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National Academy of Agricultural Science (NAAS) Rating : 3.03

Effect of oryzalin treatments on polyploidy induction, phenotypic and quantitative traits of *Zehneria capillacea* (Shumach.) C. Jeffrey

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Abstract: *The determination of ploidy level is an essential technique in plant breeding and genetics. In this study, diploid seedlings of Zehneria capillacea (2n = 2x = 22) a wild edible cucurbit in Nigeria with a narrow genetic base were treated with different concentrations of oryzalin to induce ploidy variants. The morphology, stomata and quantitative traits of the oryzalin-induced polyploids were compared with the diploid wild plants. The ploidy status of plants that survived following oryzalin treatment at the cotyledon (CT) and first leaf (FL) growth stages were evaluated putatively based on plant macro and micro-morphological observations. Significant differences (P<0.05) were observed in leaf stomatal measurements between the untreated control and induced polyploids. There was an increase in the number of pollen germinal pores and colpi. In conclusion, pre-selection or early screening of putative polyploids in Z. capillacea based on morphological alterations and leaf epidermal studies was effective and seems to be reliable for ploidy level estimation as all the putative polyploids were confirmed to be tetraploids or mixoploids with flow cytometric analysis (FCM) analysis.*

Key Words: *Zehneria capillacea, oryzalin, stomata, polyploid induction*

INTRODUCTION

Genome doubling has a lot of consequences on plant morphology, genetic composition, stomatal and pollen characteristics of the plant ([1], [2], [3], [4]). Polyploid plants have larger nuclear and cell sizes compared to diploid plants ([5], [6], [3]). The length/width ratio of leaves also decreases as a consequence of the higher ploidy level and the internode length differs in plants with different ploidy levels [7]. Ploidy manipulation is considered as a valuable tool in genetic improvement of many plants including *Solanum* spp. [2], *Citrus*[8], *Punica granatum*[9], *Allium* spp. [10] and *Rhododendron* [11]. Also in agriculture and horticulture, polyploid induction have been used to improve plant yield and fruit size[12], medicinal values of plants, resistance to pest, flower colour, size and number ([13], [2], [8],14]).

Z. capillacea is a wild edible diploid herbaceous climber that is found in West Africa[15]. In Nigeria, *Zehneria* species have several ethnobotanic values and could also be used as forage or feed for animals. The roots of *Z. cordifolia* are used by herbalists to induce abortion [16] while the powdered leaves of *Z. hallii* are used for the treatment of tapeworm and as sedatives [17]. The leaves of *Z. capillacea* are eaten as vegetables [18] and taken by nursing mothers to aid postpartum recovery ([19], [20]). It is worthy to note that this species and other members of this family are understudied [15]. Therefore this work is geared towards polyploidizing this species to enhance its economic value and domestication.

MATERIALS AND METHODS

The study was carried out using the greenhouse facilities in the BioScience center of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Seeds of *Z. capillacea* were obtained from the wild and treated with different concentrations of oryzalin at different developmental stages.

Shoot-tip seedling treatment at cotyledon stage and first true leaf stage: Seeds were planted in 9cm x 7cm nursery pots filled with top soil in the green house. At the emergence of cotyledons, various concentrations of aqueous solutions of oryzalin (0, 30, 60, 90, 120 and 150μM) were applied to the shoot-tip

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by the dropping method using a 10 ml injection needle and syringe for five consecutive days.

Data evaluation: All the plants were evaluated for percentage survival, percentage of polyploids induced, morphological traits and polyploidization efficiency was calculated according to Ajalin *et al*. [21].

Ploidy level evaluation: The ploidy levels of oryzalin treated plants were determined indirectly by assessing guard cells of the stomata (stomata size and density), pollen grain diameter and fertility, phenotypic traits such as thicker leaves and distorted leaves. The direct method involved assessment of the mitotic chromosome number in root tip cells [22] and nuclei fluorescence intensity using flow cytometer [23].

Statistical analysis: The statistical differences among means of the traits of control and induced polyploid plants were computed by analysis of variance and the t-test.

RESULTS

Shoot-tip seedling treatment at cotyledon stage: Results revealed that *Z. capillacea* seedlings at the cotyledonary growth stage were sensitive to oryzalin treatment. Despite the fact that all the cotyledons of the treated shoot-tips were necrotic, there was no immediate mortality following oryzalin application. As the first true leaves emerged, they were all morphologically distinct from the control plants displaying variations in leaf shape size and thickness. Subsequent growth revealed stunted plants with very short internodes, lobed leaves and multiple buds and branching at the apex.

Shoot-tip seedling treatment at first true leaf stage: The oryzalin treatment of *Z. capillacea* shoot-tips at the first true leaf stage also showed phenotypic variations in the morphology of treated plants but they were not as pronounced as the changes observed in the cotyledonary growth stage. The leaf deformations were observed as the second and third leaves of treated plants emerged and thereafter seedling development appeared normal at the 3rd or 4th leaf stage when visually compared to the control plants. Some of the treated seedlings had two or six apical growth points. It was also observed that seedling development was not affected by oryzalin concentration and treatment duration at this growth stage. Plants that were treated for three days and five days with 30 -150µM oryzalin expressed similar qualitative phenotypes (Table 1).

PLOIDY EVALUATION

Plant morphology: Results obtained from macro-morphology indicate that oryzalin induced *Z. capillacea* variants were phenotypically different from the wild type. The observed morphological variations recorded six weeks and 12 weeks after treatment are presented in Tables 1 and 2. All the treated seedlings at the different growth stages exhibited 100% survival irrespective of the oryzalin dosage and treatment duration. The observed percentage of phenotypic variants ranged from 90 – 100% in all treated seedlings. All the FL variants and the control exhibited a creeping plant habit in the green house while the CT variants were erect.

Seedling growth was highly inhibited in the CT variants as indicated by the plant height which was significantly shorter (1.45±0.16cm - 4.12±0.88cm) than those of the control (15.40±1.93cm) and FL (10.70±1.41 - 20.05±3.22cm) variants. The observed difference among the averages of the five treatments in the CT stage was highly significant at the 1% level with 150µM having the highest plant height of 4.12cm and 60µM having a height of 1.45cm. The number of nodes, inter-node length and number of leaves per plant was also significantly ($P<0.01$) lower in the CT seedlings than the FL and control seedlings. In the CT seedlings, most of the leaves emerged from a single node with multiple growth points unlike the control and FL seedlings where the number of nodes corresponded with the number of leaves and was statistically similar (Table 1). There was a significant dose effect ($P<0.01$) on the number of leaves at the CT stage with 60µM and 150µM producing 1.50±0.31 and 5.70±0.37 number of leaves respectively.

Oryzalin application at the cotyledonary growth stage delayed flowering as indicated by the percentage of flowering plants (Table 1). None of the CT variants flowered in the greenhouse (0%) while 40% flowering was recorded for the wild type. The flowering percentage of the FL variants ranged between 50 – 60%. Oryzalin application at the first leaf growth stage induced early flowering variants as shown by the number of days to flowering (DTF) which ranged between 31.5±2.8 – 36.6±6.7 days for the various oryzalin concentrations and 43.5±1.2 days for the control. The number of nodes to first flower (NTF) was also lower (6th – 7th node) in the FL variants than control (9th node) and was not affected by oryzalin concentration and treatment duration among the FL variants.

The fruit length was statistically similar in the control and the putative polyploids while variations

Table 1
Morphological characters of *Z. capillacea* seedlings treated with oryzalin at various concentrations and treatment duration. Traits were scored six weeks after treatment in the greenhouse

Conc. (µM)	*Duration (days)*	*% survival*	*PV[6] (%)*	*PHT[1]*	*No of nodes*	*IL[5] (cm)*	*No of leaves*	*Flowering*		*NTF[3]*	*FLT[4] (cm)*	*No of Seeds*	*Plant habit*
								(%)	*DTF[2]*				
Control													
0	0	100	0	15.4±1.9^{b}	8.7±0.5^{a}	2.4±0.1^{a}	8.7±0.5^{a}	40	43.5±1.2^{a}	9.2±0.3^{a}	1.81±0.1^{a}	13.0±3.5^{a}	creeping
Cotyledonary Growth Stage (CT)													
30	5	100	100	2.8±0.5^{e}	1.7±0.5^{b}	1.0±0.2^{b}	4.5±0.3^{b}	0	0	0	0	0	erect
60	5	100	100	1.5±0.2^{f}	1.0±0.0^{b}	0.0±0.0^{c}	1.5±0.3^{c}	0	0	0	0	0	erect
90	5	100	100	2.7±0.1^{e}	1.0±0.0^{b}	0.0±0.0^{c}	5.2±0.3^{b}	0	0	0	0	0	erect
120	5	100	90	3.9±0.5^{d}	1.7±0.4^{b}	0.8±0.4^{b}	5.2±0.3^{b}	0	0	0	0	0	erect
150	5	100	100	4.1±0.9^{d}	2.4±0.6^{b}	1.5±0.2^{b}	5.7±0.4^{b}	0	0	0	0	0	erect
First Leaf Growth Stage (FL)													
30	5	100	90	11.6±1.7^{c}	7.1±1.0^{a}	2.3±0.2^{a}	7.7±0.4^{a}	50	35.6±2.1^{ab}	6.3±0.3^{b}	1.81±0.1^{a}	13.5±0.5^{ab}	creeping
60	5	100	100	10.7±1.4^{c}	6.5±0.6^{a}	2.0±0.1^{a}	7.1±1.0^{a}	50	34.5±1.3^{b}	6.2±0.3^{b}	1.85±0.1^{a}	9.0b±2.8^{c}	creeping
90	5	100	100	11.2±1.3^{c}	7.2±0.6^{a}	2.1±0.1^{a}	6.6±0.6^{a}	50	36.6±2.1^{ab}	6.4±0.3^{b}	1.86±0.1^{a}	8.3±1.5^{c}	creeping
120	5	100	90	13.8±0.9^{b}	7.8±0.4^{a}	2.1±0.1^{a}	7.2±0.6^{a}	50	34.6±1.7^{b}	7.4±0.5^{b}	1.89±0.1^{a}	5.3±1.9^{d}	creeping
150	5	100	90	10.7±2.1^{c}	6.7±0.9^{a}	2.1±0.1^{a}	7.8±0.4^{a}	60	34.1±2.2^{b}	6.4±0.3^{b}	2.10±0.1^{a}	6.1±2.4^{d}	creeping

*Means followed by the same letter within columns are not significantly different using DMRT at the 5% level of significance, [1]Plant height, [2]Days to flowering, [3]Nodes to flowering, [4]Fruit length, , [5]Internode Length, [6]Phenotypic variants

Table 2
Morphological characters of *Z. capillacea* seedlings treated with oryzalin at various concentrations and treatment duration. Traits were scored 12 weeks after treatment in the field

Conc. (µM)	*Duration (days)*	*% survival*	*PHT[1] (cm)*	*IL[8] (cm)*	*No of branches*	*Flowering*		*LL[3](cm)*	*LW[4](cm)*	*LL/LW[5] ratio*	*FLT[6](cm)*	*No of Seeds*	*♀♂/♂[7]*
						(%)	*DTF[2]*						
Control													
0	0	100	216.9±5.1^{a}	5.8±0.3^{a}	3.6±0.2^{a}	100^{a}	43.5±1.2^{d}	5.86±0.2^{b}	6.1±0.1^{b}	0.96	1.94±0.04	29.5±0.3^{a}	10:1
Cotyledonary Growth Stage (CT)													
30	5	67	20.8±2.6^{e}	2.2±0.2^{b}	4.5±0.5^{a}	33^{d}	131.6±4.3^{a}	5.6±0.2^{b}	6.6±0.1^{b}	0.85	1.7±0.15	10.3±0.2^{b}	1:1
60	5	33	10.0±1.3^{f}	1.2±0.1^{c}	0^{c}	0^{e}	0.0±0.0^{f}	3.3±0.2^{c}	5.0±0.1^{c}	0.66	0		0
90	5	83	61.7±17.3^{b}	1.3±0.4^{c}	3.0±0.4^{a}	33^{d}	109.6±3.5^{b}	5.7±0.1^{b}	6.7±0.1^{b}	0.85	1.6±0.16	9.4±1.2^{b}	1:1
120	5	83	48.8±15.1^{c}	2.1±0.2^{b}	2.0±0.5^{b}	67^{c}	98.5±1.0^{c}	5.4±0.1^{b}	6.5±0.2^{b}	0.83	1.5±0.15	8.8±0.8^{b}	2:1
150	5	83	29.8±7.7^{d}	2.6±0.2^{b}	2.8±0.3^{a}	83^{b}	98.2±2.4^{c}	5.5±0.2^{b}	6.3±0.1^{b}	0.87	1.6±0.16	8.6±1.3^{b}	1:3
First Leaf Growth Stage (FL)													
30	5	100	215.1±6.3^{a}	5.2±0.2^{a}	3.4±0.2^{a}	100^{a}	35.6±2.1^{de}	7.0±0.2^{a}	8.0±0.1^{a}	0.88	1.9±0.06	16.7±1.2^{b}	10:1
60	5	100	216.4±5.0^{a}	5.4±0.2^{a}	3.5±0.2^{a}	100^{a}	34.5±1.3^{e}	6.9±0.2^{a}	8.0±0.1^{a}	0.86	2.1±0.04	16.0±1.6^{b}	10:1
90	5	100	216.6±4.3^{a}	5.3±0.2^{a}	3.4±0.2^{a}	100^{a}	36.6±2.1^{de}	7.3±0.2^{a}	7.9±0.3^{a}	0.92	1.9±0.07	17.5±1.2^{b}	10:1
120	5	100	216.4±5.4^{a}	5.5±0.2^{a}	3.5±0.2^{a}	100^{a}	34.6±1.7^{e}	7.2±0.2^{a}	8.2±0.2^{a}	0.88	2.0±0.02	17.2±0.8^{b}	10:1
150	5	100	215.7±4.9^{a}	5.3±0.2^{a}	3.3±0.2^{a}	100^{a}	34.1±2.2^{e}	7.1±0.2^{a}	7.9±0.3^{a}	0.90	2.3±0.04	17.4±1.0^{b}	10:1

*Means followed by the same letter within columns are not significantly different using DMRT at the 5% level of significance, [1]Plant height, [2]Days to flowering, [3]Leaf length, [4]Leaf width, [5]Leaf length/width ratio, [6]Fruit length, [7]Ratio of hermaphroditic to male flowers, [8]Internode Length

were observed in the number of seeds per fruit. The control had the same number of seeds (13.0±3.5) with the FL 30 µM (13.5±0.5) treatment but these significantly differed with the other treatments (Table 2). All the experimental plants were transferred to the field eight weeks after polyploid induction and further morphological development was recorded. One month after field establishment (12 weeks after treatment), there was a 100% survival for all the FL variants and the control while the CT variants had survival rates of 33%, 67% and 83% for 60µM, 30µM and 90-150µM oryzalin concentrations respectively. The percentage survival was the same for all treatments except for the CT treated with 60µM which exhibited 100% mortality without flowering (Table 2). The plant height for the FL variants and the control were ≥200cm with 3 - 4 branches per plant while that of CT plants varied from an average of 7.0±1.3cm to 61.7±17.3cm in height with 2 – 5 branches per plant. Significant differences were observed in the internode lengths between CT and FL variants (Tables 1 and 2). The wild type, CT and FL variants had internode lengths of 5.8±0.3cm, 1.2±0.1–2.6±0.2cm and 5.2±0.3–5.7±0.7cm respectively.

The CT variants commenced flowering in the field with DTF ranging from 98.2±2.4 – 131.6±4.3 and these were significantly different from the control and FL variants which flowered in the green house. The hermaphroditic flowers bloomed earlier than the male flowers in 95% of the variants. The ratio (10:1) of hermaphroditic to male (♀♂/♂) flowers was the same in FL variants as that observed in the wild type. However ratios of 1:1, 2:1 and 1:3 were recorded for CT variants (Table 2). The leaf length and leaf width was significantly higher in FL variants than the control and CT variants while the fruit length was similar for all the treatments. There was a high significant difference ($P<0.01$) in the number of seeds per fruit between the wild type (29.5±0.3) and the oryzalin treated plants. The CT variants had the lowest number of 8.6±1.3 – 10.3±0.2 (Table 2).

The micro-morphological characters such as stomatal density, size and pollen stainability observed between the wild type and the variants are presented in table 3. Significant differences ($P<0.05$) were observed in leaf stomatal measurements between the untreated control and oryzalin-induced polyploids. The stomatal density was higher in the diploids (18.79 ± 0.47 mm^2) than the solid tetraploids (17.40 ± 0.96 mm^2) and the $8x+16x$mixoploids (8.89 ± 0.63 mm^2). The stomatal length was 13.38 ± 0.11 µm for diploids and 15.56 ± 0.14 for tetraploids while the width was 9.50 ± 0.12 µm and 11.13 ± 0.11 µm for diploids and tetraploidsrespectively. The stomatal length of the $8x+16x$mixoploids was 17.63 ± 0.18 µm with a width of 12.63 ± 0.17 µm (Table 2).

All the pollen grains examined were 100% fertile, spherical in shape with reticulate surface and had similar polar and equatorial dimensions (28µm x 29µm) irrespective of the ploidy level. However, variations were observed in the number of colpi and germinal pores. All the diploid wild type weretricolporate while the polyploids were tricolporate, tetracolporate and pentacolporate (Table 3).

Results obtained from the flow cytometric analysis revealed histograms with significant increase in the relative amounts of DNA in the nuclei of the CT and FL variants. The three groups of ploidy level observed were diploids, tetraploids and mixoploids. The various classes of cytochimera induced were $2x+4x$, $2x+4x+8x$, $4x+8x$ and $8x+16x$ (Fig. 1). The nuclei isolated from the wild diploid plants (2n=2x=22) were used as an internal standard and the fluorescence channel set at 50. The control plants had a mean relative fluorescence (MRF) of 49.61 – 50.48 while the tetraploids had MRF values (96.1 – 102.4) double that of diploids. The mixoploids had histograms with two or three distinct MRF values (Table 3). Shoot apex

Table 3
Effect of ploidy level on micro-morphological characters of *Z. capillacea*

Character	*Diploid (2x)*	*Tetraploid (4x)*	*Mixoploid (8x + 16x)*
Stomatal density (mm^2)	18.79 ± 0.47[a]	17.40 ± 0.96[a]	8.89 ± 0.63[b]
Stomatal length (µm)	13.38 ± 0.11[c]	15.56 ± 0.14[a]	17.63 ± 0.18[a]
Stomatal width (µm)	9.50 ± 0.12[c]	11.13 ± 0.11[a]	12.63 ± 0.17[a]
Pollen stainability (%)	100	100	100
Pollen diameter (P x E)	28 x 29 µm	28 x 29 µm	28 x 29 µm
Pollen shape	Spherical	Spherical	Spherical
Pollen Colpi (Number)	3	3, 4, 5	3, 4, 5
Pollen pores (Number)	3	3, 4, 5	3, 4, 5
Pollen class	Tricolporate	Tricolporate, tetracolporate and pentacolporate	Tricolporate, tetracolporate and pentacolporate

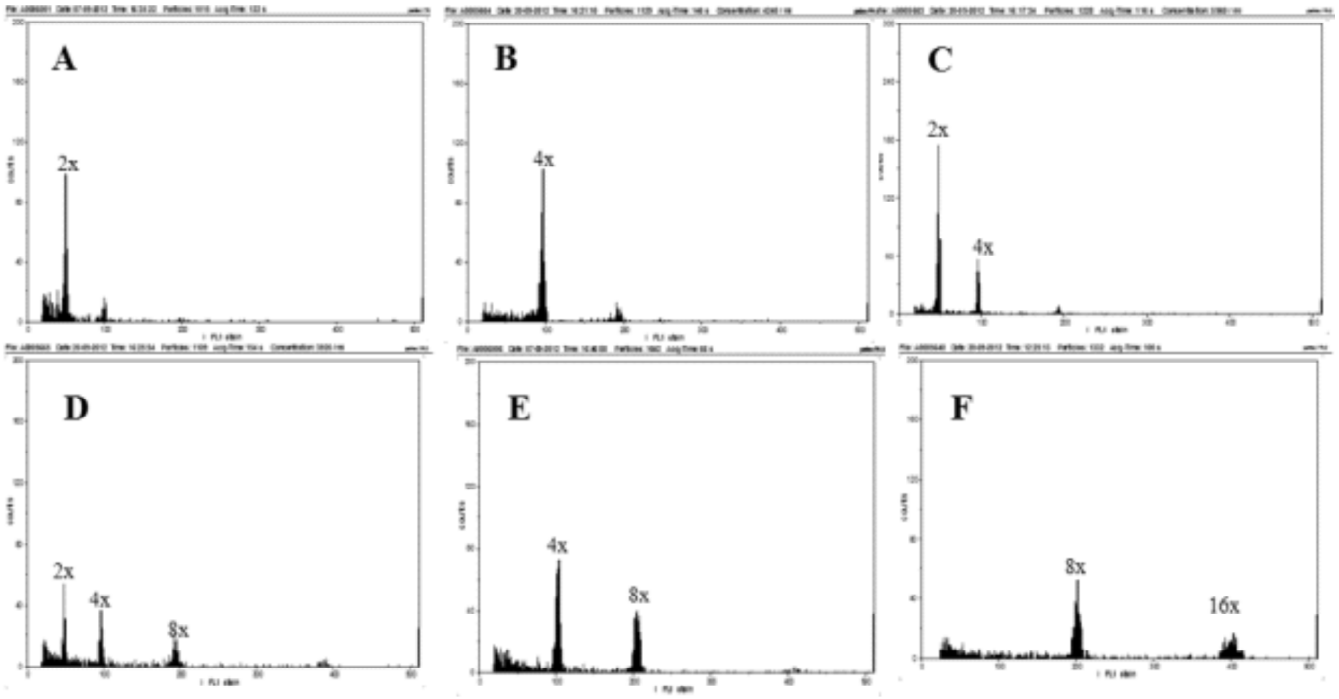

Figure 1: Flow cytometric profiles of *Z. capillacea* plants treated with oryzalin. (A) control diploid 2*x*, (B) solid tetraploid 4*x*, (C) mixoploid 2*x*+4*x*, (D)mixoploid2*x*+4*x*+8*x*, (E) mixoploid 4*x*+8*x*, (F) mixoploid 8*x*+16*x*. Diploid (control) nuclei was set to channel 50 with tetraploids resolving at channel 100, 0ctaploids at channel 200 and hexa-decaploid at channel 400

application at the cotyledon growth stage induced 100% 4*x*+8*x* cytochimera at concentrations of 30 µM and 90µM. Plants treated with 60µM oryzalin yielded 10% 4*x*+8*x* and 90% mixoploids with peaks between 6*x*/7*x* and 12*x*/13*x*. The 120µM oryzalin induced 60% 4*x*+8*x* mixoploids and 40% solid tetraploids while 150µM had 50% 4*x*+8*x* and 50% 8*x*+16*x* cytochimera (Table 3). Application of 30µM oryzalin at the first leaf growth stage induced 40% 2*x*+4*x*, 30% 2*x*+4*x*+8*x* and 20% 4*x*+8*x* cytochimeras and 10% were unaffected and remained diploids with similar peaks as the wild type. Application of 60 - 150µM induced 20% 2*x*+4*x*, 60% 4*x*+8*x*, 20% 8*x*+16*x* (Table 3).

DISCUSSION

The treatment of shoot apex at the cotyledon leaf growth stage with 30 - 150µM oryzalin induced solid tetraploids and cyto-chimeras of 4*x*+8*x* and 8*x*+16*x* with delayed seedling growth, reduced percentage of plantlet survival, increased number of days to flowering and reduced seed set. Similar results were obtained by Contreras *et al*. [24] in *Hibiscus acetosella* 'Panama Red' PP20121 and [20] who reported ineffective ploidy induction in seed treatment of *Ocimum basilicum* but obtained autotetraploids by treatment of shoot apex at the emergence of cotyledon leaves.

Application of oryzalin at the first leaf growth stage also induced solid tetraploids, cytochimeras of 2*x*+4*x,* 2*x*+4*x*+8*x*, 4*x*+8*x*, 8*x*+16*x* and plants that were unaffected and remained as diploids. The phytotoxic effect of the microtubule inhibitor was more pronounced at the cotyledon growth stage than on the first leaf growth stage. The seedling development at the true leaf stage was not affected and plantlet survival was 100%. In conformity with this result, treatment of *Dracocephalum moldavica* seedlings at the cotyledon leaf stage caused phytotoxicity, damping off and gradual death of all plantlets within 15 days while treatment at the emergence of true leaf stage was most effective for producing autotetraploids[25].

Ploidy level evaluation: The determination of ploidy level is an essential technique in plant breeding and genetics. In this research, polyploids could be visually screened as early as two weeks after oryzalin treatment and the best phenotypic indicators of early screening stages were altered leaf morphology, leaf and stem thickness. Leaves of the tetraploids had higher length, width and a smaller length-to-width ratio than the wild diploids, a phenomenon also described [7]. Stomata characteristics were also important indicators for the detection of new ploidy levels in *Z. capillacea*. Larger stomata size in the tetraploid plants observed in this study is in

accordance with similar reports in *Cucumis melo* [26], *Citrullus lanatus* [27] and in many other species where polyploid plants had larger cells than diploid plants ([28], 29], [30], [5]). Similarly, stomata length and width have been positively correlated to ploidy level in *Hibiscus* species [31], *Astragalus membranaceus* [32], *Dracocephalum moldavica* L [30] and *Tagetes erecta* [33]. Stomata parameters (length and width of guard cells) are often highly positively correlated with different ploidy levels and have been effectively used for ploidy determination ([34], [32]). Increase in genome size reduced the stomata density per area in *Z. capillacea*. The stomata number per area in the wild diploid was greater than those in the polyploid variants. Similar results have been observed in *Fragaria* species [33]. The decrease in stomata number per unit area is due to the increase in cell size in the polyploids [34].

Pollen grain diameter has been reported by several researchers to be larger in tetraploids than diploid cells and have been used as indicators in primary screening of ploidy level estimation [33], [30]. In contrast to these findings, this research found no correlation between pollen size and increase in genome size. This lack of correlation precluded using pollen size as a phenotypic marker in distinguishing the wild diploid and polyploid variants. There was also no variation in pollen fertility between the ploidy levels. Conversely, lower pollen fertility in higher ploidy level has been reported in *Cucumismelo* L. [35] and *Fragaria* species [33]. The only difference in palynological observations between the wild diploid *Z. capillacea* and the induced tetraploids was in the number of pores and colpi. Pollen grains of diploids were tricolporate while those of tetraploids were tetracolporate and pentacolporate. Similarly, pollen grains with four germinal pores and colpi were observed in autotetraploid cucumber [36] and autotetraploid watermelon [32] while the diploids had three pores and colpi. An increase in germinal pores and colpi of polyploids were also reported in *Viciavillosa* [37].

The number of seeds per fruit was fewer in the tetraploids than in the diploids. Similarly, Pradeepkumar [38] observed a significantly lower number of seeds in colchicine-induced tetraploid watermelon than the diploid control plants. Contreras *et al*. [29] also observed greatly reduced seed set in induced octoploid*Hibiscus acetosella* 'Panama Red' PP20121 that was also reported to have shorter internodes, smaller canopy volume, and shorter plant height. Stanys *et al*. [39] observed there was only one-fourth as many seeds produced in tetraploids as in diploids in colchicine-induced flowering quince (*Chaenomeles japonica*). This reduced seed set observed in polyploid *Z. capillacea* may not affect production because it is also propagated by stem cuttings.In conclusion, pre-selection or early screening of putative polyploids in *Z. capillacea* based on morphological alterations and epidermal studies was effective and seems to be reliable for ploidy level estimation as all the selected plants were confirmed to be polyploids by FCM analysis.

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