RESEARCH ARTICLE

In vitro callus induction and analysis of some phytochemical parameters of *Terminalia catappa* and *Arachis hypogaea*

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Abstract

In vitro callus induction method was developed for *Terminalia catappa* kernels and *Arachis hypogaea* nuts. They were cultured on MS medium supplemented with IAA and KiN for callus induction. The best callus induction was observed in MS medium supplemented with (IAA:KiN) (0.5:0.5) in case of *T. catappa* kernels while in case of *A. hyupogaea* nuts, it was observed with (IAA:KiN) (1.0:0.5) hormonal concentration. In addition to this, analysis of some phytochemical parameters of *T. catappa* plant parts and *A. hypogaea* nuts were evaluated in terms of Flavonoid, Anthocyanin, Chlorophyll, Proteins and Lipids. The red leaf (RL) and red epicarp (RE) showed high Antioxidants in terms of Flavonoid and Anthocyanin leading to more potent radical scavenging effect. On the other hand, Chlorophyll was found to be least in RL and RD showing that chlorophyll of the plant *T. catappa* is inversely correlated to the Antioxidants (Flavonoid and Anthocyanin). Similarly, Protein and Lipid content showed increase in red and mature parts of the plant *T. catappa.* Thus, it could be suggested that Protein and Lipids might be protected from oxidative damage due to presence of Antioxidants (Flavonoid and Anthocyanin).

Keywords: Callus, *Terminalia catappa, Arachis hypogaea,* Flavonoid, Anthocyanin, Proteins, Lipids.

INTRODUCTION

Terminalia catappa is a large tropical tree belonging to the family, Combretaceae, which is native to the tropical regions of Asia, Africa, and Australia. A deciduous or sometimes semi-evergreen tree to 15-25 m tall (Rogers and Verotta, 1996). Leaves alternate, obovate, 15-36 cm long, 8-24 cm wide, leaves turning pinkish-reddish or yellow-

brown, before falling. Fruit a somewhat compressed-ellipsoid drupe, epicarp thin, green turning yellow with a reddish blush; mesocarp fleshy, adherent to the fibrous husk of the hard-shelled stone containing the spindle-shaped seed; testa very thin, brown, enveloping the coiled cotyledons or kernel (Morton, 1985). Its fruits contain edible kernels from which high energy oil is extracted and which can also be admixed into diesel fuel (Kinoshita *et al*., 2007). It is widely planted for ornamental purposes and edible nuts (Phulwaria *et al*., 2012). The leaves contain several flavonoids, several tannins, Saponines and Phytosterols. Due to this chemical richness, the leaves (and also the bark) are used in different herbal medicines for various purposes (Hnawia *et al*., 2011). Despite its many nutritional benefits and its beneficial effects on health, processing of *Terminalia catappa* is not widespread and its consumption is limited (Biego *et al*., 2012) and means of vegetative propagation are either not known and if available have limitations. *Arachis hypogaea* L. is an annual legume belonging to family Fabaceae play a significant role in the farmers livelihoods by providing the nutritional security and fetching cash revenue (Nwokolo, 1996). Seeds have nutritional value (carbohydrates, lipids, protein, antioxidants, vitamins, essential minerals, phytochemicals and phytosterols) required for human as well as animal consumption (Atasie *et al*., 2009). Many efforts have been devoted to develop efficient *in vitro* regeneration system. It is very difficult crop to manipulate *in vitro* and only a limited success through tissue culture has been achieved (Muhammad *et al*., 2011).

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases (Kiranmai *et al*., 2011). They are important in plant for normal growth and development (Khatiwora *et al*., 2010) and in various defense reactions to protect against abiotic stresses like UV light or biotic stresses such as predator and pathogen attacks (Rispail, 2005). Anthocyanins are a subgroup of flavonoids (Markakis, 1982). Anthocyanin pigments are important to food quality because of their contribution to color and appearance (Lee *et al*., 2005). Anthocyanin biosynthesis is often initiated due to drought, insect pests, potassium deficiency, extreme temperature, and excessive light. This behavior may allow the pigment to serve as an indicator of plant stress (Steele *et al*., 2009). Anthocyanins are becoming increasing important as antioxidant properties and health benefits, including Anti-cancer, Anti-inflammatory and Vasoprotective effects, preventing Coronary heart diseases and improving Visual acuity (Arnnok *et al*., 2012).

Micropropagation of *T. catappa* using nodal segments of 15 years old mature tree has been earlier reported by Phulwaria *et al*., 2012. For large-scale *in vitro* plant production the important attributes are the quality, cost effectiveness, maintenance of genetic fidelity, and long-term storage (Filiz *et al*., 2009). Taking the above facts into consideration, the present research work was designed to study the induction of callus and also some phytochemical parameters of *T. catappa* using its various parts and also in the seeds of *A. hypogaea.*

MATERIALS AND METHODS

Explant Collection

Both the seeds and leaves of the plant were collected from the Botanical garden of G. M. Momin Women's College. The plant was authenticated in Blatter Herbarium of ST. Xaviers College, Mumbai. The plant specimen matches with the Blatter Herbarium specimen no.16063 of H. Santapau and was identified as *T. catappa*. *A. hypogaea* pods were purchased from the local market. The seeds were used for further experimentation.

Culture Media

Basal medium used for the culture was Murashige and Skoog medium (Murashige and Skoog, 1962). It was supplemented with 3% Sucrose and 1.2%

agar. Auxin and Cytokinin were used with two different combinations [IAA: 1mg/L and Kin: 0.5mg/L], [IAA: 0.5mg/L and Kin: 0.5mg/L] and added into the medium. The medium was adjusted to pH 5.8 and autoclaved at 120°C for 20 minutes.

Inoculation of Explant

The explants were sterilized properly, the leaves were taken and their edges near to midrib were trimmed and cut into bits of 1 cm (Umamaheshwari and Lalitha, 2007) they were inoculated in Cultured Tubes under strict aseptic conditions. In the case of seeds, seed coat was separated and cotyledons were inoculated on the MS basal medium supplemented with hormones for callus induction. After inoculation, Culture Tubes were transferred to the Culture Room where the temperature adjusted was 26 ± 2°C, humidity was above 60% and light was provided with tube lights with intensity varying from 2000-4000 lux. Photoperiod given was 16 hour light and 8 hour dark.

Detection of Flavonoids

The extract was prepared according to the method given by Khatiworal *et al*., 2010. The total flavonoid content of each plant extract was estimated by aluminium chloride method. Aliquots of extracts (0.1) were taken and made up volume 3ml with methanol. Then 0.1ml aluminium chloride (10%) 0.1 ml Na-k-tartarate and 2.8ml distilled water was added sequentially. The test solution was vigorously shaken. The absorbance at 415nm was recorded after 30 min of incubation. A standard calibration plot was generated at 415nm using known concentration of quercetin (Khatiworal *et al*., 2010). The content of total flavonoids was expressed in mg of Quercetin equivalents per dry weight.

Detection of Anthocyanin

The extract was prepared according to the method given Mazandarani *et al*., 2012. The total anthocyanin content was measured by the pH-differential method (Giusti and Wrolstad, 2001). Two dilutions of berry extracts were prepared, one with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5). Absorbance was measured simultaneously at 510 and 700 nm after 15 min incubation at room temperature. The content of total anthocyanins was expressed in mg of Cyanidin-3-glucoside equivalents per dry weight (Mazandarani *et al*., 2012).

Detection of Chlorophyll

It was performed according to Sadasivam and Manickam, 1996

Detection of Protein

It was performed according to Lowry *et al*., 1951

Detection of Lipids

It was performed according to Plummer, 1988

Statistical Analysis:

All assays were carried out in Triplicates and results are presented as Mean±SD.

Different parts of the plant used for various investigations are as follows:

GL(Green Leaf), RGL (Red and Green Leaf), RL(Red Leaf), RE (Red Epicarp), GE (Green Epicarp), RK (Red Kernel), GK (Green Kernel), GN (Groundnut).

RESULT AND DISCUSSION

The effect of different concentrations of IAA and KiN on callus induction of *T. catappa* and *A. hypogaea* are presented in Table.1. The best callus induction was observed when 0.5mg/l IAA and 0.5mg/l KiN was used in *T. catappa* while in *A. hypogaea* it was observed in 1.0 mg/l IAA and 0.5mg/l KiN. While in case of leaves, the nutrient medium become brown due to oxidation of phenolic compounds produced by explants.

In case of *T. catappa* both mature and immature fruit kernels were used. Results came more rapidly and efficiently in red kernel as it was more compact and mature, while layers were

separated in green kernel. In *T. catappa* (GK) with hormone concentration 1:0.5 (IAA: KiN) greening and swelling was observed after 1 week and then callus initiation was observed. Callus initiation at the edges of kernel was seen after 10-12 days. On the other hand, in RK greening and swelling was observed after 4 days and callus initiation at the edges of kernel was observed after 9-10 days. In 0.5:0.5 (IAA: KiN) hormone concentration green kernel (GK) showed no result while in red kernel (RK) greening and swelling was seen after 4-5 days and callus on the edge of kernel was seen after 1 month. Callus initiation was observed in both the kernels in the hormone concentrations 1.0: 0.5 (IAA: KiN) but callusing was little as compared to 0.5:0.5 (IAA: KiN) concentration. So it could be suggested that 0.5:0.5 is the better hormone concentration for callus induction in *T. catappa* Red fruit kernel. While in case of *A. hypogaea* in 1:0.5 concentration greening of explants was observed after 3 days and callus initiation was observed after 10 days. While in 0.5:0.5 (IAA: KiN) concentration greening of explants was seen after 4-5 days and callus initiation after 12 days. Brownish compact callus was observed in 0.5:0.5 (IAA: KiN) hormone concentration. While brown and friable callus was seen in 1:0.5 (IAA: KiN). So, it can be concluded that 1:0.5 (IAA: KiN) hormonal concentration is better for callus induction as compared to 0.5:0.5 (IAA: KiN).

Plants are conceived as sources of Antioxidants due to presence of polyphenols and flavonoids which possess wide biological properties. In the present study Flavonoid content of leaves and fruits of *T. catappa* is determined in terms of mg quercetin equivalent /g dry wt. The values were found to be high in RL i.e 23.37 mg/g as compared to RGL and GL. In case of Epicarp, it was found to be more in RE i.e 0.66 mg/g dry wt while in GE it was 0.46 mg/g dry wt. The total flavonoid content in terms of mg/g dry wt is presented in Fig 1. It was found to be absent in kernels and groundnut. Highest flavonoid content was found to be present in Red leaves. Flavonoids are most important pigments for flower coloration producing red / blue / yellow pigmentation in petals (Khatiwora *et al*., 2010). So, it can be assumed that high flavonoid content in leaves is due to the red coloration of leaves. It is reported that there is a strong correlation between the stress tolerance and antioxidant capacity in plant species (Ayşe, 2012).

Table 1: Effects of IAA and Kin in MS medium on callus induction of *T. catappa* and *A. hypogaea*

Plant	Concentration of IAA mg/L	Concentration of Kin mg/L		Morphogenetic Response
T. catappa	MS+ 0	MS+ 0		No response
	MS+1.0	MS+0.5	GK	Swelling of the explants and Callus initiation from the edges after 12 days
			RK	Swelling of the explants and Callus initiation from the edges after 12 days
	MS+0.5	MS+0.5	GK	No response
			RK	Callus observed after 1 month. Callus type : Dark green whitish
A. hypogaea	MS+1.0	MS+0.5		Callus observed after 10 days. Callus type: Brown and friable
	MS+0.5	MS+0.5		Callus observed after 12 days. Callus type: Compact brownish

GK - Green fruit Kernel; RK - Red fruit Kernel

Table 2: TLC screening of Lipids in leaves and fruits of *T. catappa* and *A. hypogaea*

Adsorbent	Solvent system	Detecting reagent	Extracts	Rf values
Silica gel Precoated sheet	Petroleum ether : diethyl ether: glacial acetic acid (80:20:1)	50% v/v Sulphuric acid	GL	0.06, 0.14, 0.31, 0.63, 0.87
			RGL	0.14, 0.33, 0.62, 0.77, 0.83, 0.95
			RL	0.05, 0.15, 0.39, 0.54, 0.73, 0.86
			RE	0.06, 0.14, 0.36, 0.6, 0.72, 0.86
			GE	0.06, 0.26, 0.71, 0.97
			RK	0.1, 0.43, 0.63, 0.78
			GK	0.08, 0.52, 0.64
			GN	0.1, 0.43, 0.54, 0.76

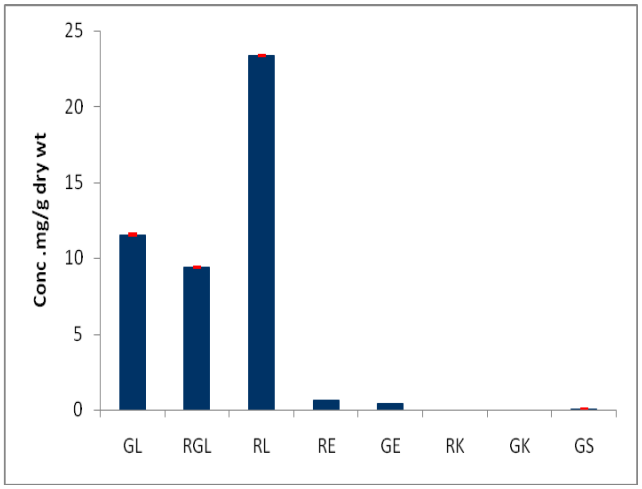

Fig 1: Determination of Flavonoid contents in various parts of *T. catappa*

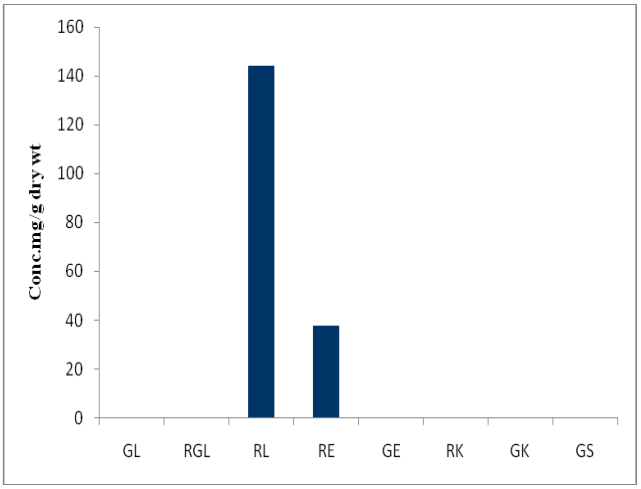

Fig. 2: Determination of Anthocyanin contents in various parts of *T. catappa*

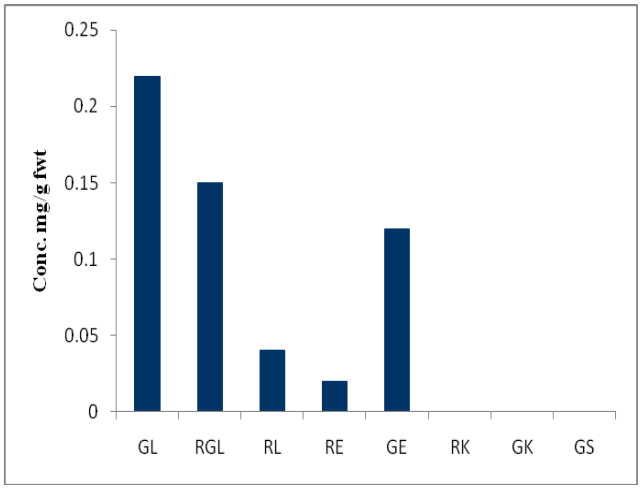

Fig. 3: Determination of Chlorophyll content in various parts of *T. catappa*

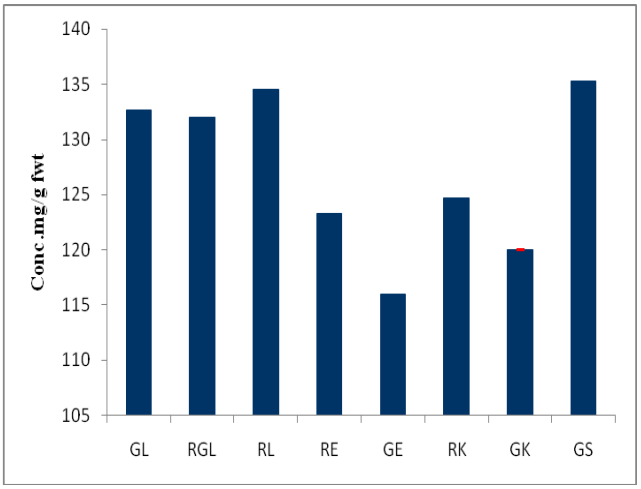

Fig. 4: Determination of protein content in various parts of *T. catappa* and *A.hypogaea*

(GL- Green leaf; RGL- Red & Green leaf; RL- Red leaf; RE – Red epicarp; GE – Green epicarp; RK – Red fruit Kernel; GK – Green fruit Kernel; GS – Groundnut seed)

Similarly Anthocyanin content was examined in various parts of *T. catappa* plant and it was found to be present only in red leaf (RL) and red epicarp (RE) and in negligible amount in kernels and groundnut. However, it has been recorded to be present in seed coat of black soyabean (Choung *et al*., 2001). It was 144 mg cyanidin-3-glucoside equivalent/g dry wt in RL while 37.5 mg/g dry wt in RE (Fig.2). In another study with rice cultivars, it was detected that under PEG induced drought stress conditions, anthocyanins, flavonoids and phenolics content increased (Basu *et al*., 2010). So, it can be concluded that plants under stress accumulates number of secondary metabolites like Anthocyanin, flavonoid etc. for its protection. Chlorophyll estimation showed reverse results to anthocyanin i.e. RL and RE contained very minute quantity of chlorophyll pigments while it was found to be highest in GL i.e. 0.22 mg/g fwt followed by RGL and GE with 0.15 mg/g fwt and 0.12 mg/g fwt. The total chlorophyll content in terms of mg chlorophyll/g tissue is presented in Fig.3. In the earlier research, it was found that under NaCl stress chlorophyll content decreased and Anthocyanin content increased (Eryilmaz, 2007). In another report, Anthocyanin accumulation increases during developmental processes (Cevahir *et al*., 2004). Present results have come in accordance with the above reports. It can be concluded that Chlorophylls and Anthocyanin are inversely proportional to each other.

Similarly, the amount of Protein and Lipid was estimated in different parts of *T. catappa* plant and it was found to be high in red parts of plant as compared to green parts. The total Protein content in terms of mg/g f wt is given in Fig. 4. It was found to be 134.66mg/g fwt in RL followed by GL and RGL with values 132.66 mg/g fwt and 132 mg/g fwt respectively. While in RE it was 123.3 mg/g fwt and in GE it was 116 mg/g fwt. In RK and GK, the values were 124.66 mg/g fwt and 120 mg/g fwt respectively. The protein content was found to be high in GN with 135.33 mg/g fwt. It can be assumed that when *T. catappa* was green and immature, protein and lipids were not synthesized but as they turn mature, its Protein and lipid synthesis increases. So, it can be assumed that protein, lipids, Anthocyanin and flavonoid are directly proportional to each other. Similarly, as Anthocyanin and flavonoids are powerful antioxidants so its increased accumulation in red leaves and fruits of *T. catappa* will protect the Proteins and lipids from any damage. On the other hand, if the plant is coming under stress, Reactive oxygen species are produced due to which cellular compartment including DNA, membrane lipids, protein may get damaged (Ayşe Ş, 2012). But due to anthocyanin and flavonoid accumulation in plants under stress condition protein and lipids are protected. Lipid estimation by TLC was done in different parts of *T. catappa* and A.hypogaea nut and to analyse number of lipid components present on the basis of their Rf values. More number of spots contributes to more number of different lipid components in different parts of *T.catappa* and *A. hypogaea* (Table.2).

Leaves and epicarp of the plant *T. catappa* showed more number of spots as compared to kernel and *A. hypogaea* nuts but they were small and thin as compared to kernel and nuts which has shown thick spots. In case of leaves, RGL and RL showed 6 spots as compared to GL which showed only 5 spots. It is possible that lipid components might have increased as leaves turn red and mature. Similarly, in case of epicarp 6 spots were visualized in RE and only 4 spots were found to be present in GE. While in fruits kernel GK showed only 3 spots and RK showed 4 spots which was much similar to GS which also showed 4 spots. The Rf values of 2 spots in RK and GS i.e. 0.1 and 0.43 were similar which might indicate the presence of same components in both the seeds. Similarly, RL showed 4 spots i.e. 0.05, 0.15, 0.73, 0.86 very much similar to the spots in RE which might also indicates the presence of same components in both RL and RE. However, lipid components in different parts of the plant *T. catappa* and *A. hypogaea* nuts are needed to be further identified.

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