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RESEARCH ARTICLE :

Callus induction from different explants in cowpea (*Vigna unguculata* L.)

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SUMMARY : Cowpea (*Vigna unguiculata* L.) is widely cultivated throughout the world. Cowpea is an important tropical herbaceous legume crop and is cultivated in dry (low rainfall) and nutritionally poor soils. Cowpea plays a major role in human nutrition not only because of its good protein quality with a high nutritional value but also because cowpea is critical for feeding animals during the dry season in many parts of West Africa. Moreover, cowpea is a valuable source of income for farmers and grain traders of this region. The genus *Vigna* currently includes around 80 species distributed throughout the tropics. It can be cultivated in diverse climatic and soil conditions. There are many reports in the cowpea tissue culture, the existing problems and the prospective in regeneration are highlighted although this plant appears to be the most recalcitrant to *in-vitro* regeneration. In the present study the explants respond to the culture medium to produced profuse callus. The maximum callus produced was in the combination of 2, 4-D+KN.

KEY WORDS :
Herbaceous, Callus, Regeneration, Harvested, Fodder

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BACKGROUND AND OBJECTIVES

Cowpea is a dicotyledonous plant belonging to the family Fabaceae and sub-family, Fabiodeae (Agbogidi, 2010). The genus *Vigna* currently includes around 80 species. The cowpea is cultivated in all tropical areas, as distributed throughout the tropics. Cowpea is called the "hungry-season crop" because it is the first crop to be harvested before the cereal crops (Gomez *et al.*, 2004). Cowpea is known as an excellent source of protein and also rich in important vitamins,minerals, including soluble and insoluble dietary fibre. Cowpea is also cultivated as a fodder, in the Sahelian area of West Africa as well as in the dry areas of Asia, and also for the fibre of its foral peduncles (Pasquet and Baudoin, 2001). The cultivated forms (var. *unguiculata*) of sp. *unguiculata* are further distinguished to cultivar groups, based mainly on pod and seed characteristics (Fang *et al.*, 2007; Pasquet, 1996). Cowpea grows in a wide range of soil pH and temperature (18-28°C) compared to other legumes and also has a considerable adaptation to high temperatures and drought compared to other crop species (Ehlers and Hall, 1996). Cowpea seeds possess high nutritive value (Ehlers and Hall, 1997).

RESOURCES AND METHODS

The seed material of all the five cultivars collected from National Seeds Corporation of India, Hyderabad, Telangana was grown in the pots near Botany Department, University College of Science, Saifabad, Osmania University. Callus tissue can be induced from different plant tissues of many plant species. So the callus culture from excised leaf is described here by the following procedure. Fresh leaves and nodal segments of *Vigna* were taken and washed thoroughly under running tap water to remove all surface detritus. The explants were then dipped into 5% 'teepol' for 10 minutes and washed.

Nutrient medium was MS medium supplemented with various combinations of auxins and cytokinins, the plant growth regulators. Incubation of culture under controlled physical conditions such as temperature, light, and humidity is indispensable for the proper initiation of callus tissue. The suitable temperature for *in vitro* callus initiation and growth is usually 25 ± 2ºC. The cultures were exposed to a photo period (14 hrs light and 8 hrs dark) for the initiation and growth of callus tissue.

This callus was taken out aseptically on a sterile Petri dish and was divided into 2 or 3 pieces. Each piece of callus tissue was transferred to tube containing medium. Prolonged culture of tissue produced large callus. Once the growth of the callus tissue was well established, portions of the callus tissue was removed and transferred directly onto fresh nutrient medium to continue growth, in this manner callus culture can be continuously maintained by serial subcultures. Callus frequency is expressed as percentage of callus induction and calculated as fallows.

$$\text{Frequency (\%) callusing} = \frac{\text{No. of explants showing response}}{\text{Total no. of explants inoculated}}$$

OBSERVATIONS AND ANALYSIS

The young disease free leaves and nodal portions were used as explants for the production of callus on the MS medium supplied with various hormones like, 2-4,D, NAA, Kinetin and BAP individually or in combination.

The explants were surface sterilized with water and sterilizing agent, mercuric chloride. The sterile explants are properly washed with sterile distilled water in the laminar air flow chamber before inoculation. The explants were incubated on the nutrient medium supplied with hormones and incubated under controlled physical condition.

The change in the explants was observed and noted. The leaf explants did not respond properly for callus formation when 2, 4-D, and kinetin were used independently as source of phytohormone in the culture medium. The explants gave response to the culture medium in the form of unorganized mass of cells in the medium when exogenous hormone supply and the endogenous hormones of the explants are in coordination with each other. Callus induction was obtained in various concentrations of 2, 4-D [1-4.5mg/l], KN [0.25-0.5mg/l] and presented in the Table 1.

Explants produced profuse, friable callus within two to three weeks. Callus was transferred to fresh media after every two weeks depending on the rate of callus growth. The hormonal concentration of 2, 4-D [3.5mg/l] and kinetin [0.5mg/l] in combination produced profuse callus (Fig. 1 and 2). The percentage of callus induction was maximum in this concentration. The fresh weight obtained after culturing the callus in a culture tube was recorded. The maximum callus produced was in the combination of 2, 4-D+KN, 3.5+0.5 (83.11%) followed by 2, 4-D+KN 4.0+0.5 (79.37%) and 2, 4-D+KN.3.0 + 0.5 (77.73%). The fresh and oven dry weights obtained after culturing the callus in culture tubes was recorded and presented in Table 1. The callus production for leaf and nodal explants was almost similar. The same hormonal combination yielded callus in a similar manner for both leaf and nodal explants (Fig. 3).

Fig. 1 : Callus from leaf explants of different cultivars

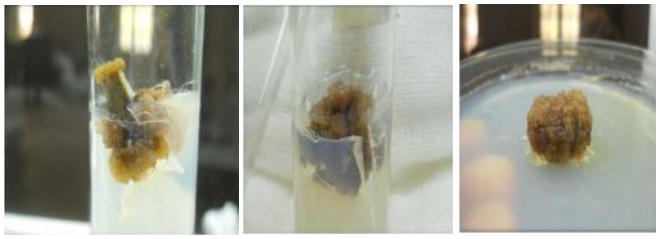

Fig. 2 : Callus produced from nodal region of different cultivars

Table 1 : Callus formation on MS medium with 2, 4-D+KN

Sr. No.	Hormone conc. (mg/l)	Callus induction (%)	Fresh weight(g)	Dry weight (g)
1.	2,4-D+KN,1.0+0.5	65.46	1.86±0.90	0.12±0.01
2.	2,4-D+KN, 1.5+0.5	68.69	1.91±0.24	0.13±0.01
3.	2,4-D+KN, 2.0+0.5	71.85	1.93±0.22	0.13±0.01
4.	2,4-D+KN, 2.5+0.5	74.06	2.12±0.36	0.18±0.05
5.	2,4-D+KN, 3.0+0.5	77.73	2.32±0.20	0.18±0.09
6.	2,4-D+KN, 3.5+0.5	83.11	2.38±0.20	0.18±0.01
7.	2,4-D+KN, 4.0+0.5	79.37	2.33±0.21	0.18±0.08
8.	2,4-D+KN, 4.5+0.5	76.79	2.22±0.39	0.17±0.013

In the present study the explants respond to the culture medium to form unorganized mass of cells in the medium when the endogenous hormone and exogenous hormone supply of the explants are in coordination with each other. The hormonal concentration of 2, 4-D [3.5mg/l] and kinetin [0.5mg/l] in combination produced profuse callus and the percentage of callus induction was maximum in this concentration. The maximum callus produced was in the combination of 2, 4-D+KN, 3.5+0.5 (83.11%) followed by 2, 4-D+KN 4.0+0.5 (79.37%) and 2, 4-D+KN.3.0+0.5(77.73%). Explants produced profuse, friable callus within two to three weeks and sub cultured to fresh media after every two weeks depending on the rate of callus growth. The fresh and oven dry weights obtained after culturing the callus in culture tubes was recorded. The leaf explants did not respond properly for callus formation when 2, 4-D, and kinetin were used independently as source of phytohormone in the culture medium. The callus from leaf explants was used for phytochemical analysis and compared with the phytochemical analysis of *in vitro* leaf extracts.

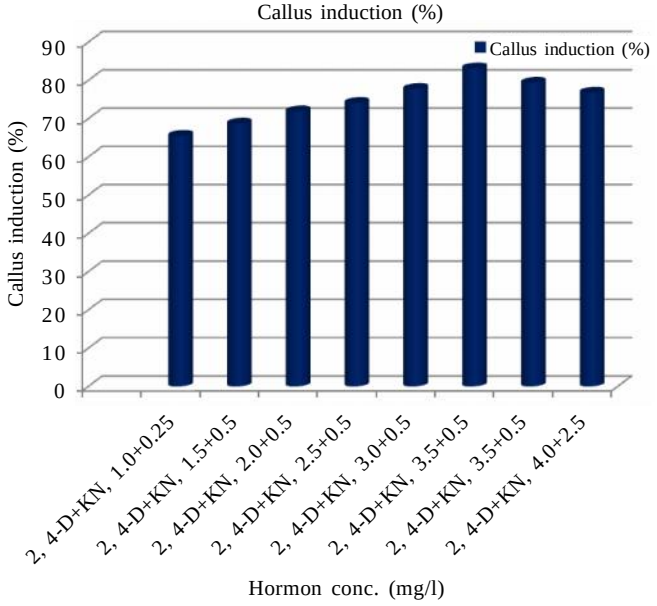

Fig. 3 : Callus formation on MS medium with 2, 4-D+KN

In cowpea, maximum callus was reported in basal MS medium containing KN (5ppm) and NAA (1ppm) (Vats *et al*., 2012). They have also reported the increased phenolic and flavoniod content in callus when compared to the seedling phytochemical evaluation. These reports support the present finding of increased tannins in leaf callus extracts compared to the *in vitro* leaf extracts when analyzed for the phytochemicals present in both.

The formation of maximum callus in presence of more auxins (Vats *et al*., 2014) also is in concurrence with the present finding of callus from leaf explants of cowpea. Callus induction with high cytokinin concentration in cowpea was also reported by Diallo *et al*. (2008). Callus in cowpea was also reported in MS medium supplemented with 1µ NAA and BAP (Odutayo *et al*., 2005).

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