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Induction of callus and somatic embryogenesis from explants of *Parkia biglobosa* (Jacq.) *Benth* and *Parkia roxburghii G.Don*.

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ABSTRACT

Legumes are very important species including herbs, shrubs and trees, they are cultivated worldwide for different economic uses. *Parkia* is genus belongs to the Legume family, Fabaceae. The most common known species are *P. biglobosa* Benth (African locust bean) and *P. roxburghii* (Tree Bean) which were introduced to Egypt since 1890. The population of the tree is rapidly declining as result conservation efforts is needed to prevent it from extinction. In an attempt to conserve this genetic resource, tissue culture studies were carried to establish a callus culture of both plants from different explants and regeneration of the developed callus and to study the effect of explant type, media composition on the callus production and the regeneration. Apical bud,axillary bud and root explants from in vitro germinated seedlings of *P. biglobosa* Benth were cultured on MS and B5 medium supplemented with (0.4., 0.8, 1.2, 1.6 or 2.0 mg l^{-1} NAA) + Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 2,4D 2.5 mg l^{-1}. Each medium was supplemented with 30 g l^{-1} sucrose, 3 g l^{-1} agar and PH 5.8.for callus induction .and (0.4.,0.8,1.2,1.6 or 2.0 mg l^{-1} NAA) + Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}. Each medium was supplemented with 30 g l^{-1} sucrose, 3 g l^{-1} agar and PH 5.8.for regeneration.The study presented here demonstrates the successful attempt at regeneration of plantlets of *P. biglobosa* Benth via indirect organogenesis. It can be concluded that the medium composition has a significant effect on the calli production together with the type of the explant which showed relatively higher significance. Seeds germination stage apical bud explants media 8 followed by media 10 showed the most favorable mean performances values for the studied characters, number of days to callus initiation (day), callus weight (mg), dead calli percentage (%), albino plants percentage (%),green plants percentage (%) and root percentage (%). Moreover, axillary bud, showed remarkable increase in the mean performance of average. However, the overall response of MS medium was found to be superior to that of B5 medium. Explants cultured on MS medium fortified with combinations of 2,4-D and BAP induced rapidly proliferating calli that turned more friable and nodular.

Indexing terms/Keywords

Parkia roxburghii G.Don.,*Parkia biglobosa*, (Jacq.) Benth, indirect organogenesis, 2,4-D and BAP, apical bud ,axillary bud and root explants.

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INTRODUCTION

Parkia is a genus of about 30–40 tree legume species of considerable evolutionary, taxonomic, biological and economic importance in Africa, Asia and South America. It has several uses, including fodder, food, medicine, green manure, fuel and timber [1].

Parkia roxburghii G. Don. is known as 'tree bean, It is a multipurpose tree with intermediate height (10-20 m) the tree known to be highly branched. The green pods and seeds of *Parkia roxburghii* have a high nutritional value and can serve as a potential source of protein and fat and also used in various local medicinal applications [2, 3].

P. biglobosa is an important tree species which generates non-timber forest products popularly known as the African locust bean tree belongs to the family Fabaceae [4, 5, 6]. It is a perennial deciduous tree occurring in a belt between 5° N and 15° N [6], 7 to 20 m tall, and in some cases it can reach up to 30 m [7].It is considered to be valuable source of nutrition and as therapeutic food [4]. The pulp of the fruit pods is rich in sucrose and the seeds are rich in carbohydrates, proteins and lipids, thus constituting an important source of energy [4]. *P. biglobosa* is rated fifth important among thirty-one woody medicinal plants used in traditional medicine in Benin. It is rated fourth from a list of eighteen priority food woody plants to preserve. In association with crops, the species help to enrich physico-chemical soil characteristics which in turn help to increase crop yields [4]. The fruit is a slightly curved, brown indehiscent pod, 30 to 40 cm long and 2 to 3 cm wide producing up to 20 seeds. The seeds when boiled and fermented is known as 'dawadawa' in Hausa language in Nigeria, a black strong smelling tasty seasoning, rich in lipid 29 per cent, protein 35 per cent, carbohydrate 16 per cent, and it is a good source of fat and calcium for rural dwellers [8]. The pods are used as sponges and strings, dyes, and for fishing, and also for preparing insecticide powder [6]. The bark is used as a mouthwash, vapour inhalant for toothache, or for ear complaints. It is macerated in baths for leprosy and used for bronchitis, pneumonia, skin infections, sores, ulcers, and washes for fever, malaria, diarrhea, and sterility. Roots are used in a lotion for sore eyes [9].

There are fews sources of these two trees (*Parkia biglobosa* and *Parkia roxburghii*) in Egypt which contains the genes of adptability to the Egyptian environment,as results efforts is needed to conserve and prevent theseindigenous plants from extinction .Vegetative propagation by means of tissue culture techniques is an important tool for plant germplasm conservation and rapid clonal multiplication as well as for reforestation and tree improvement.

Micropropagationand tissue culture studies of *Parkia roxburghii* G. Don are still not present and need efforts to establish a good protocol for plant regeneration . Also, tissue culture studies in *Parkia biglobosa* Benth are rare and obtaining shoots are still challenging, the literatures are confined to callus and embryo induction without shoot or plantlet regeneration [10, 11] or in vitro germination and multiplication of a *P.biglobosa* [11]. Plant regeneration has been successful in some non-woody horticultural and woody plants [12,13].Reviewing the current literature, it was found that a trial was made to study the effect of plant growth regulators and different media on micropropagation of *P.biglobosa (Jacq.) Benth* and *P. roxburghii G.Don.* A study showed a method for differentiation of callus, shoots and plant regeneration from hypocotyl explants of Parkia biglobosa Benth via indirect organogenesis. This work aimed to establish a callus culture of both plants from different explants and regeneration of the developed callus and to study the effect of explant type, media composition on the callus production and the regeneration of the studied plants.

Tissue culture involves the isolation of cells or tissues and placing them under controlled, aseptic environments [14,15,16]). All of the environmental factors necessary for growth (heat, light, air, water, nutrients, and support) are provided artificially with the objective being to obtain rapid asexual multiplication of plant cells or plants. Any given tissue composed of cells with competent nuclei is a suitable explant for the initiation of a plant tissue culture [17,18]. Multiplication of plant materials in vitro involves the manipulation of plant growth through modifications of the culture medium, culture environment, and the source and type of tissue taken for culture [16,18]. Multiplication can be achieved by one of three different morphogenic processes:axillary bud enhancement, adventitious shoot formation, or somatic embryogenesis [14,16,18].

MATERIALS AND METHODS

Plant materials

Trees of *Parkia. biglobosa* and *Parkia roxburghii* G. Don grown in Alzohria garden Giza, Egypt, were used as source of fresh tissue explants for *in vitro* culture techniques . A trial was made to produce callus by *in vitro* culturing of different fresh parts of the conventional plant on tissue culture media .Various types of 1-2 cm length explants {apical bud, axillary bud, and leaflet and midrib at the node (nodule explant)} from each parental species *P. roxburghii* G.Don.and *P. biglobosa* (Jacq.) Benth were used. Explants were washed thoroughly with running tap water for 7 min., then dissected, and then wrapped in sterilized lawn in sterilized Petri dish. 70 % ethanol (v/v) was used for surface sterilizing of every explant for 30 seconds. Every explant was soaked in 40 % Clorox (sodium hypochloride 5.25 %) for 20 min. After 20 min., Clorox was discarded and sterilized explants were washed by sterilized distilled water three times (3 min. each). The authors want to notify that lower concentrations of Clorox (10, 20 and 30%) which were used as trials for sterilizing the explants have been failed to supply sterile conditions.

Matured seeds of *P. biglobosa* Jack. Benth. Were obtained from Germplasm Resources Information Network (GRIN), USDA and used to develop seedling as source of fresh young explants (apical buds, axillary buds and roots). In order to chemically break the seed dormancy and reduce the testa toughness using 70% H_2SO_4 for 20 min. Surface sterilization was done by treating with 1% (v/v) sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. Seeds were then germinated on MS medium, with 10 g/l sucrose and 8 g/l agar in a growth room at 25±1℃, 16 h photoperiod, 30-40 µmol m-2 s-1 cool white fluorescent light. The pH of the medium was adjusted to 5.8 prior to the addition of agar and autoclaving at 121℃ for 15 min.The obtained in vitro seedlings (2-6 weeks old) were used as the source of the explants in the induction of callus.

Culture of explants

Four different types of length 1-2 cm plant explants [apical bud, axillary bud, and leaflet and midrib at the node (nodule explant)] from each adult parental species *P. biglobosa* (Jacq.) Benth and *P. roxburghii G*.Don have been used for callus induction. The four explants from fresh tissue were cultured in basal media B5 (Gamborg) [19] or MS (Murashige and Skoog) [20] supplemented with (0.4.,0.8,1.2,1.6 or 2.0 mg l-1 NAA) + Glutamine 0.5 mg l-1 + Proline 0.5 mg l-1 + Casein 0.3 mg l-1 + 6-BAP 2 mg l-1(Table1). Each medium was supplemented with 30 g l-1 sucrose, 3 g l-1 agar and PH 5.8 (Table1).

Three different fresh explants types (apical, axillary buds and roots) from 2-5 weeks old seedlings of *P. biglobosa* were cut with a fine sterile blade to produce small pieces of about (10 mm x 10 mm), the explants pieces were cultured in Petri-dishes containing 33 ml of callus induction medium. For each medium type, 3 replications were done, each replication with 2 Petri-dishes, and each Petri-dish contained 10-15 explants of a particular accession. An average of 160 explants (by repetition) per cultivar were used in the experiment. The cultures were maintained in dark at 25±2ºC in dark with a relative humidity of 60%. All cultures were placed at 25±1℃, 16h photoperiod, 30-40 µ mol m-2 s-1 cool white fluorescent light. All the cultures were sub cultured at two weeks intervals on fresh media. Callus induction was visually evaluated and scored to aid a more rapid screening option.The explants were sub cultured on freshly prepared media at two weeks intervals until callus developed. The chemicals used for the experiments were manufactured by M/S Himedia Company, Mumbai, India.

Table 1. Composition of media used for culture regeneration (mg/L)

Medium	Medium composition

Medium. 1	**B5 medium + 0.4 mg l^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium. 2	**MS medium + 0.4 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium. 3	**B5 medium + 0.8 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium. 4	**MS medium + 0.8 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 5	**B5 medium + 1.2 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 6	**MS medium + 1.2 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 7	**B5 medium + 1.6 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 8	**MS medium + 1.6 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 9	**B5 medium + 2.0 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}
Medium 10	**MS medium + 2.0 mg^{-1} NAA +** Glutamine 0.5 mg l^{-1} + Proline 0.5 mg l^{-1} + Casein 0.3 mg l^{-1} + 6-BAP 2 mg l^{-1}

Each medium was supplemented with 30 g l^{-1} sucrose, 3 g l^{-1} agar and PH 5.8.

Callus growth and regeneration ability characters:

Observationswere recorded on three plates taken at random from each medium used for each test entry for determination of the following criteria:-1. Number of days to callus initiation (It was counted as a number of days from date of plating to the date of first callus appear in first three plates). 2. Percentage of callus induction ability (It was calculated as apercentage of induced calli per anthers in a plate).3. Green plants percentage (%) (It was calculated as percentage of green plants regenerated from calli). 4. Albino plants percentage (%) (It was calculated as percent of albino plants regenerated from calli).5. Dead calli percentage (%) (It was calculated as percentage of calli which did not regenerate, dead calli have brown color and do not show any differentiation).6. Root percentage (%)It was calculated as percentage of roots which developed from calli and did not show any shoot differentiation**.**

Statistical analysis

The experimental design was a completely randomized design with three replications per medium type, 10-15 explants per accession per replicate. For the experiment on callus induction, 3 accessions, 3 types of explants at 4 types of media were analyzed as a 3 x 3 x 4 factorial arrangement. In this study, callus from leaf explants did not regenerate shoots, therefore, the analysis on regeneration were based on the number of hypocotyls explants cultured. Hence, 3 accessions at 4 types of media were analyzed as a 3 x 4 factorial arrangement. Data obtained were subjected to analysis of variance (ANOVA). Significant means were separated using least significant difference (LSD) test. All percent data were subjected to arc sine (√x) transformation before statistical analysis.

RESULTS

Induction of callus from the different plant organs of the conventional plant

The effect of explant types and plant growth regulators on the *in vitro* callus formation was studied and the callusing capacities, as well as, the types of explants on the different media were recorded in and **tables (2,3and 4) and fig. (1,2,3).**

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The mean performance of the different criteria of all explant for the two studied species is presented in tables 3-5. All the regeneration produced from different media and variable explants were evaluated.

Number of days to callus initiation (day): The obtained outcomes of mean performance characters of *P. biglobosa (Jacq.) Benth*, table (2) illustrated the shortest mean period to number of days to callus initiation for the apical bud explant (28.31) in media 10 followed by the axillary bud explant (29.55) in media 8. While, the nodule explant (51.39) in media 2 scored the largest number of days. On the other hand, *P. roxburghiiG.Don.*recorded the shortest mean period to number of days to callus initiation for the apical bud explant (28.33) in media 8 followed by the axillary bud explant (29.88) in media 10 While, the nodule explant (58.33) in media 5 scored the longest period (Table 2).

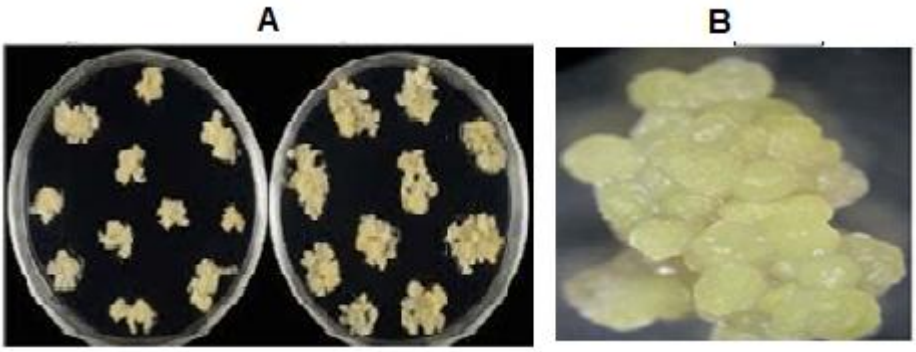

Fig 1: shows plant regeneration through callus formation (callus of the different explants). A Callus of leaflet and node explants on media2, 3 and 8. Two week old. B callus of axillary bud explants on media 2, 3 and 8.Four week old callus. Callus of apical bud Six week old callus on media 2, 3 and 8.

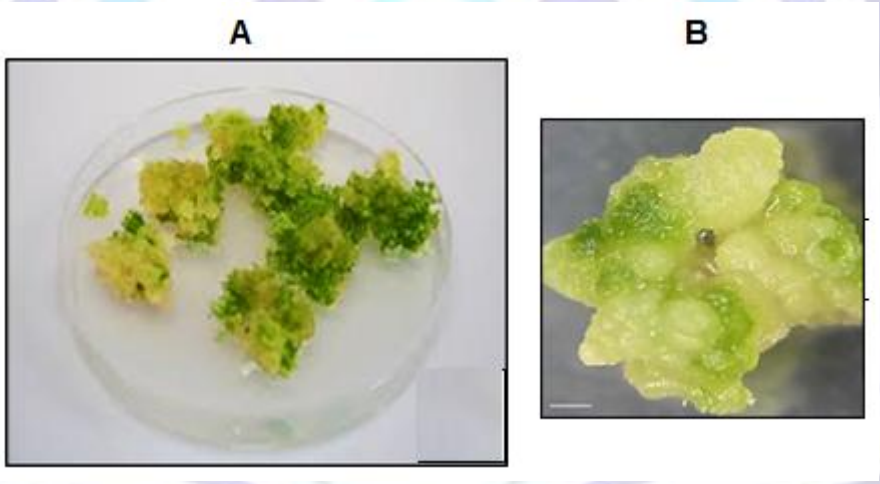

Fig 2: photographs showing the callus of apical bud explants on media 2, 3 and 8. showing green plant, A Shows (x= 2) and B Shows (x= 5).

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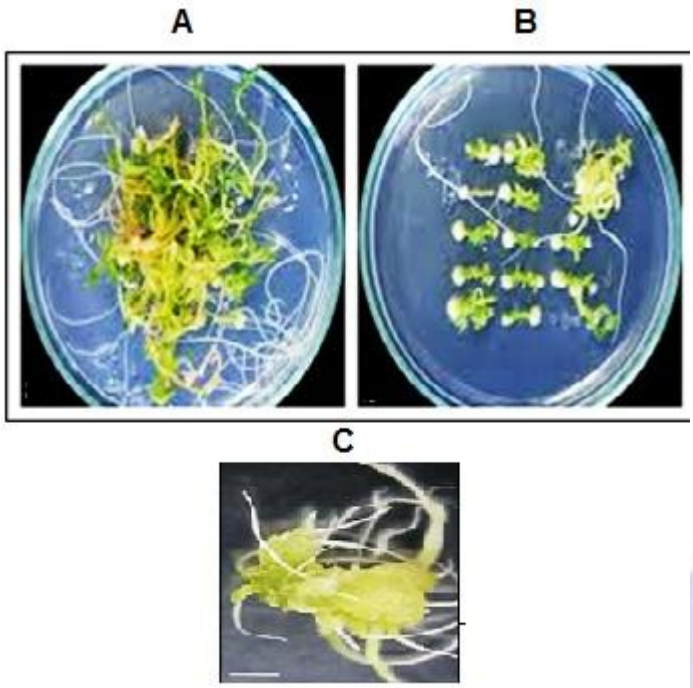

Fig3: photographs showing the shoot and root regeneration from apical, axillary buds and leaf explants on media 2, 3,8 and 10.showing green plant,A(x= 2), B (x= 2) and C- (x= 5).

Table 2.Mean performance of parental genotypes or species, mediums and explant for number of days to callus initiation (day).

Genotypes		***P. biglobosa***				***P. roxburghii***				
Explant		apical bud	axillary bud	leaflet	midrib at the node	apical bud	axillary bud	leaflet	midrib at the node	**Mean**
Medium 1		35.82	43.71	0.00	0.00	36.6	38.6	0.00	0.00	19.34
Medium 2		33.30	34.47	0.00	51.39	31.55	32.81	0.00	53.7	28.90
Medium 3		31.25	35.74	41.38	0.00	30.12	36.64	47.3	0.00	27.80
Medium 4		36.97	40.50	0.00	39.45	36.15	37.4	0.00	43.6	29.26
Medium 5		38.98	39.52	36.22	41.63	36.84	41.77	58.33	50.97	43.03
Medium 6		37.61	33.80	41.75	0.00	32.86	37.54	43.8	0.00	27.17
Medium 7		42.85	0.00	0.00	0.00	43.82	0.00	0.00	0.00	11.33
Medium 8		31.21	29.55	39.74	41.25	28.33	31.85	43.71	50.2	36.98
Medium 9		0.00	36.58	0.00	0.00	0.00	39.66	0.00	0.00	9.53
Medium 10		28.31	31.09	40.82	37.3	30.8	29.88	44.7	40.54	35.43
Mean		31.03	32.50	19.99	20.50	30.62	32.71	23.78	23.90	
LSD	Genotype	0.4483								
	Medium	0.7209								
	Genotype XMedia	1.806								

LSD: Least significant difference at 1%.

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Callus weight (mg):As for studying themean performance characters of callus weight (mg),*P. biglobosa (Jacq.) Benth,* table (4) showed the highest callus weight (mg) for the axillary bud explant (0.453) in media 8 followed by the apical bud explant (0.437) in media 8 While, the nodule explant (0.115) in media 5 scored the smallest callus weight. Another trend was observer, themean performance characters of *P. roxburghii G.Don.* proved the highest callus weight (mg) for the apical bud explant (0.475) in media 10 followed by the axillary bud explant (0.452) media 8. While, the nodule explant (0.011) media 5 showed the smallest Callus weight(Table 3).

Table 3.Mean Mean performance of parental genotypes or species, mediums and explant for callus weight

Genotypes		***P. biglobosa***				***P. roxburghii***				
Explant		apical bud	axillary bud	leaflet	midrib at the node	apical bud	axillary bud	leaflet	midrib at the node	**Mean**
Medium 1		**0.176**	**0.296**	**0.000**	**0.000**	**0.168**	**0.211**	**0.000**	**0.000**	**0.106**
Medium 2		**0.395**	**0.406**	**0.000**	**0.196**	**0.346**	**0.339**	**0.000**	**0.110**	**0.224**
Medium 3		**0.427**	**0.386**	**0.199**	**0.000**	**0.485**	**0.463**	**0.202**	**0.000**	**0.270**
Medium 4		**0.321**	**0.292**	**0.000**	**0.201**	**0.207**	**0.389**	**0.000**	**0.158**	**0.196**
Medium 5		**0.269**	**0.157**	**0.115**	**0.289**	**0.155**	**0.297**	**0.008**	**0.011**	**0.163**
Medium 6		**0.329**	**0.308**	**0.256**	**0.000**	**0.315**	**0.376**	**0.243**	**0.000**	**0.228**
Medium 7		**0.286**	**0.000**	**0.000**	**0.000**	**0.217**	**0.000**	**0.000**	**0.000**	**0.063**
Medium 8		**0.437**	**0.453**	**0.218**	**0.225**	**0.398**	**0.452**	**0.199**	**0.238**	**0.321**
Medium 9		**0.000**	**0.354**	**0.000**	**0.000**	**0.000**	**0.301**	**0.000**	**0.000**	**0.082**
Medium 10		**0.403**	**0.389**	**0.275**	**0.209**	**0.475**	**0.411**	**0.237**	**0.243**	**0.337**
Mean		**0.304**	**0.304**	**0.106**	**0.112**	**0.277**	**0.324**	**0.089**	**0.076**	
LSD	**Genotype**	**0.00994**								
	Medium	**0.00374**								
	Genotype XMedia	**0.05683**								

LSD: Least significant difference at 1%.

Dead calli percentage (%): On comparing the dead calli percentage % it was cleared that the mean performance characters of *P. biglobosa (Jacq.) Benth,* showed thelowest percentage of dead callifor the apical bud explant (31.68) in media 8 followed by the axillary bud explant (33.71) in media 2. While, the nodule explant scored the highest percentage (71.25) in media 7. Meanwhile, the mean performance characters of *P. roxburghii G.Don.* Showed the lowest percentagefor the apical bud explant (31.17) in media 8 followed by the axillarybud explant (32.58) in media10. While, the nodule explant scored the highest percentage of dead calli (70.35) in media 10 (Table 4).

Theresults as shown in tables 2, 3 and 4 recommended that media 2, 3,8 and 10 as they were the most suitable media for callus production as revealed from the three mean performance of the measured factors (number of days to callus initiation (day), callus weight and dead callus percentage). Concerning explants, apical bud and axillary bud showed appropriate callus result as demonstrated in the previous results. That's why we decide to continue using those explants from the germinated seeds. Accordingly to these results in our present study we decided to continue using those explants from the germinated seeds.

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Table 4.Mean performance of parental genotypes or species, mediums and explant for number of days to callus initiation (day).

Genotypes		*P. biglobosa*				*P. roxburghii*				
Explant		apical bud	axillary bud	Leaflet	midrib at the node	apical bud	axillary bud	Leaflet	midrib at the node	**Mean**
Medium 1		50.35	43.87	0.00	0.00	57.76	51.52	0.00	0.00	25.44
Medium 2		36.36	33.71	0.00	51.96	42.17	40.81	0.00	70.35	34.42
Medium 3		35.49	38.36	61.89	0.00	41.22	38.67	62.58	0.00	34.78
Medium 4		44.84	52.69	0.00	53.87	43.65	48.39	0.00	50.71	36.77
Medium 5		41.36	43.57	58.39	50.27	57.36	51.87	70.25	68.35	55.18
Medium 6		51.24	39.88	55.37	0.00	61.85	49.71	59.62	0.00	39.71
Medium 7		60.25	0.00	0.00	71.25	58.74	0.00	0.00	0.00	14.87
Medium 8		31.68	38.65	67.55	0.00	31.17	39.65	58.67	61.36	50.17
Medium 9		0.00	57.98	0.00	0.00	0.00	47.66	0.00	0.00	13.21
Medium 10		35.81	39.67	55.34	51.28	36.70	32.58	52.36	56.82	45.52
Mean		38.47	39.10	29.85	27.86	43.20	40.45	30.35	30.76	
LSD	Genotype	0.882								
	Medium	1.367								
	GenotypeXMedium	3.507								

LSD: Least significant difference at 1%.

Induction of callus from the in vitro germinated seedlings:

The mean performance of selected parts as source of explant from *P. biglobosa*(Jacq.) Benth are presented in tables 5, 6 and all. the regeneration formation were evaluated.

Number of days to callus initiation (day): The obtained outcomes of mean performance characters of *P. biglobosa (Jacq.) Benth*illustrated the shortest mean period of **number of days to callus initiation (day)** for the **apical bud** explant (28.363) media 8 followed by the root explant (30.557) media2. On the other hand, the a**xillary bud** explant (36.207) media 2 showing the longest period as shown in table 6.

Callus weight (mg) It could be concluded from table 6 that, the mean performance characters of *P. biglobosa (Jacq.) Benth*demonstrated the highest mean performance of c**allus weight (mg)**,for the **apical bud** explant (0.683) media 8 followed by media 3 (0.623) for the same explant. On the other hand, the a**xillary bud** explant (0.459) media 2 scored the lowest callus weight (mg).**Green plants percentage (%)**

Table 5.mean performance to Days to callus initiation and Callus weight of *P. biglobosa*, media and explant .

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Plant species	*P. biglobosa*							
Criteria	**Days to callus initiation**				**Callus weight**			
Explant	**Apical bud**	**Axillary bud**	**Root**	**Mean**	**Apical bud**	**Axillary bud**	**Root**	**Mean**
Medium 2	33.170	36.207	34.530	34.635	0.537	0.459	0.507	0.501
Medium 3	32.883	32.780	31.360	32.341	0.623	0.570	0.467	0.553
Medium8	28.363	30.727	30.557	29.882	0.683	0.590	0.583	0.619
Medium10	32.037	34.467	33.863	33.455	0.607	0.473	0.463	0.514
Mean	31.613	33.545	32.577		0.613	0.523	0.505	
LSD	**Explant**	0.6509			0.0107			
	Media	0.8552			0.0086			
	Explant×Media	1.9264			0.0795			

LSD: Least significant difference at 1%.

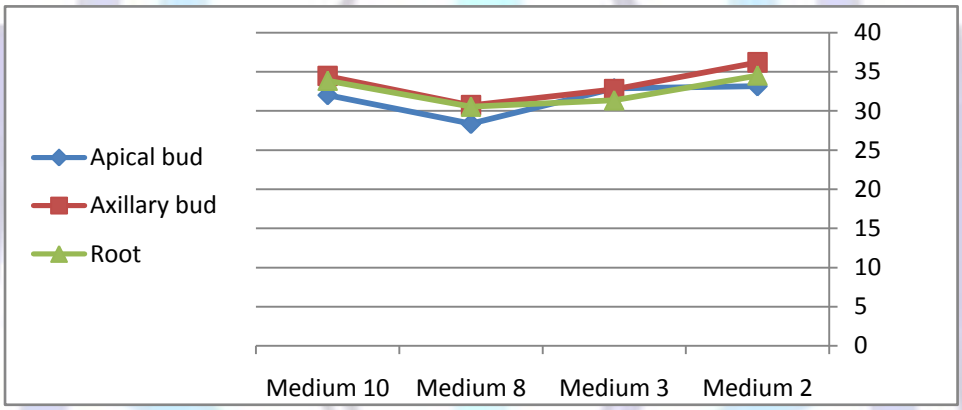

Fig 4: Mean performance of *P. biglobosa* explants to Days to callus initiation on the used media

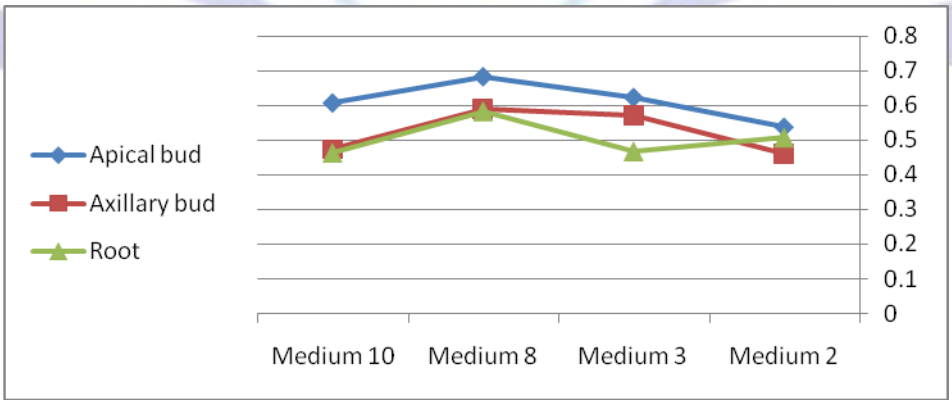

Fig 5: Mean performance of *P. biglobosa* explants to Callus weight on the used media

Table 6. Mean performance of *P. biglobosa* explants to Dead calli percentage % and Albino plants percentage % on the recommended media

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Plant species		*P. biglobosa*							
Criteria		Dead calli percentage %				Albino plants percentage %			
Explant		Apical bud	Axillary bud	Root	mean	Apical bud	Axillary bud	Root	mean
Medium2		40.457	36.840	35.887	37.728	25.517	33.267	19.723	26.169
Medium3		30.107	41.440	36.497	36.015	35.447	30.303	30.490	32.080
Medium8		24.473	27.047	32.630	28.050	11.400	21.300	24.473	19.058
Medium10		31.087	34.960	33.830	33.292	39.573	26.850	20.697	29.040
Mean		31.531	35.072	34.711		27.984	27.930	23.846	
LSD	Ex	0.573				1.574			
	M	1.187				1.604			
	ExM	2.895				3.036			

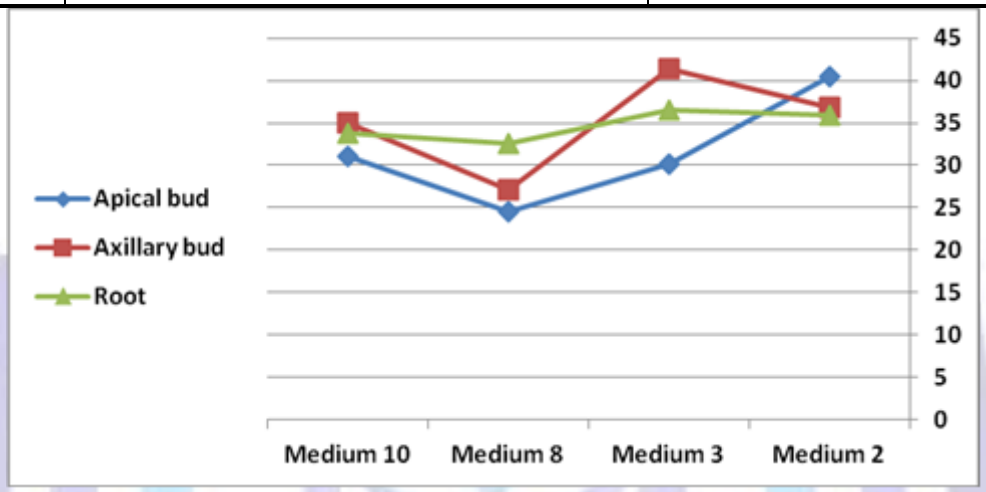

Fig. (6) Mean performance of *P. biglobosa* explants to dead calli percentage % on the recommended media

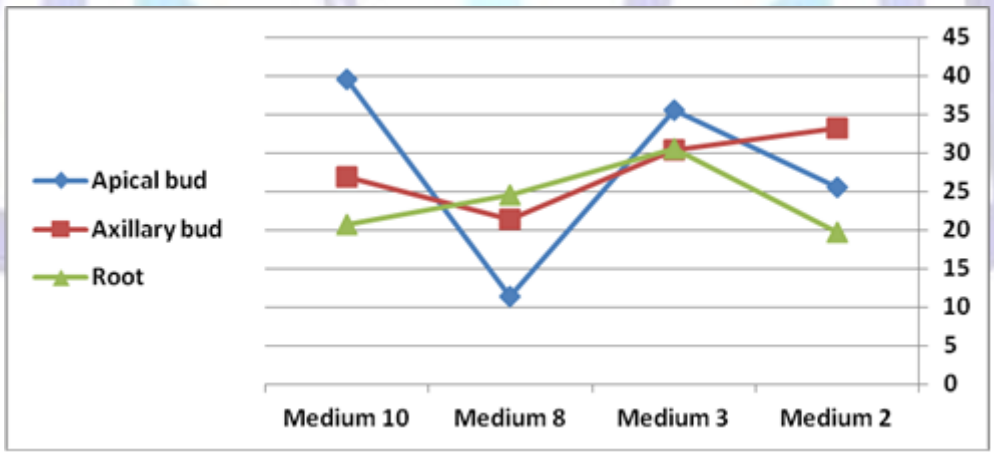

Fig. (7) Mean performance of P. biglobosa explants to Albino plants percentage % on the recommended media

Dead calli percentage (%): As for the mean performance characters of dead calli percentage of the studied species,it was shown that the lowest **dead calli percentage** table 48, for the **apical bud** explant (**24.473**) media 8 followed by the axillary bud explant (**27.047**) in the same media. Meanwhile, the a**xillary bud** explant (**41.440**) media 3 scored the highest mean performance of dead calli percentage.as shown in table 6. Fig.6

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Albino plants percentage (%): Data in table 48, proved that the lowest mean performance characters **percentage of Albino plants,** wasfor the **apical bud** explant (11.40) media 8 followed by the root explant (19.723) media2 while, the **apical bud** explant (**39.573**) media 10 scored the highest percentage of Albino plants.as shown in table 6. Fig.7

Considering the **mean performance** characters of the studied plant, it was illustrated that the highest **percentage of green plants was** for the **apical bud** explant (61.750) media 8 followed by the root explant (57.120) media10. On the other hand, the a**xillary bud** explant (22.413) media 2 scored the lowest percentage of green plants as shown in table 7, fig 8.

Root percentage (%) It is obvious that, the mean performance characters of*P. biglobosa (Jacq.) Benth*recorded the highest **percentage of root** for the **apical bud** explant (53.140) media 8 followed by the **axillary bud** explant (49.513) in the same media.At the same time, the **axillary bud** explant (27.373) media 2 showed the lowest percentage of root as shown in table 7, Fig. 9.

Generally, **Seeds germination stage apical bud** explants media 8 followed by media 10 showed the most favorable mean performances values for the studied characters, **Number of days to callus initiation (day) , Callus weight (mg)**, **Dead calli percentage (%),Albino plants percentage (%),Green plants percentage (%)** and **Root percentage (%)**. Moreover, a**xillary bud**, showed remarkable increase in the mean performance of average.

However, the overall response of MS medium was found to be superior to that of B5 medium. Explants cultured on MS medium fortified with combinations of 2,4-D and BAP induced rapidly proliferating calli that turned more friable and nodular. These results are in accordance with those reported by **[21], [22]and [23].**

Table 7. Mean performance of the green plants percentage and root percentage of *P. biglobosa*, media and explants

Plant species		*P. biglobosa*							
Criteria		Green plants percentage%				Root percentage%			
Explant		Apical bud	Axillary bud	Root	mean	Apical bud	Axillary bud	Root	Mean
Medium2		38.650	22.413	31.377	30.813	40.593	27.373	36.403	34.790
Medium3		42.457	33.957	31.750	36.055	39.153	34.510	29.920	34.528
Medium8		61.750	45.140	34.407	47.099	53.140	49.513	43.617	48.757
Medium10		55.463	42.417	57.120	51.667	39.687	37.733	32.763	36.728
Mean		49.580	35.982	38.664		43.143	37.282	35.676	
LSD	explants	1.195				1.227			
	Medium	1.487				1.542			
	ExplantXMedium	1.985				1.894			

LSD: Least significant difference at 1%, M: Medium

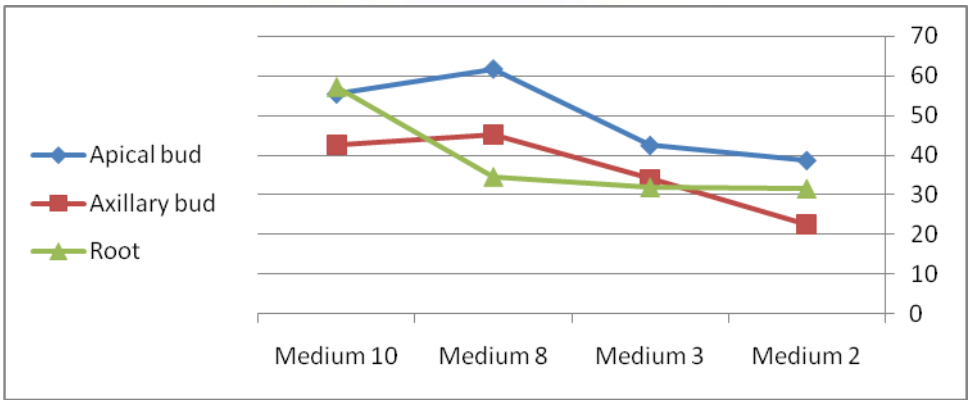

Fig 8: Mean performance of *P. biglobosa* explants to green plants percentage%on the used media

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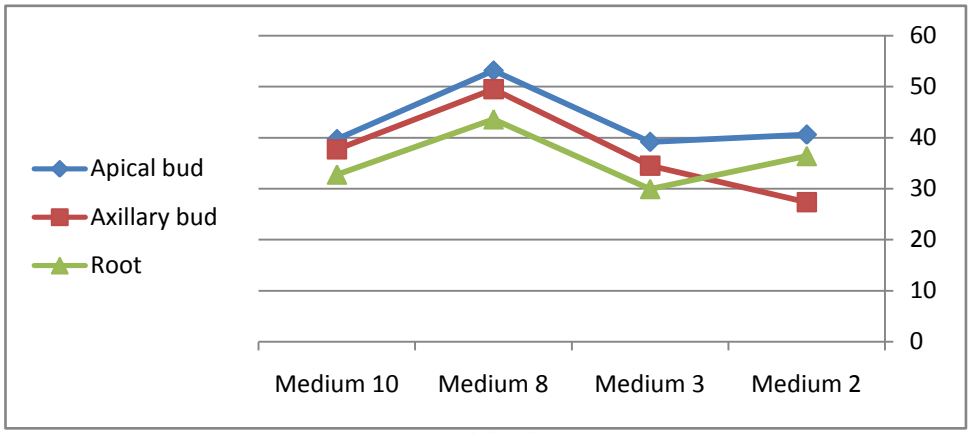

Fig 8: Mean performance of *P. biglobosa* explants to Root percentage%on the used media

DISCUSSION

Tissue culture studies on seeds of *P. biglobosa* Benth to prevent it from extinction. Leaf and hypocotyl explants from in vitro germinated seedlings were cultured on MS medium containing different concentrations of 6-benzylaminopurine (BA) either alone or in combination with (NAA) for callus induction. Seeds produced the highest percentage (80%) of callus than from leaf explants.**[10]** and in vitro conservation of *P. biglobosa* in Senegal through tissue culture technique. Different concentrations of growth regulators were added alone or in combination in a MS basal medium. During acclimatization achievement, survival rates were respectively 80% for apex and cotyledonary explants and 86.66% for axillary explants.**[11]** .Cotyledon explants of *P. timoriana* for in vitro rapid regeneration, on MS and B5 basal media supplemented with various concentrations of 2,4-D, NAA and BAP. Successful callus induction was observed in all the treatments. Maximum percentage of callus induction was obtained in the 2, 4-D supplemented basal media. Explants cultured on MS medium fortified with combinations of 2, 4-D and BAP. **[3]**

CONCLUSION

The study presented here demonstrates the successful attempt at regeneration of plantlets from apical and axillary buds together with root explants of *P. biglobosa*Benth via indirect organogenesis. Further studies are needed to increase the regeneration frequency and to induce shoots from other explants. Our results suggest that this protocol would provide useful information for mass propagation and germplasm conservation, and would help in the further work concerning genetic transformation to incorporate useful genes and for the production of valuable secondary metabolites.

It can be concluded that the medium composition has a significant effect on the calli production together with the type of the explant which showed relatively higher significance. Medium 3, 8 and 10 gave the highest yield of the cultured callus. Concerning the types of explants, the most viable explants used for callus production and for further regeneration were apical and axillary buds together with root explants.

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