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Comparison between Cowpea, *Vigna radiate* L., Plants Differentiated from Explain Callus and Seeds Plants in their Ability to Produce Nitrogen-Fixing Nodules

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ABSTRACT

In this study, callus was produced from leaves and stems explants of cowpea axenic seedlings grown on agar_ solidified MS medium supplemented with growth regulators. Medium used for callus production was MS+NAA 1.0 mg^{-1} +BA 0.5 mg^{-1}. It sustioned shoots differentiation. Regenerants shoots were rooted easily in MS medium+ NAA 1.0 mg^{-1} +BA 0.5 mg^{-1}, Roots were inoculated with cowpea *rhizobium* 321H inoculum for 10 minutes. The inoculated plants were placed on the surface of 15 ml agar solidifed NF medium, nodules were developed after 50 days of inoculation. They used for measurement of amino acids. Also, sections were prepared from these nodules to follow anatomical lvariations between nodules produced on seed-plants and plants produced from callus. seedlings growing on NF media were transferred to a mixture of soil and peatmoss.

Keywords- Root nodule, *Vigna radiata* L., *Cowpea Rhizobium*321H.

I. INTRODUCTION

Cowpea plants *Vigna radiata* L. (Fabaceae) are important economic crops, It had a high nutrional value. This crop is sensitive to climate variability and environmental stressors salinity stress (Sehrawat *et al*.,2021). Cowpea is an annual creeping crop that can grow widely on all types of soil but is largely cultivated by farmers in arid and semi-arid regions, and requires a warm climate for seed germination (28-30°C).The best growth performance (30-35°C) throughout. Its life cycle, up to 90 cm high, trifoliate, pale green or green in color. The flowers are pale yellow to greenish yellow. The color of the pods varies from green to black (Ganesan,2018). Cowpea *Vigna radiata* L. a source of easily digestible carbohydrates and proteins. They also improve soil nitrogen, and yield better quality beans due to the nitrogen added to the soil (Schafleitner *et al*., 2015). Proteins found in cowpea seeds is rich in amino acids and tryptophan. However, it lacks methionine and cysteine when compared to animal proteins (Gonçalves *et al*., 2016). Regarding its medicinal activity, it is used as an anticancer due to the presence of Lectin (Gautami and Iyer, 2018).

Tissue culture technology is invested to produce disease-free plants and others characterized by rapid production of completely identical plants, resulting from planting the seed, part of the root, leaf, anther or pollen on a nutritious environment that may sometimes be prepared by adding some growth regulators including auxins that stimulate Root formation and growth, and cytokinins to encourage stem growth, or are added to direct the cultivated plant part to create callus (Al-Bayati, 2002). Various studies have been conducted on the response of Explant to various interventions of phytohormones to obtain undifferentiated mass of callus, or regeneration processes (Gautami and Iyer, 2018). Tissue culture provided the opportunity to produce plants that are resistant or tolerant to different

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environmental conditions such as high or low temperatures, salt stress, humidity, exposure to radiation and other various environmental stresses. Production of plants resistant to heat, the effects of which are reflected in some characteristics of the whole plant, as in the sunflower plant (Rasheed and Qasim, 2006). It was successful to develop callus from different parts of pea plants, *Pisum sativum* L. and chickpeas, *Cicer arietinum* L., as well as lentils, *Lens culinaris* L. (Lulsdorf *et al*., 2012). Callus was created from the leaves and stems of Lotus glaber plants and vegetative branches were formed (Morris *et al*.,1999). The use of MS medium prepared at 1.0 mg / L BA stimulated the development of subcotyledonous stem callus in Clitoria ternate L plants. Vegetative shoots formed and then rooted on MS medium with half its synthetic strength in the presence of IBA at a concentration of 0.25 mg / L. Likewise, the formation of callus leaves of bean Vicia faba on MS medium containing 0.2 mg / L Kin and 2.0 mg / L 2,4-D, produced From the use of MS medium with the addition of 2.0 mg / L BA and 0.2 mg / L IAA to the development of white callus and turning it green after a period of growth and brittle, but failed (Fakhrai and Evans, 1989). Cowpea are herbaceous plants with a main root and many lateral roots spread in the surface soil. Its root system carries nodules containing specialized symbiotic bacteria *Cowpea Rhizobium* 321 H that contribute to the fixation of atmospheric nitrogen in the form of nitrates in the soil (Sheahan, 2012). Rhizobium bacteria are unique in their highly specialized symbiotic relationship, *Rhizobium* - host specificity, with plants of the *Leguminaceae* family(Doyle and Lukow,2003). These symbiotic relationships result in the production of N2-stabilized nodules that are carried on the roots of their specialized hosts and converted into amino compounds that plants benefit from and contribute to soil fertility (Yokota and Hayashi, 2011).

For development and reproduction, plants need relatively high levels of N (nitrogen). N is really the third most crucial element for agricultural plants' growth and development (Thilakarathna et al., 2016). In a mutualistic connection, plants provide bacteria a habitat and fixed carbon in return for fixed nitrogen in symbiotic nitrogen fixation. A highly O2-sensitive protein complex termed nitrogenase reduces N2 to NH3 in a bacterial group as part of biological nitrogen fixation, or BNF. Rather than attaching to microbes, the BNF route may be transported directly into plants, which can result in crop production that is less reliant on synthetic nitrogen fertilizers and boosts agricultural output. sustainability Buren and Rubio, 2018).

This study aimed to compare between nodules activity produced on both types of cowpea plants produced from callus explants from cowpea and those produced from seeds as inoculated by *cowpea Rhizobium*321H.

II. MATERIALS AND METHODS

2.1 *Preparation of culture media*

• MS (Murashige and Skoog 1962).

MS medium was prepared by dissolving 4.4 g of medium powder, MSP09 – (Caisson – USA), in 500 ml of distilled water, then adding sucrose 30 g / L. adjust pH to 5.8 - 6.0. Add 8 g of agar (NEOGEN, UK) in 400 ml of distilled water completely dissolve. Mix the two volumes and bring the final volume up to one liter by adding distilled water. Distribute 30 ml of the medium into a set of 70 ml test tubes. Mouths of tubes were covered with Aluminum foil and tube were autoclaved (121°C and 1 atmosphere pressure for 20 minutes).

• NF Nitrogen Free medium (Fahraeus, 1957)

Medium was prepared by dissolving its components in one liter of distilled water and adjusting pH to 6.5, Then adding 8 g of agar. This medium was used to grow cowpea seedlings inoculated with *cowpea rhizobium*321H.

• Yeast extract mannitol YEM (Vincent, 1970).

This medium was prepared by dissolving its components in one liter of distilled water, adjusting pH to 6.8 pH, then adding 8 g of agar. It was used in its liquid state to prepare rhizobial suspension.

Seed garmination

Cowpea seeds were sown on the surface of filter paper (No.11cm./Brightsign/China) moistened with strerilized water in a plastic Petri dish (9 cm diameter). Seeds began to germinate after 5 days. Germination percentage was calculated according to this formula below:

Germination = number of germinated seeds x 100
(Willi, 1990).

(%) Total of sterile

2.2 *Production of sterile seedlings*

Thirty cowpea seeds were sterilized by immersed in 50 ml of 96% ethyl alcohol with continuous stirring for two minutes. Then transfered to 50 ml of sodium hypochlorite NaOCl (Babylon Detergent Factory / Baghdad) at a ratio of 1:2 (v:v) of water (Al-Jawary, 2004) for 15 minutes. Seeds were removed and washed with sterile water 3 times to remove traces of the sterilizer (Arife and Muhammad, 2019).

Sterilized seeds were planted on the surface of 20 ml of agar solidified MS medium provided with growth regulators in 70 ml test tubes with two seeds/ tube. Samples were incubeted in culture room conditions in darkness for the first three days (25 ± 2 °C) until the emergence of roots and the beginnings of shoot. Samples were transferred to light and dark reglem 16 hours light /

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8 hours darkness illumination 2000 lux, until the formation of the intact seedlings (Badrani and Al-Bakr, 2021).

2.3 Preparation of explants

Axenic healthy cowpea seedlings produced from sterilized seeds were removed from MS0 medium at the age of 10-15 days. Stems were cut into pieces of 1.0 cm length using a sterile scalpel. Leaves were cut into pieces of 0.5-1.0 cm^2 to be used for callus production.

2.4 Induction of callus from explants

Stems and leaves explants were placed on the surface of 30 ml of the induction medium in 100 ml glass flasks. at a rate of three pieces flasks were sealed with aluminum foil. The following media were tested.

MS0 (control)

MS+ 0.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA

MS + 1.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA

MS + 1.5 mgl^{-1} NAA + 1.0 mgl^{-1} BA (Al-Jawari, 2004).

Specimens were kept in culture room at conditions previously mentioned.

2.5 Callus subculture

Callus cultures were subcultured as callus was acquired brown color and the medium cracked. Before callus reached this stage was removed from the flask and the dead parts were removed. The remaining callus piece was divided into pieces weighing 1.0 gm / piece. Each piece was transferred to a glass flask containing 30 ml of the fresh culture medium. Samples are kept in culture room condition previously mentioned.

2.6 Regeneration of shoots

Many pieces callus (one gram /each) was taken from both types of calluses induced from leaves and stems independently and placed individually on the surface of 30 ml of shoot regeneration media mentioned below.

MS0 (control)

MS + 1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA

MS + 1.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA

MS + 2.0 mgl^{-1} IAA

2.7 Shoot rooting

The differentionted shoots were cut from the callus of lengths 2-3 cm and their bases were planted upright in selected rooting media. They were:

MS0 (control).

MS + IBA 0.5 mgl^{-1} +NAA 1.0 mgl^{-1}.

Many single shoots were each planted in a bottle containing 30 ml of rooting medium. The bottles were closed with a double layer of aluminum foil.

Samples were kept in the culture room conditions mentioned previously.

2.8 Acclimatization of cowpea plants produced from callus.

Cowpea plants produced from both leaf and stem calli were grown in agar solidified MSO medium, were each covered with transparent coverings and gradually removed. Later plants were transferred to greenhouse conditions to monitor their growth and finally were transport to field conditions.

2.9 Production of sterile cowpea seedlings

Surface sterilized cowpea seeds were sown on the surface of 20 ml of agar solidified NF medium in 9 cm diameter plastic Petri dish at a rate of 3 seeds / dish. Dishes were covered with their lids and kept in complete darkness for 48 hours in culture - room conditions (25 ± 2°C). Germinated seeds were transferred to the conditions of light and dark system (16 light / 8 dark). The produced seedlings were used in inoculation experiments with ***Cowpea rhizobium*** 321H.

2.9 Isolation of cowpea Rhizobium 321H from nodules

A number of root nodules were separated from roots with part of the root from cowpea plants at age of 3 months growing in the soil. Nodules were thoroughly washed with water. The excised nodules were surface sterilized by immersing in 5.0 ml of 96% ethyl alcohol for 3 min. then were immersed in sterilizing solution (FAS, Production of Babylon Detergent Factory / Baghdad) sodium hypochlorite (NaoCl) 6% conc. for 5 min. Nodules were washed with sterile water three times for 3 minutes to remove traces of the sterile substance. Each sterile nodule was mashed in the presence of 1.0 ml of liquid YEM medium by sterile glass rod. Dishes (9.0 cm diam) each contaning 20 ml of agar solidified YEM medium were inoculated with -the bactreial suspension. Dishes were covered with their lids, sealed with Parafilm, kept in incubator at 28 °C in the dark until the beginning of the emergence of colonies. ***Cowpea Rhizobium* 321** H bacteria were prepared on the YEM nutrient medium after streaking method to obtain a single colony on a solid YEM incubated in incubator at 28 °C for 48 hours. (Al-Barhawi, 1999)

2.10 Preparation of the bacterial inoculum

Bacterial suspension was prepared by taking a loopful of ***cowpea Rhizobium 321***H growing on the surface of solid YEM medium and inoculating 25 ml of liquid YEM medium into 50 ml capasity flasks. Flasks were placed in a shaking incubator (New Brunswick Scientific, Co. Inc. Edision, N.J.USA). at 150 rpm, in dark conditions at 28 °C. The bacterial culture was harvested by centrifugation (Jouan BR311) at 1200 rpm for 15 min. The precipitated bacteria were resuspended by adding 1.0 ml of fresh YEM liquid medium (Al-Mallah *et al*., 1987).

2.11 Inoculation of cowpea seedlings with *cowpea Rhizobium* 321 H

Roots of sterile cowpea seedlings, one week old containing abundance of root hairs were inoculated by immersing their root system in 1.0 ml of the inoculum for 10-15 minute. The inoculated seedlings were placed on the surface of 15 ml of agar solidified NF medium in Petri dishes(9.0 cm diameter) a rate of 3 seedlings / dish. Dishes were closed with their lids and sealed with parafilm. They were kept vertically, with the root area covered with a dark cover. Samples were kept in culture room conditions (25 ± 2 m for 16 h light / 8 h darkness and intensity of light. 3000 lux) .(Khaitov *et al*., 2016**)**.

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2.12 Estimation of protein content in each part of cowpea plant

The standand method (Lowry et al., 1951) was used to estimate the protein levels extracted from callus samples for each treatments in all experiments.

2.13 Preparation of cross-sections of root nodules

The method of embedding in paraffin wax (Al-Hajj, 1998) was followed in preparation of permanent preparation of root nodules.

III. RESULTS

3.1 Determination of seed viability

The results of cultivating cowpea seeds grown on solid MS0 medium showed their ability to germinate, and germination approached 93%.

3.2 Surface sterilization of cowpea seeds and axenic seedlings production

Data showed that the method of surface sterilization of cowpea seeds with a sterilization solution of NaOCl for 15 min. after treating them with ethyl alcohol 96% for one min. was efficient. Cultivation of sterilized seeds on MS0 medium in dark for 3 days, and they were transferred to light, germination reached 93%, and healthy seedlings were obtained. (Table1).

Table 1: Efficiency of different sterilization solutions of seeds of cowpea *Vigna radiata L.*

Solution	Sterilization (v :v)	Germination
1.	Water + NaOCl (2 : 1)	93%
2.	Water + NaOCl (1 : 1)	66%
3.	Water + NaOCl (1: 2)	40%

3.3 Get a cut of the sub cotyledonous stems

Data were obtained from collecting a large number of sub cotyledonous stems, which were taken from healthy and sterilized 10-day-old cowpea seedlings to produce a callus.

3.4 Formation of callus cultures

The results indicate the development of callus from cotyledonous stems provided with various proportions of growth media confirmed (**Figure1)** the formation of stems callus involved 30 days. (Table2). This medium was consisting of solid MS medium supplemented with growth regulators NAA and BA .

Table 2: Production of callus from stem explants of media used for callus development in cowpea plant *Vigna radiata* L.

Media	Createa			Description
MS	0	0	0	0
MS + 0.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA	0	0	0	0
MS + 1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA	95	30	Green	coherent
MS + 1.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA	83	35	Green	coherent
MS + 2.0 mgl^{-1} NAA + 1.0 mgl^{-1} BA	76	35	Green	coherent

The sub cotyledonous stems obtained from the cultivated sterilized cowpea seedlings at the age of 12 days began to show a clear ability to create callus, and it took a period of 25-30 days for the callus to develop. The sub cotyledonous stem pieces turned into an undifferentiated cell mass after 18 days of cultivation. Callus was characterized by its fragile texture and clear green color. Sub cotyledonous stems failed to develop callus when cultured on MS0 medium free from growth regulators (control) (Figure 1).

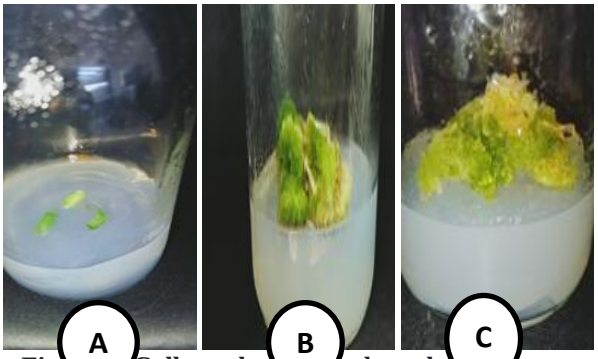

Figure 1: Callus culture of subcotyledonous stems taken from cowpea seedlings. *Vigna radiata* L.

(A) Explant taken from sterilized cowpea seedlings cultivated on MS+ 1.0 NAA mgl^{-1} + BA 0.5 mgl^{-1}.

(B,C) Callus of subcotyledonous stems, 18 days old grown in MS+ 1.0 NAA mgl^{-1} + BA 0.5 mgl^{-1}.

3.5 Callus of subculture

Observations indicate the need of callus to subculture of callus every 25-30 in the same culture media.

3.6 Plant regeneration from callus differentiation

Shoot regeneration

The results of cultivating a group of subcotyledonous stems callus of 1.0 g/ piece in a group of differentiation media expressed a differential response. The vegetative branches were distinguished by their abundant growth and short stems. The medium MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1}Kin succeeded in differentiating vegetative branches by 60% and characterized by a longer period and did not show any differentiation. (Figure2).

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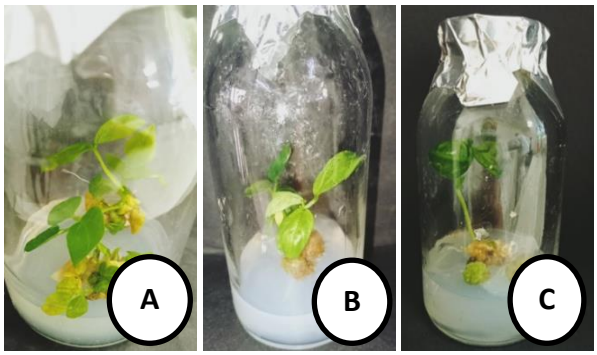

Figure 2: Shoots differentiation of cowpea *Vigna radiata* L. From callus of stems calli growing in MS medium supplemented with growth regulators.

(A) Differentiation of shoots from subcotyledonous callus grown in MS + 1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA

(B) Differentiation of shoots from subcotyledonous callus grown in MS + 1.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA

(C) Differentiation of branches from subcotyledonous stems callus grown in MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1}Kin

3.7 Rooting of shoots formed from callus

The results showed that shoots were differentiated branches consisting transferred to solid MS0 medium encouraged the rooting of shoots produced from callus subcotyledonous calli, and the percentage was 65%, which led to the formation of a group of dense roots. While solid MS0 medium containing growth regulators IBA 0.5 mgl^{-1} and NAA 1.0 mgl^{-1} and 30 g/l sucrose stimulated the rooting of these shoots and the percentage was 20% within 25-30 days of cultivation on this medium **(Figure 3**).

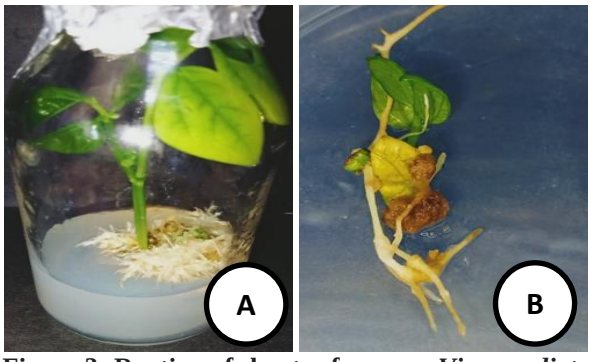

Figure 3: Rooting of shoots of cowpea *Vigna radiata* L. produced from calli developing subcotyledonous stems.

(A) Rooting of shoots on MS0.

(B) Rooting of shoots on MS medium supplemented with the addition of IBA 0.5 mgl^{-1} and NAA 1.0 mgl^{-1} and 30 g / L sucrose.

3.8 Acclimatization of the regenerated plants and their transfer to the soil

Cowpea plants that were successfully rooted were transferred to plastic pots consisting of peatmoss and soil. They were and covered after a week of transfer, they were gradually removed, then completely removed and kept in the growth room, after which they were transferred to the plastic house (Figure4).

Figure 4: Acclimatization of plants formed in the laboratory and their transfer to the soil

3.9 Isolation of cowpea Rhizobium 321H from root nodules

The root nodules of rhizobium bacteria were isolated from the roots of the cowpea plant grown in the soil. The root nodes located in the upper part of the root are larger and of different sizes and spherical shapes. The color of the root nodes was white to tan.

Inoculated with solid YEM medium, cream- coloured colonies were obtained. The colonies were circular in shape, with a raised surface of mucous texture and smooth edges.

3.10 *Inoculation of root seedlings with cowpea Rhizobium* 321 H

Data indicate that inoculated seedlings of Cowpea ***Vigna radiata*** L.at the age of one week that root hairs were seen to produce small root nodules. They grew on the solid NF medium. without going through the middle. The number of the resulting nodules ranged from 5-8 nodule/plant. Also, plants grown in the field formed nodules. The number of these nodules in field plants ranged from 8-10 nodule/plant, while plants resulting from the differentiation from stems and leaves callus were 4-6 nodule/plant. **(Figure5)**.

Figure 5: A group of cowpea plants inoculated with *cowpea Rhizobium* 321 H

(A) Forms of root nodules of cowpea plants grown in the field.

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(B) Root nodules of cowpea plants differentiated from callus.

3.11 Protein Measurement of proteins in parts of cowpea plant.

Table 3: Measuremet of proteins of in parts of cowpea plant grown in the field.

NO.	Leaf	Stem	Root	Nodules
1	0.412	0.216	0.229	0.095
2	0.253	0.119	0.128	0.086
3	0.265	0.136	0.129	0.101
4	0.190	0.205	0.235	0.098
5	0.275	0.119	0.132	0.080
6	0.248	0.221	0.142	0.110
7	0.197	0.129	0.167	0.093
8	0.218	0.154	0.290	0.569
9	0.227	0.208	0.145	0.104
10	0.224	0.136	0.215	0.096

Table 4: Measuring of proteins of parts of the cowpea plant grown on NF

Protein conc. mgl^{-1}				
NO.	Leaf	Stem	Root	Nodules
1	0.186	0.283	0.179	0.206
2	0.142	0.250	0.204	0.247
3	0.197	0.301	0.288	0.229
4	0.314	0.160	0.259	0.139
5	0.209	0.184	0.272	0.124
6	0.107	0.198	0.221	0.117
7	0.148	0.177	0.301	0.148
8	0.117	0.283	0.284	0.219
9	0.147	0.225	0.251	0.181
10	0.211	0.225	0.168	0.132

Also, the callus protein of the cowpea plant grown on MS medium containing growth regulators 1.0 NAA mgl^{-1} + BA 0.5 mgl^{-1} was measured, and the result was 0.394.

***3.12 Anatomy of root nodules produced by inoculation Cowpea Rhizobium* 321H**

The results of light microscopy examinations of cross sections of cowpea plant roots that inoculated with rhizobium showed swelling in the root penetration area of the bacteria. The examinations confirmed that the epidermal region consists of one row of coherent and compact cells, followed by the cortex cells region consisting of multiple rows of cells containing interspaces that are necessary for respiration infection and food. The area of the infection thread is very clear and is associated with the cells of the inner cortex as it is a distinct and wide area of the multiplicity of dividing cells.

As for the transverse sections of the mature root nodules, the affected cortex cells are enlarged, due to the hormonal imbalance between auxins and cytokinins on the one hand, and on the other hand, a change in the concentration of Ca^{+2} ions inside the cortex cells, due to the frequent divisions in them and because they contain large numbers of irregular rhizobium bacteria. The shape is surrounded by a membrane called the Bacteriod, after which the cytoplasm of the cortex is released and it becomes more mature, clustered next to the wall of the root nodule.

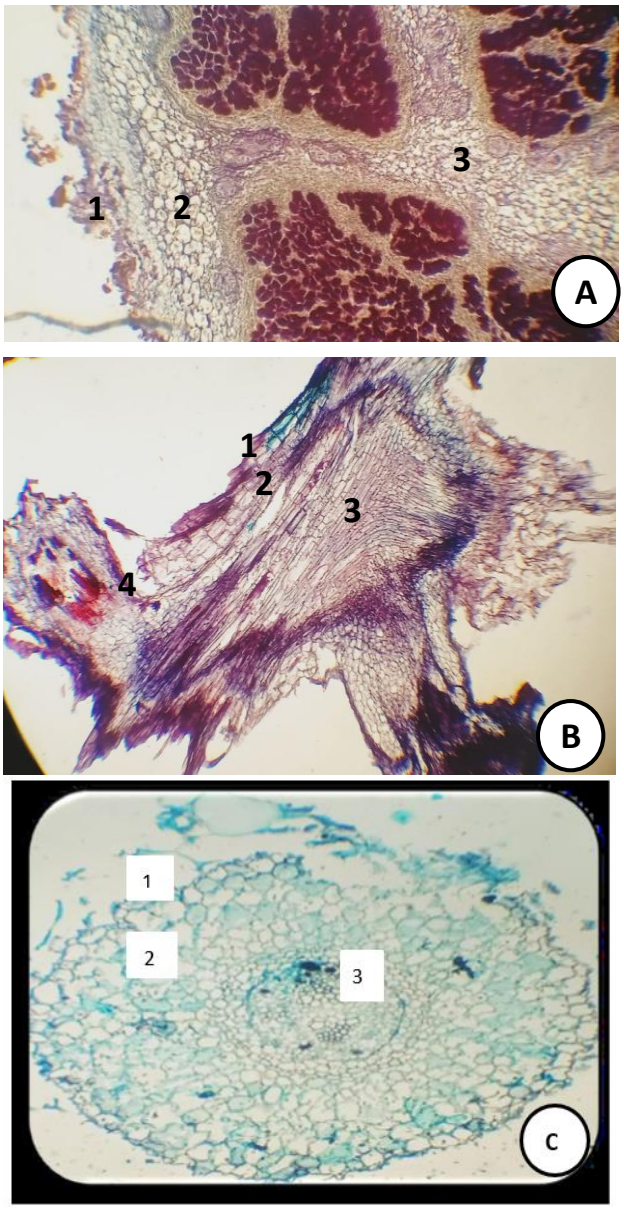

Figure 8: Cross-sections of root nodules in the roots of cowpea (A) Plants grown from seed in soil (B) tissue cultured on NF medium at the level of light microscopy. (C) Plants resulting from differentiation of callus subcotyledonous stems.1:Epidermis,2:cortex, 3:inner cortex , 4;root nodules protrusion.

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IV. DISCUSSION

Cultivation of excised plants on MS medium initially resulted in green calluses on MS medium containing BA alone and greenish nodular callus was obtained when BA was used in combination with NAA. Callus growth was higher in the medium containing a concentration of BA along with NAA. MS media supplemented with 3.0 mg/L BA combined with 0.5 mg/L NAA produced 92% callus from plant explants of stems and leaves. MS media supplemented with 2.5 mg/L BA along with 0.5 mg/L NAA produced a better pathway with higher growth. Callus regeneration was found in MS medium containing 2.5 mg/L BA (Aparna *et al*.,2018)**.** In general, Vigna regeneration *invitro* is more limited than that of other legumes. In *Vigna radiata* L. plants are directly regenerated from explants, for example, cotyledon tips, cotyledons, primary leaf and hypocotyl. Regeneration of shoot buds from cotyledon tissues of Gram green were grown on MS medium supplemented with various concentrations of BA or Kinetin with NAA or IAA. There was no callus formation and bud budding without a growth regulator in MS medium. Among the cytokines tested, Kinetin with BA showed a higher rate of cotyledon bud regeneration((Varalaxmi *et al.*,2007) The success of establishing cellular suspensions from the fragile callus of stems is explained by the ease of separation of its cells and its containment of large numbers of single cells and their divisions and formation of callus facilities are likely to continue. This is due to the abundance of essential nutritional needs in the presence of NAA (Mohammed and Al-Mallah, 2013; Neumann *et al*., 2009).

The symbiosis between legumes and roots initiates the exchange of signals between the host plant and its microsymbiont (Oldroyd, 2013).

A compatible bacterium is recognized by the host and the cortex induces cell divisions to form the primitive root nodule, and at the same time initiates the infection process to deliver the bacteria to the nodule cells. Infection of most legumes involves the development of root hairs infection of the plant. Infection of the root hairs leads to division. The bacteria grow through the epidermal cell layer into the nodule cells, where the bacteria are released and internalized in a process resembling endocytosis. In the nodule cells, individual bacteria are encapsulated by a membrane of plant origin, forming an organelle-like structure called the innard which the bacterium secretes into nitrogen-fixing bacteria (Jones *et al*., 2007; Oldroyd *et al.,* 2011). The symbiotic relationship between the rhizobium bacteria that fix atmospheric nitrogen and the leguminous plant is the result of this relationship, the formation of nodules that fix atmospheric nitrogen N_2 on the roots of their specialized plant hosts, where the rhizobium bacteria convert the fixed N_2 into amino products that the host plants benefit from and work to improve soil fertility and in return get compounds carbohydrates (Yokota and Hayashi, 2011). The formation of nitrogen-fixing nodules on legumes requires the integration of infection by roots into the root epidermis and the initiation of cell division in the cortex, which is several cell layers distant from the infection sites. Several recent advances have added to our understanding of the signaling events in the epidermis associated with the perception of root nodule factors and the role of phytohormones in stimulating cell division leading to nodule formation (Oldroyd and Downie, 2008).

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