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# A PRELIMINARY STUDY ON DISINFECTION AND IN VITRO PROPAGATION OF *CELOSIA TRIGYNA* LINN.

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## Article Info

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**Abstract**

*Celosia trigyna* is an underutilized indigenous vegetable in Nigeria with numerous medicinal uses. Information is sparse on any attempt to conserve it. In other to conserve it through in vitro propagation, a disinfection protocol was developed using Clorox (NaClO) and Mancozeb (C8H12MnN4S8Zn) (Treatment T1 to T9) and a preliminary investigation testing its responsiveness in vitro. Nodal explants of *Celosia trigyna* were collected from the mother plants at Obafemi Awolowo University Estate, Ile – Ife (Latitude 7º32'N and Longitude 4º31'E). The nodal explants of *C. trigyna* were cultured on Murashige and Skoog (MS) medium supplemented with various levels of plant growth regulators (PGRs). The clean cultures obtained were monitored for degree of callus formation, morphology of callus, shoot and root regeneration. Treatment T6 (5% v/v Clorox for 9 minutes and 1% w/v mancozeb for 5 minutes) was best for disinfecting nodal explants of *C. trigyna* resulting into 81.47 ± 2.14a % aseptic cultures. Nodal explants cultured on MS with 0.5mg/L 2, 4 –D produced callus. This study established that using 5% v/v Clorox for 9 minutes followed by 1% w/v mancozeb for 5 minutes is effective for producing clean cultures of nodal explants and that *C. trigyna* can be propagated *in vitro*.

**Keyword**: Celosia, disinfection, explant, in vitro, MS, trigyna.

## Introduction

*Celosia trigyna* commonly referred to as wool flower, is one of the neglected and underutilized leafy vegetables in Nigeria. It is the most widespread species of Celosia in tropical Africa (Denton, 2004). Various parts of *Celosia trigyna* plants have medicinal values (Aregheore, 2012). In Sierra Leone, the leaf is eaten raw for heart treatment

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and to relieve costal pain (Mabona et al., 2013). It has been reported to be diuretic and hemostatic; and used to hasten childbirth (Mabona et al., 2013). In Congo, it is used to treat ovarian cancer as it has shown a potential cytotoxic activity (Sowemimo et al., 2009). In addition, *Celosia trigyna* has proven to be effective in the treatment of gastric ulcers due to the presence of secondary metabolites (Ofusori et al., 2018). The plant is reported to be useful in the treatment of women's disorders and diseases. Despite this array of usefulness, no information exists on its collection and conservation (Denton, 2004). This work has therefore sought to provide preliminary data on the micropropagation of this leafy vegetable by developing a suitable protocol for its disinfection. To date, no report is documented on the disinfection viz a viz the micropropagation of *Celosia trigyna*, despite its diverse medicinal benefits.

*Celosia trigyna* is rarely cultivated but always harvested without replacement and on many occasions treated as a weed on agricultural lands (Denton, 2004). Traditional propagation can be difficult because the seeds of *Celosia trigyna* become readily dormant and have a low germination percentage (Orwa et al., 2009). To prevent the genetic erosion of this nutrient-rich indigenous vegetable, there is an urgent need to conserve its germplasm. Micropropagation is an alternative propagation method since it bypasses the use of seeds as propagules (Saurabh et al., 2015); thus, it is recommended for plants with hard-to-germinate and/or dormant seeds. A suitable disinfection procedure is a prerequisite to achieve success in micropropagation. Therefore, the main aim of this study is to develop a disinfection protocol for the explants of *Celosia trigyna* and test the responsiveness of this plant to micropropagation.

**Materials and Methods**

The experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Plants of *Celosia trigyna* were collected from Obafemi Awolowo University Campus, Ile-Ife (Latitude 7°32'N and Longitude 4°31'E). *Celosia trigyna* was deposited in the IFE Herbarium, Obafemi Awolowo University, Nigeria and was given a deposition number of IFE – 17466. Ready-made powder of Murashige and Skoog medium (MS, 1962) with vitamins and sucrose (M5501) was purchased and prepared following standard procedures. Freshly cut 1 cm nodal explants of *C. trigyna* were rinsed under running tap water for about 3 minutes and rinsed twice using sterile distilled water, to remove any surface dirt or attached dust particles. Inside the laminar airflow cabinet, the nodal explants were then pre-disinfected with 70% ethanol for 10 minutes followed by further treatment with NaOCl alone and then Mancozeb. The following concentrations of NaOCl and Mancozeb with exposure periods were tested as shown in Table 1. After disinfection of the explants with each of the treatments, they were rinsed 3 times with sterile distilled water to eliminate all traces of disinfectants. Then, each of the nodal explants were sectioned into 1cm length with sterile forceps and scalpels and inoculated vertically on MS basal media. The cultures were maintained in the incubator at a temperature of 25 ± 2°C. The percentage of clean cultures, percentage of contaminated cultures, and percentage of necrotic cultures were observed and recorded after two weeks. This experiment was repeated three times with nine explants per treatment for each trial.

To test the responsiveness of *C. trigyna* to in vitro propagation, benzylaminopurine (BA) and 2,4 - Dichlorophenoxyacetic acid (2, 4 – D) alone and each in combination with naphthalene acetic acid (NAA) were added to the culture media. Nodal explants were excised from healthy mother plants and disinfected with the best disinfection treatment obtained from the disinfection experiment above. The experiment was repeated twice with 5 explants per treatments indicated below:

i. MS Only
ii. MS + 1mg/L BA
iii. MS + 2mg/L BA
iv. MS + 3mg/L BA
v. MS + 1mg/L BA + 0.5mg/L NAA
vi. MS + 1mg/L BA + 1mg/L NAA
vii. MS + 2mg/L NAA + 1mg/L BA
viii. MS + 0.5mg/L NAA
ix. MS + 1mg/L NAA
x. MS + 2mg/L NAA
xi. MS + 0.5mg/L 2, 4-D
xii. MS + 1mg/L 2, 4-D
xiii. MS + 2mg/L 2, 4-D

All data were subjected to arc-sine transformation (Gomez and Gomez, 1984) and then analyzed with one-way analysis of variance (ANOVA), and least significant difference (LSD) test was used to separate the means where required at a probability level of 5%. The results were expressed as mean ± standard error (S.E.)

**Table 1**: **Different Concentrations of Disinfectant and Different Exposure Time on Nodal Explants**

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| **TRT** | **DF** | **CONC.** | **DUR** | **DF** | **CONC.** | **DUR** |
|---|---|---|---|---|---|---|
| $T_1$ | NaOCl | 5% v/v | 5 mins | - | - | - |
| 9 | | 5% v/v | 5 mins | MCZ | 1% w/v | 5 mins |
| $T_2$ | NaOCl | | | | | |
| $T_3$ | NaOCl | 5% v/v | 5 mins | MCZ | 1% w/v | 10 mins |
| $T_4$ | NaOCl | 5% v/v | 6 mins | MCZ | 1% w/v | 5 mins |
| $T_5$ | NaOCl | 5% v/v | 7 mins | MCZ | 1% w/v | 5 mins |
| $T_6$ | NaOCl | 5% v/v | 8 mins | MCZ | 1% w/v | 5 mins |
| $T_7$ | NaOCl | 5% v/v | 9 mins | MCZ | 1% w/v | 5 mins |
| $T_8$ | NaOCl | 5% v/v | 9 mins | MCZ | 1% w/v | 5 mins |
| $T_9$ | NaOCl | 5% v/v | 10 mins | MCZ | 1% w/v | 10 mins |
| $T_{10}$ | NaOCl | 5% v/v | - | MCZ | 1% w/v | 10 mins |

Key DF Key DF-

tant, CONC. -concentration, DUR- duration, MCZ- Mancozeb

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**Result and Discussion**

Disinfection of nodal explants of *Celosia trigyna*

To establish a successful in vitro regeneration protocol, an effective disinfection protocol needs to be developed as contamination of explants can prevent their successful growth in vitro. All concentrations and combinations of chemicals tested were significantly different, when compared with T1, T2 and T3 (Table 2). Explants disinfected with Treatment T1 (5% v/v NaOCl for 5 minutes) resulted in the lowest percentage sterility and alive cultures indicating that treatment with NaOCl alone was not effective in disinfection of the explants. Addition of 1% (w/v) Mancozeb for 5 minutes to the disinfection mixture (T2) resulted into a significant increase in the percentage clean culture (40.67 ± 2.14) when compared to T1 (18.47 ± 2.14). This indicates that the addition of 1% w/v mancozeb for 5 minutes was effective to further removing contaminants from the explants of *C. trigyna*. Mancozeb is a fungicide. Afridi et al., (2015) reported a remarkable reduction in fungal contamination by using fungicides. A decrease in the percentage clean and alive culture (25.87 ± 2.14) was observed (cultures became necrotic) when the exposure period of explants to Mancozeb increased; treatment T3 (Figure 1). This showed that exposing nodal explants of *C. trigyna* to 1% mancozeb for 10 minutes had a damaging effect on the cells of the explants; hence affected the vigor of explants. Treatment T6 gave the highest percentage clean and alive culture (81.47± 2.14). This treatment was found to be best for removing bacterial and fungal contaminants from the explant of *C. trigyna* (Figure 2), as it gave a result that was significantly higher than the other treatments: T1, T2, T3, T4, T5 and T7 (Table 2).

Nodal explants of *Celosia trigyna* sterilized with T4 (59.27 ± 2.14), T5 (59.27 ± 2.14) and T6 (81.47± 2.14) resulted in a significantly higher percentage clean and alive culture compared to T2 (40.67 ± 2.14). The increased exposure period to NaOCl further disinfected the explants. Shahrzad and Linden (1997) reported an increase in percentage sterile culture of Zingiber officinale explants when their exposure period to NaOCl increased. Exposure to treatment T8 increased the percentage clean and alive culture from 62.97 ± 2.14 to 74.07 ± 2.14, although the increase was not significant. A decrease resulted with treatment T9 when explants were exposed to a longer exposure to 1% (w/v) Mancozeb for 10 minutes. This trend was observed in the use of treatments T2 and T3. Thus, exposing nodal explants of *Celosia trigyna* to 1% (w/v) Mancozeb for 10 minutes has an adverse effect on the explant.

**Table 2: Effect of Different Concentrations of Disinfectants and Their Exposure Time on The Percentage of Clean and Alive Culture of Nodal Explants of *Celosia trigyna*.**

| S/N | Treatment | % Clean and alive cultures (Mean ± S. E.) |
|---|---|---|
| **1** | T1 | 18.47 ± 2.14e |
| **2** | T2 | 40.67 ± 2.14d |
| **3** | T3 | 25.87 ± 2.14e |
| **4** | T4 | 59.27 ± 2.14c |
| **5** | T5 | 59.27 ± 2.14c |
| **6** | T6 | 81.47 ± 2.14a |
| **7** | T7 | 62.97 ± 2.14bc |
| **8** | T8 | 74.07 ± 2.14ab |
| **9** | T9 | 70.37 ± 2.14abc |
| **10** | T10 | 74.07 ± 2.14ab |

*Values with the same superscript in the columns are not significantly different from each other at $P < 0.05$

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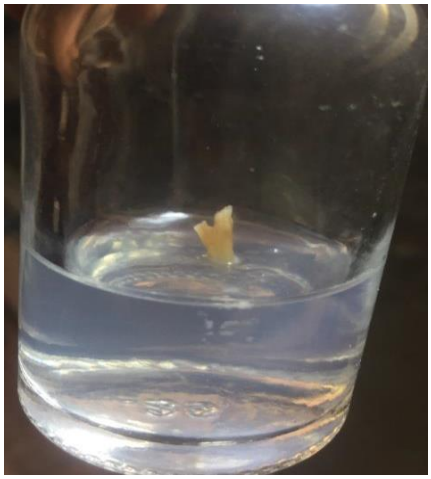

**Figure 1. Necrotic culture of nodal explant of *Celosia trigyna***

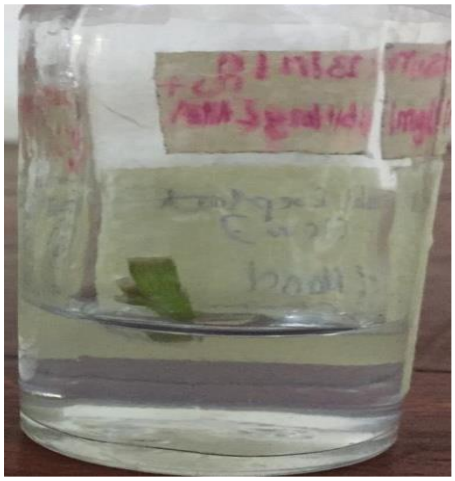

**Figure 2. Sterile and alive culture of explant of *Celosia trigyna***

**Callus Induction and Root Initiation from Nodal Explants**

Nodal explants of *Celosia trigyna* cultured on MS basal media alone showed neither callogenesis nor organogenesis response (Table 3). The concentration of endogenous plant growth regulators present in nodal explants of *C. trigyna* was not high enough to initiate any response. In contrast to this report, Bakar et al., (2014) observed shooting response from culturing explants of Celosia argentea on MS basal medium alone. In *C. trigyna*, the addition of BA (1 mg/L) to the MS basal media initiated callogenesis, a small-sized white friable callus grew (Table 3). The inclusion of BA into the medium stimulated the action of endogenous auxin to initiate callus formation. Hemmati et al., (2020) reported a similar finding of callus formation from *Salvia tebesana* in BA-supplemented media. However, in this study, as the concentration of BA increased to 2 mg/L and 3 mg/L, callus induction was suppressed and completely inhibited (Table 3). This inhibition of callus induction can be attributed to the effect of BA at concentrations higher than the optimal required for callus induction. This BA effect is a species-dependent response as Ogbimi et al., (2016) reported callus inhibition in nodal explants of *Solanecio biafrae* at lower concentrations of BA.

Another combination of plant growth regulator in the culture media also elicited callus growth. The addition of NAA to the BA in the basal medium resulted also in callus initiation. Nodal explants of *C. trigyna* cultured on MS + 1 mg/L BA + 0.5 mg/L NAA formed callus (1.09 ± 0.38) (Figure 3), though a reduction in the percentage response was observed with respect to nodal explants cultured on MS + 1 mg/L BA (1.47± 0.76). Increasing the concentration of NAA to 1mg/L, that is culturing explants on MS + 1 mg/L BA+ 1 mg/L NAA, stimulated root initiation alone without callusing (Figure 4), while a further increase in the concentration of NAA to 2 mg/L inhibited rooting but enhanced callusing (Figure 5). It can be explained that the explant concentrated more on

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callus formation at the availability of a higher auxin concentration. Andaryani et al., (2019) reported a similar response with the explants of *Jatropha curcas*. This response depicts the effect of auxin to cytokinin ratio in determining morphogenesis and dedifferentiation. 1 mg/L NAA was optimal for stimulating the cells of *Celosia trigyna* to root; while at higher concentrations such as 2 mg/L, rooting was inhibited. A similar response was reported in *Hemarthria compressa* where the application of higher concentration of NAA resulted in a significant decrease in root formation (Yan et al., 2014).

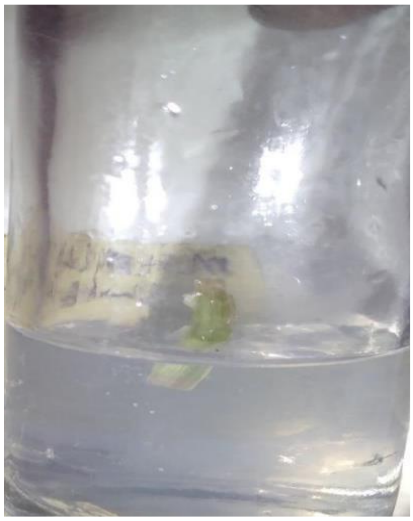

**Figure 3. Nodal explant of *Celosia trigyna* showing off white callus on MS supplemented with 1mg/L BA + 0.5mg/L NAA**

Just as a low concentration of BA enhanced callus initiation from nodal explants of *Celosia trigyna*, the addition of a low concentration of NAA also gave a similar response. However, the addition of a low concentration of 2,4-D (0.5 mg/L) alone elicited the best response of callus size; moderate-sized callus (C++). A similar response was observed in Rumex pictus where a low concentration of 2,4-D in combination with a lower concentration of BAP produced the highest mass of callus (El-Shafey et al., 2019).

**Figure 4. Nodal explant of *Celosia trigyna*showing a root on 1mg/L BA + 1.0 mg/L NAA**

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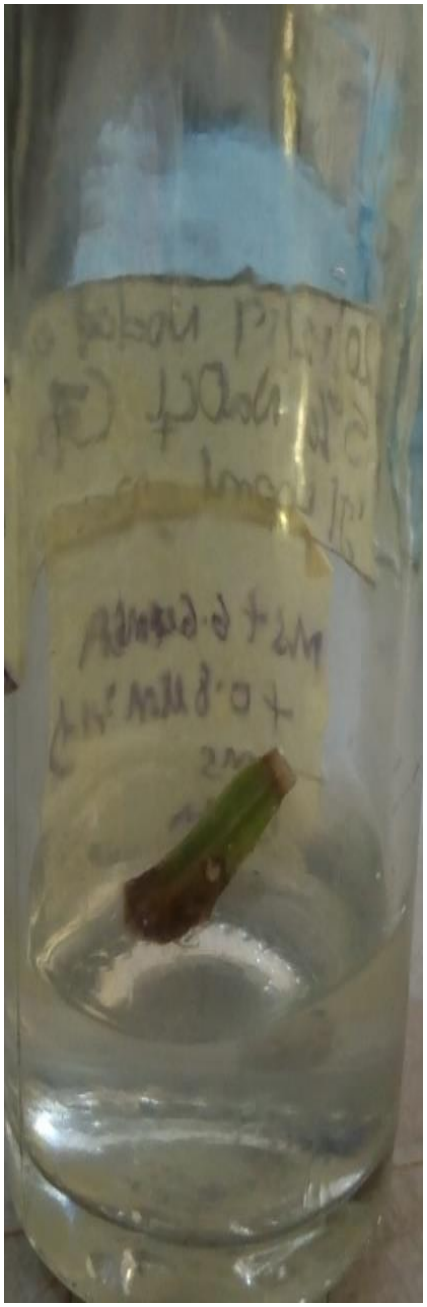

**Figure 5. Nodal explant of *Celosia trigyna* showing brown callus on 1mg/L BA + 2mg/L NAA respectively**

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**Table 3: Effect of Various Concentrations of Ba, Naa, 2, 4-D Alone and NAA Combined with Ba on Callus/Root Formation from Nodal Explants of *Celosia trigyna***

| S/N | BA | NAA | 2,4-D | TOR | PRE | MOC |
|---|---|---|---|---|---|---|
| **1** | - | - | - | N | 0.00± 0.00b | - |
| **2** | 1 | - | - | C+ | 1.47± 0.76ab | White and friable |
| **3** | 2 | - | - | N | 0.00 ± 0.00b | - |
| **4** | 3 | - | - | N | 0.00 ± 0.00b | - |
| **5** | 1 | 0.5 | - | C+ | 1.09 ± 0.38ab | White and friable |
| **6** | 1 | 1 | - | R | 1.09 ± 0.38ab | - |
| **7** | 1 | 2 | - | C+ | 1.09 ± 0.38ab | Brown and soft |
| **8** | - | 0.5 | - | C+ | 1.85 ± 0.85ab | Cream and soft |
| **9** | - | 1 | - | C+/R | 2.24 ± 0.93a | White and soft |
| **10** | - | 2 | - | C+ | 1.47± 0.76ab | Cream and soft |
| **11** | - | - | 0.5 | C++ | 1.85 ± 0.47ab | Cream and friable |
| **12** | - | - | 1 | C+ | 1.09 ± 0.38ab | Cream and friable |
| **13** | - | - | 2 | C+ | 1.09 ± 0.38ab | Cream and friable |

Values with the same superscripts in the columns are significantly different from each other at $P < 0.05$.

**Keys:** N: No Response, R: Root Initiation, C+: Slight Callus Formation, C++: Moderate Callus Formation, TOR: Type of Response, PRE: Percentage Response of Explants, MOC: Morphology of Callus.

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Nodal explants cultured on MS + 1 mg/L NAA alone responded showing both callogenesis and rhizogenesis (Figure 6); only this treatment induced callus and root formation simultaneously. This treatment was optimal for organogenesis and callogenesis since it initiated the highest percentage response from explants. Comparing treatments of MS + 1 mg/L BA, MS + 1 mg/L BA + 1 mg/L NAA and MS + 1 mg/L NAA, showed the callus-stimulatory effect of MS + 1 mg/L BA when used alone and its callus-inhibitory effect when used in combination with the same concentration of NAA. BA has been observed in this study to elicit different responses in different media combinations. For organogenesis to occur in *C. trigyna* without an intervening callogenic response, a combination of NAA and BA in the same ratio is required.

**Figure 6. Nodal explant of *Celosia trigyna* showing root with callus on 1mg/L NAA alone**

No organogenesis response was obtained from the addition of 2, 4-D to the media, but only callus formation. However, a low concentration of 2, 4-D (0.5 mg/L) in the media favored callus formation (Figure 7). 2, 4-D is commonly used for induction of callus growth because it reverts cells in the explant by de–differentiating already differentiated cells, thus initiating cell division again in these cells (George et al., 2008).

**Figure 7. Nodal explant of *Celosia trigyna* showing friable callus on MS supplemented with 0.5 mg/L 2,4 – D alone**

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**Conclusion**

Nodal explants of *C. trigyna* can be disinfected adequately with treatment T6 - 5% (v/v) NaOCl for 9 minutes + 1% (w/v) mancozeb for 5 minutes without damaging the cells of the nodal explants. *Celosia trigyna* has the potential to be regenerated through in vitro propagation. The study reports that 2, 4-D is a better auxin for callus initiation from nodal explants of *C. trigyna*, however, further research is required to optimize the callus initiation protocol, as well as to develop a protocol for plantlet regeneration from the nodal explants of *C. trigyna*. This research has confirmed that *C. trigyna* is amenable to in vitro propagation.

**Authors' Contributions**

ER and MS designed and experimented, with data analysis. ER wrote the manuscript, while AMA and SE were involved in reviewing and editing the manuscript. All authors read and approved the final manuscript.

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**Conflict of Interest**

The author (s) declare there are no conflicts of interest related to this article.

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