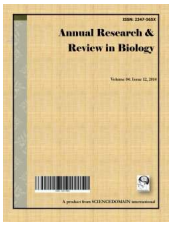

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In vitro Multiple Shoots Formation in Wild Lettuce (*Launaea taraxacifolia)* (Willd.) Amin ex C. Jeffrey

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OA, OAB and OSA managed the literature searches. Authors OAB and OSA wrote the first draft of the manuscript. Author MNO designed the study and wrote the protocol. Author JOP managed the analyses of the study. Author BIO managed the editorial work and author OOO supervised the study from design to completion of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Launaea taraxacifolia* (Willd) Amin Ex.C Jeffrey is an undervalued traditional leafy vegetable, which is economically important as food and medicine. It also has several therapeutic and nutritive values. The cultivation is hampered by low seed viability, seed dormancy and poor vegetative propagation.
Aim: The study aimed to develop an efficient protocol for micropropagation of *L. taraxacifolia.*
Place and Duration of Study: The study was conducted at the tissue culture laboratory of National Centre for Genetic Resources and Biotechnology, Moor Plantation, Ibadan. Nigeria.
Methodology: One centimetre (1.00 cm) double-sterilized single-node explants of *L. taraxacifolia*

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cultured on Murashige & Skoog (MS) medium amended with a constant concentration (0.5 mg/l) of 1-Naphthaleneacetic acid (NAA) in combination with varying concentration (0.5 mg/l, 1.0 mg/l, 1.5 mg/l, 2.0 mg/l and 2.5 mg/l) of two cytokinins, 6-Benzylaminopurine (BAP) and kinetin were investigated for their effects on shoot proliferation, multiplication and rooting. Growth parameters were observed and recorded and the data subjected to statistical analysis.

Results: Among the cytokinin treatments, significant increase in means was observed with increase in the concentration of BAP. There was no significant difference among the treatments with kinetin. BAP treatments at 2.5 mg/l gave significantly highest number of shoots (3.33 and 4.67) and number of leaves (16.70 and 30.00) at 4 and 8 weeks of culture, respectively as compared to the control. Shoot length was highest in treatment with MS+2 mg/l BAP+0.5 mg/l NAA and MS+1 mg/l KIN+0.5 mg/l NAA (2.33 and 0.30) and these were higher than that of control at 4 weeks and equal at 8 weeks. Residual effect of the Plant growth regulators (PGRs) was observed in treatment with MS+0.5 mg/l KIN+0.5 mg/l NAA alone.

Conclusion: The results from the study revealed that *L. taraxacifolia* nodal explants resulted in multiple shoots in BAP treatments than in kinetin treatments. Double disinfection with treatment with MS+2.5 mg/l BAP+0.5 mg/l NAA which produced the highest number of clean multiple shoots and leaves is thereby proposed as shoot multiplication medium for this species.

Keywords: Launaea taraxacifolia; wild lettuce; Yanrin; plant growth regulators; treatments.

1. INTRODUCTION

Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey, commonly referred to as Wild Lettuce (English), "Efo Yanrin' (Yoruba), 'ugu' (Igbo) and 'nonon barya' (Hausa), is economically important as food, medicine and animal fodder [1]. The Asteraceae member plant is an underutilized leafy vegetable found in the wild in tropical Africa [1]. It grows well in moist soil, under direct sun, tolerates drought and can also grow on poor soils with low water content [1,2]. The plant is considered to be medicinally useful as it is rich in vitamins, minerals, proteins, essential fatty acids, fibre contents and flavonoids [1,3–8]. The leaves are fed to lactating cows to increase milk production in northern part of Nigeria and to sheep and/or goats to induce multiple births. Among the Yoruba people of southwestern Nigeria, the leaves are mixed with local black soap to prevent and/or cure skin diseases [3]. The milky latex it produces could be used to cure eye disease known as conjunctivitis/apollo. Its pharmacological properties include antibiotic, anti-venimous, anti-poison, anti-anemic, anti-inflammatory, anti-nausea, fungicide, nematocide, antalgic, febrifuge, sedative, cholesterol reduction, anti-diabetic, anti-cough, galactogen and laxative [9]. However, low seed viability, seed dormancy and lack of variability imposed by vegetative mode of propagation have been identified as factors militating against the cultivation of the plant [5].

Previous papers described its ethnobotanical uses, morphological, ecological, nutritional, phytochemical, therapeutic and biological activity studies [1,2,5,6,8–12]. The only plant tissue culture procedure previously reported for the species was on its *in vitro* potential using stem and leaf explants [5]. However, successful clonal micropropagation of most species of the family Asteraceae has been achieved via tissue culture techniques using different types of explants [13]. To therefore enhance all year round availability of this nutritious and medicinally important vegetable, there is need to employ plant tissue culture techniques for this species. This technique enhances propagation and conservation of important and rare plants. It also contributes towards the enhanced production of high quality planting material [14]. To the best our knowledge, this is the first report on *in vitro* propagation of this species via multiple shoot formation. In this study, we present protocol for micropropagation of *L. taraxacifolia via* multiple shoots induction using nodal explants.

2. MATERIALS AND METHODS

2.1 Source of Primary Biological Material and Disinfection

Plants of *L. taraxacifolia* were collected from natural habitat of the Institute of Agriculture, Research and Training (IAR&T), Obafemi Awolowo University, located on longitude 03°51E, latitude 07°23N and altitude 65°, Ibadan, Oyo state. It was authenticated by a taxonomist at the herbarium of the Forestry Research Institute of Nigeria. Surface sterilization of the

plant material was carried out under sterile conditions of laminar air flow cabinet.

2.2 Explant Source

Double disinfection was adopted for the surface sterilization of the plant material. The nodal segments (1.00 cm long) of *L. taraxacifolia* were immersed in 70% ethanol for 5 min, and then transferred into 10% sodium hypochlorite (NaOCl) for 10 min, followed by 15% sodium hypochlorite with periodic shaking for 15 min. The nodal cuttings were then rinsed three times with sterile distilled water.

2.3 *In vitro* Culture of *L. taraxacifolia*

All the sterilized explants were trimmed and 1 cm long single node cultured on Murashige and Skoog (MS) basal medium [10] supplemented with 3% sucrose. The pH was adjusted to 5.7 and 0.7% agar added before autoclaving at 121℃ for 15 min. All cultures were maintained at 8 h dark and 16 h photoperiod provided by cool white fluorescent tubes of 90 $\mu molm^{-2}s^{-1}$ intensity at 25±2℃ of temperature.

2.4 Effect of Cytokinin on Shoot Multiplication

Interactions between the auxin, Naphthalene acetic acid (NAA) and Cytokinins, 6-Benzylaminopurine (BAP) and kinetin (KN) on morphogenic response of *L. taraxacifolia* was investigated. The plant growth regulators (PGRs) were added to the medium before the pH was adjusted. A constant concentration of NAA was set at 0.5 mg/l in combination with varying concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) or kinetin (KN) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l). The treatments including control (MS basal medium only) are presented in Table 1. Medium devoid of PGRs was used as control and evaluated. A total of 11 treatments were assessed for the explant. The cultures were examined periodically and morphogenic responses were evaluated by visual observation.

2.5 Study Design and Statistical Analysis

The experiment had a completely randomized design (CRD). A minimum of ten replicates were prepared for each treatment. Three explants were cultured per treatments. In order to choose the best interactions of PGRs and medium for micropropagation, the number of shoots and roots per explants and length of shoots and leaves were evaluated at 4 and 8 weeks of initial culture. The data were subjected to one-way analysis of variance (ANOVA) and significant differences of means was determined using Tukey's multiple range test (P=.05). All statistical analyses were performed with GraphPad Prism 5 software.

Table 1. Media treatments with different concentrations of PGR used for this study

Treatments	Media
TC	MS only
TB1	MS+0.5 mg/l BAP+0.5 mg/l NAA
TB2	MS+1 mg/l BAP+0.5 mg/l NAA
TB3	MS+1.5 mg/l BAP+0.5 mg/l NAA
TB4	MS+2 mg/l BAP+0.5 mg/l NAA
TB5	MS+2.5 mg/l BAP+0.5 mg/l NAA
TK1	MS+0.5 mg/l Kinetin+0.5 mg/l NAA
TK2	MS+1 mg/l Kinetin+0.5 mg/l NAA
TK3	MS+1.5 mg/l Kinetin+0.5 mg/l NAA
TK4	MS+2 mg/l Kinetin+0.5 mg/l NAA
TK5	MS+2.5 mg/l Kinetin+0.5 mg/l NAA

3. RESULTS AND DISCUSSION

To investigate the influence of increasing cytokinin concentrations on shoot proliferation, the responses of nodal explants of *L. taraxacifolia* cultured in MS supplemented with a constant concentration (0.5 mg/l) of NAA in combination with varied concentrations of BAP or kinetin were investigated. 51% aseptic nodal segments were successfully established using double-disinfection protocol (10% and 15% NaOCl). Treatment containing 4 mg/l Kinetin (TK4) was completely lost to contamination, as such, no results is presented for this treatment. Number and length of shoots, number of leaves and number and length of roots were analyzed at 4 and 8 weeks of culture. All cultures initiated shoots at the second week of initial culture. Clean shoots were observed to have proliferated from the axillary buds of the explants. *In vitro* grown shoots began to wither after 8 weeks.

3.1 Shoot Proliferation Rate

One shoot bud per explants emerged within the first two weeks of culture initiation. However, the number of shoot buds per explants gradually increased with culture duration. BAP treatments gave significantly higher shoot bud emergence and multiplication rate than the control and kinetin treatments. MS medium alone produced

one shoot per explants. As the concentration of BAP was increased, a gradual increase in the number of shoots per culture was observed except in 1 mg/l BAP (TB2). MS medium supplemented with NAA (0.5 mg/l) in combination with BAP (2.5 mg/l) i.e., TB5 (Figs. 1 and 3A) gave the best response (3.33 shoots/explants at 4 weeks and 4.67 shoots/explants at 8 weeks) when compared to medium with kinetin treatment i.e., TK5 (Fig. 3B). Whereas these results conform to the concept that a higher cytokinin:auxin ratio is needed for multiple shoot induction, however, Obembe et al. [15] reported *in vitro* multiple shoot formation when nodal explants from *Cucurbita pepo* were cultured on PGR-free medium. There was no significant difference between TB2, treatments with kinetin (TK1, TK2, TK3, and TK5) and the control (1.00 shoots/explants at 4 and 8 weeks). The number of shoots in treatments with BAP increased significantly at 8 weeks of culture while treatments with kinetin did not increase significantly (Fig. 1). Number of new shoots per explants was highest when BAP was used as the cytokinin whereas, the control (TC) had single shoot per explants till 8 weeks of culture (Fig. 1). This influence of BAP on *L taraxacifolia* may be due to cytokinin's involvement in multiple developmental processes [16]. Similar result was reported by Ashraf et al. [17] that BAP alone (8.88–26.6 µM) was significantly effective on shoot multiplication from young shoot buds of *C. borivilianum* compared to control containing only MS basal salt. Arab et al. [18] also documented that the number of micro-shoots per explants increased significantly when nodal cuttings of G x N15 (hybrid almond x peach) were cultured on MS medium amended with increasing concentrations of BAP up to 1 mg/l.

3.2 Shoot Elongation

It was observed that shoot length increased with the duration of culture but there was no significant difference between the control and other concentrations or treatments. Only TB4 and TK2 (0.23 cm and 0.3 cm at 4 and 8 weeks respectively) had higher shoot length than TC (0.20 cm at 4 weeks but the same (0.3 cm) at 8 weeks). From the present study, treatments with BAP and Kinetin did not significantly influence shoot elongation (Fig. 2).

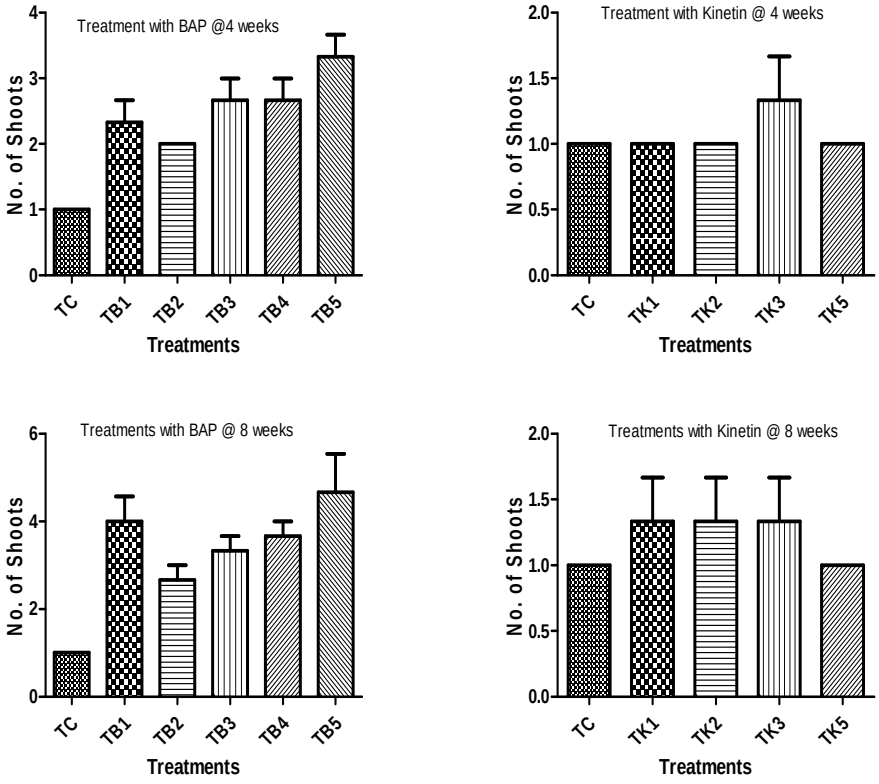

Fig. 1. Effect of BAP and kinetin on shoot number of *L. taraxacifolia* at 4 and 8 weeks

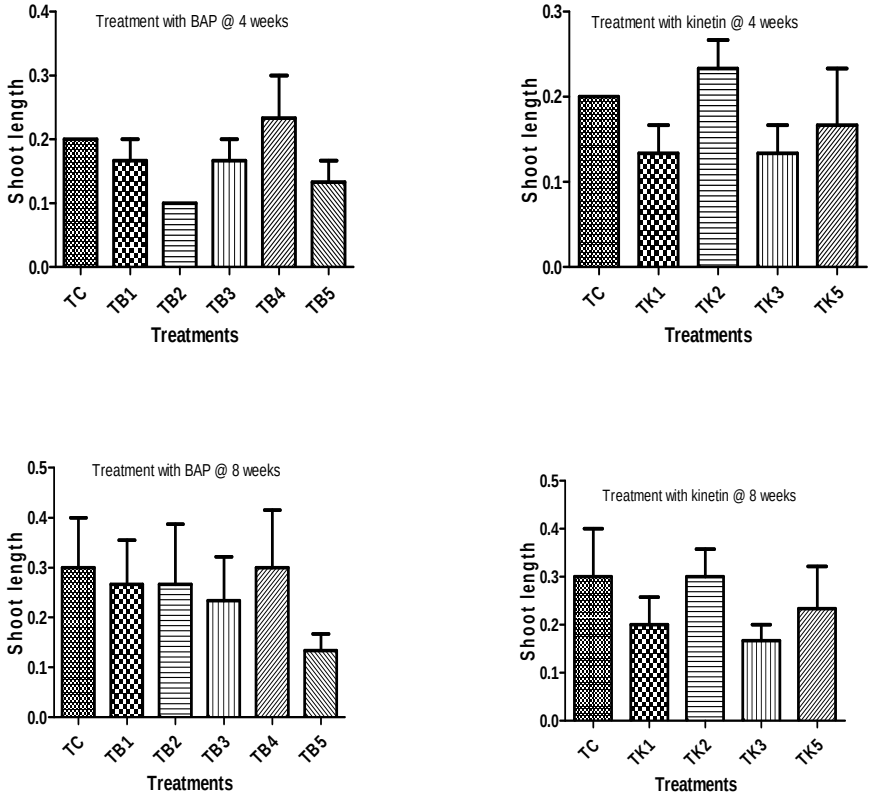

Fig. 2. Effect of BAP and kinetin on shoot length of *L. taraxacifolia* at 4 and 8 weeks

3.3 Effect of Cytokinin on Leaf Number

All the treatments recorded higher number of leaves than the control (TC). TB5 had significantly highest number of leaves (16.70 and 30.00 at 4 and 8 weeks) (Table 1 and Fig. 3A). All treatments with BAP were significantly higher than TC with only TK2 being significantly different from the control but not from other concentration in treatments with kinetin. This finding corroborates the report that BAP applied during the vegetative stage of Medicago x varia T Martyn, increased number of leaves [19]. Additionally, foliar application of kinetin based PGRs (Biozyme) spray to mulberry plant before onset of water logging, showed that biozyme partially compensated the water logging effect and increased the leaf yield by 30% [20]. *L. taraxacifolia* is utilized as a leafy vegetable, as such; treatments with highest number of leaves would be advantageous for its propagation.

3.4 Residual Effect of Plant Growth Regulators on Rooting

Some explants formed roots on shoot induction medium. The treatment with the lowest concentration of kinetin (TK1) produced 2.33 and 3.33 roots per explants at 4 and 8 weeks of culture. This observation substantiates the report that Kinetin and NAA had a positive effect on root formation from leaf explants of *Matthiola incana* [21]. The roots formed, elongated with length ranging from 0.50 cm at week 4 to 0.83 cm at week 8 (Fig. 3E). Though, statistical analysis shows that there was no significant difference between all the treatments and control. This suggests that if the explants had been cultured on a root induction medium, it would have led to a 100% rhizogenesis. Meanwhile, Table 3 shows that the treatments with BAP did not produce any roots. This agrees with the result obtained when higher concentrations of BAP (15 µM) used alone proved ineffective to induce rooting from shoot tip explants of *Prunella vulgaris* [22].

Table 2. Effect of cytokinins on number of leaves of *L. taraxacifolia*

Treatment	No of leaves	
	4 weeks	8 weeks
TC	3.67±0.33 ^{c}	4.33±0.88 ^{cd}
TB1	10.70±0.67 ^{b}	26.70±4.41 ^{ab}
TB2	10.00±1.00 ^{b}	14.30±1.45 ^{bc}
TB3	10.00±0.58 ^{b}	18.70±1.76 ^{abc}
TB4	13.30±1.45 ^{ab}	29.3±4.81 ^{a}
TB5	16.70±1.67 ^{a}	30.00±5.77 ^{a}
TK1	7.00±1.53 ^{bc}	9.33±2.33 ^{cd}
TK2	10.00±1.73 ^{b}	13.00±2.08 ^{bd}
TK3	7.33±0.67 ^{bc}	13.70±1.45 ^{bd}
TK5	5.67±0.67 ^{bc}	9.00±1.00 ^{cd}

Data (±SE) are the mean values of ten replicates. Different lowercase letters indicate significant differences among treatments within each column (Tukey's multiple range test, P = .05)

Table 3. Residual effect of cytokinins on *L. taraxacifolia*

Treatment	Number of roots		Root length	
	4 weeks	8 weeks	4 weeks	8 weeks
TC	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TB1	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TB2	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TB3	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TB4	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TB5	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TK1	2.33±2.33 ^{a}	3.33±3.33 ^{a}	0.50±0.50 ^{a}	0.83±0.83 ^{a}
TK2	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TK3	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
TK5	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}

Data (±SE) are the mean values of ten replicates. Different lowercase letters indicate significant differences among treatments within each column (Tukey's multiple range test, P = .05)

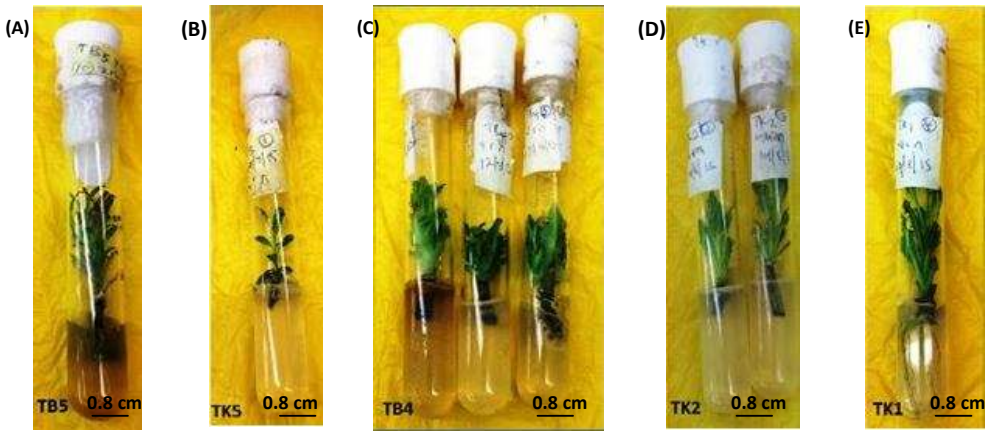

Fig. 3. *In vitro* grown plants of *L. taraxacifolia* showing: (A.) Treatment with 2.5 mg/l BAP giving the highest number of shoots and leaves; (B.) Treatment with 2.5 mg/l kinetin forming single shoots; (C&D.) Treatment with 2.0 mg/l BAP and kinetin giving higher shoot length than the control; (E.) treatment with 0.5 mg/l kinetin forming roots

The use of plants as sources of natural medication for treating ailments [23] and the production of recombinant proteins (including pharmaceuticals and industrial proteins) and other secondary metabolites [24] will subsist as long as man exists. This study therefore sets a

pace for further tissue culture research to overcome propagation limitations of the plant as proposed by Obembe et al. [25] for plants generally and for formation of higher number of shoots to ensure sustenance of this plant species and to avoid its extinction. However, withering of the in vitro grown plants commenced after eight (8) weeks in culture.

4. CONCLUSION

This study serves as a pace-setting protocol for the establishment of multiple shoots of L. taraxacifolia and it is expected to inspire further research efforts to overcome its propagation limitations.

It was established in the study that 51% clean culture resulted from double disinfection compared to single disinfection, which gave no clean culture (unreported). Also, treatments with 6-Benzylaminopurine performed better than treatments with kinetin in all the recorded growth parameters such as number of shoots and length, length of root and number of leaves except for number of root. It was also established that cultures began to wither after eight (8) weeks in culture.

Therefore, double disinfection with combination of 1-Naphthaleneacetic acid and 6-Benzylaminopurine at ratio 1:5 is recommended for multiple shoot formation of *L. taraxacifolia* species. Treatment containing MS supplemented with 2.5 mg/l 6-Benzylaminopurine and 0.5 mg/l 1-Naphthaleneacetic acid, which produced the highest number of multiple shoots and leaves is thereby proposed as shoot multiplication medium for this species. *In vitro* grown plants, not later than eight (8) weeks in culture, are considered appropriate for subculture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sakpere AMA, Aremu OA. The growth of *Launaea taraxacifolia* (Asteraceae) and its response to shading. Res. J. Bot. 2008;3: 90–96.
2. Adebisi AA. *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffrey. In PROTA 2: Vegetables/Légumes. [CD-Rom], O. A. Grubben, G. J. H. and Denton, Ed. PROTA, Wageningen, Netherlands; 2004.
3. Burkill HM. The useful plants of West Tropical Africa. (Families A-D). Royal Botanical Gardens, Kew. 1985;1.
4. Larson RA. The antioxidants of higher plants. Phytochemistry. 1988;27(4):969–978.
5. Sakpere AMA, Ayisire ER, Abioye OI. Potential of *Launaea taraxacifolia* (Willd) Amin Ex. C. Jeffrey for *in vitro* regeneration. Not Sci Biol. 2011;3(3):93–96.
6. Adinortey MB, Sarfo JK, Quayson ET, Weremfo A, Adinortey CA, Ekloh W, Ocran J. Phytochemical screening, Proximate and Mineral composition of *Launaea taraxacifolia* leaves. Res. J. Med. Plant. 2012;2:171–179.
7. Dickson RA, Anna K, Fleischer TC, Amponsah IK, Nsiah K, Oteng JA. "Phytochemical investigations and nutritive potential of eight selected plants from Ghana. J. Pharm Nutr. Sci. 2012;2:172–177.
8. Gbadamosi IT, Okolosi O. Botanical galactogogues: Nutritional values and therapeutic potentials. J. Appl. Biosci. 2013;61:4460–4469.
9. Sanoussi F, Ahissou H, Dansi M, Hounkonnou B, Agre P, Dansi A. "Ethnobotanical investigation of three leafy vegetable (*Alternanthera sessilis* (L. DC)., *Bidens pilosa* L., *Launaea taraxacifolia* Willd.) widely consumed in southern and central Benin. J. Biodiv. Env. Sci. 2015; 6(2):187–198.
10. Yang RY, Keding GB. Nutritional contributions of important African vegetables; 2009.
11. Gbadamosi IT, Alia AE, Okolosi O. *In-vitro* antimicrobial activities and nutritional assessment of roots of ten Nigerian vegetables. New York Sci. J. 2012;5(12): 234–240.
12. Odufuwa TK, Atunnise AK, Olukanni OD, Salau BA. Juicing alters oxalates contents in commonly consumed leafy vegetables in South West Nigeria. Int. J. Nutr. Food Sci. 2014;3(3):183–186.
13. Trejgell A, Michalska M, Tretyn A. Micropropagation of *Senecio macrophyllus*

M. Bieb. Acta Biol. Cracoviensia Ser. Bot. 2010;52(1):67–72.
14. Siddique I, Bukhari NAW, Perveen K, Siddiqui I. Influence of plant growth regulators on *in vitro* shoot multiplication and plantlet formation in *Cassia angustifolia* vahl. Brazilian Arch. Biol. Technol. 2015;58(5):686–691.
15. Obembe OO, Aworunse OS, Bello OA, Ani AO. Multiple shoots induction from Indigenous Nigerian Pumpkin (*Cucurbita pepo* L.). Ann. Res. & Rev. in Biol. 2017; 17(5):1-10
16. Haberer G, Kieber J. Cytokinins. New insights into a classic phytohormone. Physiol. Plant. 2002;128(2):354–362.
17. Ashraf MF, AbdAziz M, Kemar N, Ismail I. Effect of cytokinin types, concentrations and their interaction on in vitro shoot regeneration of *Chlorophytum boriyilianum* Sant. and Fernandez. Electron J. Biotechnol. 2014;17(16):275–279.
18. Arab MM, Yadollahi A, Shojaeiya A, Shokri S, Ghojah SM. Effect of nutrient media, different cytokinin types and their concentrations on *in vitro* multiplication of G x N15 (hybrid of almond x peach) vegetative root stock. J. Genet. Eng. Biotechnol. 2014;12(2):81–87.
19. Sosnowski J, Malinowska E, Jankowski K, Krol J, Redzik P. An estimate of the effects of synthetic auxin and cytokinin and the time of their application on some morphological and physiological characteristics of Medicago x varia T Martyn. Saudi J. Biol. Sci.; 2017.
20. Dhiraj K, Kumar RV. Application of foliar nutrients to increase productivity in sericulture. J. Entomol; 2011.
21. Kaviani B, Hesar AA, Tarang A, Zanjani SB, Hashemabadi D, Ansari MH. Effect of kinetin (Kn) and naphthalene acetic acid (NAA) on the micropropagation of *Matthiola incana* using shoot tips, and callus induction and root formation on the leaf explants. Afr. J. Agric. Res. 2013; 8(30):4134–4139.
22. Rasool R, Kamili AN, Ganai BA, Seema A. Effect of BAP and NAA on shoot regeneration in *Prunella vulgaris*. J. Nat. Sci. Math. Qassim Univ. 2009;3(1):21–26.
23. Obembe OO. Plant biotechnology flight: Is Africa on board? African J. Biotechnol. 2010;9(28):4300–4308.
24. Obembe OO, Popoola JO, Leelavathi S, Reddy SV. Advances in plant molecular farming. Biotechnol. Adv. 2011;29(2):210–222.
25. Obembe OO, Khan T, Popoola JO. Use of somatic embryogenesis as a vehicle for cotton transformation. J. Med. Plant. Res. 2011;5(17):4009–4020.

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