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Effect of Phytohormones on Shoot Proliferation of Doubled Haploid Lines of African Marigold (*Tagetes erecta* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In vitro propagation of marigold has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. Shoot proliferation is a very important phase of micropropagation. This paper describes an efficient protocol for *In vitro* micropropagation of Doubled haploid lines of marigold (*Tagetes erecta* L.) using nodal explants. Murashige and Skoog medium (MS) supplemented with various concentrations and combinations of Benzylaminopurine (BAP), Naphthylacetic acid (NAA), Gibberellic acid (GA3) and Thidiazuron (TDZ) were employed for shoot regeneration in this study, maximum percent sprouting (100%) and minimum days to sprout break (1.48) were found in MS media supplemented with BAP 2mg/L + NAA 0.05mg/L + GA3 1mg/L, while maximum number of sprouts (1.73) and maximum average shoot length (3.98cm) were observed in case of media supplementation with BAP 1mg/L + NAA 0.05mg/L + GA3 0.5mg/L. However, in case of Thidiazuron (TDZ) minimum days taken for shoot

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proliferation (2.66) and maximum number of shoots per micro shoot (7.04) were observed in media supplemented with BAP 1mg/L + NAA 0.1mg/L + TDZ 0.5mg/L, while maximum length of shoots (4.2cm) was observed in control devoid of any hormones.

Keywords: Marigold; doubled haploids; Thidiazuron (TDZ); Gibberellic acid (GA3).

1. INTRODUCTION

Marigold (*Tagetes* spp.) is one of the economic ornamental crops grown worldwide. It is gaining popularity on account of its free flowering habit, short duration, extensive use as loose flower for making garlands, beautification, [1], mosquito and nematode repellent [2], as a feed additive for poultry industry [3] against menstrual irregularities etc. [4]. The pharmacological activity of marigold is related to the content of several secondary metabolites *viz* flavonoids [5]. There are about 33 species in genus *Tagetes* [6], out of which, *Tagetes erecta* L. (African marigold) and *Tagetes patula* L. (French marigold) are highly important for loose flower production.

Doubled haploids (DH's) are produced by the process of chromosome doubling of the haploids. Though haploidy was identified much earlier [7] and attempted in commercial crop improvement [8], it was not until the work of several researchers [9; 10; and 11] that the potential of anther culture to create haploid plants revived plant breeders' interest. *In vitro* production of doubled haploids has been successfully done in crops like tall fescue [12]; sugar beet [13], African violet ([14], *Pelargonium roseum* [15], *Lilium davidii* var. Willmottiae [16], *Lilium longiflorum* [17], *Narcissus tazetta* [18] etc.

Earlier, few workers demonstrated techniques of multiplication of marigold through shoot tip and axillary bud proliferation [19, 20, 21 and 22]. There are several published studies describing successful protocols for micropropagation of marigold [23, 24 and 25] however, thus far no research has been carried out specifically on micropropagation of Doubled haploid lines of African marigold. Doubled haploid lines once developed must be multiplied in a large scale to carry out further experiments. Hence, to obtain a high multiplication ratio and a good quality of micro-shoots, a comprehensive propagation protocol was developed in this study.

2. MATERIALS AND METHODS

African marigold variety Local orange was used for the present study. Experiments involved buds in the size range of 2-2.5cm [23] which were thoroughly washed with tap water and sterilized with 70% ethanol and were pretreated 0.3M mannitol solution for 4 days [26]. After which, excised anthers were cultivated as per the study [23]. Shoots thus obtained were assessed for ploidy analysis as per the protocol given [23]. Haploid shoots were further taken for chromosome doubling by using colchicine [23] and this way doubled haploid shoots of marigold were obtained from which nodal segments measuring 2–2.5 cm in length were excised. The explants were pre-treated with carbendazim (0.2%) + metalaxyl (0.2%) + 8-hydroxy quinoline citrate (200 mg/l) on a horizontal shaker (100 rpm) for 60 minutes and surface sterilized using $HgCl_2$ (0.1%) for 3 minutes under laminar air-hood.

The preparation of media used for inoculation of nodal segments, was done using the standard MS salts. The solidification of the media was done with 0.8% agar (w/v). The explants were inoculated in culture tube (15 cm in height × 2.5 cm in diameter) containing 20mL of solid media and closed with a polypropylene cap. All cultures were maintained at 60%–65% relative humidity and 25℃ temperature.

Two sets of media combinations were used for multiplication; 1. MS medium supplemented with BAP at 1, 2, 3, 4, 5 mg/L plus NAA at 0.05 mg/L plus GA3 at 0.5 and 1 mg/L. 2. MS medium supplemented with BAP at 0.5, 1 mg/L plus NAA at 0.1 mg/L plus TDZ at 0.5 and 1 mg/L. control in every case consisted of zero phytohormones.

3. RESULTS AND DISCUSSION

Gibberellic Acid (GA_3) is a tetracyclic diterpenoid compound and a plant hormone stimulating plant growth and development, which trigger transitions from meristem to shoot growth, [27]. In our experiments GA_3 was used in combination with BAP and NAA in several combinations. It is clearly evident from the Table 1 that, maximum (100%) percent sprouting was recorded on MS medium supplemented with BAP 2mg/L + NAA 0.05mg/L + GA_3 1mg/L, (Fig. 1) followed by 93.32 % sprouting in media composed of MS medium supplemented with BAP 3mg/L + NAA 0.05mg/L + GA_3 0.5mg/L. GA_3 was found to be more

effective at higher concentrations of 1mg/L, while increasing the concentration of BAP from 2 to 5 mg/L highly reduced the percent sprouting from 100 % to 33.3 %. In case of days to sprout break, minimum days to sprout break (1.48) were taken when nodal segments were inoculated in MS media supplemented with BAP 2mg/L + NAA 0.05mg/L + GA_3 1mg/L followed by 2.14 in media supplemented with BAP 2mg/L + NAA 0.05mg/L + GA_3 0.5mg/L and BAP 3mg/L + NAA 0.05mg/L + GA_3 0.5mg/L. Maximum number of sprouts (1.598) were obtained when inoculation was done in MS media supplemented with BAP 1mg/L + NAA 0.05mg/L + GA_3 1mg/L (Fig 3) followed by 1.530 in MS media supplemented with BAP 3mg/L + NAA 0.05mg/L + GA_3 0.5mg/L. Higher concentrations of BAP highly effected the days to sprout break and number of sprouts per nodal segment. In case of average shoot length maximum shoot length (3.978cm) was obtained when inoculation was done in MS media supplemented with BAP 1mg/L + NAA 0.05mg/L + GA_3 0.5mg/L (Fig 5) followed by 2.078cm in media supplemented with BAP 2mg/L + NAA 0.05mg/L + GA_3 0.5mg/L. The above observations showed that increasing the concentration of BAP proved to be ineffective in case of shoot proliferation while GA_3 was much effective at a highest concentration of 1mg/L. All pre-treatments gave significantly better response compared to control. These findings are in close confirmation with earlier results reported by Kumar et al. [23] who reported that shoot tip explants of marigold cultured on MS medium supplemented with 2 mg/l BAP gave maximum axillary bud sprouting. 10.0 mg/l BAP and 0.2 mg/l IAA produced maximum shoots in proliferation. Gupta et al. [21] showed that maximum establishment was recorded when shoot tips cultured on MS + 10 µM BAP + 2 µM NAA. Highest shoot proliferation was reported in MS + 10 µM BAP + 2 µM NAA. Majumder *et al*. [22] observed highest proliferation when the shoot tips were transferred to MS + 2.0 mg/l BAP + 0.1 mg/l NAA + 0.5 mg/l GA3. Many researchers reported plant regeneration in marigold from different explant sources *viz*., from leaf [28, 29, 30], Misra and Dutta [29] developed a protocol for differentiation of shoot buds directly from leaf segments of white marigold (*Tagetes erecta* L.) in their experiments shoot buds were induced directly in MS medium supplemented with 14.43 mM GA and 4.44 mM 6-benzyladenine in the absence of any auxin.

Thidiazuron was first reported to have cytokinin activity in 1982. Since then, it has been used successfully *in vitro* to induce adventitious shoot formation and to promote axillary shoot proliferation [31], in *in vitro* propagation of plants including medicinal and horticultural crops [32] and for the micropropagation of several plant species [33].

In our experiments, it is clear from the Table 2 that, minimum days taken for shoot proliferation 2.460 was observed in MS media supplemented with BAP 1mg/L + NAA 0.1mg/L + TDZ 0.5mg/L which was followed by 2.680 in media supplemented with BAP 0.5mg/L + NAA 0.1mg/L + TDZ 1mg/L (Fig 2). In case of average number of shoots per micro shoot, maximum number of shoots (7.040) were obtained in MS media supplemented with BAP 1mg/L + NAA 0.1mg/L + TDZ 0.5mg/L (Fig 4) followed by 5.240 in control with no hormones. In case of mean length of

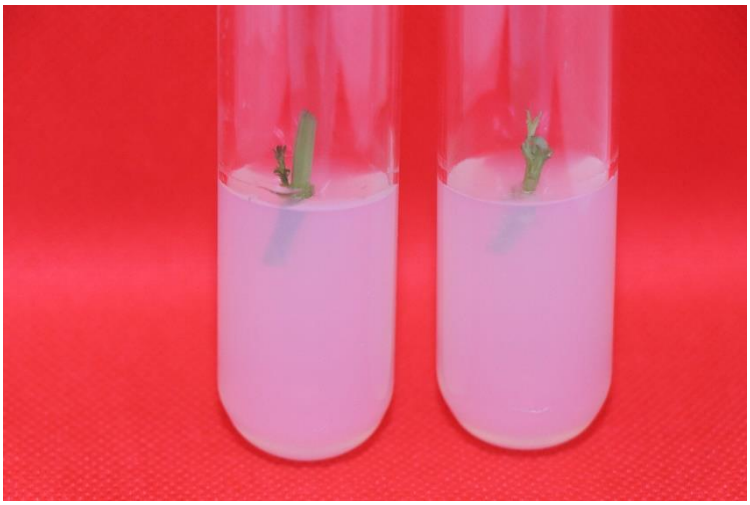

Fig. 1. Sprouting in media supplemented with BAP 2mg/L + GA_3 Img/L

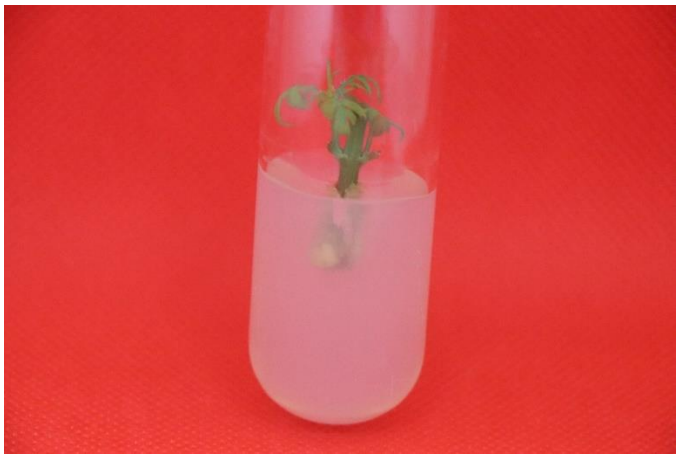

Fig. 2. Sprouting in media supplemented with BAP 1mg/L + NAA 0.1mg/L + TDZ 05mg/L

Fig. 3. Number of shoots per nodel segment in media supplemented with BAP 1mg/L + NAA 0.05mg/L + GA_3 05mg/L

Fig. 4. Number of shoots per nodel segment in media supplemented with BAP 1mg/L + NAA 0.1mg/L + TDZ 0.5mg/L

shoots maximum length (4.200cm) was observed when nodal segments were inoculated with MS media supplemented with no hormones (control) followed by 3.400cm in media supplemented with BAP 0.5mg/L + NAA 0.1mg/L + TDZ 1mg/L, (Fig 6). From the above observations it was clear that

TDZ is more effective at a lower concentration of 0.5mg/L. higher concentrations of TDZ tend to increase the number of days taken for shoot proliferation and reduce the average number of shoots per micro shoot. Furthermore, TDZ had harmful effect on the shoot length where maximum length was observed in control devoid of TDZ.

In their experiments conducted by Deepa, A.V *et al*, [32], TDZ was found to be more effective in multiple shoot induction, as compared to other cytokinin's. In some systems the synergistic effect of TDZ with other cytokinin/auxin was found more effective than using TDZ alone. In other experiments on blueberry conducted by Roberto Cappelletti *et al*, [34], they found out that the addition of both 0.2 mg L−1 or 0.5 mg L−1 of TDZ in the medium led to improved callus formation. The addition of 15 mg L−1 of 2iP in the same medium promoted blueberry stem elongation. Singh P and Dwivedi P [35] used varying concentrations of cytokinin's, supplemented in the nutrient media of *Stevia rebaudiana* Bertoni, to observe their effects on shoot development. Their results showed that best response was observed in the TDZ (0.5 mg/l). Certain experiments were carried out by Lu, C Y [31] on carnation and rose, the results confirmed that the highest number of shoots per explant was observed in carnation media supplemented with TDZ. While in case of rose TDZ was found to be most effective cytokinin giving 100% shoot formation and an average of 2.3 shoots per explant. In yet another experiment conducted by Barna, K.S., Wakhlu, A.K [36] on effect of TDZ on tissue culture of rose, maximum number of micro shoots per shoot tip explant were obtained on MS medium supplemented with 5-20 µM TDZ.

Fig. 5. Avearge shoot length (cm) in media supplemented with BAP 1mg/L + NAA 0.05mg/L + GA_3 0.5mg/L

Fig. 6. Avearge shoot length(cm) in media supplemented with BAP 1mg/L + NAA 0.1 mg/L + TDZ 0.5mg/L

Table 1. Effect of different concentrations of BAP, NAA in combination with GA_3 on shoot proliferation of double haploid line of African marigold

Treatment (s)	Percent sprouting (%)	Days to sprout break	Number of sprouts	Average shoot length (cm)
T^0 (BAP 0mg/L + NAA 0mg/L + GA_3 0mg/L)	22.200 (28.083) ±0.860	5.218±0.219	0.132±0.081	0.412±0.223
T1 (BAP 1mg/L + NAA 0.05mg/L + GA_3 0.5mg/L)	66.500 (57.777) ±10.547	2.426±0.750	1.730±0.221	3.978±0.311
T2 (BAP 1mg/L + NAA 0.05mg/L + GA_3 1mg/L)	93.320 (82.935) ±6.680	2.180±0.398	1.598±0.266	1.430±0.189
T3 (BAP 2mg/L + NAA 0.05mg/L + GA_3 0.5mg/L)	93.320 (82.935) ±6.680	2.140±0.398	1.130±0.170	2.078±0.328
T4 (BAP 2mg/L + NAA 0.05mg/L + GA_3 1mg/L)	100.000 (90.000) ±0.000	1.480±0.235	1.528±0.081	1.182±0.187
T5 (BAP 3mg/L + NAA 0.05mg/L + GA_3 0.5mg/L)	93.320 (82.935) ±6.680	2.140±0.382	1.530±0.170	0.937±0.163
T6 (BAP 3mg/L + NAA 0.05mg/L + GA_3 1mg/L)	73.320 (64.935) ±19.438	2.640±0.331	1.132±0.326	0.397±0.111
T7 (BAP 4mg/L + NAA 0.05mg/L + GA_3 0.5mg/L)	79.980 (71.981) ±13.343	3.000±0.207	0.996±0.235	0.542±0.185
T8 (BAP 4mg/L + NAA 0.05mg/L + GA_3 1mg/L)	79.960 (68.804) ±8.181	3.540±0.201	1.064±0.163	0.594±0.107
T9 (BAP 5mg/L + NAA 0.05mg/L + GA_3 0.5mg/L)	59.940 (50.784) ±6.660	4.026±0.361	0.996±0.235	0.350±0.097
T10 (BAP 5mg/L + NAA 0.05mg/L + GA_3 1mg/L)	33.300 (35.230) ±0.000	5.026±0.300	0.530±0.290	0.724±0.253
±SE(m)	9.110	0.374	0.217	0.210
C.D. (P≤ 0.05)	26.053	1.071	0.621	0.599

**Values in parenthesis are angular values*

Table 2. Effect of different concentrations of BAP, NAA in combination with TDZ on proliferation of double haploid line of African marigold

Treatment(s)	Days taken for shoot proliferation	Av. No. of shoots per micro shoot	Mean length of shoots (cm)
T^0 (BAP 0mg/L + NAA 0mg/L + TDZ 0mg/L)	4.100±1.027	5.240±0.103	4.200±0.071
T^1(BAP 0mg/L + NAA 0.1mg/L + TDZ 0.5mg/L)	4.160±0.081	4.200±0.071	2.200±0.071
T^2(BAP 0mg/L + NAA 0.1mg/L + TDZ 1mg/L)	3.400±0.851	4.400±0.071	2.400±0.071
T^3(BAP 0.5mg/L + NAA 0.1mg/L + TDZ 0.5mg/L)	3.100±0.063	5.200±0.071	3.300±0.071
T^4(BAP 0.5mg/L + NAA 0.1mg/L + TDZ 1mg/L)	2.680±0.678	5.400±0.071	3.400±0.071
T^5(BAP 1mg/L + NAA 0.1mg/L + TDZ 0.5mg/L)	2.460±0.244	7.040±0.051	1.480±0.373
T^6(BAP 1mg/L + NAA 0.1mg/L + TDZ 1mg/L)	4.520±0.250	2.000±0.837	0.940±0.414
±SE(m)	0.582	0.324	0.219
C.D. (P≤ 0.05)	N/A	0.942	0.638

**Values in parenthesis are angular values*

In one of the research, on micropropagation of tea (*Camellia sinensis* (L.) O. Kuntze) using nodal segments as explant, The results showed that the best treatment for nodal segment multiplication in terms of the number of shoots per explant and shoot elongation was obtained using 3 mg/L BAP in combination with 0.5 mg/L GA_3. TDZ was found to be inappropriate for multiplication of tea clone Iran 100 as it resulted in hyperhydricity especially at concentrations higher than 0.05 mg/L [37].

4. CONCLUSION

Shoot proliferation is a very important phase of micropropagation. This study describes an efficient protocol for *in vitro* micropropagation of Doubled haploid lines of marigold (*Tagetes erecta* L.) using nodal explants. Several concentrations and combinations of Benzylaminopurine (BAP), Naphthylacetic acid (NAA), Gibberellic acid (GA3) and Thidiazuron (TDZ) were employed for shoot regeneration in this study. It was concluded that phytohormones showed positive effect on several aspects including percent sprouting, days to sprout break, number of sprouts per explant and average shoot length etc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta P, Vasudeva N. Marigold: A potential ornamental plant drug. Hamdard Medicus. 2012;55(1):45-59.
2. Wanga Koon-Hui, Hooksa Cerruti R, Ploeg Antoon. Protecting crops from nematode pests: using marigold as an alternative to chemical nematicides. Plant Disease. 2007;1-6.
3. Nuraini Mirzah, Ade D. Marigold Flower Extract as a Feed Additive in the Poultry Diet: Effects on Laying Quail Performance and Egg Quality. International Journal of Poultry Science. 2017;16:11-15.
4. Wichtl M. Herbal drugs and phytopharmaceuticals. Stuttgart: Medpharm Scientific Publisher. 199; 446.
5. Vidal-Ollivier E, Elias R, Faure F, Babadjamian A, Crespin F, Balansard G, Boudon G. Flavonal Glycosides from Calendula officinalis flowers. Planta Medica. 1989;55:73–74.
6. Rydberg PA. Tagetes, North Am. Flora. 1915;34:148-159
7. Blakeslee AF, Belling J, Farnham ME, Bergner AD. A haploid mutant in the Jimson weed, Datura stramonium. Science. 1922;55:646-647.
8. Chase SS. Monoploids and monoploid derivatives of maize. Bot. Rev. 1969;35:117–167.
9. Guha S and Maheshwari SC. *In vitro* production of embryos from anthers of Datura. Nature. 1964;204:497
10. Nitsch JP, Nitsch C. Haploid plants from pollen grains. Science. 1969;163:85-87.
11. Kasha KJ, Kao KN. High frequency haploid production in barley (*Hordeum vulgare* L.). Nature. 1970;225:874–876.
12. Kasperbaeur MJ and Eizenga GC. Tall fescue doubled haploids via tissue culture and plant regeneration. Crop Science. 1985;25: 1091-95.
13. Klimek-Chodacka M, Baranski R. Comparison of haploid and doubled haploid sugar beet clones in their ability to micro propagate and regenerate. Electronic Journal of Biotechnology. 2013; 16(2):1-10.
14. Hughes KW, Bell SL and Caponetti JD. Anther derived haploids of the African violet. Canadian Journal of Botany. 1975;53(14):1442-1444.
15. Kato M, Suga T, Tokumasu S. Effect of 2, 4-D and NAA on callus formation and haploid production in anther culture of *Pelargonium roseum*. Memoirs of the College of Agriculture, Ehime University. 1980;24(2):199-207.
16. Gu ZP and Cheng KC. Studies on induction of pollen plantlets from the anther cultures of lily. Acta Botanica Sinica. 1982;24:28-32.
17. Qu Y, Mok MC, Mok DW, Stang JR. Phenotypic and cytological variation among plants derived from anther cultures of *Lilium longiflorum*. *In vitro* Cellular And Developmental Biology. 1988;24(5):471-476.
18. Chen L, Zhu X, Gu L, Wu J. Efficient callus induction and plant regeneration from anther of Chinese narcissus (*Narcissus tazetta* L. var. Chinensis Roem). Plant Cell Reports. 2005;24(7): 401-407.
19. Misra P, Datta SK. *In vitro* maintenance of F_1 hybrid. Current Science. 2000;78(4): 383-385.

20. Kumar A, Raghava SPS, Singh SK, Misra RL. Micropropagation of male sterile marigold plants for F1 hybrid seed production. Indian Journal of Ornamental Horticulture. 2003;6(1):1-6.
21. Gupta YC, Vaidya P, Dhiman SR, Sharma P. *In vitro* propagation and maintenance of genetic male sterility in marigold. Progressive Horticulture. 2013;45(1):152-159.
22. Majumder J, Singh KP, Singh SK, Prasad KV, Verma M. *In vitro* morphogenesis in marigold using shoot tip as explant. Indian Journal of Horticulture. 2014;71(1):82-86.
23. Kumar A. 2002. *In vitro* production of male sterile lines of marigold (*Tagetes erecta* L.) for F_1 hybrid seed production. Ph.D. thesis submitted to Indian Agricultural Research Institute, New Delhi-12.
24. Manjusha V, Majumder J, Shiv KS, Prasad, KV, Kanwar PS. *In vitro* morphogenesis in marigold using shoot tip as explant. Indian Journal of Horticulture. 2015;71:82-86.
25. Shumaila K, Jaskani M, Muhammad ZI, Rafiq A, Muhammad ZS, Ali S, Huma, B. Effects of Growth Regulators on Micropropagationand callogenesis of Marigold. Basic Research Journal of Agricultural Science and Review. 2016;5(1).
26. Mehraj U, Singh KP, Kumar G and Panwar S. Influence of anther pretreatment on the efficiency of haploid production in marigold (*Tagetes erecta* L.), AMA, Agricultural Mechanization in Asia, Africa and Latin America. 2022;54(11):16287-16295.
27. Pradeep CM, Kumar SY, Chandrashekar GB, Kavana BV, Supriya. A review on role and use of gibberellic acid (GA3) in flower production. International Journal of Chemical Studies. 2020;8:3076-3084.
28. Kothari SL and Chandra N. *In vitro* propagation of African marigold. Hort Science., 1984;19:703-705.
29. Misra P and Datta SK. Direct differentiation of shoot buds in leaf segments of white marigold (*Tagetes erecta* L.). In Vitro Cellular and Development Biology. 2001;37:466-470.
30. Qi Y, Ye Y, Manzhu Bao. Establishment of plant regeneration system from anther culture of *Tagetes patula*. African Journal of Biotechnology. 2011;10 (75):17332-17338.
31. Lu CY. The use of thidiazuron in tissue culture. In Vitro Cell Dev Biol – Plant. 1993;29: 92–96.
32. Deepa AV, Anju M, Dennis Thomas T. The Applications of TDZ in Medicinal Plant Tissue Culture. In: Ahmad N, Faisal M. (eds); Thidiazuron: From Urea Derivative to Plant Growth Regulator. Springer, Singapore; 2018.
33. Mazri MA, Belkoura I, Meziani R. Use of TDZ for Micropropagation of Some Mediterranean Crop Species. In: Ahmad, N, Faisal M, (eds) Thidiazuron: From Urea Derivative to Plant Growth Regulator. Springer, Singapore; 2018.
34. Roberto Cappelletti, Silvia Sabbadini, Bruno Mezzetti. The use of TDZ for the efficient *in vitro* regeneration and organogenesis of strawberry and blueberry cultivars, Scientia Horticulturae. 2016;207: 117-124.
35. Singh P, Dwivedi P. Two-stage culture procedure using thidiazuron for efficient micropropagation of *Stevia rebaudiana*, an anti-diabetic medicinal herb. 3 Biotech. 2014;4(4): 431-437.
DOI: 10.1007/s13205-013-0172-y
36. Barna KS, Wakhlu AK. Effects of thidiazuron on micropropagation of rose. In Vitro Cell Dev Biol – Plan.t. 1995;31:44–46.
37. Reza Azadi Gonbad, Uma Rani Sinniah, Maheran Abdul Aziz, Rosfarizan Mohamad. Influence of Cytokinins in Combination with GA_3 on Shoot Multiplication and Elongation of Tea Clone Iran 100 (*Camellia sinensis* (L.) O. Kuntze), The Scientific World Journal. 2014;1-9.

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