Journal of Advances in Biology & Biotechnology

13(2): 1-13, 2017; Article no.JABB.28686
ISSN: 2394-1081

In vitro Polyploidization of *Zehneria capillacea* (Shumach.) C. Jeffrey Using Nodal Explants

Josephine U. Agogbua[1] and Chimezie Ekeke[1*]

[1]*Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author JUA designed the study. Authors JUA and CE performed the statistical analysis, wrote the protocol and the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/28686
Editor(s):
(1) Cosmas Nathanailides, Department Fisheries and Aquaculture Technology, Technological Educational Institute of West Greece, Greece.
Reviewers:
(1) Hakan Sevik, Kastamonu University, Turkey.
(2) Elisabeth Wischnitzki, AIT Austrian Institute of Technology, Austria.
(3) Nidhal Marzougui, National Research Institute of Rural Engineering, Tunisia.
(4) Mustika Mukti, Gadjah Mada University, Yogyakarta, Indonesia.
(5) César Luiz Da Silva Guimarães, Federal Universy of Rondônia State – Unir, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/19506

Original Research Article

Received 30th July 2016
Accepted 20th March 2017
Published 13th June 2017

ABSTRACT

Zehneria capillacea (Schumach.) C. Jeffrey is a wild diploid (2n = 2*x* = 22) andromonoecious climber that is eaten as vegetable in Nigeria. *In vitro* polyploidy induction was conducted using oryzalin as a microtubule inhibitor at various concentrations (10, 20 and 30 µM) and two time durations (24 and 48hrs). This research was conducted to develop a protocol for induction of polyploids through *in vitro* technique in order to create variability and broaden the genetic base of this species. Shoot length was significantly affected by oryzalin concentration at 24 and 48 hours treatment duration. The nodal segments immersed in 10, 20 and 30 µM oryzalin for 24 hrs had shoot lengths of 7.56 ± 0.06 cm, 7.24 ± 0.06 cm and 7.31 ± 0.07 cm respectively which were statistically similar while the shoot length of those treated for 48 hrs significantly decreased with increase in oryzalin concentration ranging from 7.02 ± 0.07 cm for 10 µM to 2.71 ± 0.11 cm for 30 µM. 10 µM oryzalin for 24 hrs and 48 hrs induced 100% 2*x*+4*x* and 2*x*+4*x*+8*x* cytochimera respectively. Application of 20 µM for 24 hrs induced 75% 2*x*+4*x* and 25% solid tetraploids while 20

**Corresponding author: E-mail: ekeke.uche@uniport.edu.ng;*

µM for 48 hrs induced 25% 2*x*+4*x* and 75% solid tetraploids. Application of 30 µM oryzalin for 24 hrs induced 100% 2*x*+4*x*+8*x* and 48 hrs induced 20% 2*x*+4*x*+8*x* and 80% 4*x*+8*x*. The results obtained indicate that polyploids (tetraploid, 4x and octoploids, 8x) can be induced using nodal explants from *Z. capillacea* grown *in vitro* thereby conserving and enhancing the ethno-botanical and economic values the species.

Keywords: In vitro; polyploidization; Zehneria capillacea; oryzalin; tetraploid; octoploid.

1. INTRODUCTION

Plants are the source of life for living beings, and they carry out many functions at the places where they exist. In the place they grow, plants reduce air pollution [1]; Reduce noise [2]; Increase aesthetic value [3]; have a positive psychological effect [4]; provide energy conservation [5]; prevent erosion [6]; reduce wind speed and hold the soil with their roots, thus preventing washing away of the soil with rainfalls and streams, and protect wildlife and hunting resources. Open-green areas with plantation are important activity areas for both adults and children [7].

Such advancement of the plants market made the researchers to be interested in various issues such as defining the distribution areas of plants [8]; protection of plants [9–11]; cultivation of plants [12,13]; resistance of plants to stress factors [14,15]; economic value [16]; various areas of use [2]; genetic variability of plants [6,17,18], their relationship with the environment [19,20], thus resulting in various studies on these issues.

Due to these different uses of plant, plant production is therefore of great importance to man and animals. Plants are extensively produced by seed or vegetative way. However; micro-culture techniques of production in the plant in recent years has been quite widespread [21,22,18]. These techniques have been used to improve the yield of plants [23,24], enhance/increase the chemical constituents (secondary metabolites) and medicinal values [25,26].

Polyploidy which is a condition whereby a biological cell or organism or plant has more than two sets of homologous sets of chromosomes can arise spontaneously in nature by several mechanisms, including meiotic or mitotic failures, and fusion of unreduced (2n) gametes [27]. Both autopolyploids in potato [28] and allopolyploids (e.g. canola, wheat, cotton) can be found among both wild and domesticated plant species. Most polyploids display novel variation or morphologies relative to their parental species, that may contribute to the processes of speciation and eco-niche exploitation [18,27,29]. The mechanisms leading to novel variation in newly formed allopolyploids may include gene dosage effects (resulting from more numerous copies of genome content), the reunion of divergent gene regulatory hierarchies, chromosomal rearrangements, and epigenetic remodeling, all of which affect gene content and/or expression levels [29–36]. Huge explosions in angiosperms species diversity appear to have coincided with the timing of ancient genome duplications shared by many species.

In West Africa and Nigeria in particular, *Zehneria* species are used for several purposes including food [37,38], treatment of tapeworm and as sedatives [39], taken by nursing mothers to aid postpartum recovery [40,41] and to induce abortion [42]. This plant is sourced from the wild by the locals. The impacts of anthropogenic activities have led to depletion in the biodiversity of Niger Delta [31]. In view of the biodiversity loss and the ethno-botanical potential of this *Zehneria* species there is need to broaden the genetic base by creating variability through *in vitro* polyploid induction. This study was therefore carried out to induce polyploids in *Zehneria capillacea* using nodal explants from *in vitro* plantlets and oryzalin as a doubling agent.

2. MATERIALS AND METHOD

This study was carried out in the tissue culture laboratory of the BioScienec Center, International Institute of Tropical Agriculture Ibadan, Nigeria.

2.1 Culture Medium Preparation

The medium formulation described by Murashige and Skoog [43] referred to as MS medium was selected as the optimal culture medium for *in vitro* studies. The medium was supplemented with plant growth regulators. The plant growth regulators used in this experiment were 6-benzyl

amino purine (BAP), and indole-3-acetic acid (IAA). The preparation of the oryzalin, shoot multiplication, root induction, culture incubation, Flow Cytometry (FCM) Analysis using Partec PAS 11 flow cytometer (Partec GmbH, Germany), plant acclimatization and establishment is as stated in [18,44,45].

2.2 *In vitro* Polyploidization

The *in vitro* mitotic polyploidization performed under aseptic conditions involved culture of donor plants, preparation of nodal segments, application of microtubule inhibitor and culture of treated nodes. *In vitro* plantlets were cultured on hormone free Murashege and Skoog (MS) medium for three weeks in a culture chamber. The experimental design was a 2-way factorial design with four concentrations of oryzalin (0, 10, 20 and 30 µM) and two time duration (24 and 48 hours). Nodal segments were excised from the upper part (2nd nodal segment under the shoot apex) of the *in vitro* grown plants, defoliated and submerged in sterilized baby food jars containing 10mls of aqueous solutions of the various concentrations of oryzalin. Two jars with ten nodes each were used for the two treatment duration giving a total of 20 nodes per treatment. The jars containing the explants were continuously agitated on a Gallenkamp orbital shaker at 90 rpm for the given treatment duration after which the oryzalin solution was poured out and the nodes rinsed 5 - 6 times with sterile distilled water under a laminar flowhood. The treated rinsed nodal segments were transferred into fresh gelrite-solidified hormone free MS medium and cultured under standard conditions [18]. After two weeks, necrotic stem tips were trimmed off from the nodal segments and sub-cultured into fresh gelrite-solidified MS medium supplemented with 1.0 mg/l BAP to obtain shoots for ploidy determination. The nodal segments were visually examined after one month to determine percentage contamination/survival, phenolics exudation and overall growth response. The nodal segments of 5-explants that survived and proliferated were analyzed for ploidy level using a flow cytometer [44,46].

2.3 Data Collection

The parameters evaluated were percentage of explants survival, seedling length, ploidy level and induction frequency after 21 days.

The induction frequency of plant was calculated as follows:

Frequency induction (%) = (No. of explants showing response/ Total No. of explants inoculated) X 100

The number of plantlets that survived were recorded and the percentage calculated.

2.4 Statistical Analysis

All the data obtained from the *in vitro* studies were expressed as mean ± SE and were subjected to one way analysis of variance and Duncan's multiple range test (DMRT) to test the significance of the treatment difference.

3. RESULTS

The treatment of nodal segments with lateral buds in various concentrations of aqueous solution of oryzalin for 24 and 48 hours revealed a significant interaction between oryzalin concentration and treatment duration. It was observed that all the micro-shoots in the control had mean shoot lengths of 15.53 ± 0.08 cm and 15.51 ± 0.07 cm which differed significantly from that exhibited by the oryzalin treated shoots (Table 1 and Fig. 1). Shoot length was significantly affected by oryzalin concentration and 48 hours treatment duration. The nodal segments immersed in 10, 20 and 30µM oryzalin for 24 hrs had shoot lengths of 7.56 ± 0.06 cm, 7.24 ± 0.06 cm and 7.31 ± 0.07 cm respectively which were statistically similar while the shoot length treated with 10 µM and 30 µM oryzalin for 48 hrs are significantly shooter with values ranging from 7.02 ± 0.07 cm for 10 µM to 2.71 ± 0.11 cm for 30 µM respectively (Table 1 and Fig. 2). These were significantly shorter than those treated for 24 hrs time duration.

Factors that inhibit culture growth such as contamination, hyper-hydricity and phenolic exudation were not observed in all the treatments throughout the culture period. Necrosis was observed in the oryzalin treated micro-shoots after two weeks of transfer to MS solid medium but only 5% of the micro-shoots treated with 30 µM oryzalin for 48 hrs were lost to necrosis (Table 1).

3.1 Flow Cytometer (FCM) Analysis

The three groups of ploidy level observed were diploids, tetraploids and three classes of

mixoploids (2*x*+4*x*, 2*x*+4*x*+8*x* and 4*x*+8*x*). The control plants had a mean relative fluorescence (MRF) of 49.18 – 49.67 while the tetraploids had MRF values (95 – 99) which double that of the diploid samples. The mixoploids had histograms with two or three distinct MRF values (Fig. 3, Table 2).

Table 1. Effect of different concentrations of oryzalin and treatment duration on shoot length of *Z. capillacea* in MS proliferation medium

Treatments	Oryzalin conc. (µM)	Treatment duration (Hours)	Shoot length (cm)*	Shoot survival (%)
T_1	0	24	15.53 ± 0.11^a	100
T_2	10	24	7.56 ± 0.11^b	100
T_3	20	24	7.24 ± 0.08^b	100
T_4	30	24	7.31 ± 0.09^b	100
T_5	0	48	15.51 ± 0.10^a	100
T_6	10	48	7.02 ± 0.09^c	100
T_7	20	48	5.67 ± 0.10^d	100
T_8	30	48	2.71 ± 0.16^d	95

**Means followed by the same letter within columns are not significantly different using DMRT at the 5% level of significance*

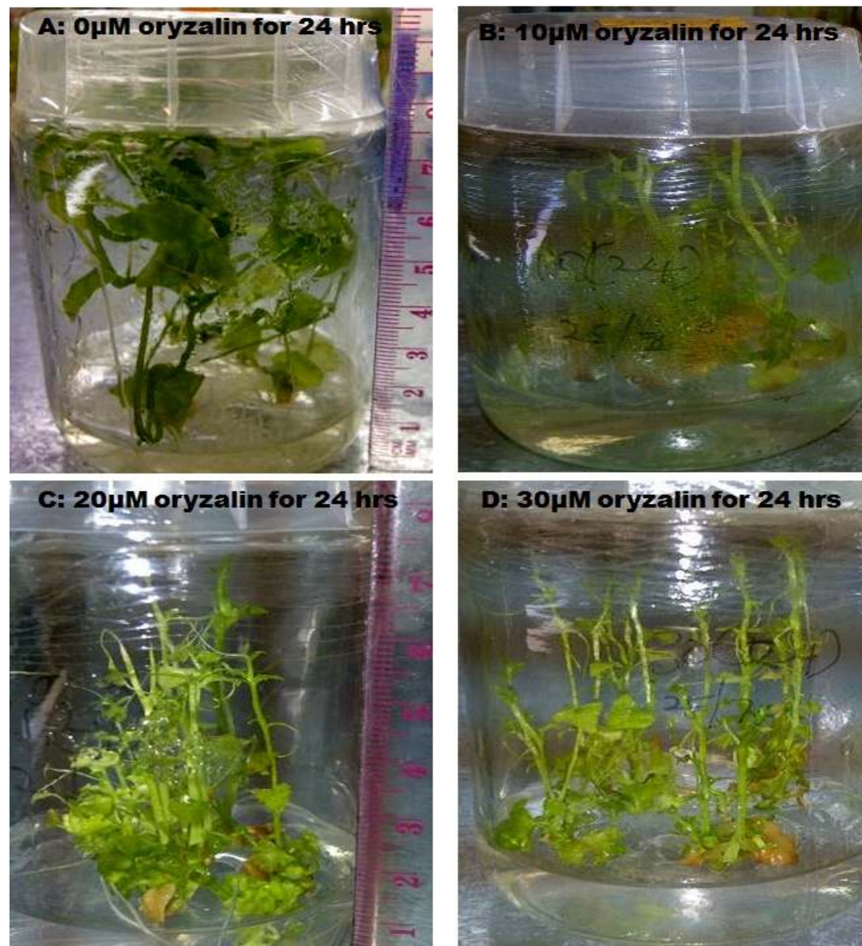

Fig. 1. Effect of different concentrations of oryzalin on micro-shoot development of *Zehneria capillacea*. (Nodal segments were treated for 24 hours)

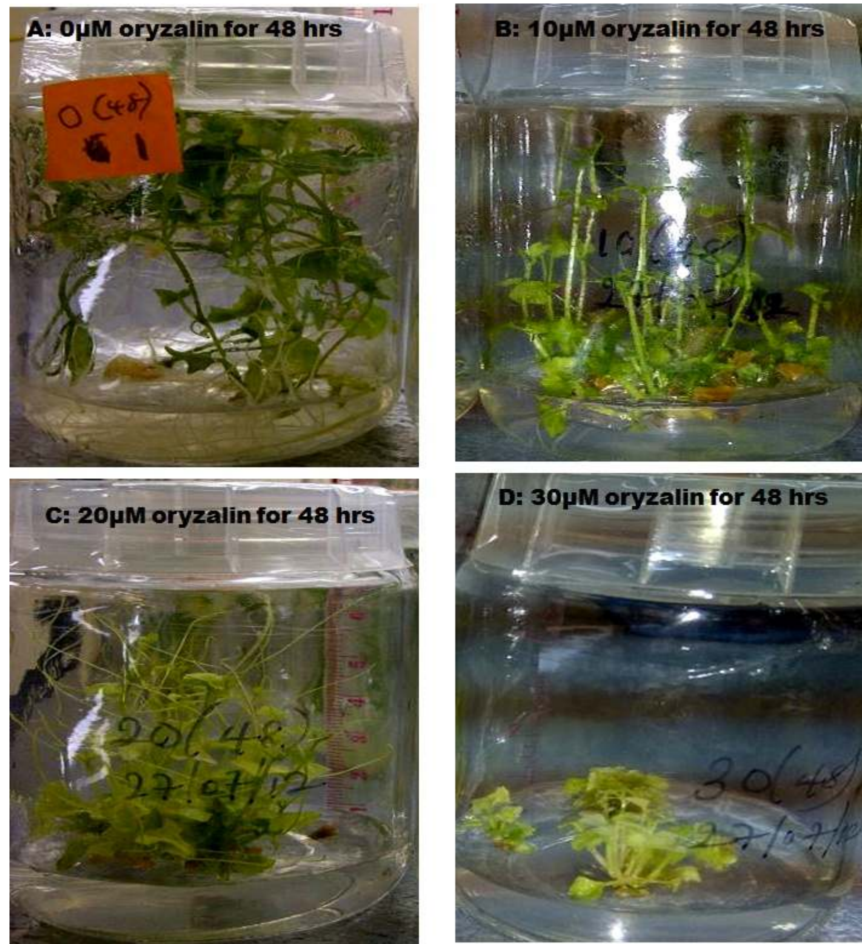

Fig. 2. Effect of different concentrations of oryzalin on micro-shoot development of *Zehneria capillacea*. (Nodal segments were treated for 48 hours)

Table 2. Ploidy level, MRF values, coefficient of variation and polyploidization efficiency induced by various oryzalin concentration on *Z. capillacea*

Oryzalin conc. (µM)	Induction efficiency (%)					MRF*	CV** (%)
	2*x*	4*x*	2*x*+4*x*	2*x*+4*x*+8*x*	4*x*+8*x*		
			***In vitro* induction (24 hrs)**				
0	100	0	0	0	0	49.18	2.3
10	0	0	100	0	0	50, 99	1.7, 2.8
20	0	0	75	25	0	48, 96	1.6, 1.3
30	0	0	0	100	0	47, 95, 193	1.4, 1.5
			***In vitro* induction (48 hrs)**				
0	100	0	0	0	0	49.67	1.5
10	0	0	0	100	0	50, 99, 196	1.7, 2.8, 2.2
20	0	75	25	0	0	48, 96	1.6, 1.8
30	0	0	0	20	80	48, 97, 193	1.3, 1.4, 1.5

MRF = Mean Relative Fluoresence and CV** = means coefficient of variation*

The percentage of various ploidy levels induced by different oryzalin concentration, the MRF values, coefficient of variation and polyploidization efficiency are presented in Table 2. The *in vitro* application of 10 µM oryzalin for 24hrs and 48hrs induced 100% 2*x*+4*x* and

2*x*+4*x*+8*x* cytochimera respectively. Application of 20 µM for 24 hrs induced 75% 2*x*+4*x* and 25% solid tetraploids while 20µM for 48 hrs induced 25% 2*x*+4*x* and 75% solid tetraploids. Application of 30 µM oryzalin for 24 hrs induced 100% 2*x*+4*x*+8*x* and 48hrs induced 20% 2*x*+4*x*+8*x* and 80% 4*x*+8*x* (Table 2).

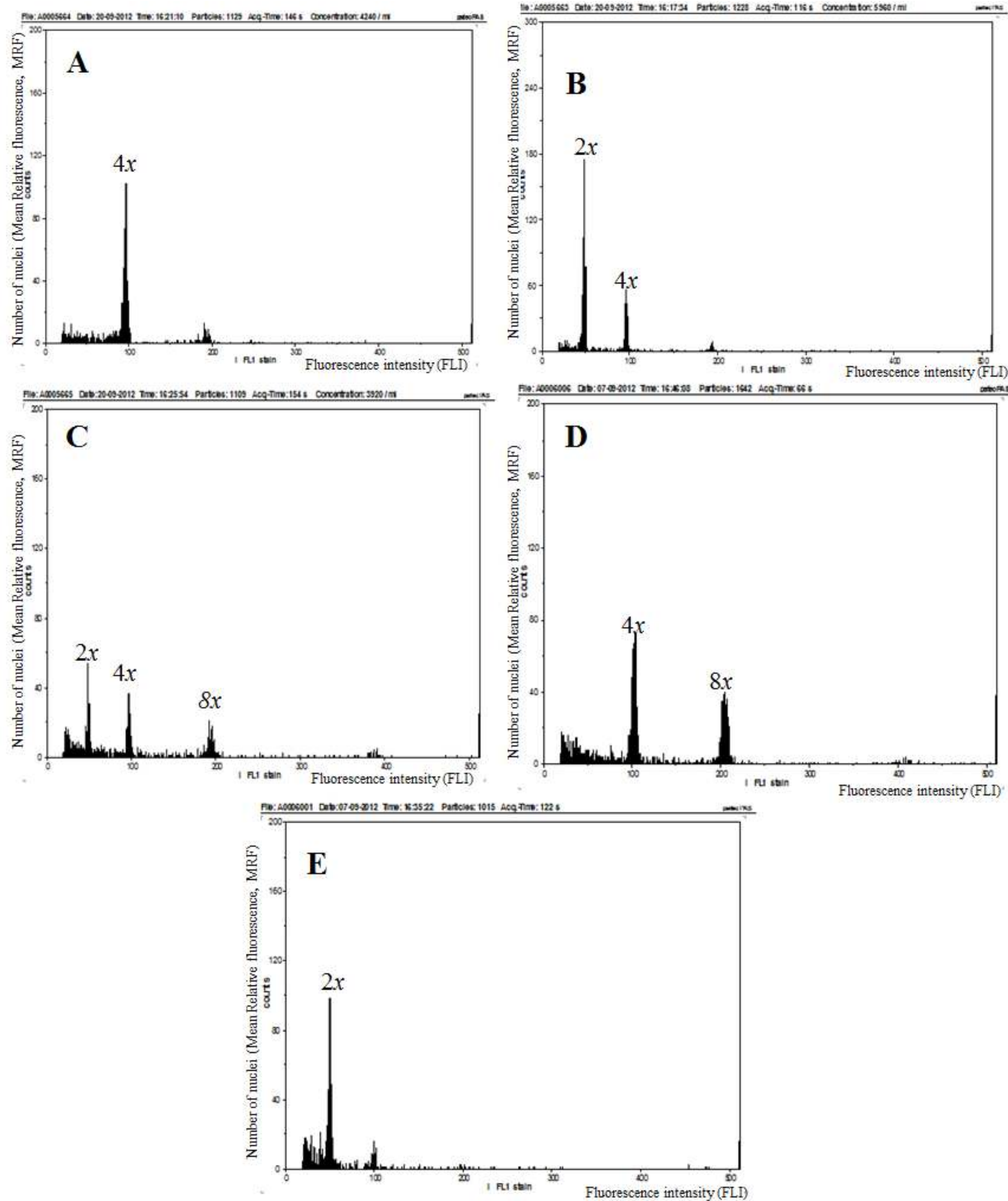

Fig. 3. Flow cytometric profiles of *Z. capillacea* plants treated with oryzalin (The peaks of the horizontal y-axis correspond to relative nuclear DNA content, which is expressed as the fluorescence intensity (FLI). The number of nuclei is shown on the vertical x-axis: (A) solid tetraploid 4*x*, (B) mixoploid 2*x*+4*x*, (C) mixoploid 2*x*+4*x*+8*x*, (D) mixoploid 4*x*+8*x*, (E) control diploid 2*x*, (Diploid/control nuclei was set to channel 50 with tetraploids resolving at channel 100, 0ctoploids at channel 200)

4. DISCUSSION

The increase in nuclear ploidy affects the structural and anatomical characteristics of the plant [18]. In general, polyploidy results in increased leaf and flower size, stomatal density, cell size and chloroplast count [47], decrease in stomatal density and increase in stomatal size [18]. These phenomena are collectively referred to as the gigas effect [33]. Physiological changes are also known to accompany genome duplication. These mainly result from change of metabolism resulting in a general increase in secondary metabolites [25]. This property has found application in the breeding of medicinal herbs in the production of pharmaceuticals. Hybrid vigor resulting from interspecific crosses in allopolyploids is one of the most exploited advantages of polyploid in plant breeding.

Genome doubling under *in vitro* controlled conditions is a tool for exploration and domestication of wild germplasm [48]. *In vitro* polyploidization has become an almost routinely applied breeding technique in many ornamentals and other crops [49,50]. In watermelon, induced autotetraploids are used for the production of seedless triploid hybrids [51]. *In vitro* polyploidization can be achieved through the addition of chemicals that inhibit mitotic spindle fibre formation directly into the culture medium [50,52], as an aqueous solution onto nodal segments [53]. Success has been reported using embryogenic and non-embryogenic callus [54,55], nodal segments [56], cotyledons [57] and hypocotyls [52]. *In vitro* polyploidization has been induced in many species such as banana [58,59], *Alocasia* [60], *Bacopa monnieri* [53] and cotton [61].

Oryzalin is often more effective than colchicine because it has a higher affinity for plant tubulins [62-64]. The ploidy level of several plant species which have been altered using oryzalin include *Pyrus* L. [65], *Solanum* L. [66], *Tulipa* L. [67], *Rosa* L. [68] and *Miscanthus sinensis* Anderson [69]. Treating shoot apices of *M. sinensis* in 15 *µ*M oryzalin solution for 96 hours was the most effective treatment for inducing polyploids while 60 *µ*M oryzalin prevented callus initiation in immature inflorescences of *M. sinensis* that were cultured *in vitro* [69]. Bouvier [18] observed that 200 *µ*M – 300 *µ*M concentrations of oryzalin were required to induce polyploidy in *Pyrus* L while in *Solanum* L. the most effective treatment for tetraploid induction was 24 hour treatment with 28.8 *µ*M oryzalin solution applied to apical buds [66]. In agriculture and horticulture, polyploid induction have been used to improve plant yield and fruit size [70], medicinal values of plants, resistance to pest, flower colour, size and number [71-74].

In the present study, we used an *in vitro* method to investigate the effect of oryzalin treatment at different concentrations and time durations on polyploid induction in *Z. capilllacea*. The *in vitro* application of 10µM oryzalin for 24hrs induced 2x+4x, 20 µM oryzalin for 24 and 48hrs to nodal segments produced the optimal number (75%) of solid tetraploids while application of 30µM for 48hrs resulted in stunted shoots that redoubled yielding cytochimeras with 4*x*+8*x* cells. This suggests that for a breeder whose interest is solid tetraploids, the best treatment for him is to apply 20 µM oryzalin for 24 and 48 hrs and 10 µM oryzalin for 24 hrs to nodal segments of *Z. capilllacea.* On the other hand, for tetraploids and octoploids, the breeder will pretreat the nodal segment of the explant in 30µM for 48hrs. This is consistent with reports that high concentrations and prolonged exposure times result in lethal or redoubling effects [75]. Also among the Cucurbitaceae, *In vitro* technique has been well demonstrated and the regeneration of plants has been reported from excised plant parts such as hypocotyls [34], cotyledons [34–36,76–81], shoot tips [82,83], nodal segments [84-86], leaf [80,87-89], embryonal axis [90] and anthers [91].

In other plant families and genera, ploidy manipulation is considered as a valuable tool in genetic improvement of many plants including *Solanum* spp. [92], citrus [93], pomegranate [94], *Allium* spp. [95] and azaleas [96]. An attempt to increase ploidy level has been conducted with different objectives in various plants. In citrus, tetraploid (4x) parents were produced to create seedless triploids by crossing (4x) and (2x) parents [93]. In medicinal plants, such as, *Scutellaria* spp. [97] and *Artemisia* spp. [98], tetraploidy increases the amounts of the secondary metabolites, baicalin and artemisinin. In *Azalea*, chromosome doubling was used to obtain new ornamental characteristics [96]. In *Acacia mearnsii*, tetraploids were produced for increased bark and tannin production and for generating sterile triploids [99]. In addition, the polyploids also provide a wider germplasm base for breeding studies [100].

The enhanced production of secondary metabolites such as alkaloids and terpenes in polyploids may concurrently offer resistance to pests and pathogens [101,102]. Experiments with diploid *Glycine tabacina*, a forage legume

established that 42% of the tetraploid plants were resistant to leaf rust, *Phakopsora pachyrhizi* compared to 14% of the diploid plants [27]. Similar results were observed while comparing resistance to insects and the clover eel nematode between *Trifolium pratense* (red clover) tetraploids and diploids [103]. Given these possible beneficial consequences, chemical polyploidization has become an almost routinely applied breeding technique in many ornamentals and other crops [104,105]. Breeders have harnessed the process of chromosome doubling through induced polyploidy to produce superior crops. For example in watermelon, induced autotetraploids are used for the production of seedless triploid hybrids [106]. The polyploid induction of *Z. capilllacea* could enhance its medicinal properties. This is attributed to the fact that increase in ploidy level corresponds to increase in chemical constituents of plants [101,102].

In vitro polyploid induction provide a more controlled standardized environment, increases efficiency and decreases the occurrence of chimaeras compared with conventional *in vivo* methods [107,108]. For *in vitro* induction, a reliable regeneration system must be established and potential explants that are commonly utilized are shoot tips [61,109] and nodal segments [76,110] because of their regeneration ability and lower levels of somaclonal variation than calli [55] and somatic embryos [50].

5. CONCLUSION

The finding of this work indicates that this species can be grown *In vitro* and for efficient induction of solid 2x+4x, the nodal explant from *Z. capilllacea* should be treated with 10 or 20 μM of oryzalin for 24 hrs and 20 μM for 48 hrs were the best treatments while for 2x+4x+8x, 10μM and 30 μM oryzalin for 48rs were the best treatments. Also the conservation, ethno-botanical and economic values of *Z. capilllacea* could be enhanced through *In vitro* culture and polyploidization.

ACKNOWLEDGEMENTS

The authors wish to thank the International Institute of Tropical Agriculture (IITA) Ibadan**,** Nigeria more especially Delphine Amah and the entire staff of Tissue Culture Laboratory of the Bioscienec Center, for providing enabling environment for this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sevik H, Cetin M, Belkayali N. Effects of Forests on Amounts of CO2: Case Study of Kastamonu and Ilgaz Mountain National Parks. Polish Journal of Environmental Studies. 2015;24(1):253-256.
2. Yiğit N, Öztürk A, Sevik H. Ecological impact of urban forests (Example of Kastamonu urban forest). International Journal of Engineering Sciences & Research Technology. 2014;3(12):558-562.
3. Sevik H, Belkayalı N, Aktar G. Change of chlorophyll amount in some landscape plants. Journal of Biotechnological Sciences. 2014;2(1):10-16.
4. Cetin M. Evaluation of the sustainable tourism potential of a protected area for landscape planning: A case study of the ancient city of Pompeipolis in Kastamonu. International Journal of Sustainable Development & World Ecology. 2015a;22 (6):490-495.
   DOI: 10.1080/13504509.2015.108165
5. Cetin M. Determining the bioclimatic comfort in Kastamonu City. Environmental Monitoring and Assessment. 2015b;187 (10):640.
   DOI: 10.1007/s10661-015-4861-3
6. Turna I, Güney D. Altitudinal variation of some morphological characters of scots pine (*Pinus sylvestris* L.) in Turkey. African Journal of Biotechnology. 2009;8(2):202-208.
7. Tekce N, Belkayali N, Oğuz D, Baştemur CT. A survey on recreational use of domestic water supply reservoirs: A case study from Kurtboğazi–Ankara, Turkey. African Journal of Agricultural Research. 2010;5(14):1897-1907.
8. Šálek L, Güney K. New host plant for the species *Agapanthia lateralis* Gangl. (Coleoptera; Cerambycidae). Entomological News. 2014;124(1): 29-32.
9. Canbulat S. Checklist of Turkish *Raphidioptera* on the basis of distribution pattern and biogeographical analysis. Turkish Journal of Zoology. 2015;39(2): 225-234.

10. Kravkaz İS, Vurdu H. Botany of *Crocus ancyrensis* through domestication. 3rd IS on Saffron, Eds. Tsimidou MZ, et al. Acta Hort. 850, ISHS. 2010;61-65.
11. Guloglu Y. The establisment of forest ownership and the legal regulations on the forests until the tanzimat (reform) period ın the ottoman state. Kastamonu Univ Journal of Forestry Faculty. 2015;10(2): 180-194.
12. Sevik H, Guney K. Effects of IAA, IBA, NAA, GA3 on rooting and morphological features of *Melissa officinalis* L. Stem Cuttings. The Scientific World Journal; 2013.
13. Cetin M. Sevik H. Evaluating the recreation potential of Ilgaz Mountain National Park in Turkey. Environmental Monitoring and Assessment. 2016;188(1):52. DOI: 10.1007/s10661-015-5064-7
14. Topacoglu O, Sevik H, Akkuzu E. Effects of water stress on germination of *Pinus nigra* subsp. *pallasiana* Arnold. Seeds. Pakistan Journal of Botany. 2016;48(2): 447-453.
15. Sevik H, Cetin M. Effects of water stress on seed germination for select landscape plants. Polish Journal of Environment Studies. 2015;24(2):689-693.
16. Ozden S, Erkan Bugday S. As a factor of production forest villagers' population movement: Kastamonu sample, Journal of Forestry Faculty. 2015;15(2):231-240.
17. Sevik H, Topacoglu O. Variation and inheritance pattern in cone and seed characteristics of scots pine (*Pinus sylvestris* L.) for evaluation of genetic diversity. Journal of Environmental Biology. 2015;36(5):1125-1130.
18. Agogbua UJ, Ekeke C, Okoli BE. Effect of oryzalin treatments on polyploidy induction, phenotypic and quantitative traits of *Zehneria capillacea* (Shumach.) C. Jeffrey. International Journal of Tropical Agriculture. 2015;33(3):2067-2073.
19. Cetin M. Consideration of permeable pavement in landscape architecture. Journal of Environmental Protection and Ecology. 2015c;16(1):385–392.
20. Belkayalı N, Güloğlu Y, Sevik H. What Affects perceptions of local residents toward protected areas? A case study from Kure Mountains National Park. Turkey. International Journal of Sustainable Development & World Ecology. 2016; 23(2):194–202.
21. Guney K, Cetin M, Sevik H, Guney KB. Influence of germination percentage and morphological properties of some hormones practice on *Lilium martagon* L. seeds. Oxidation Communications. 2016; 39(1-II):466-474.
22. Sevik H, Çetin M. Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. Onion Scales; 2016.
23. Kermani MJ, Sarasan V, Roberts AV, Yokoya A, Wentworth J, Sieber VK. Oryzalin-induced chromosome doubling in Rosa and its effects on plant morphology and pollen viability. Theory of Applied Genetics. 2003;107:1195-1200.
24. Allum JF, Bringloe DH, Roberts AV. Chromosome doubling in a *Rosa rugosa* Thunb. Hybrid by exposure of *in vitro* nodes to oryzalin: The effects of node length, oryzalin concentration and exposure time. Plant Cell Rep. 2007;26: 1977-1984.
25. Levin D. Polyploidy and novelty in flowering plants. American Naturalist, 1983;122:1-25.
26. Ahmad N, Anis M. *In vitro* mass propagation of *Cucumis sativus* L., from nodal segments. Turk. J. Bot. 2005;29: 237-240.
27. Comai L. The advantages and disadvantages of being polyploid. Nature Reviews Genetics. 2005;6:836-846.
28. Xu Y, Guo SG, Zhang HY, Gong GY, Huang SW, Ye HP, Wu MZ, Zheng Y, Fei ZJ. Latest advances in watermelon genomics. Abstract of 4th Intl. Cucurbitaceae Symp. Changsha, China. 2009;38.
29. Chen ZJ, Ni ZF. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bio Essays. 2006;28:240-252.
30. Chen LL, Gao SL. *In vitro* tetraploid induction and generation of tetraploids from mixoploids in *Astragalus membranaceus*. Sci. Hort. 2007;112:339-344.
31. Agbagwa IO, Ndukwu BC. *Cucurbita* L. species in Nigeria: Underexploited food and vegetable crops. Niger Delta Biologia. 2004;4(2):11-15.
32. Dhawan OP, Lavania UC. Enhancing the productivity of secondary metabolites via induced polyploidy: A review. Euphytica. 1996;87:81-89.

33. Acquaah G. Principles of plant genetics and breeding Wiley-Blackwell, Malden; 2007.
34. Ugandhar T, Venkateshwarrlu M, Begum G, Srilatha T, Jaganmohanreddy K. *In Vitro* plant regeneration of Cucumber (*Cucumis sativum* (L.) from cotyledon and hypocotyl explants. Science Research Reporter. 2011;1(3):164-169.
35. Liu EE, Leung-David WM, Xia QH, Zheng JR, Peng XX, He XM. Efficient plant regeneration *in vitro* from cotyledon explant of Chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua). Science Asia. 2013a;39:134-138.
36. Kim KM, Kim CK, Han JS. *In vitro* regeneration from cotyledon explants in figleaf gourd (*Cucurbita ficifolia* Bouché), a rootstock for Cucurbitaceae. Plant Biotechnology Report. 2010;4:101-107.
37. Jeffrey C, De Wilde WJJO. A review of the subtribe Thladianthinae (Cucurbitaceae). Bot. Zhurn. 2006;91:766–776.
38. Edwin–Wosu NL, Ndukwu BC. The ethno-botany and utility evaluation of some species of cucurbits among the people of Niger Delta Nigeria. Global Journal of Pure and Applied Sciences. 2008;14(3):279-284.
39. Burkill HM. The useful plants of West Tropical Africa. (families A-D). 2nd Ed. Royal Botanical Gardens, London; 1985.
40. Agogbua J. *In vitro* studies, mitotic polyploidization and morgenetic characterization of *Zehneria capillacea* (Schumach) *C. Jeffrey* (Cucurbitaceae). Ph. D Thesis. University of Port Harcourt; 2014.
41. Okoli BE. Fluted Pumpkin, *Telfairia*-the under-exploited golden treasure. University of Port Harcourt Press, Nigeria; 2013.
42. Chike CPR, Dapper DV, Odee P. Possible abortifacient effect of Zehneria cordifolia methanol extract on female wistar rat. African Journal of Applied Zoology and Environmental Biology. 2006;8:140-145.
43. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiolgia Plantarum. 1962;15:437-497.
44. Stipp LCL, Mendes BMJ, Piedade SMDS, Rodriguez APM. *In vitro* morphogenesis of *Cucumis melo* var. Inodorus. Plant Cell Tiss. Org. Cult. 2001;65:81-89.
45. Otto FJ. DAPI staining of fixed cells for high resolution flow cytometry of nuclear DNA in: Crissman HA, Darzynkiewic Z. eds. Methods in cell biology Vol. New York, NY: Academic Press. 1990;33: 105-110.
46. Agogbua JU, Okoli BE, Osuji JO. Procedure for *In vitro* seed sterilization, germination, and aseptic seedling establishment of *Zehneria capillacea* (Shumach) C. Jeffrey. In: Biotechnology as a Tool for National Peace and Development Proceedings of 25th Annual National Conference of Biotechnology Society of Nigeria. Abuja, Nigeria. 2012; 94-97.
47. Kathiravan K, Vengedesan G, Singer S, Steinitz B, Paris H, Gaba V. Adventitious regeneration *in vitro* occurs across a wide spectrum of squash (*Cucurbita pepo*) genotypes. Plant Cell Tiss. Org. Cult. 2006;85:285-295.
48. Escandon AS, Miyajima I, Alderete M, Hagiwara JC, Facciuto G, Mata D, Soto S. Wild ornamental germplasm exploration and domestication based on biotechnological approaches. *In vitro* colchicine treatment to obtain a new cultivar of Scoparia montevidiensis. Electronic Journal of Biotechnology. 2005; 8:2.
49. Cohen D, Yao JL. (*In vitro* chromosome doubling of nine *Zantedeschia* cultivars. Plant Cell Tissue and Organ Culture. 1996; 47:43-49.
50. Eeckaut TGR, Werbrouck SPO, Leus LWH, Van Bockstaele EJ, Debergh PC. Chemically induced polyploidization in *Spathiphyllum wallisii* regel through somatic embryogenesis. Plant Cell, Tissue and Organ Culture. 2004;78(3):241-246.
51. Wehner TC. Watermelon. Springer Science, New York; 2008.
52. De Carvalho JFRP, de Carvalho CR, Otoni WC. *In vitro* induction of polyploidy in annatto (*Bixa orellana*). Plant Cell, Tissue and Organ Culture. 2005;80:69-75.
53. Escandon AS, Hagiwara JC, Alderete LM. A new variety of *Bacopa monnieri* obtained by *in vitro* polyploidization. Electronic Journal of Biotechnology. 2006;9(3):181-185.
54. Gmitter FG, Ling XB, Cai CY, Grosser JW. Colchicine-induced polyploidy in citrus embryogenic cultures, somatic embryos and regenerated plantlets. Plant Sci. 1991;74:135-141.
55. Gao SL, Chen BJ, Zhu DN. *In vitro* production and identification of

autotetraploids of *Scutellaria baicalensis.* Plant Cell, Tissue and Organ Culture. 2002;70:289-293.
56. Chen LL, Gao SL. *In vitro* tetraploid induction and generation of tetraploids from mixoploids in *Astragalus membranaceus*. Sci. Hort. 2007;112:339-344.
57. Stanys V, Weckman A, Staniene G, Duchovskis P. *In vitro* induction of polyploidy in Japanese quince (*Chaenomeles japonica*). Plant Cell Tiss. Org. Cult. 2006;84:263–268.
58. Azhar M, Mak C, Mohd-Nasir B. Polyploidy induction in pisang mas, a diploid banana (*Musa* spp). Asia Pacific Journal of Molecular Biology and Biotechnology Supplement. 2000;1:11.
59. Rodrigues FA, Soares JDR, dos Santos RR, Pasqual M, Silva SO. Colchicine and amiprophos-methyl (APM) in polyploidy induction in banana plant. African Journal of Biotechnology. 2011;10(62):13476-13481.
60. Thao NTP, Ureshino K, Miyajima I, Ozaki Y, Okubo H. Induction of tetraploids in ornamental Alocasia through colchicine and oryzalin treatments. Plant Cell Tissue and Organ Culture. 2003;72(1):19-25.
61. Alishah O, Bagherieh-Najjar MB. Polyploidization effect in two diploid cotton (*Gossypium herbaceum* L. and *G. arboreum* L.) species by colchicine treatments. African Journal of Biotechnology. 2008;7(2):102-108.
62. Brisibe EA, Ogbu-Udensi O, Ntui VO, Otu PA, Chukwurah PN. Sensitivity of some quantitative and yield characters of 'Egusi' melon (*Colocynthis citrullus* L.) to treatment with microtubule inhibitors. African Journal of Plant Science. 2011; 5(13):759-766.
63. Dolezel J, Bartos, J. Plant DNA flow cytometry and estimation of nuclear genome size. Ann. Bot. 2005;95:99-110.
64. Zlesak DC, Thill CA, Anderson NO. Trifluralin mediated polyploidization of *Rosa chinensis* minima (Sims) Voss seedlings. Euphytica. 2005;14:281-290.
65. Bouvier LP, Guerif P, Djulbic M, Durel C, Chevreau E, Lespinasse Y. Chromosome doubling of pear haploid plants and homozygosity assessment using isozyme and microsatellite markers. Euphytica. 2002;123:255-262.
66. Chauvin JE, Souchet C, Dantec JP, Ellissache D. Chromosome doubling of 2x *Solanum* species by oryzalin: Method development and comparison with spontaneous chromosome doubling *in vitro.* Plant Cell, Tissue Organ Cult. 2003;73:65-73.
67. Chauvin JE, Label A, Kermarrec MP. In vitro chromosome – doubling in tulip (*Tulipa gesneriana* L.). Hort Science Biotechnol. 2005;80:693-698.
68. Kermani MJ, Sarasan V, Roberts AV, Yokoya A, Wentworth J, Sieber VK. Oryzalin-induced chromosome doubling in Rosa and its effects on plant morphology and pollen viability. Theory of Applied Genetics. 2003;107:1195-1200.
69. Petersen KK, Hagberg P, Kristiansen K. Plant *in vitro* doubling of *Miscanthus sinensis* A. Plant Breeding. 2002;121:445-450.
70. Hannweg K, Penter M, Sippel A. Use of polyploidy in tropical and subtropical plant improvement programmes. ISHS Acta Horticulturae 935: XXVIII International Horticultural Congress on Science and Horticulture for People. International Symposium on New Developments in Plant Genetics and Breeding; 2010.
71. Van Bogaert G. A comparison between colchicine induced tetraploid and diploid cultivars of *Lolium* species. In ploidy in fodder crops, Neusch B. (ed.). Eucarpia Report, Zurich; 1975.
72. Chauvin JE, Souchet C, Dantec JP, Ellissache D. Chromosome doubling of 2x *Solanum* species by oryzalin: Method development and comparison with spontaneous chromosome doubling *in vitro*. Plant Cell, Tissue Organ Cult. 2003; 73:65-73.
73. Wu J, Mooney P. Autotetraploid tangor plant regeneration from in vitro citrus somatic embryogenic callus treated with colchicine. Plant Cell, Tissue Organ Cult. 2002;70:99-104.
74. Leus L, Baert J, Van Laere K, Van Huylenbroeck J. Polyploidy in breeding: Examples in horticultural and agricultural crops. International conference on Polyploidy, Hybridisation and Biodiversity, Pruhonice, Czech Republic; 2012.
75. Allum JF, Bringloe DH, Roberts AV. Chromosome doubling in a *Rosa rugosa* Thunb. Hybrid by exposure of *in vitro* nodes to oryzalin: The effects of node length, oryzalin concentration and exposure time. Plant Cell Rep. 2007;26: 1977-1984.

76. Halder T, Gadgil VN. Morphogenesis in some species of the family cucurbitaceae. In Rao AN, (Ed.). Tissue Culture of Economically Important Plants. Singapore: Natl. University. 1982;98-103.
77. Gambley RL, Dodd WA. An *in vitro* technique for the production of *de novo* multiple shoots in cotyledon explants of cucumber (*Cucumis sativus* L.). Plant Cell Tissue Organ Cultue. 1990;20:177-183.
78. Singh MN, Kathal R, Bhatnagar SP. Regeneration of plants from hypocotyl and cotyledon cultures of *Cucumis melo* cv. pusa maduras. Phytomorphology. 1990; 40:401-405.
79. Singh MN. Mishra AK, Bhatnagar SP. *In vitro* production of plants from cotyledon explant of *Cucumis melo* L. and their successful transfer to field. Phytomorphology. 1996;46:395-402.
80. Stipp LCL, Mendes BMJ, Piedade SMDS, Rodriguez APM. *In vitro* morphogenesis of *Cucumis melo* var. Inodorus. Plant Cell Tiss. Org. Cult. 2001;65:81-89.
81. Kathiravan K, Vengedesan G, Singer S, Steinitz B, Paris H, Gaba V. Adventitious regeneration *in vitro* occurs across a wide spectrum of squash (*Cucurbita pepo*) genotypes. Plant Cell Tiss. Org. Cult. 2006;85:285-295.
82. Vasudevan A, Selvaraj N, Ganapathi S, Kasthurirengan V, Ramesh-Anbazhagan V. Manickavasagam M. Glutamine: A suitable nitrogen source for enhanced shoot multiplication in *Cucumis sativus* L. Biol. Plant. 2004;3:125-128.
83. Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Ramesh-Anbazhagan V, Manickavasagam M, Choi CW. Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). *In vitro* Cell. Dev. Biol.-Plant, 2008;44:300-306.
84. Anand SP, Jeyachandran R. *In vitro* multiple shoot regeneration from nodal explants of *Zehneria scabra* (L. F.) Sonder - An important medicinal climber. Plant Cell and Tissue Culture. 2004;14(2):101-106.
85. Bekele F, Abera B, Getahun M. *In vitro* propagation of anchote (*Coccinia abyssinica)* (Lam.) Cogn. African Journal of Plant Science. 2013;7(6):253-264.
86. Ahmad N, Anis M. *In vitro* mass propagation of *Cucumis sativus* L., from nodal segments. Turk. J. Bot. 2005;29: 237-240.
87. Kathal R, Bhatnagar SP, Bhojwani SS. Regeneration of plants from leaf explant of *Cucumis melo* cv. pusa sharbati. Plant Cell Reports. 1988;7:449-451.
88. Mishra AK, Bhatnagar SP. Direct shoot regeneration from the leaf explant of cucumber (*Cucumis sativus* L.). Phytomorphology. 1995;45:47-55.
89. Liu LZ, Chitrampalam PR, Zhai WQ, Chen YY, Zhu WM, Shi B. Efficient plant regeneration in three cultivars of Hami melon [*Cucumis melo* L., ssp. *melo* convar. *ameri* (Pang.) Greb] via organogenesis. The J. of Hort. Sci. and Biotecnology. 2013b;88(4):415-420.
90. Vasudevan A, Selvaraj N, Ganapathi A, Choi CW, Manickavasagam M, Kasthurirengan S. Direct plant regeneration from cucumber embryonal axis. Biol. Plant. 2007;3:521-524.
91. Kumar HGA, Murthy HN, Paek KY. Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L. Scientia Hort, 2003;98:213-222.
92. Chauvin JE, Souchet C, Dantec JP, Ellissache D. Chromosome doubling of 2x *Solanum* species by oryzalin: Method development and comparison with spontaneous chromosome doubling *in vitro*. Plant Cell, Tissue Organ Cult. 2003;73:65-73.
93. Wu J, Mooney P. Autotetraploid tangor plant regeneration from *in vitro* citrus somatic embryogenic callus treated with colchicine. Plant Cell, Tissue Organ Cult. 2002;70:99-104.
94. Shao J, Chen, C, Deng X. *In vitro* induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell, Tissue Organ Culture. 2003;75:241-246.
95. Jakse M, Havey MJ, Bohanec B. Chromosome doubling procedures of onion (*Allium cepa* L.) gynogenic embryos. Plant Cell Rep. 2003;21:905-910.
96. De Schepper S, Leus L, Eeckhaut T, Van Bockstaele E, Debergh P, De Loose M. Somatic polyploid petals: Regeneration offers new roads for breeding Belgian pot azaleas. Plant Cell, Tissue Organ Cult. 2004;76:183-188.
97. Gao SL, Chen BJ, Zhu DN. *In vitro* production and identification of autotetraploids of *Scutellaria baicalensis*. Plant Cell, Tissue and Organ Culture. 2002;70:289-293.
98. De Jesus-Gonzalez L, Weathers PJ. Tetraploid *Artemisia annua* hairy roots

produce more artemisinin than diploids. Plant Cell Rep. 2003;21:809-813.

99. Beck SL, Dunlop RW, Fossey A. Evaluation of induced polyploidy in *Acacia mearnsii* through stomatal counts and guard cell measurements. S. Afr. J. Bot. 2003;69:563-567.
100. Thao NTP, Ureshino K, Miyajima I, Ozaki Y, Okubo H. Induction of tetraploids in ornamental *Alocasia* through colchicine and oryzalin treatments. Plant Cell Tissue and Organ Culture. 2003;72(1):19-25.
101. Burdon JJ, Marshall DR. Inter-and intraspecific diversity in the disease response of glycine species to the leaf-rust fungus *Phakopsora pachyrhizi.* Journal of Ecology. 1981;69:381-390.
102. Thompson JN, Cunningham BM, Srgraves KA, Althoff DM, Wagner D. Plant polyploid and insect/plant interactions. The American Naturalist. 1997;150(6):730-743.
103. Mehta R, Swaminathan M. Studies on induced polyploids in forage crops. 1. Survey of previous work. Ind. J. Genet. PL Breed. 1957;17:27-57.
104. Cohen D, Yao, JL. *In vitro* chromosome doubling of nine *Zantedeschia cultivars.* Plant Cell Tissue and Organ Culture. 1996; 47:43-49.
105. De Schepper S, Leus, L, Eeckhaut T, Van Bockstaele E, Debergh P, De Loose M. Somatic polyploid petals: Regeneration offers new roads for breeding Belgian pot azaleas. Plant Cell, Tissue Organ Cult. 2004;76:183-188.
106. Wehner TC. Watermelon. Springer Science, New York; 2008.
107. Dhooghe E, Van Laere K, Eeckhaut T, Leus L, Van Huylenbroeck J. Mitotic chromosome doubling of plant tissue *in vitro.* Plant Cell Tiss. Organ Cult. 2011;104:359-373.
108. Shao J, Chen C, Deng X. *In vitro* induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell, Tissue Organ Culture. 2003;75:241-246.
109. Zhang Y, Zhou J, Wu T. Cao J. Shoot regeneration and relationship between organogenesis capacity and endogenous hormonal contents in pumpkin. Plant Cell Tiss. Org. Cult. 2008; 93:323-331.
110. Khosravi P, Jafarkhani Kermani M, Nematzade GA, Bihamta MR. Role of mitotic inhibitors and genotype on chromosome doubling of Rosa. Euphytica. 2008;160:267-275.

© 2017 Agogbua and Ekeke; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19506