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*Full Length Research Paper*

# Effect of water stress on growth of colchicine induced polyploid *Coccinia palmata* and *Lagenaria sphaerica* plants

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**Polyploid induction was conducted by different colchicine concentration with seedlings of *Coccinia palmata* and *Lagenaria sphaerica*. Colchicine induced polyploid seedlings were grown in pots and subjected to varying levels of irrigation until they reached a flowering stage. The survival rate was best at colchicine concentration of 0.001 g/L in seedlings treated after three days of germination. Polyploidy induction was best at colchicine concentration of 0.01 g/L in seedlings treated after three days of germination. The induction rates for *C. palmata* and *L. sphaerica* were 90 and 86%, respectively. Compared with normal diploid plants, the polyploid plants of *C. palmata* and *L. sphaerica* inducted in our experiments had short stems. The polyploid induced plants had many active shoot apices, increased leaf chlorophyll content and were more drought resistant compared with normal diploid plants.**

**Key words:** Colchicine, *Coccinia palmate*, *Lagenaria sphaerica*, water stress.

## INTRODUCTION

*Coccinia palmata* (Sond.) Cogn. and *Lagenaria sphaerica* (Sond.) Naud. belongs to the Cucurbitaceae family and are both indigenous to southern Africa (Fox and Norwood Young, 1982; Pooley, 1998). Both species are used as indigenous leafy vegetables and as traditional medicines. Their leaves are cooked as vegetables (Peters et al., 1992; Williams, 1997; Pooley, 1998; Jacobs, 2002) and roots of *C. palmata* are also edible (Fox and Norwood Young, 1982; FAO, 1988). Both species are used for stomach complaints and *L. sphaerica* also serves as a remedy for glandular swellings (Hutchings, 1996; Pooley, 1998). The African homestead (Muzi) garden contains a mixture of spiritual, protective and medicinal plants including *C. palmate* and *L. sphaerica* (Zobolo and Mkabela, 2006). The improvement of these species through polyploidy can make them commercially imperative in both food and pharmaceutical industries.

The treatment of seeds with polyploid inducing agents such as colchicine retards seed germination and increases seedling mortality (Takamura and Miyajima, 1996; Beck et al., 2003; Rubuluza et al., 2007). An increase in the number of genomic complements is often associated with the emergence of new phenotypes not present in diploid progenitors (Martelotto et al., 2005). Polyploidy usually results in an increase in cell volume and therefore of the plant parts (Sliwinska and Lukaszewska, 2005).

Polyploids exhibit at least one of the following characteristics: larger tuber, rhizome or root size, larger and/or thicker leaves (Romero-Aranda et al., 1997; Choi et al., 2000; Shao et al., 2003), larger guard cell size and fewer stomata (Cohen and Yao, 1996; Takamura and Miyajima, 1996; Beck et al., 2003; Hansen et al., 2007), improved photosynthetic capacity associated with increase in the amount of photosynthetic enzymes and pigments per cell (Romero-Aranda et al., 1997; Martelotto et al., 2005), improved drought tolerance, viral, fungal and pest resistance (Al Hakimi et al., 1998; Fock et al., 2000 and 2001; Martelotto et al., 2005; Nouri-Ellouz et al., 2006; Xiong et al., 2006), enhanced flower, fruits and seed size and weight (Shao et al., 2003; Martelotto et al., 2005), many and large, but less viable pollen grains (Cohen and Yao, 1996; Takamura and

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Miyajima, 1996; Shao et al., 2003), improved flower colour intensity (Takamura and Miyajima, 1996), vigorous growth (Sreekumari et al., 1999; Yang et al., 1999) or retarded growth resulting in dwarfism (Choi et al., 2000; Väinölä, 2000; Beck et al., 2003).

The objectives of this paper were to identify a suitable colchicine concentration for polyploidy induction, to investigate the survival rate of colchicine treated seedlings and to investigate the performance of colchine induced polyploid *C. palmata* and *L. sphaerica* plants under water stress conditions

## MATERIALS AND METHODS

Seeds of *C. palmata* and *L. sphaerica* were collected from Eshowe and KwaDlangezwa, KwaZulu-Natal, South Africa and stored in sealed plastic bottles at 22°C room temperature. Seeds were germinated in Petri dishes for one day (1DT) three days (3DT) and six days (6DT), and thereafter treated with 0.001, 0.01 and 0.1 g/l (w/v) colchicine for 24 h. The experiment was carried out at Kwadlangezwa (28°51'S, 31°50'E) where the climate is sub-tropical. The percentage of polyploid formation was determined using flow cytometry (Dolezel, 1991). Seedlings with well developed plumule and green cotyledons were transferred to seedling trays and hardened at 80% shade in the nursery. Seedlings with three developed leaves were transferred into 10 L plant bags with a river sand-vermiculite mixture (1:1) and grown in the nursery at 40% shade.

Potted plants were divided into two groups and subjected to two levels of irrigation, namely plants watered every third and tenth day, respectively.

Plants were harvested at 90 and 120 days after planting (DAP). Growth parameters such as fresh and dry weight of root and shoot, number of shoot apices and chlorophyll content were investigated. Approximately 1 $cm^2$ of fresh leaf was cut and weighed for chlorophyll extraction, and each disc was immersed in 5 $cm^3$ of 90% (v/v) acetone in a snap cap test tube and refrigerated overnight (about 14 - 15 h) (Hewitson, 2004). The chlorophyll was also extracted through grinding of leaf discs with mortar and pestle.

Optical Density (OD) of the solution was read at 645 and 663 nm and the measurements were substituted in the following equation:

Concentration of chlorophyll extract ($mg/cm^3$) = ($20.2 \times OD_{645\ nm}$) + ($8.02 \times OD_{663\ nm}$).

## RESULTS AND DISCUSSION

### Growth of radicle and plumule

The radicles of the control seedlings were significantly longer than those of plants treated with various colchicine concentrations in both *C. palmate* and *L. sphaerica*. In each colchicine concentration there was an increase in radicle length as the incubation period increased from 1 to 6DT. The increase in colchicine concentration from 0.001 to 0.1 g/L resulted in an increase in the length of radicle in all the treatments (Table 1). The results of the present study agree with reports on Viola x wittrockiana and *Punica granatum* where the hypocotyls of colchicine treated plants became thicker and shortened (Ajalin et al., 2002; Shao et al., 2003).

The plumules of control seedlings were significantly longer than those that were treated with colchicine (Table 1). There was a significant decline in plumule length as the concentration increased from 0.001 to 0.1 g/L. The increase in incubation period from 1 to 6DT resulted in a slight increase in the length of the plumule in all the treatments for *C. palmate* and *L. sphaerica*. The shortening of plumule in colchicine treated seedlings has been reported in *Triticum aestivum* and *Platanus acerifolia* (Yang et al., 1999; Liu et al., 2007).

### Seedling survival and polyploidy formation

There was a high rate of survival in control seedlings as compared to the ones treated with colchicine (Table 2). Seedling survival decreased as the colchicine concentration increased. Seedlings that were incubated for a longer period prior to colchicine treatment (3 and 6DT) had a significantly higher survival rate than the ones that received one day incubation (1DT). Several studies have shown an increase in mortality of colchicine treated seedlings (Takamura and Miyajima, 1996; Vainola, 2000; Beck et al., 2003; Nair, 2004; Rubuluza et al., 2007). The survival rates of shoot tips were affected by the concentration of colchicine and the duration of the treatment. High concentrations and longer duration reduced survival of shoot tips. According to Zhang and coworkers, the most efficient condition for inducing tetraploids was to treat shoot tips with 0.005% colchicine for 20 days, with 30% survival rate (Zhang et al., 2008). In the present study, the best condition for inducing tetraploids was to treat *C. palmate* and *L. sphaerica* seedlings that had been germinated for 6DT with 0.01 g/L colchicine for 24 h, with 70 and 68% survival rate, respectively.

There was a significant increase in polyploid formation as the colchicine concentration increased from 0.001 to 0.1 g/L (Table 2). Seedlings that were germinated for a longer period prior to colchicine treatment (3 and 6DT) produced a higher percentage of polyploidy than the ones that were germinated for one day (1DT). The increase in polyploid formation as the colchicine concentration increased from 0.001 to 0.1 g/L in the present study agrees with the reports obtained from (Ajalin et al., 2002). Tetraploids of *Phlox subulata* were induced successfully by treating shoot tips *in vitro* with colchicine. Tetraploids were obtained in all the concentrations used, but the percentage of tetraploids varied among different treatments, from 25 - 75% (Zhang et al., 2008).

In the Viola x wittrockiana, ploidy formation was 87% in apex treatment (Ajalin et al., 2002). In the present study, the average ploidy formation for both *C. palmate* and *L. sphaerica* was 98% (Table 2).

### The effect of irrigation frequency and incubation period on plant growth

#### *Shoot dry weight*

In both *C. palmate* and *L. sphaerica*, control plants that

**Table 1.** Effect of colchicine concentration on the length of radical and plumule of *C. palmata* and *L. sphaerica* seedlings at 14 days after incubation.

| Colchicine (g/L) | *C. palmate* (N = 20) | | | *L. sphaerica* (N = 20) | | |
|---|---|---|---|---|---|---|
| | 1DT | 3DT | 6DT | 1DT | 3DT | 6DT |
| **Length of radical (cm)** | | | | | | |
| Control | 79.50 ± .258d | 79.50 ± 1.258d | 79.50 ± 1.258d | 71.00 ± 1.932d | 71.00 ± 1.932d | 71.00 ± 1.932c |
| 0.001 | 16.83 ± 0.945c | 21.00 ± 0.258c | 20.33 ± 0.919c | 12.00 ± 0.258c | 15.00 ± 1.065c | 15.00 ± 0.258b |
| 0.01 | 10.83 ± 0.703b | 13.00 ± 0.258b | 15.67 ± 0.882b | 8.00 ± 1.065b | 10.00 ± 0.258b | 12.17 ± 1.195a |
| 0.1 | 5.00 ± 0.258a | 7.33 ± 0.918a | 12.00 ± 0.258a | 4.50 ± 0.342a | 5.00 ± 0.258a | 11.00 ± 0.258a |
| **Plumule length (mm)** | | | | | | |
| Control | 19.67 ± 0.882c | 19.67 ± 0.882d | 19.67 ± 0.882c | 14.0 ± 1.065c | 14.0 ± 1.065c | 14.0 ± 1.065b |
| 0.001 | 10.88 ± 0.601b | 12.00 ± 0.258b | 15.83 ± 0.601b | 8.0 ± 1.065b | 10.0 ± 0.258b | 12.0 ± 1.065b |
| 0.01 | 10.00 ± 0.258b | 14.33 ± 0.802c | 15.00 ± 0.258b | 9.0 ± 0.258b | 11.0 ± 1.065b | 11.0 ± 0.258a |
| 0.1 | 5.67 ± 0.557a | 7.00 ± 0.258a | 10.83 ± 0.601a | 4.0 ± 0.258a | 5.0 ± 0.258a | 10.5 ± 0.342a |

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Values that are followed by different letters within a column differ significantly ($P < 0.05$).

**Table 2.** Effect of colchicine concentration on survival and polyploidy formation in *C. palmate*, *L. sphaerica* seedlings at 30 days after treatment.

| Colchicine (g/L) | *C. palmate* (N = 20) | | | *L. sphaerica* (N = 20) | | |
|---|---|---|---|---|---|---|
| | 1DT | 3DT | 6DT | 1DT | 3DT | 6DT |
| **Survival (%)** | | | | | | |
| Control | 99.33 ± 0.33c | 99.33 ± 0.33d | 99.50 ± 0.34d | 98.17 ± 0.31d | 98.17 ± 0.31d | 98.17 ± 0.31d |
| 0.001 | 19.50 ± 0.89b | 70.17 ± 2.02c | 81.17 ± 0.95c | 17.17 ± 0.83c | 67.33 ± 2.28c | 78.17 ± 1.28c |
| 0.01 | 12.67 ± 1.31a | 53.67 ± 1.20b | 70.33 ± 1.98b | 11.17 ± 1.38b | 51.17 ± 1.28b | 67.67 ± 2.09b |
| 0.1 | 10.17 ± 0.87a | 18.33 ± 1.02a | 24.67 ± 0.95a | 8.00 ± 1.00a | 16.33 ± 1.02a | 22.17 ± 0.87a |
| **Polyploid (%)** | | | | | | |
| Control | 2.50 ± 0.56a | 3.67 ± 0.33a | 4.50 ± 0.43a | 0.17 ± 0.40a | 2.50 ± 0.43a | 2.67 ± 0.33a |
| 0.001 | 5.12 ± 0.87b | 13.67 ± 1.17b | 19.33 ± 1.15b | 0.83 ± 0.70a | 11.67 ± 1.17b | 17.33 ± 1.15b |
| 0.01 | 10.33 ± 0.99c | 81.33 ± 0.71c | 99.17 ± 0.31c | 0.67 ± 0.84b | 78.00 ± 0.89c | 94.67 ± 0.61c |
| 0.1 | 19.00 ± 1.10d | 89.66 ± 1.28d | 99.50 ± 0.34c | 16.50 ± 0.92c | 87.00 ± 1.26d | 97.00 ± 0.58d |

Values that are followed by different letters within a column differ significantly ($P < 0.05$).

were watered every 3rd day gave a significantly higher shoot dry weight than the colchicine treated plants. The colchicine treated plants that were germinated for 6 days (6DT) and watered every 10th day showed a significantly higher shoot dry weight than the controls. These findings agree with the report on work done in *Lolium perenne* and *Triticum aestivum* where tetraploids plants produced the best yield under drought stress (Sugiyama and Nikara 2004; Xiong et al., 2006). In both genera, 6DT was significantly higher than both 1 and 3DT in terms of shoot dry weight (Table 3). In other studies, colchicine-treated plants of *Sesame indicum* showed a higher shoot dry weight than control plants (Mensah et al., 2007). Polyploid leaves of *Gossypium arboretum* and *Citrus sinensis* were much larger than those of diploid plants ranging from 100 to 125% (Romero-Aranda et al., 1997; Wongpiyasatid et al., 2003; Rauf et al., 2006; Liu et al., 2007).

## Root dry weight

In both *C. palmate* and *L. sphaerica*, control plants that were watered every 3rd day showed a significantly higher root dry weight than in 3 and 6DT plants. Plants that were watered every 10th day showed a significantly high root dry weight in 6DT plants (Table 3). The same trend was obtained in studies of colchicine treated *Sesame indicum* plants (Mensah et al., 2007).

## Number of shoot apices

In *C. palmata* plants that were watered every 3rd day, 3DT showed a significantly higher number of shoot apices than the other treatments. *L. sphaerica* plants that were watered every 3rd day showed no significant difference between the treatments.

**Table 3.** Effects of colchicine concentration (0.01 g/L) on some agronomic characters of *C. palmate* and *L. sphaerica* at 120 days after planting.

| Parameter | | Control | 1DT | 3DT | 6DT |
|---|---|---|---|---|---|
| ***C. palmate* (N = 20)** | | | | | |
| Chlorophyll (mg/g leaf) | Watered 3rd day | 7.351 ± 0.092a | 7.775 ± 0.166b | 8.023 ± 0.113b | 9.382 ± 0.115c |
| | Watered 10th day | 5.173 ± 0.162a | 9.635 ± 0.108b | 10.465 ± 0.109c | 11.388 ± 0.072d |
| Dry wt of shoot (g/plant) | Watered 3rd day | 6.443 ± 0.477c | 1.707 ± 0.109a | 2.413 ± 0.188a | 3.617 ± 0.289b |
| | Watered 10th day | 2.413 ± 0.188b | 1.497 ± 0.117a | 3.162 ± 0.796b | 6.443 ± 0.477c |
| Dry wt of root (g/plant) | Watered 3rd day | 1.535 ± 0.222c | 0.340 ± 0.078a | 0.813 ± 0.100b | 0.525 ± 0.132a |
| | Watered 10th day | 0.517 ± 0.136a | 0.525 ± 0.132a | 0.798 ± 0.101a | 1.535 ± 0.222b |
| No. shoot apices/branch | Watered 3rd day | 2.00 ± 0.00a | 2.00 ± 0.00a | 2.83 ± 0.17b | 2.33 ± 0.33a |
| | Watered 10th day | 1.50 ± 0.22a | 2.50 ± 0.22a | 4.17 ± 0.17b | 4.83 ± 0.17c |
| ***L. sphaerica* (N = 20)** | | | | | |
| Chlorophyll (mg/g leaf) | Watered 3rd day | 8.232 ± 0.100a | 9.755 ± 0.200b | 10.182 ± 0.22b | 11.26 ± 0.072c |
| | Watered 10th day | 5.173 ± 0.162a | 10.245 ± 0.158b | 11.592 ± 0.048c | 12.697 ± 0.203d |
| Dry wt of shoot (g/plant) | Watered 3rd day | 9.293 ± 0.436c | 1.113 ± 0.048a | 1.188 ± 0.076a | 2.078 ± 0.041b |
| | Watered 10th day | 1.290 ± 0.026a | 2.842 ± 0.206b | 5.762 ± 0.271c | 9.293 ± 0.436d |
| Dry wt of root (g/plant) | Watered 3rd day | 0.840 ± 0.126b | 0.787 ± 0.335b | 0.368 ± 0.138a | 0.300 ± 0.057a |
| | Watered 10th day | 0.307 ± 0.057a | 0.782 ± 0.335b | 0.837 ± 0.122b | 0.840 ± 0.126b |
| No. shoot apices/branch | Watered 3rd day | 2.00 ± 0.00a | 2.00 ± 0.00a | 2.00 ± 0.00a | 2.00 ± 0.00a |
| | Watered 10th day | 1.50 ± 0.22a | 3.50 ± 0.22b | 4.50 ± 0.22c | 5.00 ± 0.00c |

Values that are followed by different letters within a row differ significantly ($P < 0.05$).

In both *C. palmate* and *L. sphaerica*, plants that were watered every 10th day gave a significantly higher number of shoot apices in 6DT plants (Table 3). Studies on egusi melon cultivar L5 (*Citrullus lanatus*) and other genera showed a significant increase in the number active shoot apices in tetraploids when compared with diploid plants (Idehen et al., 2006; Mensah et al., 2007; Rubuluza et al., 2007).

## Chlorophyll content

In both *C. palmate* and *L. sphaerica*, plants that were watered every 3rd day showed the lowest chlorophyll content in the controls and the highest in 6DT. The same trend was found in plants that were watered every 10th day (Table 3). These results agree with the findings of other researchers where colchicine treated plants such as *Sesame indicum*, *Triticum species*, *Citrus sinensis* and *Acacia mearnsii* showed a higher chlorophyll content than the controls (Kaminski et al., 1990; Romero-Aranda et al., 1997; Ajalin et al., 2002; Mathura et al., 2006; Mensah et al., 2007).

## Conclusions

The high rate of survival of colchicine treated seedlings suggest that this technique can be used to make drought resistant seedlings available to small scale rural farmers. These farmers are in remote areas of northern KwaZulu Natal, South Africa and have no access to irrigation. The high shoot dry weight, high chlorophyll content , large leaves and many active shoot apices obtained under water stress conditions suggests the suitability of these plants for growth in rural dry habitats. The utilization of colchicine-treated seedlings by rural farmers will ensure availability of leafy vegetables even during dry seasons and will improve food security.

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### REFERENCES

Ajalin I, Kobza F, Dolezel J (2002). Ploidy identification of doubled chromosome number plants in Viola x wittrockiana Gams. M1-generation. Hort. Sci. 29(1): 35-40.

Al Hakimi A, Monneveaux P, Nachit MM (1998). Direct and indirect selection for drought tolerance in alien tetraploid wheat x durum wheat crosses. *Euphytica* 100(1-3): 287-294.

Beck SL, Dunlop RW, Fossey A (2003). Evaluation of Induced Polyploidy in *Acacia mearnsii* Through Stomatal Counts and Guard Cell Measurements. S. Afr. J. Bot. 69(4): 563-567.

Choi HW, Lemaux PG, Cho MJ (2000). Increased chromosomal variation in transgenic versus nontransgenic barley (*Hordeum vulgare* L.) plants. Crop Sci. 40: 524-533.

Cohen D, Yao JL (1996). *In vitro* chromosome doubling of nine *Zantedeschia* cultivars. Plant Cell, Tissue Organ. Cult. 47: 43-49.

Dolezel J (1991). Flow cytometric analysis of nuclear DNA content in higher plants. Phytochem. Anal. 2: 143-154.

FAO (1988). Traditional Food Plants: A Resource Book for Promoting the Exploitation and Consumption of Food Plants in Arid, Semi-Arid and Sub-Humid lands of Eastern Africa. FAO Food Nutr. Paper 42. FAO. Rome.

Fock I, Collonnier C, Purwito A, Luisetti J, Souvannavong V, Vedel F, Servaes A, Ambroise A, Kodja H, Ducreux G, Sihachakr D (2000). Resistance to bacterial wilt in somatic hybrids between *Solanum tuberosum* and *Solanum phureja*. Plant Sci. 160: 165-176.

Fock I, Collonnier C, Luisetti J, Purwito A, Souvannavong V, Vedel F, Servaes A, Ambroise A, Kodja H, Ducreux G, Sihachakr D (2001). Use of *Solanum stenotomum* for introduction of resistance to bacterial wilt in somatic hybrids of potato. Plant Physiol. Biochem. 39: 899-908.

Fox FW, Norwood-Young ME (1982). Cucurbitaceae. *Food from the Veld.* Edible Wild Plants of Southern Africa: South African Institute for Medical Research. Cape Town, pp. 164-179.

Hansen DL, Lambertini C, Jampeetong A, Brix H (2007). Clone-specific differences in *Phragmites australis*: Effects of ploidy level and geographic origin. Aquat. Bot. 86(3): 269-279.

Hewitson J (2004). Question and Answer Archive: How Can I Quantify the Chlorophyll in Leaves? Science and Plants for Schools (SAPS). Web site: http://www-saps.plantsci.co.uk/records/rec1.htm

Hutchings A (1996). Cucurbitaceae. *Zulu* Medicinal Plants – An Inventory: 303-306. University of Natal. Pietermaritzburg.

Idehen EO, Kehinde OB, Adegbite AE (2006). Somatic chromosome counts and yield performance of some accessions of ‘egusi’ melon (*Citrullus lanatus*). Afr. J. Biotechnol. 5(22): 2049-2052.

Jacobs TV (2002). Underutilized edible plants from South Africa: a perspective. Managing Plant Genetic Diversity: IPGRI. University of Transkei. Umtata, pp. 371-337.

Kaminski A, Austin RB, Ford MA, Morgan CL (1990). Flag leaf anatomy of Triticum and Aegilops species in relation to photosynthetic rate. Ann. Bot. 66: 359-365.

Liu G, Li Z, Bao M (2007). Colchicine induced chromosome doubling in Platanus acerifolia and its effect on plant morphology. Euphytica 157(1-2): 145-154.

Martelotto LG, Ortiz JPA, Stein J, Espinoza F, Quarin CL, Pessino SC (2005). A comprehensive analysis of gene expression alterations in a newly synthesized *Paspalum notatum* autotetraploidy. Plant Sci. 169: 211-220.

Mathura S, Fossey A, Beck SL (2006). Comparative study of chlorophyll content in diploid and tetraploid black wattle (Acacia mearsii). Forest J. 79(4): 381-388.

Mensah JK, Obadoni BO, Akomeah PA, Ikhajiagbe B Ajibolu J (2007). The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indica* L). Afr. J. Biotechnol. 6(5): 534-538.

Nair RM (2004). Developing tetraploid ryegrass (*Lolium perenne* L) populations. New Zealand J. Agric. Res. 47: 45-49.

Nouri-Ellouz O, Gargouri-Bouzid R, Sihachakr D, Triki MA, Ducreux G, Drira N, Lakhoua L (2006). Production of potato intraspecific somatic hybrids with improved tolerance to PVY and *Pythium aphanidermatum*. J. Plant Physiol. 163: 1321-1332.

Peters CR, O’Brien EM, Drummond RB (1992). Edible Wild Plants of SubSaharan Africa: Royal Botanic Gardens. KEW. pp. 98-103.

Pooley ES (1998). Cucurbitaceae. A Field Guide to Wild Flowers of Kwazulu-Natal and the Eastern Region: Natal Flora Publication Trust. Durban. 78(81): 558-559.

Rauf S, Khan IA, Khan FA (2006). Colchicine induced tetraploidy and changes in allele frequency in colchicine-treated populations of diploids assessed with RAPID markers in *Gossypium arboretum* L. Turk. J. Biol. 30: 93-100.

Romero-Aranda R, Bondada BR, Syvertsen JP, Grosser JW (1997). Leaf characteristics and net gas exchange of diploid and autotetraploid *Citrus*. Annals Bot. 79: 153-160.

Rubuluza T, Nikolova RV, Smith MT, Hannweg H (2007). *In vitro* induction of tetraploids in *Colophospermum mopane* by colchicine. S. Afr. J. Bot. 73(2): 259-261.

Shao J, Chen C, Deng X (2003). *In vitro* induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell, Tissue Organ Cult. 75(3): 241-246.

Sliwinska E, Lukaszewska E (2005). Polysomaty in growing in vitro sugar-beet (*Beta vulgaris* L.) seedlings of different ploidy level. Plant Sci. 168: 1067-1074.

Sreekumari MT, Jos JS, Nair SG (1999). ‘Sree Harsha’: A superior triploid hybrid in cassava. Euphytica 106(1): 1-6.

Sugiyama S, Nikara C (2004). Differential contribution of avoidance and tolerance to dehydration resistance in populations of perenntial ryegrass, Lolium perenne. Austr. J. Agric. Res. 55(10): 33-37.

Takamura T, Miyajima I (1996). Colchicine induced tetraploid in yellow-flowered cyclamens and their characteristics. Sci. Horticult. 65: 305-312.

Väinölä A (2000). Polyploidization and early screening of *Rhododendron* hybrids. Euphytica 112(3): 239-244.

Wongpiyasatid A, Hormchan P, Rattanadilok N (2003). Preliminary test of polyploidy induction in cotton (Gossypium arboretum) using colchicine treatment. Kasetsart J. Nat. Sci. 37: 27-32.

Xiong YC, Li FM, Zhang T (2006). Performance of wheat crops with different chromosome ploidy: root-source signals, drought tolerance, and yield performance. Planta 224(3): 710-718.

Yang YF, Furuta Y, Nagata SI, Watanabe N (1999). Tetra Chinese spring with AABB genomes extracted from the hexaploid common wheat, chinese spring. Genes. Genet. Syst. 74: 67-70.

Zhang Z, Dai H, Xio M, Lui X (2008). *In Vitro* induction of tertaploids in *Phlox subulata*. J. Euphytica 159(1,2): 59-65.

Zobolo AM, Mkabela QN (2006). Traditional knowledge transfer of activities practiced by Zulu women to manage medicinal and food plant gardens. Afr. J. Range Forage Sci. 23(1): 77-80.