1.408	1.900	1.001	0.052	4.590	1.808	5.062	3.905	1.669	3.055	1.228	1.339	1.718	0.810
Seasons x Trs.		27.310	22.155	20.819	18.390	25.382	34.592	20.212	29.806	18.793	24.351	32.757	19.883	24.110	25.609
Trs. x S. x Anti. Oxid. (Alive) =			20.347					30.416				21.390			
Trs. X S. x Anti-oxid. (Asept.) =			23.482					23.173				30.099			

Exps. X Anti-oxid. (Asept.) = 20909 and Exps. X Anti-oxid. (Alive) = 17.00

1: Asept. %, 2: Alive %

studied factors revealed the same previous trends as shown in the table. Data presented in Table 2 and 3 almostly showed the same previous trends (Table 1), with exceptions of the treatment of 0.3% $HgCl_2$ for 3 minutes gave the higher significant values of the aseptic explants and the apical growth explants with anti-oxidants recorded the highest significant value of the alive explants. According to the previous results, it could be recommended with using either the apical growth explants in December-January and for 3 month old seedlings of jackfruit or using new leaves explants in June-July, also using the anti-oxidants beside the previous recommended sterilization treatment for obtaining a satisfied results in sterilizing the jackfruit's explants.

There were a significant differences between the explants used in response to the sterilization treatments,these differences may be due to a variant their anatomical structures,specially the surface tissues.

These results were in harmony with those obtained by Chavan *et al.* [15] who mentioned that $HgCl_2$ at 0 .2% for 4 minutes or at 0.1% for 10 minutes gave a good aseptic establishment of 10-20 mm explants of jackfruit axillary buds and shoot tips. In the same subject. Aydieh *et al.* [16] assured that soaking pineapple explants in Na O Cl 1% for 20 min., $HgCl_2$ 0.1% for 30 second and ethanol 70% for 5 second resulted in totally microbial-free explants.

Callus formation and differentiation: Data represented in Table 4 and Fig. 1 revealed that explants of the apical growth gave the highest value of callused explants (16.66%), followed by the nodal segments (14.42%) and without significant difference in between. On the contrary, explants of cotyledons segments gave the lowest significant value (0%). On the other hand, explants of the nodal segments recorded the higher values of the number of shootlets/callused explant (2.59) and the callus diameter (0.57 cm), while explants of cotyledons segments gave the lowest significant values (zero), regardless the media.

Considering the media, regardless the explants: media devoided of NAA and kinetin failed to from any callus. Besides, MS medium+2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin produced the higher significant values of both the callused explants (30 and 33.25% for the first and the second season, respectively) and the callus diameter (1.05 and 1.02 cm for the first and the second season, respectively) and considered the recommended medium, besides MS medium+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. Adenine sulphate showed the higher significant values of the number of shootlets/callused explant (5.97 for the two studied seasons). The interaction effect between the explants and the media indicated the same previous trends. Data presented in Table 6 showed the same previous trends, while those presented in Table 5 followed the same previous trends, except that explants

Fig. 1: Callus formation and shootlets regenerated and proliferated on the callus produced on MS medium+2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. Kinetin+25 mg/L. Adenine sulphate: A: nodular segments explants collected on Dec.-Jan., B: nodal segments explants of 3 months old seedlings and C: nodular segments explants collected on June-July

Table 4: Effect of the explant's type, seasons and the media on the callused explants percentages (A), number of shootlets / callused explant (B) and callus diameter (cm) (c) of the recovered explants of the jackfruit trees, co llected on June-July of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)			Nodal segments (10-15 mm)			New leaves segments			Cotyledons segments			AVs.		
Measurements																
Media (mg/L.) (M)		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
MS.	S_1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS+5 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA+0.5 NAA+	S_1	47	3.1	1.1	40	3.9	1.6	33	2.8	1.5	0	0	0	30.00	2.45	1.05
0.5 kinetin (kin.)	S_2	53	3.7	0.9	40	4.2	1.9	40	3.8	1.3	0	0	0	33.25	2.92	1.02
MS+5 BA+0.5 NAA+	S_1	33	1.5	1.0	27	1.8	1.2	20	1.3	1.1	0	0	0	20.00	1.15	0.80
0.5 kin.	S_2	27	1.7	1.1	33	2.0	1.1	27	1.4	0.9	0	0	0	21.75	1.02	0.77
MS+5 BA+0.5 NAA+	S_1	20	6.9	1.3	20	9.7	0.9	13	7.3	0.9	0	0	0	13.25	5.97	0.77
0.5 kin.+25 Adenine sulphate	S_2	20	7.5	1.2	13	9.5	1.2	13	6.9	1.0	0	0	0	11.50	5.97	0.85
AVS.	S_1	16.66	1.92	0.57	14.50	2.57	0.62	11.00	1.9	0.58	0	0	0	10.54	1.59	0.44
	S_2	16.66	2.15	0.53	14.33	2.62	0.53	13.33	2.02	0.53	0	0	0	11.08	1.65	0.44
General Avs.		16.66	2.03	0.55	14.42	2.59	0.57	12.16	1.96	0.55	0	0	0	10.81	1.62	0.44
LSD (0.05)																
M.	S_1	10.110	1.396	0.266	16.715	4.860	0.586	10.615	3.850	0.408	0.001	0.001	0.001	8.186	1.004	0.616
	S_2	11.960	1.817	0.275	18.287	6.359	0.618	11.498	3.008	0.356	0.001	0.001	0.001	9.751	0.989	0.801
S		0.001	0.385	0.100	0.316	0.106	0.131	3.158	0.391	0.917	0.001	0.001	0.001	1.719	0.112	0.001
M. x S.		12.106	1.921	0.290	19.193	7.005	0.788	12.801	3.695	0.484	0.001	0.001	0.001	9.817	0.995	0.719

Exps. (A) = 4.119 ; EXPs. (B) = 0.595 and EXPs. (C) = 0.105

Table 5: Effect of the explant's type, seasons and the media on the callused explants percentages (A), number of shootlets / callused explant (B) and callus diameter (cm) (c) of the recovered explants of the jackfruit trees, collected on December-January of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)			Nodal segments (10-15 mm)			New leaves segments			AVs.		
Measurements													
Media (mg/L.) (M)		A	B	C	A	B	C	A	B	C	A	B	C
MS.	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+5 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA+0.5 NAA+	S_1	53	3.8	1.1	33	3.3	1.4	33	2.3	1.2	39.67	3.13	1.23
0.5 kinetin (kin.)	S_2	53	4.0	1.1	40	4.0	1.7	27	3.0	1.1	40.00	3.67	1.30
MS+5 BA+	S_1	33	1.6	1.2	27	1.5	1.1	20	1.1	0.9	26.67	1.40	1.07
0.5 NAA+0.5 kin.	S_2	33	1.8	1.3	27	1.8	1.0	20	1.1	0.7	26.67	1.57	1.00
MS+5 BA+	S_1	27	7.3	1.4	13	8.7	0.9	0	6.8	1.0	13.33	7.60	1.10
0.5 NAA+0.5 kin.	S_2	20	7.9	1.4	13	8.8	1.1	7	6.4	0.8	13.13	7.70	1.10
+25 Adenine sulphate													
AVS.	S_1	18.83	2.12	0.62	12.17	2.25	0.57	8.83	1.70	0.52	13.28	2.02	0.57
	S_2	17.67	2.28	0.63	13.33	2.43	0.63	9.0	1.75	0.43	13.30	2.16	0.57
General Avs.		18.25	2.20	0.62	12.75	2.34	0.60	8.91	1.72	0.47	13.15	2.09	0.57
LSD (0.05)													
M.	S_1	18.159	3.146	1.027	17.059	3.212	0.875	12.190	2.154	0.764	22.191	2.003	1.311
	S_2	19.661	3.346	1.062	18.136	3.863	0.942	16.368	2.778	0.655	23.511	2.998	1.215
S.		2.192	0.215	0.081	2.056	0.293	0.158	0.357	0.020	0.112	0.100	0.188	0.011
M. x S.		19.797	3.458	1.081	18.201	3.790	0.962	15.018	2.565	0.786	23.801	3.215	1.351

Exps. (A) = 3.542 ; EXPs. (B) = 0.394 and EXPs. (C) = 0.089

Table 6: Effect of the explant's type, seasons and the media on the callused explants percentages (A), number of shootlets / callused explant (B) and callus diameter (cm) (c) of the recovered explants of the jackfruit seedlings of 3 month old, collected on September-October of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)			Nodal segments (10-15 mm)			New leaves segments			AVs.		
Measurements													
Media (mg/L.) (M)		A	B	C	A	B	C	A	B	C	A	B	C
MS.	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+5 BA	S_1	0	0	0	0	0	0	0	0	0	0	0	0
	S_2	0	0	0	0	0	0	0	0	0	0	0	0
MS+2 BA+0.5 NAA+	S_1	60	2.9	1.0	33	3.2	1.4	33	2.6	1.4	42	2.9	1.27
0.5 kinetin (kin.)	S_2	53	3.1	0.8	40	4.0	1.6	33	3.6	1.1	42	3.57	1.17
MS+5 BA+0.5 NAA+	S_1	40	1.3	0.9	20	1.3	1.1	13	1.1	1.0	24.33	1.23	1.0
0.5 kin.	S_2	33	1.5	1.0	27	1.8	0.9	20	1.1	0.7	26.67	1.47	0.87
MS+5 BA+0.5 NAA+	S_1	27	6.6	1.1	13	9.1	0.7	7	7.0	0.8	15.67	7.57	0.87
0.5 kin.+25 Adenine sulphate	S_2	27	7.0	1.2	20	9.2	1.0	7	6.6	0.9	18.0	7.60	1.03
AVS.	S_1	21.17	1.80	0.50	11.00	2.27	0.53	8.83	1.78	0.53	13.67	1.95	0.52
	S_2	18.83	1.93	0.50	14.50	2.50	0.58	10.00	1.88	0.45	14.44	2.11	0.51
General Avs.		20.00	1.86	0.50	12.75	2.38	0.55	9.41	1.83	0.49	14.05	2.03	0.51
LSD (0.05)													
M.	S_1	18.701	3.271	0.819	11.592	1.751	0.582	12.021	2.109	0.507	14.213	3.909	0.800
	S_2	19.056	3.752	0.967	14.309	2.350	0.469	11.069	3.501	0.616	12.011	3.886	0.916
S.		3.851	0.201	0.001	4.879	0.391	0.091	2.907	0.282	0.187	1.019	0.557	0.110
M. x S.		19.319	3.792	0.989	13.639	2.291	0.501	11.869	2.314	0.629	13.312	3.950	0.921

Exps. (A) = 6.059 ; EXPs. (B) = 0.401 and EXPs. (C) = 0.139

Fig. 2: Indirect shoot formation and multiplication on MS medium+2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. Kinetin+25 mg/L. Adenine sulphate, the multiplicated shootlets regenerated from : A-explants of new leaves segments of shootlets produced on explants of 3 month old seedlings, B-explants of apical growth of shootlets produced on explants of 3 months old seedlings and C: explants of new leaves segments of shootlets produced on explants collected on June-July

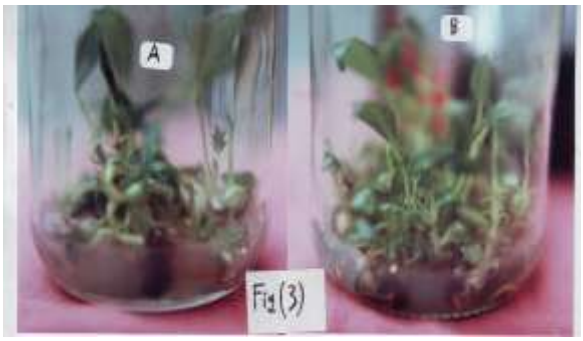

Fig. 3: Indirect organogenesis on MS medium+2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. Adenine sulphate, the organs initiated from: A-explants of apical growth of shootlets produced on explants collected on June-July and B-explants of nodal segments of shootlets produced on explants of 3 month old seedlings

of the apical growth gave the highest callus diameter (0.62 cm). It could be recommended with the previous medium that included MS medium+BA+NAA+ Adenine sulphate for obtaining a good callus and recommended with explants of either the apical growth or the nodal segments for a well callus formation at all the studies dates. The differences in the callus formation between the explants may be due to both the physiological state of the explants and the media. NAA

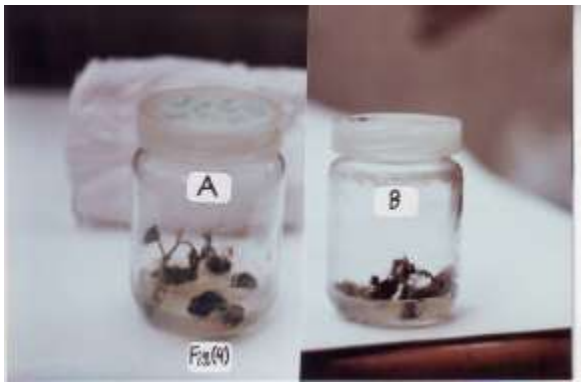

Fig. 4: Hyperhydric vegetative growth on MS medium+2 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin, A-vegetative growth produced on explants of 3 month old seedlings and B-vegetative growth produced on explants collected on Dec-Jan

Fig. 5: Sprouting of shootlet's lateral buds on MS medium+2 mg/L. BA

Fig. 6: Growth of shootlet's lateral buds as a result of horizontal culture on MS medium

and kinetin seems to be necessary for callus formation on the jackfruit's explants. The previous results were in agreement with those obtained by Chavan *et al.* [15]

Table 7: Effect of the explant's type, seasons and the media on number of the multiplicated shootlets / explants of shootlet (A), shoot let's length (cm) (B), number of leaves / shootlet (c) and the hyperhydric shoolets percentages (D) of shoot lets produced of the recovered explants of the jackfruit trees, collected on June-July of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)				Nodal segments (10-15 mm) shootlets				New leaves segments shootlets				AVs.			
Measurements / Media (mg/L.) (M)		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
MS.	S_1	1.30	2.20	1.30	0.00	1.00	1.30	1.10	0.00	5.10	3.20	1.40	0.00	1.85	1.67	0.95	0.00
	S_2	1.70	2.10	1.50	0.00	1.10	1.20	1.20	0.00	5.30	3.00	1.10	0.00	2.02	1.57	0.95	0.00
MS+2 BA	S_1	8.00	6.80	1.80	0.00	0.00	0.00	0.00	0.00	10.60	7.60	2.40	0.00	4.65	3.60	1.05	0.00
	S_2	8.20	6.90	2.00	0.00	0.00	0.00	0.00	0.00	10.80	7.70	2.50	0.00	4.75	3.65	1.12	0.00
MS+5 BA	S_1	2.00	2.90	2.40	0.00	3.60	6.20	2.30	0.00	7.00	4.00	3.50	0.00	3.15	3.27	2.05	0.00
	S_2	2.10	3.00	2.60	0.00	3.80	6.40	2.50	0.00	7.60	4.50	3.20	0.00	3.37	3.47	2.07	0.00
MS+2 BA+0.5 NAA+	S_1	4.00	9.80	3.90	10.00	4.60	8.70	2.30	2.70	3.60	8.00	3.30	47.00	3.05	6.62	2.37	21.00
0.5 kinetin (kin.	S_2	4.30	9.00	3.70	10.00	4.40	8.50	2.60	2.70	3.10	7.90	3.00	47.00	2.95	6.35	2.37	21.00
MS+5 BA+0.5 NAA+	S_1	4.30	5.70	3.10	10.00	8.20	8.00	3.30	7.00	8.30	7.10	3.90	33.00	5.20	5.20	2.57	12.50
0.5 kin.	S_2	4.10	5.60	3.80	10.00	8.60	7.80	3.80	7.00	8.70	7.80	3.30	33.00	5.35	5.30	2.72	12.50
MS+5 BA+	S_1	11.60	6.80	4.30	0.00	6.00	8.60	3.30	0.00	9.60	7.20	4.50	20.00	6.80	5.65	3.02	5.00
0.5 NAA+0.5 kin.+	S_2	11.40	6.90	4.10	0.00	6.30	8.80	3.20	0.00	10.20	7.60	4.90	20.00	6.97	5.82	3.50	5.00
25 Adenine sulphate																	
AVS.	S_1	5.20	5.70	2.80	3.33	3.90	5.47	2.05	5.67	7.37	6.18	3.170	16.67	4.12	4.33	2.00	6.42
	S_2	5.30	5.58	2.95	3.33	4.03	5.45	2.22	5.67	7.62	6.42	3.000	16.67	4.23	4.36	2.11	6.42
General Avs.		5.25	5.64	2.87	3.33	3.96	5.46	2.13	5.67	7.49	6.30	3.080	16.67	4.17	4.34	2.05	6.42
L.S.D. (0.05)																	
M.	S_1	2.337	2.557	1.172	8.176	2.358	2.416	0.942	18.194	1.395	3.821	1.058	11.909	1.517	1.828	0.813	7.106
	S_2	2.094	2.836	1.098	8.176	2.189	2.303	1.079	18.194	1.602	3.060	1.801	11.909	1.731	1.790	0.901	7.106
S.		0.292	0.336	0.303	0.001	0.306	0.109	0.301	0.001	0.333	0.300	0.225	0.001	0.330	0.194	0.210	7.106
M. x S.		2.408	2.717	1.200	8.176	2.417	2.465	1.110	18.194	1.400	3.603	1.446	11.909	1.769	1.933	1.181	

Exps. (A) = 2.039 ; EXPs. (B) = 0.676 ; EXPs. (C) = 1.007 and EXPs. (D) = 6.305

Table 8: Effect of the explant's type, seasons and the media on number of the multiplicated shootlets/ explants of shootlet (A), shoot let's length (cm) (B), number of leaves / shootlet (c) and the hyperhydric shoolets percentages (D) of shootlets produced of the recovered explants of the jackfruit trees, collected on December-January of 2004/2005 (S_1) and 2005 /2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)				Nodal segments (10-15 mm) shootlets				New leaves segments shootlets				AVs.			
Measurements / Media (mg/L.) (M)		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
MS.	S_1	1.1	1.9	1.0	0	1.0	1.6	1.0	0	1.0	1.7	1.0	0	1.03	1.73	1.00	0.00
	S_2	1.5	2.0	1.1	0	1.2	1.2	1.0	0	1.0	1.9	1.1	0	1.23	1.70	1.07	0.00
MS+2 BA	S_1	7.2	6.2	1.5	0	1.1	2.0	1.2	0	2.8	4.8	1.7	0	3.70	4.33	1.47	0.00
	S_2	7.8	6.5	1.2	0	1.4	1.9	1.0	0	3.0	4.1	1.6	0	4.07	4.17	1.27	0.00
MS+5 BA	S_1	1.7	2.5	1.9	0	2.5	4.2	2.2	0	4.2	3.5	2.6	0	2.80	3.40	2.23	0.00
	S_2	1.9	2.7	2.2	0	3.0	4.6	2.2	0	4.3	2.7	2.8	0	1.73	3.33	2.40	0.00
MS+2 BA+0.5 NAA+	S_1	3.7	8.3	2.3	13	4.0	7.2	2.5	33	1.1	8.0	3.0	53	2.93	7.83	2.60	33.00
0.5 kinetin (kin.	S_2	3.9	9.2	2.5	20	3.8	7.7	2.7	33	1.4	8.3	3.1	47	3.03	8.40	2.77	33.33
MS+5 BA+0.5 NAA+	S_1	4.0	4.5	3.2	20	6.5	7.2	3.0	13	4.5	6.1	2.9	40	5.00	5.93	3.03	24.33
0.5 kin.	S_2	3.8	5.2	3.3	20	7.6	7.8	3.4	13	5.0	6.6	3.2	33	5.47	6.53	3.30	22.00
MS+5 BA+	S_1	10.2	6.4	4.1	0	5.2	8.0	4.0	7	7.0	8.5	3.8	27	7.47	7.63	3.97	11.33
0.5 NAA+0.5 kin.+	S_2	10.4	6.5	3.5	0	6.0	8.1	3.9	0	6.2	8.3	3.6	33	7.53	7.63	3.67	11.00
25 Adenine sulphate																	
AVS.	S_1	4.65	4.97	2.33	5.50	3.38	5.03	2.82	8.83	3.43	5.43	2.50	20.00	3.82	5.14	2.38	11.44
	S_2	4.88	5.35	2.30	6.67	3.78	5.22	2.37	7.67	3.48	5.27	2.57	18.83	3.84	5.29	2.41	11.05
General Avs.		4.76	5.16	2.31	6.08	3.58	5.12	2.59	8.25	3.45	5.35	2.53	19.41	3.83	5.21	2.39	11.24
LSD (0.05)																	
M.	S_1	2.515	1.892	0.636	6.683	1.458	2.051	1.158	5.107	1.608	1.801	1.315	11.393	2.105	1.408	1.156	10.582
	S_2	2.326	2.200	0.795	13.805	2.316	2.480	1.094	9.680	1.760	2.112	1.100	9.401	2.200	1.002	1.238	9.875
S.		0.385	0.556	0.083	1.415	0.605	0.313	0.719	1.389	0.110	0.362	0.090	3.506	0.066	1.103	0.116	0.772
M. x S.		2.719	3.010	0.858	14.108	2.409	2.518	1.209	9.889	1.816	2.510	1.410	11.602	2.296	1.500	1.281	10.918

Exps. (A) = 1.016 ; EXPs. (B) = 0.395 ; EXPs. (C) = 0.197 and EXPs. (D) = 6.536

Fig. 7: The suitable stage of the proliferated shootlets before culturing on the rooting media

who reported that MS or WPM with BA (10 ppm)+NAA (5 ppm)+GA_3 (5 ppm), gelrite (0.25%) and large explant (20 mm) all were better for a good establishment. Also, Amin and Jaiswal [17] decided that tissue culture of 5-10 mm long of jackfruit's apical buds on MS gave a good establishment, also, MS with BA and kinetin (4.5-9 μ M) gave a good proliferation and multiplication when used 1-2 nodes from *in vitro* shoots. November-January was the best season for initiation. The callus formation of the jackfruit's explants in this trial consider a new technique of microppagation of the jackfruit plants.

Multiplication, elongation and vitrification phenomenon: It is shown from Table 7-9 and Fig. 2-7

Table 9: Effect of the explant's type, seasons and the media on number of the multiplicated shootlets / explants of shootlet (A), shoot let's length (cm) (B), number of leaves / shootlet (c) and the hyperhydric shoolets percentages (D)of shootlets produced of the recovered explants of the jackfruit seedlings of 3 month old, collected on September-October of 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Explants (exps.)		Apical growth without leaves (5-10 mm)				Nodal segments (10-15 mm) shootlets				New leaves segments shootlets				AVs.			
Measurements / Media (mg/L.) (M)		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
MS.	S_1	2.0	2.5	1.4	0	1.0	2.3	2.0	0	1.1	4.2	1.3	0	1.37	3.00	1.57	0.00
	S_2	2.2	2.8	1.6	0	1.0	2.6	1.8	0	1.5	4.5	1.1	0	1.57	2.30	1.50	0.00
MS+2 BA	S_1	10.0	7.7	2.5	0	5.3	3.2	1.5	0	4.0	8.2	2.0	0	6.43	6.37	2.00	0.00
	S_2	9.2	8.5	2.2	0	4.2	3.5	1.7	0	3.5	8.3	1.8	0	5.63	6.77	1.90	0.00
MS+5 BA	S_1	2.6	3.4	3.8	0	3.1	7.1	2.7	0	8.2	5.3	3.1	0	4.63	5.27	3.20	0.00
	S_2	3.0	4.2	3.1	0	3.3	7.4	2.5	0	8.3	6.0	3.3	0	4.87	5.87	2.97	0.00
MS+2 BA+0.5 NAA	S_1	4.3	10.2	3.8	33	8.2	9.6	3.0	20	3.2	8.7	3.4	40	5.23	9.50	3.40	31.00
+0.5 kinetin (kin.)	S_2	4.5	10.5	4.0	33	6.5	9.3	2.7	20	3.0	8.8	3.6	40	4.67	9.53	3.43	31.00
MS+5 BA+0.5 NAA	S_1	6.3	6.0	3.9	13	4.0	8.3	3.3	7	7.9	8.0	3.4	40	6.07	7.43	3.53	20.00
+0.5 kin.	S_2	6.7	6.1	4.0	13	3.8	9.0	4.1	0	8.2	8.1	3.5	40	6.23	7.73	3.87	17.67
MS+5 BA+	S_1	14.0	7.5	4.8	13	6.3	9.5	4.5	0	10.2	8.3	4.0	20	10.17	8.43	4.43	11.00
0.5 NAA+0.5 kin.+	S_2	15.3	7.8	5.1	13	5.8	10.2	4.0	0	10.5	8.5	4.2	20	10.53	8.83	4.43	11.00
25 Adenine sulphate																	
AVS.	S_1	6.53	6.22	3.37	9.83	4.65	6.67	2.83	4.50	5.77	7.12	2.87	16.67	5.65	6.67	3.02	8.67
	S_2	6.82	6.65	3.33	9.83	4.10	7.00	2.80	3.33	5.83	7.37	2.92	16.67	5.58	6.77	3.02	9.94
General Avs.		6.67	6.43	3.35	9.83	4.37	6.83	2.81	3.91	5.80	7.24	2.89	16.67	5.61	6.72	3.02	9.30
LSD (0.05)																	
M.	S_1	3.228	2.356	2.202	16.104	2.153	2.418	1.356	11.960	2.705	1.986	1.593	17.531	2.801	2.758	1.411	8.969
	S_2	3.562	2.501	1.920	16.108	2.208	2.651	1.492	15.112	3.318	2.156	1.514	17.531	2.617	3.055	1.314	6.009
S.		0.457	0.706	0.101	0.001	0.695	0.513	0.100	1.506	0.129	0.425	0.339	0.001	0.219	0.251	0.001	1.670
M. x S.		3.618	2.680	2.114	16.301	2.314	2.693	1.553	14.517	3.502	2.236	1.601	17.531	2.916	2.897	1.551	7.851

Exps. (A) = 2.111 ; EXPs. (B) = 1.152 ; EXPs. (C) = 0.669 and EXPs. (D) = 4.006

that the new leaves of the shootlets produced on the callus of the explants cultured on June-July recorded the higher significant values of number of the multiplicated shootslets/ explants of shootlet (7.49), shootlet's length (6.30 cm), number of leaves/ shootlet (3.08) and the hyperhydric shootlets (16.67%) which caused a big loss in the tissue culture; the explants of apical growth came in the second rank in the economic traits (5.25 shootlet/ explants of shootlet, 5.64 cm of shootlet's length and 2.87 leaf/shootlet) and with a less significant hyperhydric shootlets% (3.33%).

In respect to the effect of media, MS medium+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. Adenine sulphate showed the higher significant values of shootlet's number / explants of shootlet (6.8 and 6.97 in the first and the second season, respectively) and leaf's number/ shootlet (3.02 and 3.50 in the first and the second season, respectively); as well as, MS medium+2 mg/ L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin gave the higher significant values of shootlet's length (6.62 and 6.35 cm in the first and the second season, respectively) and the hyperhydric shootlets (21% in the two studied season); while, MS medium produced the lower significant values of all studied traits, as shown in Table 7. Considering the effect of interactions between the explants of shootlets of explants cultured on June-July and the media, they took the same previous trends, almostly. Data of Table 8 and 9 clearly showed the same mentioned trends found in Table 7, with except that the of apical growth of shootlets of the explants cultured on December-January (Table 8) and those of 3 month old seedlings (Table 9) produced the higher significant values of shootlets number/ explants of shootlet (4.46 and 6.67 in Table 8 and 9, respectively), the shootlets apical growth produced of 3 month old seedlings (Table 9) achieved the highest significant number of leaves/ shootlet (3.35) and the shootlet's nodal segments produced of explants cultured on December-January (Table 8) recorded the highest significant number of leaves/shootlet (2.59).

From the previous results, it could be recommend with using the explants of apical growth and the nodal segments, for obtaining a good multiplication of the jackfruit, where they recorded an acceptable results, especially in producing a lower significant percentages of the hyperhydric shootlets. It must be exclude the explants of leaf's segments and cotyledons in micropropagation of the jackfruit because of they resulted in a higher significant percentages of the hyperhydric shootlets. Also, it is recommended with MS medium+5 mg/L. BA+0.5 mg/L. NAA+0.5 mg/L. kinetin+25 mg/L. Adenine sulphate, which included a lower concentrations of NAA and kinetin that seems to be caused an vitrification (hyperhydricity) phenomenon, as it noticed in Table 7-9, where the media devoided of NAA and kinetin produced hyperhydric free shootlets. The previous results were in accordance with those obtained by Nower *et al.* [18] who decided that the jackfruit seeds could be cultured on MS with 1 mg/L. of each BA and NAA and produced shoots, which could be used *in vitro* culture. The highest number of shoots achieved on MS with 3 mg/L. BA and 0.1 mg/L. NAA, while the highest number of leaves / culture achieved on MS with 1 mg /L. BA and 0.1 mg/L. NAA. Also, Roy *et al.* [2] said that the most shoot proliferation (87.4% of cultures with 10.3 shoots/culture) of shoot tips and nodal

Table 10: Effect of media, type of the shootlets and seasons on percentage of the rooted shootlets (A), roots number / shootlet (B), root length (cm) (c) and the hyperhydric shoolets percentages (D) of the jackfruit during 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Types of shootlets		Shootlets of apical growth Without leaves (5 -10 mm)				Shootlets of nodal segments (10-15 mm)				Shootlets of new leaves segments				AVs.			
Media (mg/L.) (M)	Seasons (S.)	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
½ MS	S_1	13	2.2	4.0	0	13	2.4	3.8	0	7	1.7	5.3	0	11.00	2.10	4.37	0.00
	S_2	20	2.4	4.8	0	13	2.5	4.2	0	0	0	0.0	0	11.00	1.60	3.00	0.00
½ MS+0.5 IBA	S_1	40	2.8	4.6	0	33	3.0	4.1	0	27	1.7	5.4	0	33.33	2.50	4.70	0.00
	S_2	40	2.6	4.5	0	40	2.8	4.2	0	27	1.8	5.5	0	35.66	2.43	4.73	0.00
½ MS+1 IBA	S_1	73	3.2	4.4	7	66	3.0	4.2	0	60	2.5	3.2	0	66.33	2.90	3.93	2.33
	S_2	66	3.8	4.2	0	66	3.8	4.0	0	53	3.0	3.3	0	61.66	3.53	3.83	0.00
½ MS+3 IBA	S_1	80	4.2	3.8	13	73	4.0	4.0	7	66	3.2	4.5	7	73.00	3.80	4.10	9.00
	S_2	80	4.5	4.0	7	66	4.4	4.3	7	60	3.3	4.3	7	68.66	4.06	4.20	7.00
½ MS+0.5 NAA	S_1	60	3.0	4.1	27	47	3.0	4.2	20	33	2.1	5.0	20	46.66	2.70	4.43	22.33
	S_2	53	3.6	4.3	27	40	3.5	4.4	27	33	2.4	6.0	20	42.00	3.16	4.90	24.66
½ MS+2 NAA	S_1	73	3.8	4.7	100	66	4.1	4.3	93	60	3.0	5.2	87	66.33	3.63	4.73	93.33
	S_2	73	3.6	4.8	100	73	3.8	4.5	100	60	2.8	5.4	93	68.66	3.40	4.90	97.66
½ MS+1 NAA+1 IBA	S_1	80	4.2	3.3	53	73	4.5	3.0	47	66	3.2	4.3	40	73.00	3.97	3.53	46.66
	S_2	73	4.0	3.6	53	73	4.2	3.2	53	66	3.0	4.8	47	70.66	3.73	3.87	51.00
½ MS+0.5 NAA+0.5 IBA	S_1	66	3.5	4.2	13	60	3.8	4.0	13	47	2.3	4.9	20	57.66	3.20	4.37	15.33
	S_2	60	3.3	4.5	7	53	3.5	4.2	13	40	2.4	5.2	13	51.00	3.07	4.63	11.00
½ MS+2 NAA+3 IBA	S_1	93	5.8	2.8	87	93	6.1	2.6	73	87	5.0	3.5	73	91.00	5.63	2.97	77.66
	S_2	87	5.3	2.6	87	93	5.6	2.4	80	80	4.8	3.7	73	86.66	5.23	2.90	80.00
½ MS+1 NAA+3 IBA	S_1	87	4.8	2.7	66	80	4.2	3.0	60	73	4.0	3.0	53	80.00	4.33	2.90	59.67
	S_2	80	5.0	2.9	73	80	4.7	3.0	66	73	4.2	3.2	60	77.66	4.63	3.03	66.33
MS	S_1	7	2.0	5.2	0	0	0.0	0.0	0	0	0.0	0.0	0	2.33	0.66	1.73	0.00
	S_2	7	1.8	4.7	0	0	0.0	0.0	0	0	0.0	0.0	0	2.33	0.60	1.57	0.00
AVS.	S_1	61.1	3.59	3.98	33.27	54.90	3.46	3.38	28.45	47.81	2.61	4.03	27.27	54.60	3.22	3.80	29.66
	S_2	58.1	3.63	4.10	32.18	54.27	3.53	3.49	31.45	44.73	2.52	3.78	28.45	52.36	3.22	3.78	30.69
General Avs.		59.6	3.61	4.04	32.73	54.58	3.49	3.43	29.95	46.27	2.56	3.91	27.86	53.48	3.22	3.79	30.17
LSD (0.05)																	
M.	S_1	10.218	1.415	1.019	25.611	31.515	0.981	1.105	19.150	24.510	1.519	1.281	19.711	20.191	0.915	1.791	20.010
	S_2	14.119	1.371	1.286	26.151	30.731	0.899	1.310	18.981	23.801	1.622	1.309	19.011	19.815	1.159	1.816	19.815
S.		4.017	1.212	0.615	1.336	0.523	0.391	0.201	4.061	4.819	0.919	1.152	1.905	3.416	0.010	0.065	2.116
M. x S.		16.811	1.592	1.325	28.516	32.701	0.992	1.410	19.851	26.101	1.762	1.415	19.900	23.092	1.319	1.906	22.111

Shootlets type (A) = 4.295 ; Shootlets type (B) = 0.697 ;Shootlets type (C) = 0.508 and Shootlets type (D) = 3.517

segments of jackfruit were obtained on MS with 2.5 mg/L. BA+0.5 mg/L. NAA, after 28 days. The number of shoots increased to 40 by adjusting media into MS+1.25 mg/L. BA+0.25 mg/L. NAA+15% (v/v) coconut milk. Roy *et al*. [19] stated that clonal of jackfruit identified as flood tolerant could be micropropagated by culturing shoot buds on MS with 8.88 µ M BA+2.68 µM NAA, as this medium gave average number of shoot bud of 10 per transfer through 7 subcultures and explants collected on June-August increased the multiplication. Besides, Rajmohan and Mohanakumaran [6] reported that multiplication rate of 4.5 was obtained with jackfruit's shoot apices cultured for 5 weeks on MS contained 5 mg/L. BA+0.2 mg/L. NAA. Adenine sulphate at 20 mg/L. increased the multiplication rate by 27.3%, shoot elongation occurred on MS with 2 mg/L. BA and 0.2 mg/L. NAA. Roy *et al*. [20] indicated that multiplication of jackfruit achieved by culturing nodal explants on MS with 1 mg/L. BA and 0.5 mg/L. kinetin. In the same trend, Rajmohan and Mohanakumaran [7] denoted that the physiological age of jackfruit explants significantly influenced shoot growth, seedling (2 month old) apices showed a 17.4 fold multiplication rate in 5 weeks on MS+0.2 mg/L. NAA+500 mg/L. PVP (Ployvidone)+8 g agar+10 mg/L. BA. The multiplication rate of adult tree shoot apices ranged from 2.09-4.5 fold (according to the age) in 5 weeks on MS+5 mg/L. BA. Explants of 6 month old grafts failed to multiplicate. Activated charcoal used during stages of shoot multiplication.

The rooting stage

Effect of the NAA and IBA: Data of Table 10 and Fig. 8-11 indicated that NAA may be cause a vitrification phenomenon, where this phenomenon disappeared in NAA-free media as shown in Table 10. The explants (shootlets produced of different explants) showed a significant differences in their response to the studied media, as the shootlets produced of apical growth recorded the higher significant values of the rooted shootlets (59.6%), roots number/shootlet (3.61), root length (4.04 cm) and the highest significant hyperhydric shootlets (32.73%), while the shootlets produced of either nodal segments or new leaf segments gave a satisfied rooting with a lower significant values of hyperhydric shootlets as shown in Table 10.

Also, the media appeared a significant differences in their effect on the studied traits, where the media devoided of NAA resulted in a hyperhydricity-free shootlets; besides the medium of ½ MS+2 mg/L. NAA+3 mg/L. IBA gave the higher values of rooted shootlets (91 and 86.66% for the first and second season, respectively) and roots number/shootlet (5.63 and 5.23 for the first and the second season,

Fig. 8: The root formation on ½ MS medium+2 mg/L. NAA+3 mg/L. IBA

Fig. 10: The root formation on ½ MS medium+2 mg/L. NAA

Fig. 9: The root formation on ½ MS medium+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. coumarin+1 mg/L. paclobutrazol

Fig. 11: The suitable stage of the plantlet before starting the acclimatization stage

respectively), as well as, the medium of ½ MS+2 mg/L. NAA showed the higher significant values of root length (4.73 and 4.90 cm on the first and the second season, respectively) and the hyperhydric shootlets (93.33 and 97.66% on the first and the second season, respectively). On the contrary, MS medium recorded the lower significant values of all studied traits.

It is recommended with ½ MS+3 mg/L. IBA for producing a good rooting and the lowest significant hyperhydric shootlets %. The interactions followed the same trends.

Effect of coumarin, paclobutrazol, IBA and NAA: Similarly, data of Table 11 indicated that the shootlets produced of the apical growth showed the higher significant values of rooted shootlets (71.56%), roots number/shootlet (4.63) and the highest significant hyperhydric shootlets (8.97%), so it must be exclude these shootlets, which caused a big loss in the micropropagation of jackfruit, especially at the rooting stage (the last) and replace them with the shootlets produced of either a nodal segments or a new leaf segments whose produced a good rooting with a lower vitrification percentages.

Considering the media, it is noticed that all media included paclobutrazol devoided of the vitrification (hyperhydricity), which lead to velieve that the paclobutrazol may be prevent this harmful phenomenon. On the contrary, all media included, NAA, IBA or coumarin have an vitrification, which believe that these growth regulators may be cause the hyperhydricity in *in vitro* culture of jackfruit. The pronounced medium was ½ MS+2 mg/L. NAA+3 mg/L. IBA+1 mg/L. paclobutrazol+1 mg/L. coumarin, as it showed the least significant values of the hyperhydric shootlets (zero % for both studied season) and the higher significant values of the rooted shootlets (97.66 and 93.33% for the first and the second season, respectively), roots number/shootlet (7.27 and 7.03 for the first and the second season, respectively) and the root length (2.47 and 2.07 on the first and the second season, respectively).

Table 11: Effect of media, type of the shootlets and seasons on percentage of the rooted shootlets (A), roots number / shootlet (B), root length (cm) (c) and the hyperhydric shoolets percentages (D) of the jackfruit during 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Type of shootlets		Shootlets of apical growth without leaves (5-10 mm)				Shootlets of nodal segments (10-15 mm)				Shootlets of new leaves segments				AVs.			
Seasons (S.)																	
Media (mg/L.) (M)		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
½ MS+1 Coumarin	S_1	80	4.7	3.7	13	73	4.2	4.0	7	66	4.0	4.2	7	73.00	4.30	3.97	9.00
	S_2	73	4.8	3.5	7	73	4.0	3.8	7	60	3.8	4.4	7	68.66	4.20	3.90	7.00
½ MS+0.5 Coumarin (co.)	S_1	66	3.4	4.2	7	66	3.0	4.5	7	53	2.8	4.8	7	61.66	3.07	4.50	7.00
	S_2	66	3.2	3.9	7	60	3.0	4.2	0	53	3.0	4.6	7	59.66	3.07	4.23	4.67
½ MS+1 Paclobutrazol	S_1	0	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0.00	0.00	0.00	0.00
	S_2	0	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0.00	0.00	0.00	0.00
½ MS+0.5 Paclobutrazol (Pac.)	S_1	0	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0.00	0.00	0.00	0.00
	S_2	0	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0.00	0.00	0.00	0.00
½ MS+1 NAA+1 IBA+1 Co.	S_1	93	5.6	3.0	27	87	5.2	3.3	27	80	4.8	3.6	27	86.66	5.20	3.30	27.00
	S_2	87	5.8	2.8	33	80	5.4	3.2	27	73	4.0	3.4	20	80.00	5.07	3.13	26.67
½ MS+1 NAA+1 IBA+0.5 Co.	S_1	87	5.2	3.2	27	80	5.0	3.4	27	73	4.6	3.8	20	80.00	4.93	3.47	24.67
	S_2	87	5.0	3.3	27	73	4.7	3.6	20	66	4.6	3.8	20	75.33	4.77	3.57	22.33
½ MS+1 NAA+1 IBA+1 Pac.	S_1	47	3.1	4.5	0	33	2.0	5.8	0	27	1.0	7.2	0	35.66	2.03	5.83	0.00
	S_2	40	2.9	4.2	0	33	1.8	6.2	0	20	1.2	7.5	0	31.00	1.97	5.97	0.00
½ MS+1 NAA+1 IBA+0.5 Pac.	S_1	53	3.0	4.3	0	47	2.6	4.8	0	40	2.2	5.2	0	46.66	2.60	4.77	0.00
	S_2	47	2.8	4.0	0	40	2.4	4.4	0	33	2.4	5.0	0	40.00	2.53	4.47	0.00
½ MS+2 NAA+3 IBA+1 Co.	S_1	100	8.7	2.0	33	93	5.2	2.4	33	80	4.0	2.7	40	91.00	5.97	2.37	35.33
	S_2	100	9.0	1.7	40	87	5.6	2.2	33	73	3.8	2.5	40	86.66	6.13	2.13	37.67
½ MS+2 NAA+3 IBA+0.5 Co.	S_1	100	7.0	2.2	33	87	7.2	2.7	33	80	7.5	3.0	27	89.00	7.23	2.63	31.00
	S_2	100	6.8	2.3	33	87	7.0	2.5	27	73	7.2	2.8	27	86.66	7.00	2.53	29.00
½ MS+2 NAA+3 IBA+1 Pac.	S_1	73	3.8	4.0	0	66	4.0	4.3	0	60	4.2	4.6	0	66.33	4.00	4.30	0.00
	S_2	66	3.5	3.8	0	60	3.8	4.0	0	60	4.0	4.3	0	62.00	3.77	4.03	0.00
½ MS+2 NAA+3 IBA+0.5 Pac.	S_1	73	4.0	4.3	0	73	4.3	4.5	0	66	4.6	4.7	0	70.66	4.30	4.50	0.00
	S_2	73	4.2	4.5	0	66	4.5	4.8	0	60	4.6	5.0	0	66.33	4.43	4.77	0.00
½ MS+1 NAA+1 IBA+	S_1	100	7.1	1.8	0	100	6.0	1.4	0	93	5.2	1.5	0	97.66	6.10	1.57	0.00
1 Co.+1 Pac.	S_2	100	7.6	1.7	0	100	5.8	1.5	0	100	4.9	1.6	0	100.00	6.10	1.60	0.00
½ MS+1 NAA+1 IBA+	S_1	93	6.0	2.5	0	87	6.2	2.7	0	80	6.4	3.0	0	86.66	6.20	2.73	0.00
0.5 Co.+0.5 Pac.	S_2	93	5.7	2.2	0	80	6.0	2.4	0	80	6.2	2.8	0	84.33	5.97	2.47	0.00
½ MS+2 NAA+3 IBA+	S_1	100	7.0	2.3	0	100	7.2	2.5	0	93	7.6	2.6	0	97.66	7.27	2.47	0.00
1 Co.+1 Pac.	S_2	100	6.7	1.8	0	93	7.0	2.0	0	87	7.4	2.4	0	93.33	7.03	2.07	0.00
½ MS+2 NAA+3 IBA+	S_1	100	5.9	2.0	0	87	6.2	2.3	0	73	6.5	2.6	0	86.66	6.20	2.30	0.00
0.5 Co.+0.5 Pac.	S_2	93	5.6	2.2	0	80	5.8	2.5	0	66	6.0	2.7	0	79.66	5.80	2.47	0.00
AVS.	S_1	72.81	4.66	2.75	8.75	67.44	4.27	3.02	8.37	60.25	4.09	3.34	8	66.83	4.34	3.04	8.37
	S_2	70.31	4.60	2.62	9.19	63.25	4.30	2.96	7.12	56.50	3.94	3.30	7.56	63.35	4.28	2.96	7.96
General Avs.		71.56	4.63	2.68	8.97	65.34	4.28	2.99	7.74	58.37	4.01	3.32	7.78	65.09	4.31	3.00	8.16
LSD (0.05)																	
M.	S_1	31.115	1.588	1.817	15.516	38.151	0.902	1.198	13.731	35.911	1.659	2.119	11.381	34.161	1.460	1.909	14.101
	S_2	35.801	1.892	1.543	14.681	39.011	1.060	1.007	12.192	37.513	1.518	2.200	10.861	36.417	1.616	1.840	12.915
S.		3.091	0.215	0.201	0.561	5.196	0.117	0.112	2.011	6.515	0.306	0.016	0.610	6.199	0.182	0.156	0.510
M. x S.		33.356	1.709	1.693	15.236	37.950	0.980	1.105	12.876	36.713	1.580	2.708	11.183	35.131	1.560	1.865	13.550

Shootlets type (A) = 4.205 ; Shootlets type (B) = 0.459 ; Shootlets type (C) = 0.515 and Shootlets type (D) = 0.908

The interactions between the explants and the media showed the same previous trends, not in the rooting stage only, but during all stages of jackfruit's micropropagation, which indicated that a relative stable behaviour of the jackfruit's explants during all stages of the micropropagation process.

The results of Table 10 and 11 were in agreement with those obtained by Wongmaneeroj *et al.* [21] whose obtained a good rooting of jackfruit cv. paisarn Taksin shoots, derived from tissue culture and using 2-3 cm segments on solid MS with 0.2 mg/L. NAA and 15% v/v coconut water, then transferred onto solid Woody Plant Medium (WPM) with 10% coconut water for few weeks and the basal end of shoot was dipped in 5000 mg/L. IBA for 10 minutes.

Roy *et al.* [2] obtained a good rooting of *in vitro* shoots of jackfruit on ½ MS+1mg/L. IBA+1 mg/L. NAA. Besides, Roy *et al.* [19] decided that 80% of *in vitro* multiplicated shoots of jackfruit rooted within 3 weeks on ½ MS+5.37 μ M NAA+4.92 μ M IBA. Amin and Jaiswal [17] stated that 60-80% of *in vitro* shoots of jackfruit rooted on ½ MS+10 μ M IBA or NAA. Rajmohan and Mohanakumaran [6] stated that *in vitro* rooting of jackfruit occurred on ½ MS+2 mg/L. NAA+2 mg/L. IBA+30 g sucrose+6 g agar for 6 days, followed by transfer into ½ MS only. Roy *et al*. [20] obtained a rooting of jackfruit's shootlets onto ½ MS+1 mg/L. each of IBA and NAA. Rajmohan and Mohanakumaran [7] achieved the best rooting of jackfruit's shootlets produced of 2 month old seedling apices on ½ MS+0.2-0.8 ppm IBA, ½ MS+0.4 ppm each of NAA and IBA or ½ MS+0.5 ppm NAA+2 ppm IBA (100% rooting and 6 roots / explant in 20.75 day). *In vitro* shoots produced of stem shoot apices of adult jackfruit trees gave 70% rooting with 5.43 roots/explant in 13.43 day, while explants of 6 month old grafts gave 50% rooting with 2 roots/explant in 20.5 day all on ½MS+0.4 ppm NAA+1.6 ppm IBA and ½ MS+2 ppm NAA+2 ppm IBA.

Table 12: Effect of the cultivation media, time after the planting date and the seasons on the leaf number / acclimatized plantlet (A), shoots number / acclimatized plantlet (B), survival percentages (c) and height (cm) of the vegetative growth (D) of the acclimatized jackfruit plantlets during 2004/2005 (S_1) and 2005/2006 (S_2) seasons

Time of P.D.		Planting date (P. d.)				One month of P. d.				2 months of P.d.				3 months of P. d.				AVs.				
Cultivation media (CM)	S	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	D
1 V. / 1 V. of sand / soil	S_1	3.50	2.0	100	5.6	4.8	2.2	37	6.2	7.2	3.3	23	8.5	9.8	4.0	17	13.2	6.32	2.87	44.25	8.37	
	S_2	4.60	1.3	100	8.1	5.1	3.0	33	10.1	6.8	3.2	17	12.3	10.8	4.2	13	14.7	6.82	2.85	40.75	11.30	
1 V. / 1 V. of sand : peat	S_1	4.00	2.1	100	6.3	6.0	3.1	23	8.8	9.1	3.5	17	10.3	10.4	3.8	13	18.4	7.37	3.12	38.25	10.95	
	S_2	3.10	2.3	100	7.0	5.2	3.0	27	9.0	11.7	4.3	17	11.1	12.8	4.7	10	16.2	8.20	3.57	38.50	10.82	
1 V. / 1 V. /1V. of soil :	S_1	4.10	1.4	100	7.2	5.5	3.2	60	8.0	10.3	3.4	33	9.2	15.3	51.0	27	11.4	8.80	3.27	55.00	8.95	
burned rice hull : fibrous sheath of date palm	S_2	3.30	2.5	100	6.5	6.0	4.6	53	7.2	13.5	5.1	40	9.5	15.8	6.0	17	12.1	9.40	4.55	52.50	8.82	
AVS.	S_1	3.87	1.83	100	6.37	5.43	2.83	40.00	7.67	8.87	3.4	24.33	9.33	11.83	4.30	19.00	14.33	7.50	2.94	45.83	9.42	
	S_2	3.67	2.03	100	7.20	5.10	3.53	37.67	8.77	10.67	4.2	24.67	10.97	13.13	4.97	13.33	14.33	8.14	3.66	43.92	10.31	
General Avs.		3.77	1.93	100	6.78	5.26	3.18	38.83	8.22	9.77	3.80	24.50	10.15	12.48	4.63	16.16	14.33	7.82	3.30	44.87	9.86	
LSD (0.05)																						
CM.	S_1	0.315	0.531	0.001	0.606	1.100	0.901	12.118	1.690	1.890	0.617	8.195	0.806	2.519	1.051	15.166	5.219	1.009	0.391	9.907	1.313	
	S_2	0.459	0.765	0.001	0.850	0.899	1.350	14.511	1.709	2.700	0.398	11.081	1.315	2.706	1.259	6.056	3.850	1.195	0.556	10.705	1.860	
S.		0.401	0.279	0.001	0.995	0.566	0.818	3.116	1.315	2.159	1.051	0.451	1.970	1.515	0.867	6.160	0.001	0.911	0.956	2.217	1.115	
M. x S.		0.486	0.667	0.001	0.767	1.002	1.193	13.201	1.602	2.226	0.506	10.113	1.103	2.593	1.102	9.976	4.662	1.104	0.416	10.321	1.581	

Time P.D. (A) = 2.551; Time P.D. (B) = 0.759; Time P.D. (C) =20.915 and Time P.D. (D) = 1.754

Fig. 12: Acclimatization stage on a growing mixture of sand: peatmoss (1:1). Notice: height of the acclimatized plantlet

Fig. 13: Acclimatization stage on a growing mixture of soil: burned rice: fibrous sheath of date palm (1:1:1). Notice: branch of the acclimatized plantlet

Nower *et al*. [18] obtained the highest rooting (100%) of *in vitro* seedling's shoots of jackfruit on MS+0.5, 1 or 2 mg/L NAA, while the highest number of roots were on MS+2 mg/L. IBA followed by MS+2 mg/L. NAA. The higher root length observed on MS only and MS+0.5 mg/L. NAA.

Zaied [22] mentioned that adding of 90-150 μ M coumarin to the culture medium of swingle citrumelo (citrus) enhanced root formation,and found that adding 1 mg/L. coumarin to the medium of banana induced a maximum *in vitro* growth parameters. Chen and Ziv [10] found that plant growth retardants used in a liquid cultures to overcome the hyderhydric malformation.

The acclimatization stage: Data of Table 12 and Fig. 12, 13 evinced that the culture mixture of soil: burned rice hull: fibrous sheath of date palm (1:1:1, v/v/v) recorded the highly significant values of all studied growth parameters in the two studied seasons, while mixture of sand: soil gave the lower significant values except the survival %, besides the mixture of sand: peatmoss gave the lowest significant survival %. Considering the dates, all studied growth parameters increased with increase of the dates and reached to the maximum significant values after 3 months of the planting date. The effect of interaction was similar as the previous effects and took the same trends. The results of Table 12 were in accordance to those obtained by Nower *et al*. [18] whose decided that the highest survival (95%) of jackfruit's plantlets recorded in mixture of peatmoss : perlite (2:1, v/v) in the greenhouse. Also, Wongmaneeroj *et al*. [21] stated that the plantlets without roots of jackfruit transplanted into the soil mixtures and the roots induced after 31 days on mixture of sand: coconut husk : burned rice hull at 2:2:1 gave 70% rooting. Roy *et al*. [2,19] reached to 75% survival plantlets of jackfruit in the acclimatization by transplanting the plantlets into pots contained a sterile sand, soil and humus (1:2:1) and covered by transparent plastic bags. Amin and Jaiswal [17] reported that regenerated plantlets of jackfruit transferred to the soil and about 50% survived Roy *et al*. [20] decided that the acclimatization of jackfruit successed in pots under greenhouse.

Finally, it could be concluded that there are insignificant differences between the two studied seasons; the explants collected from 3 months old seedlings have a highly survival percentages during the sterilization, while those of June-July were less contamination. Explants of the seedlings and those of December-January were the best in callus formation and proliferation. Explants of the seedlings gave a good multiplication and less hyperhydric shootlets during the multiplication. During the rooting, the shootlets produced of nodal segments achieved a good rooting and the least hyperhydricity. All growth estimations during the acclimatization were increased with increase of dates recorded these estimations and reached to the maximum values after 3 months of the planting date, beside, the best growing mixture in the acclimatization was soil: burned rice hull: Fibrous sheath of the date palm.

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