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OPTIMIZING PLANT TISSUE CULTURE OF *CASSIA FISTULA* L. FOR ENHANCED PHENOLIC PRODUCTION THROUGH AUXIN AND CYTOKININ MODULATION

Sushil Kumar
Assistant Professor
Department of Botany
Shaheed Mangal Pandey Govt Girls PG College, Madhavpuram, Meerut

Abstract: This study investigated the potential of plant tissue culture for the sustainable production of secondary metabolites from *Cassia fistula L.* (Indian Laburnum). Callus cultures were established and optimized using a combination of plant growth regulators (PGRs) to maximize the production of phenolics, which are the known active ingredients with medicinal properties. The experiment identified contrasting effects between individual auxins (NAA, 2,4,5-T, IBA, IAA) and their interactions with cytokinins (Kn, BAP) on callus metabolism. NAA generally promoted both carbohydrate and phenolic accumulation, while IBA specifically favored phenolics. IAA, at lower concentrations, promoted sugar synthesis but potentially induced stress at higher levels. The type and concentration of cytokinin further influenced these responses. Combinations of BAP with NAA or IBA showed promise for balancing carbohydrate and phenolic production, whereas Kn often led to stress responses with variable effects on phenolics. The study revealed that the highest total phenolic content was achieved in calli derived from media supplemented with 2.0 mg/L of the synthetic auxin 2,4,5-T. However, this treatment resulted in poorer callus growth. Conversely, callus with superior growth attributes exhibited lower phenolic content. These findings suggest a trade-off between callus growth and targeted metabolite production, highlighting the importance of optimizing PGR combinations for the desired outcome. In conclusion, this research demonstrates the feasibility of utilizing plant tissue culture for the sustainable production of phenolics from *C. fistula*. The study emphasizes the importance of selecting appropriate PGR combinations to achieve a balance between callus growth and the targeted production of medicinally valuable secondary metabolites.

Keywords
Medicinal plants, secondary metabolites, growth index, fresh weight, dry weight, plant growth regulators

I. INTRODUCTION

The pharmaceutical industry is embracing the development of plant-based therapies with renewed vigor, potentially leading to a new wave of more sustainable and accessible medications. This initiative leverages the wisdom gleaned from established traditional medicinal systems. With a history of use in traditional medicine, *Cassia fistula* L. (Caesalpiniaceae) is a versatile medicinal plant showing promise in treating skin conditions, liver problems, and even tuberculous glands (Ali 2014). *Cassia fistula* L. boasts a impressive array of health benefits, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antitumor, and liver-protective properties (Mwangi et al., 2021). Scientists found 120 different chemicals in *C. fistula* using chromatography. These chemicals include flavonoids, anthraquinones, chromones, coumarins, alkaloids, phenolics, phytosterols, triterpene, and long-chain hydrocarbons (Aminah et al., 2021). Phenolics are dominant biochemical components of *C. fistula* and responsible for various biological activities (Goldson Barnaby et al., 2016). It exhibits a diverse array of pharmacologically active constituents, demonstrating efficacy against a broad spectrum of bacterial, fungal, and viral pathogens (Rahmani et al., 2015). Furthermore, it possesses antitussive, anti-inflammatory, and antineoplastic properties, suggesting potential applications in the treatment of coughs, inflammatory conditions, and neoplastic disorders (Hu et al., 2021). Additionally, *C. fistula* demonstrates antioxidant and antidiabetic activity, with potential benefits for nephroprotection (Singh et al., 2023).

Crucially, alternative production methods are needed for plant-based drugs to protect dwindling wild populations (AlSheikh et al., 2020). Plant cell cultures offer a controlled environment to produce valuable metabolites, bypassing the limitations of growing whole plants (Wu et al., 2021). While maximizing production and cost reduction require further research, the potential is well-established. We assessed the effects of individual and combined hormonal treatments at varying concentrations on the physical and biochemical attributes of calli after four weeks of subculture. Plant growth regulators (PGRs), particularly auxins and cytokinins, are critical factors influencing growth, development, and stress tolerance. *In vitro* and *in vivo*, a precise balance of PGR concentrations is paramount for optimal plant growth and development. Altering PGR concentrations can impact the biochemical profile of primary and secondary metabolites (Sosnowski et al., 2023). This influence may occur through the enhancement of specific metabolic pathways or, conversely, by inducing stress in the treated

tissues. This study investigates callus induction in *C. fistula* L. young leaf explants and the effect of varying concentrations of PGRs on phenolics accumulation in calli raised from young leaves.

II. MATERIALS AND METHODS

Tender leaves of *C. fistula* of 5 - 10 mm were collected from naturally growing plants in May. The calli were raised using the basic plant tissue culture protocol described in the literature (Kumar et al., 2022). Several hormone combinations were employed to induce calli based on MS medium (Murashige and Skoog, 1962). The MS medium was standardized with 30 g/L sucrose and 8.0 g/L agar-agar, supplemented with different growth regulators was used for culture establishment. The best calli produced were subcultured on different auxins and cytokinins-only supplementations along with their combinations to evaluate the impact on physical parameters, carbohydrate, and total phenolics content in four-week-old calli.

2.1 Measurement of Moisture Content and Growth Index

Callus health and growth heavily depend on proper water balance. Excess moisture can lead to rot and contamination, while insufficient moisture restricts growth. The fresh weight (FW) of the callus is measured after wiping off surface moisture. The callus is then dried in an oven at a specific temperature (at 60°C) until constant dry weight (DW) is achieved. Moisture per cent (%) is computed as

$$\text{Moisture Per cent} = \frac{\text{FW} - \text{DW}}{\text{FW}} \times 100$$

Monitoring callus growth is crucial for optimizing culture conditions and assessing the success of experiments. The measurement of the growth index (GI) was by measuring the fresh weight for different treatment and their volume was measured by filling it into a graduated tube and calculated as-

$$\text{GI} = \frac{\text{FW of Callus}}{\text{Vol. of Callus}} \times 100$$

By monitoring moisture content and growth index, researchers can optimize callus cultures for secondary metabolite production (Jain et al., 2012).

2.2 Determination of Reducing, Non-reducing, and Total sugar

For estimation of reducing sugar (RS), non-reducing sugars (NRS), and total sugars (TS) 50 mg dry calli were homogenized with 80% alcohol and heated in a water bath to evaporate the alcohol. Then 5 ml saturated lead acetate was added to remove the tannins, proteins, etc. The extract was centrifuged and the supernatant was mixed with 6.0 ml of saturated Na_2HPO_4 to dislodge excess lead. After centrifugation, supernatant is made of 8 ml with distilled water (DW). Three ml of this extract is used to estimate the RS while the remaining is for TS. The 5 ml extract was mixed with 1 ml N HCl and heated in a water bath for 20 min. One ml of this hydrolysate was cooled and pH maintained at 7 using 1N NaOH and made 8 ml by adding DW. One ml of hydrolysate was incubated with 1.0 ml alkaline copper tartrate (20 min, water bath), cooled, and then mixed with 1.0 ml arsenomolybdate. The resulting blue color's optical density was measured at 660 nm. A standard curve was drawn using 100 µg/ml D-glucose solution (Nelson, 1944).

2.3 Estimation of Total Phenolics

Fresh calli 50 ml homogenized with 2 ml 80% alcohol and extraction step repeated five times. The extract evaporated and the palette dissolved in DW to make it 5 ml. A series of aliquots was prepared and made 1 ml with DW. The 0.5 ml double diluted Folin and Ciocalteu's reagent was added to each sample. The mixture was boiled with 20% Na2CO3 for 1 minute, cooled, and absorbance read at 650 nm against the blank. Total phenolic content was expressed as mg trans-cinnamic acid equivalents (mg TCE)/g fresh weight based on a calibration curve with a 3.0 mg/ml trans-cinnamic acid stock solution (Sadasivam and Manickam, 1992).

III. RESULTS AND DISCUSSION

3.1 Callus Induction and Physical Attributes of Calli

Stem, cotyledons, and leaf explants were inoculated over a range of PGRs supplemented to MS medium. Leaf explants on MS medium supplemented with 0.5 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid), 1.0 mg/L 2,4-D, and 2.0 mg/L 2,4-D along with 0.5 mg/L BAP. The best callus induction was recorded on MS medium supplemented with 1.0 mg/L 2,4-D + 0.5 mg/L BAP. High phenolics contents in explants led to the browning of callus induction medium. To avoid this problem we used anti-oxidants like activated charcoal (AC) and citric acid (Table-1; Figure 1a). *In vitro*, callus induction in *Cassia angustifolia* was investigated by Zafar et al. (2018). They found that a combination of 2,4-D and Kinetin (Kn) was effective for callus formation. In current investigation we found amongst individual indole, non-indole auxins, synthetic and natural cytokinins best callus growth was observed in 1.0mg/L 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid) and 50 ml/L coconut milk (CM). Coconut milk is a natural source of cytokinins, auxins, and other bioactive compounds (Kong et al., 2023). These PGRs can promote seed germination by alleviating seed dormancy and stimulating cell division and shoot development. However, the composition of CM could not be maintained standard hence, 1.0 mg/L 2,4,5-T was selected as an individual auxin for induction of callus growth. GI in 1.0 mg/L 2,4,5-T was although not the highest yet fresh weight, dry weight, and moisture percent were the highest along with callus growth. Thus, 1.0mg/L 2,4,5-T has also been treated as a control due to superior physical attributes (Figure 1b). Gharyal and Maheshwari (1990) achieved callus initiation and subsequent shoot differentiation in Albizia lebbeck, *Cassia fistula*, and *C. siamea*. Their study used B5 medium supplemented with either 0.5mg/L IAA and 1.0 mg/L BAP or 2.0 mg/L NAA and 0.5 mg/L BAP, with explants derived from stem and petiole tissues of mature trees.

Table: 1 Callus induction response from different explants of *Cassia fistula* on

S. No.	MS + Supplements	Stem	Cotyledon	Leaf
1	1.0 mg/L 2,4-D	---	+++	---
2	2.0 mg/L 2,4-D	---	++	---
3	0.5 mg/L 2,4-D + 1% AC	---	+	---
4	1.0 mg/L 2,4-D+1%AC	---	++	---
5	2.0 mg/L 2,4-D+1%AC	---	++	---
6	1.0 mg/L 2,4-D + 0.5 mg/L Kn + 25mg/L Citric Acid	---	---	---

7	1.0 mg/L 2,4-D + 0.5 mg/L Kn + 1%AC	---	++	---
8	0.5 mg/L 2,4-D + 0.5 mg/L BAP + 50mg/L Ascorbic Acid \	++	---	+++
9	1.0 mg/L 2,4-D + 0.5 mg/L BAP + 50mg/L Ascorbic Acid	++	---	+++
10	2.0 mg/L 2,4-D + 0.5 mg/L BAP + 50 mg/L Ascorbic Acid	++	---	+++

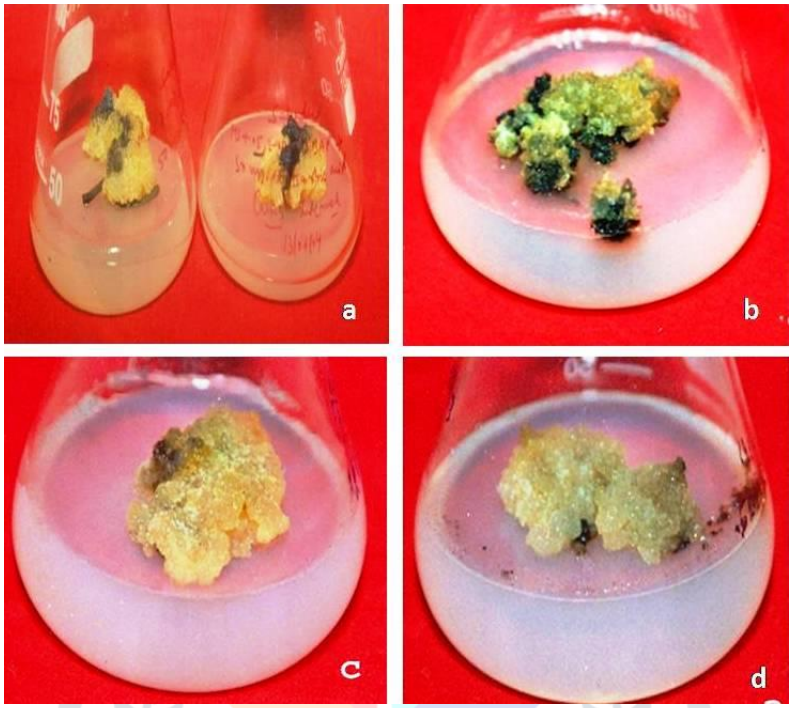

Figure 1: (a) Callus induction 1.0 mg/L 2,4-D + 0.5 mg/L BAP + 50mg/L Ascorbic Acid (b) Four weeks old callus sub-cultured on 1.0 mg/L 1.0mg/L 2,4,5-T (c) Four weeks old callus sub-cultured on 1.0 mg/L 1.0mg/L 2,4,5-T + 0.5 mg/L BAP (d) Four weeks old callus sub-cultured on 1.0 mg/L 1.0mg/L 2,4,5-T + 1.0 mg/L BAP

Amongst combinations of auxins and cytokinins, 1.0mg/L 2,4,5-T with 0.5- 2.0mg/L BAP and IAA with 0.5-2.0mg/L BAP displayed higher GI, fresh weight, dry weight, and moisture percent. Except for IBA and BAP combinations which exhibited poor callus growth, NAA and BAP combinations exhibited no callus growth, in opposition to the findings of Gharyal and Maheshwari (1990). The rest of the combinations NAA, 2,4,5-T, IBA, and IAA with Kn exhibited moderate callus growth to maintain a comparative relationship with standard (control). 1.0mg/L 2,4,5-T + BAP combinations were preferred for secondary metabolite studies later (Table-2; Table-3).

Table: 2 Physical growth attributes of *Cassia fistula* calli subcultured on MS media Supplemented with individual auxins

S.No.	MS + Supplements	GI (g/ml)	FW (g)	DW (g)	Moisture (%)
1	1.0 mg/L NAA	0.67	1.608	0.358	77.86
2	2.0 mg/L NAA	0.5	1.352	0.218	88.87
3	5.0 mg/L NAA	0.5	1.355	0.4	70.47
4	1.0 mg/L 2,4,5-T	0.4	5.043	0.636	88.29
5	2.0 mg/L 2,4,5-T	0.5	4.081	0.453	88.89
6	5.0 mg/L 2,4,5-T	0.5	2.19	0.289	41.14
7	1.0 mg/L IBA	0.444	3.319	1.022	69.2
8	2.0 mg/L IBA	0.444	4.986	1.146	77.01
9	5.0 mg/L IBA	0.465	3.614	0.992	72.55
10	1.0 mg/L IAA	0.51	2.302	0.371	86.55

11	2.0 mg/L IAA	0.5	2.231	0.38	82.96
12	5.0 mg/L IAA	0.5	2.341	0.367	84.32

Table: 3 Physical growth attributes of *Cassia fistula* calli subcultured on MS supplemented with auxins and synthetic cytokinins

S.No.	MS + Supplements	Growth Index (g/ml)	Fresh Weight (g)	Dry Weight (g)	Moisture (%)
1	1.0 mg/L NAA + 0.5 mg/L BAP	0.44	3.116	0.882	71.69
2	1.0 mg/L NAA + 1.0 mg/L BAP	0.5	3.343	0.677	92.27
3	1.0 mg/L NAA + 2.0 mg/L BAP	0.5	4.907	0.992	79.78
4	1.0 mg/L NAA + 0.5 mg/L Kn	0.54	12.767	1.175	90.76
5	1.0 mg/L NAA + 1.0 mg/L Kn	0.54	13.923	1.209	91.31
6	1.0 mg/L NAA + 2.0 mg/L Kn	0.5	4.689	0.722	84.6
7	1.0 mg/L 2,4,5-T + 0.5 mg/L BAP	0.5	20.525	2.306	88.76
8	1.0 mg/L 2,4,5-T + 1.0 mg/L BAP	0.5	14.893	1.892	87.37
9	1.0 mg/L 2,4,5-T + 2.0 mg/L BAP	0.53	28.043	3.082	89
10	1.0 mg/L 2,4,5-T + 0.5 mg/L Kn	0.5	9.028	1.025	88.64
11	1.0 mg/L 2,4,5-T + 1.0 mg/L Kn	0.5	7.611	0.996	86.91
12	1.0 mg/L 2,4,5-T + 2.0 mg/L Kn	0.5	9.495	1.125	88.15
13	1.0 mg/L IBA + 0.5 mg/L BAP	0.55	8.422	1.44	82.9
14	1.0 mg/L IBA + 1.0 mg/L BAP	0.53	5.514	1.037	81.19
15	1.0 mg/L IBA + 2.0 mg/L BAP	0.5	29.048	3.405	88.39
16	1.0 mg/L IBA + 0.5 mg/L Kn	0.5	7.245	1.77	75.56
17	1.0 mg/L IBA + 1.0 mg/L Kn	0.44	7.245	0.994	86.28
18	1.0 mg/L IBA + 2.0 mg/L Kn	0.47	10.28	1.725	83.21
19	1.0 mg/L IAA + 0.5 mg/L BAP	0.5	41.582	5.021	87.93
20	1.0 mg/L IAA + 1.0 mg/L BAP	0.51	25.832	3.339	87.07
21	1.0 mg/L IAA + 2.0 mg/L BAP	0.54	38.44	3.797	90.12
22	1.0 mg/L IAA +0.5 mg/L Kn	0.57	13.413	2	85.08
23	1.0 mg/L IAA +1.0 mg/L Kn	0.5	19.306	2.75	85.75
24	1.0 mg/L IAA +2.0 mg/L Kn	0.57	16.718	2.39	85.7

3.2 Effect of Individual Auxins on Carbohydrate and Phenolics Contents

Callus cultures initiated with 1.0 mg/L 2,4-D and 0.5 mg/L BAP were transferred to fresh MS media to assess the effects of auxin type and concentration on callus carbohydrate and phenolic content. These cultures received various concentrations (0.5, 1.0, and 2.0 mg/L) of indole-based (IAA, IBA) and non-indole-based auxins (NAA, 2,4,5-T). NAA promoted carbohydrate and phenolic accumulation, while 2,4,5-T had a mixed effect. NAA increased RS and TS with increasing concentrations. Maximum reducing sugars were observed at 5.0mg/L NAA (0.80 mg glu eq/gdw). NRS and TS increased with increasing NAA concentration, but a dip was recorded at 1.0mg/L NAA, indicating maximum mobilization of NRS to RS for growth activities with no fresh synthesis at this concentration. Total phenolic content increased with increasing NAA concentration. Reducing sugars decreased with increasing 2,4,5-T concentrations. Maximum accumulation of reducing sugars observed in 1.0mg/L 2,4,5-T (0.51 mg glu eq/gdw). NRS and TS increased with increasing concentrations of 2,4,5-T. Total phenolic content increased with increasing concentration but decreased at the highest concentration (5.0 mg/L). Maximum phenolics were recorded at a medium concentration of 1.0mg/L 2,4,5-T (71.90 mg ca eq/gdw).

Indole butyric acid (IBA) decreased RS, NRS, and TS compared to control (1.0mg/L 2,4,5-T). There were increased phenolics at all concentrations, with a peak at 2.0 mg/L IBA, suggesting decreased sugar synthesis for increased phenolic production. IAA increased RS and TS with increasing concentration. The highest levels of TS were at 2.0mg/L IAA. NRS declined at higher concentrations, suggesting utilization for growth and energy. IAA treatment increased phenolics at all concentrations with a peak at 2.0mg/L IAA, potentially due to stress response. 5.0mg/L IAA led to the highest levels of all measured compounds (sugars & phenolics). For maximizing phenolic production, 2.0mg/L 2,4,5-T appears most favorable. These results suggest contrasting effects of indole auxins on callus metabolism. IBA prioritizes phenolics over sugars, while IAA promotes sugar synthesis and may induce stress at higher concentrations (Table-4).

Table: 4 Carbohydrate and total phenolics contents of 4 week-old calli of *Cassia fistula* subcultured on MS supplemented with non-indole and indole auxins

S. No.	MS + Supplements	Carbohydrates			Phenolics
		RS	NRS	TS	
1	1.0 mg/L NAA	0.53*	3.4	3.93**	10.38**
2	2.0 mg/L NAA	0.79**	0.02	0.81	32.18**
3	5.0 mg/L NAA	0.80**	9.1	9.90**	36.48**
4	1.0 mg/L 2,4,5-T	0.51	0.257	0.77	34.65
5	2.0 mg/L 2,4,5-T	0.44*	1	1.43**	71.90**
6	5.0 mg/L 2,4,5-T	0.31**	1.186	1.49*	63.67**
7	1.0 mg/L IBA	0.99**	0.2	1.19**	7.27**
8	2.0 mg/L IBA	0.59**	0.01	0.60*	10.57*
9	5.0 mg/L IBA	0.43	0.01	0.44**	7.86**
10	1.0 mg/L IAA	0.51	1.09	1.60**	6.84**
11	2.0 mg/L IAA	0.37**	3.05	3.43**	38.57**
12	5.0 mg/L IAA	2.01**	0.11	2.12**	30.19

3.3 Effects of Indole and Non-indole Auxins in Combination of Cytokinins on Carbohydrates and Phenolics Accumulation

Each auxin was taken at 1.0 mg/L along with increasing concentrations of BAP and Kn for four weeks. Increased carbohydrates (RS, NRS, TS) up to 1.0mg/L NAA + 1.0mg/L BAP, then declined. Phenolics increased with all BAP concentrations. Decreased carbohydrates (RS, TS) with all Kn concentrations in combination with 1.0 mg/L NAA. NRS remained stable or increased slightly. Phenolics peaked at 1.0mg/L NAA + 1.0 mg/L Kn. Reduced, carbohydrates (RS, NRS, TS) with increasing BAP concentrations. Phenolics peaked at the lowest BAP concentration (0.5 mg/L). Decreased carbohydrates (RS, TS) and phenolics with all Kn concentrations. NRS showed a gradual decline. Overall, NAA + BAP promoted carbohydrate synthesis, while NAA + Kn or 2,4,5-T with either BAP or Kn favored phenolic accumulation. The combination of 1.0mg/L NAA + 1.0 mg/L BAP resulted in the highest levels of carbohydrates, while 1.0mg/L NAA + 1.0mg/L Kn led to the highest phenolics among non-indole auxin combinations. The lowest levels of both carbohydrates and phenolics were observed with 1.0 mg/L 2,4,5-T + 2.0mg/L Kn (Table-5).

Table: 5 Carbohydrate and total phenolic contents of 4 week-old calli of *Cassia fistula* subcultured on MS supplemented with non-indole auxins with cytokinins

S. No.	MS + Supplements	Carbohydrates			Phenolics
		RS	NRS	TS	
1	1.0 mg/L NAA + 0.5mg/L BAP	0.49	1.19	1.72**	8.19**
2	1.0 mg/L NAA + 1.0mg/L BAP	0.66**	3.33	3.99**	9.81**
3	1.0 mg/L NAA + 2.0mg/L BAP	0.49	2.92	3.41**	13.04**
4	1.0 mg/L NAA + 0.5mg/L Kn	0.43	0.77	1.20**	35.08**
5	1.0 mg/L NAA + 1.0mg/L Kn	0.27**	0.78	1.11*	79.47**
6	1.0 mg/L NAA+2.0mg/L Kn	0.30**	0.89	1.19*	22.26**
7	1.0 mg/L 2,4,5-T + 0.5mg/L BAP	0.61*	2.5	3.11**	4.18**
8	1.0 mg/L 2,4,5-T + 1.0mg/L BAP	0.49	2.3	2.79**	2.99**
9	1.0 mg/L 2,4,5-T + 2.0mg/L BAP	0.52*	1.85	2.37**	3.09**
10	1.0 mg/L 2,4,5-T + 0.5mg/L Kn	0.20**	1.34	1.56**	13.46**
11	1.0 mg/L 2,4,5-T + 1.0mg/L Kn	0.20**	1.12	1.23**	11.46**
12	1.0 mg/L 2,4,5-T + 2.0mg/L Kn	0.17-	1.25	1.41**	9.71**

Indole butyric acid (1.0 mg/L) with increasing BAP concentration RS, NRS, and TS initially decreased but sharply increased at higher BAP. Phenolics steadily increased with BAP, indicating stress tolerance. IBA (1.0 mg/L) with Kn (0.5/1.0/2.0 mg/L) RS remained constant but NRS and TS decreased with increasing Kn suggesting Kn intolerance. Phenolics peaked at intermediate Kn concentration. Indole acetic acid (IAA) and BAP-supplemented MS media resulted in higher quantities of RS and NRS at lower BAP but decreased at higher BAP (intolerance). Phenolics initially decreased but rose sharply at the highest BAP. IAA in combination with varying Kn concentrations led to a decrease in RS with increasing Kn concentration, but NRS, TS, and

phenolics all increased, with phenolics highest at the highest Kn (stress response). Overall, IBA with BAP showed initial carbohydrate decline followed by recovery and stress tolerance for phenolics. Conversely, Kn with IBA or IAA led to Kn intolerance for carbohydrates but varied responses for phenolics, suggesting stress-induced accumulation at higher Kn (Table-6).

Table: 6 Carbohydrate and total phenolic contents of 4 week-old calli of *Cassia fistula* subcultured on MS supplemented with indole auxins with cytokinins

S. No.	MS + Supplements	Carbohydrates			Total Phenolics
		RS	NRS	TS	
1	1.0 mg/L IBA + 0.5mg/L BAP	0.54**	2.92	3.46	10.69**
2	1. 0 mg/L IBA + 1.0mg/L BAP	0.51**	2.55	3.06	13.59**
3	1.0 mg/L IBA + 2.0mg/L BAP	0.57**	4.77	4.84	16.72**
4	1.0 mg/L IBA + 0.5mg/L Kn	0.59**	3.01	3.6	10.10**
5	1.0 mg/L IBA + 1.0mg/L Kn	0.57**	2.08	2.65	16.45**
6	1.0 mg/L IBA + 2.0mg/L Kn	0.59**	1.68	2.08	9.58**
7	1.0 mg/L IAA + 0.5mg/L BAP	0.14**	1.55	1.69	13.74**
8	1.0 mg/L IAA + 1.0mg/L BAP	0.30**	4.8	5.1	10.59
9	1.0 mg/L IAA + 2.0mg/L BAP	0.37*	2.5	2.95	18.12**
10	1.0 mg/L IAA + 0.5mg/L Kn	0.28**	1.05	1.33	12.80**
11	1.0 mg/L IAA + 1.0mg/L Kn	0.19**	1.35	1.54	20.12**
12	1.0 mg/L IAA + 2.0mg/L Kn	0.19**	1.66	1.85	25.30**

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IV. CONCLUSIONS

The study concludes that optimizing callus cultures for desired metabolite production requires balancing auxin and cytokinin types and concentrations. For high total phenolics content, using 2,4,5-T or a combination of NAA and Kn is most effective, but this may come at the cost of poorer callus growth. Conversely, using BAP with auxin may improve the balance between carbohydrate and phenolic production, potentially allowing for good callus growth and some phenolic content.

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