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Standardization of Protocol for Mass Proliferation and Rooting of Gynogenically induced Regenerants in African Marigolds (*Tagetes erecta* L.)

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ABSTRACT: *In vitro* gynogenesis has been widely used for the induction of haploids and doubled haploids in many petaloid-type genotypes of marigold. The *in vitro* mass multiplication and rooting of gynogenically induced shoots is still the hardest part of their production. The high percentage of mortality in gynogenically induced shoots encountered during proliferation, rooting, and hardening, often limits their wider utility in the breeding of marigold. Therefore, in the present study efforts have been made to develop an efficient protocol for *in vitro* shoot proliferation and rooting of gynogenically induced regenerates of African marigold. Micro-shoots of 2-3 cm in length from healthy gynogenically induced shoots of genotypes 'DAMH-24' and 'DAMH-55' were cultured on modified MS medium supplemented with different concentrations of cytokinins and auxins. The minimum days required for shoot emergence and maximum increase in fresh weight of cultured shoots were observed on modified MS medium enriched with BAP (0.5 mgl^{-1}), the longest shoot length and the number of leaf pairs per branch were recorded on MS medium fortified with 1.0 mgl^{-1} BAP. The maximum increase in the number of micro-shoots from gynogenically induced shoots was recorded on MS medium supplemented with KIN (0.25 mgl^{-1}). The earliest rooting, highest rooting percent, better root growth character, and highest number of roots per micro-shoots were recorded on the treatment comprising of ½ MS medium supplemented with NAA (0.5 mgl^{-1}). Among both the genotypes 'DAMH-24' responded better than 'DAMH-55' during *in vitro* proliferation and rooting of gynogenically induced shoots. This protocol is highly useful for the development of plants for further hardening after rooting with cutinized, strengthened shoots from *in vitro* gynogenically developed plantlets, which will further help in development of high-yielding F1 hybrids.

Keywords: *Tagetes erecta* L., *In vitro* proliferation, Rooting, Gynogenesis, Growth regulators.

INTRODUCTION

Marigold (*Tagetes* spp.) is the most important flower crop among the loose flowers; it ranks first in terms of both area and production among all flower crops grown in India. It has been growing in an area of 66.13 thousand hectares, with a production of 603.18 thousand metric tonnes (Anonymous, 2017). It belongs to the Asteraceae family and is native to South and Central America, specifically Mexico. Among the 33 species documented in the genus *Tagetes*, only two species namely, *T. erecta* L. (African marigold) and *T. patula* L. (French marigold) are being grown commercially worldwide. The flowers of African marigold are mostly used for the preparation of wreaths, garlands, and ornamental flower decorations in religious rituals and ceremonies (Kumar *et al*., 2017). Besides, they are also used for garden display, landscape use, and cut flower production. They are also in great demand for culinary use (in the food industry as a source of dye and Vitamin-A substitute due to the presence of carotenoid), poultry industrial (for strengthening of yolk and skin colour), and medicinal purposes (Dwyer *et al*., 2001). It is commercially propagated through seeds, but ornamentally valuable petaloid-types sterile lines can also be maintained through vegetative propagation (Kumar *et al*., 2018) and tissue culture. The demand for F_1 hybrids is extensively increasing in this crop due to their uniform, early, and synchronous flowering. To exploit the hybrid vigor through F_1 development and to maintain the hybrid uniformity, the development of completely homozygous lines is the basic prerequisite. The development of completely homozygous inbreds through conventional techniques of selfing is a tedious and time-consuming process, hence induction of

haploids and their subsequent diploidization is frequently used. *In vitro* induction of maternal haploids through gynogenesis is the only suitable option for haploid induction in double-flowered petaloid-type sterile genotypes of marigolds. Gynogenesis involves the induction of haploids by the culture of unfertilized ovaries and their subsequent diploidization. The resultant doubled haploid (DH) lines can be directly used as new cultivars or as a parental line to produce high-yielding F1 hybrids (Kumar *et al*., 2020).

Gynogenesis has been used for the induction of haploidy in a few cultivars of marigold. The albinism and abnormal growth of gynogenically induced shoots is a huge concern during the production of haploid and doubled haploids in marigold. The high percentage of mortality in gynogenically induced shoots encountered during proliferation, rooting, and hardening, often limits the wider utility of haploids and doubled haploids in the breeding of marigold. The modification of culture media can play an important role in improving the vigor of gynogenically induced shoots during multiplication and rooting. Hence, the present investigation was undertaken to optimize the growth regulators for mass multiplication and rooting of *in vitro*-induced gynogenic shoots of African marigold genotypes.

MATERIALS AND METHODS

The present study was carried out during 2021–2022, in the tissue culture laboratory of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, 110012. The gynogenically induced shoots from two marigold genotypes, DAMH-24, and DAMH-55 were utilized to undertake this study. The unfertilized ovary culture technique was employed for the induction of haploids and gynogenesis in marigold. The gynogenically induced micro-shoots, 2-3 cm in length were used in various experiments.

For *in-vitro* shoot proliferation, a fortified MS medium comprising of MS basal salts, 0.25 mgl^{-1} GA_3, 0.1mgl^{-1} NAA, 125 mgl^{-1} PVP, 40 gl^{-1} Sucrose, 8 gl^{-1} agar was utilized as basal media to undertake various experiments. The effects of varying concentrations (0.25 to 1.0 mgl^{-1}) of two different cytokinins *viz*., Benzyl amino purine (BAP) and Kinetin (KIN) were evaluated for *in vitro* growth and proliferation of gynogenically induced shoots. For rooting, the medium consisting of ½ MS (Murashige and Skoog 1962), 125mgl^{-1} PVP, 45gl^{-1} Sucrose, and 8 gl^{-1} agar supplemented with different concentrations (0.25 to 1.0) of auxins, *viz.*, Indole Butyric Acid (IBA) and 1-Naphthalene Acetic Acid (NAA). Prior to autoclaving, the pH of the media was adjusted to 5.7. The gynogenically induced micro-shoots measuring 2 to 3 cm in length were cultured on different hormone-fortified media for shoot proliferation and rooting. In each treatment, 3 replications were used and three culture vessels with three shoots in each vessel was used for each replication. All the culture vessels were incubated at 25±2°C. With the aid of four cool white fluorescent tubes fixed to the top of each culture rack, illumination was adjusted to 47µmol/m²/s photosynthetic photon flux intensity and a photoperiod of 16:8 (light: dark) hours was maintained inside the lab.

The experiment was conducted using a Completely Randomized Design (CRD). For each treatment, there were three replicates. Opstat software was used to analyze the recorded data and the data were subjected to standard analysis of variance (ANOVA) to test significance among different lines (Anonymous, 2022). Each experiment was repeated at least twice, and the reported data are the means of two experiments. The percentage data were subjected to an Arc Sine transformation.

RESULTS and DISCUSSION

A. In-vitro shoot proliferation of gynogenically induced micro-shoots

One of the key steps in haploid plant production is the proliferation of quality shoots through rapid multiplication. An appropriate ratio of cytokinins and auxins is the key factor to obtain maximum shoot proliferation clearly depicted in Table 1. MS media fortified with different levels of BAP and KIN significantly reduced the number of days required for shoot emergence from gynogenically derived micro-shoots. The minimum number of days (5.00 days) required for shoot emergence was observed in the treatment comprising of modified MS medium enriched with 0.5 mgl^{-1} BAP, which was at par with the medium containing 1.0 mgl^{-1} KIN (6.67 days) and significantly earlier than the treatment with 0.25 mgl^{-1} BAP (8.00 days). Among the two genotypes, the earliest shoot emergence (8.48 days) was recorded in DAMH-55, which was found to be earlier than DAMH-24 (8.57 days). The interaction between genotypes and treatments were found to be non-significant for days to shoot emergence during mass proliferation of gynogenically induced shoots. Similar result revealed that MS medium fortified with different levels of BAP and KIN significantly increased the fresh weight of culture from gynogenically developed shoots over devoid of hormone. The maximum percent increase in fresh weight (0.64g) from the cultured gynogenic shoots was noted in MS with 0.5 mgl^{-1} BAP (Fig. 1b), which was statistically higher than the treatment devoid of any growth hormone and treatment having MS with 0.25 mgl^{-1} BAP which recorded (0.57g) and (0.53g) increase in fresh weight of shoot culture, respectively. While, comparing the genotypes for their percent increase in fresh weight, it was observed DAMH-24 (0.65g) showed a significantly higher increase in fresh weight than the DAMH-55 (0.33g). Genotype x

treatment interactions for the percent increase in fresh weight of culture was found non-significant. Enrichment of MS media with specific doses of BAP and KIN lead to a significant increase in the number of micro-shoots from gynogenically developed shoots over MS devoid of hormones. The highest increase in the number of micro-shoots from gynogenic shoots (3.5) was noted in treatment having MS medium with 0.25 mgl^{-1} KIN, which was statistically higher than the treatment having MS medium enriched with 0.5 mgl^{-1} KIN (2.67) (Fig. 1d). While comparing the genotypic response during the proliferation of gynogenic shoots, it was observed that DAMH-24 produced a significantly higher number of micro-shoots (2.33) than DAMH-55 (1.48). Genotype × treatment interactions for the proliferation of gynogenically induced was found to be non-significant. MS media supplemented with different levels of BAP and KIN increased the shoot length of gynogenically induced shoots more than those devoid of hormones. Among the various levels of BAP and KIN used for shoot proliferation, the longest (9.56 cm) shoot length was recorded on MS medium fortified with 1.0 mgl^{-1} BAP, which was statistically higher than MS medium having 0.5 mgl^{-1} BAP (6.97 cm). Among two genotypes of African marigold, a significantly higher shoot length (7.72 cm) was recorded in DAMH-24 than in the DAMH-55 (6.46 cm) **(**Fig. 1). Genotype × treatment interaction was found non-significant for the shoot length. MS medium fortified with different levels of BAP and KIN randomly increased and decreased the number of leaf pairs per branch from gynogenically developed shoots over the treatment devoid of hormones. The maximum increase in the number of leaf pairs per branch (5.17) was noted in treatment having 1.0 mgl^{-1} BAP which was statistically higher than the treatment having MS medium devoid of any growth hormone (4.67) and treatment having MS medium with 0.5 mgl^{-1} BAP (3.50) (Fig. 1b). While comparing both the genotypes for the increase in the number of leaf pairs per branch, it was observed that DAMH-24 produced higher number of leaf pairs per branch (3.52) than DAMH-55 (3.10). Genotype × treatment interactions for the number of leaf pairs per branch were found non-significant. These results are in conformity with the previous work done by Kumar *et al.* (2018), where the maximum number of micro-shoots per explant (1.88) was recorded in 2.0 mgl^{-1} BAP + 0.05 mgl^{-1} NAA and earliest axillary bud emergence (4.45 days) on a modified MS medium supplemented with BAP (2.0 mgl^{-1}) and NAA (0.05 mgl^{-1}) as well as Gupta *et al.* (2013) reported Pusa Narangi Gainda had the highest shoot proliferation (3.43) as compared to the male sterile line (3.00) in African marigold. The present findings lend support from the previous work done by Sadhu *et al.* (2021) in *Callistephus chinensis* and Misra and Datta (2000) in White marigold.

Table 1: *In vitro* shoot proliferation from gynogenically developed shoots.

Treatment			Days to shoot emergence/sprouting			Percent increase in fresh weight of culture (g)			No. of micro-shoots per shoot			Height of shoot (cm)			No. of leaf pair per branch		
	NAA	IBA	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean
T_0	0	0	12.33	10.33	11.33	0.74	0.41	0.57	1.33	1.00	1.17	9.07	8.37	8.72	4.67	4.67	4.67
T_1	0.5		7.33	8.67	8.00	0.68	0.37	0.53	1.67	1.00	1.33	6.43	5.23	5.83	2.33	1.67	2.00
T_2	1		6.00	4.00	5.00	0.82	0.47	0.64	2.33	1.67	2.00	7.67	6.27	6.97	3.33	3.67	3.50
T_3	1.5		6.67	9.67	8.17	0.70	0.29	0.49	2.00	1.00	1.50	9.77	9.36	9.56	5.33	5.00	5.17
T_4		0.5	9.00	11.67	10.33	0.79	0.24	0.52	4.33	2.67	3.50	7.00	6.80	6.90	2.67	2.33	2.50
T_5		1	10.67	9.67	10.17	0.43	0.26	0.34	3.33	2.00	2.67	8.00	4.87	6.43	3.33	2.33	2.83
T_6		1.5	8.00	5.33	6.67	0.42	0.30	0.36	1.33	1.00	1.17	6.10	4.33	5.22	3.00	2.00	2.50
Mean			8.57	8.48		0.65	0.33		2.33	1.48		7.72	6.46		3.52	3.10	
			C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±
Genotype			N/A		0.626	0.052		0.018	0.5		0.172	N/A		0.555	N/A		0.259
Treatment			3.412		1.172	N/A		0.033	0.454		0.321	N/A		1.039	1.409		0.484
Genotype × Treatment			N/A		1.657	N/A		0.047	N/A		0.454	N/A		1.47	N/A		0.684

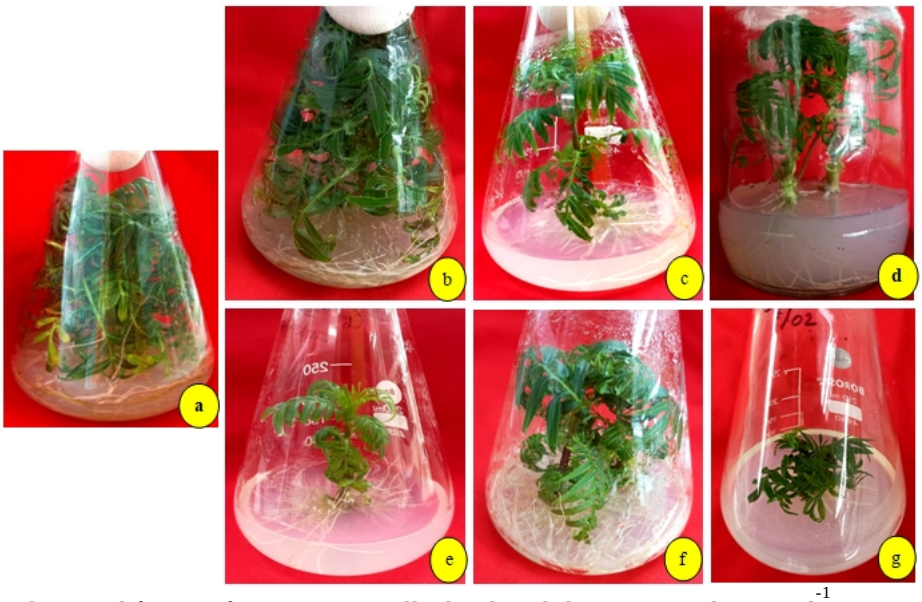

Fig. 1. *In vitro* shoot proliferation from gynogenically developed shoots. Control- 0.0 mgl^{-1} BAP + 0.0 mgl^{-1} KIN (a); DAMH-24 { 0.5 mgl^{-1} of BAP (b), 1.0 mgl^{-1} of BAP (c), 0.25 mgl^{-1} of KIN (d)}, DAMH-55 { 0.5 mgl^{-1} of BAP (e), 1.0 mgl^{-1} of BAP (f), 0.25 mgl^{-1} of KIN (g)}.

B. In-vitro rooting of gynogenically induced micro-shoots

As shoot proliferation, root induction is also an important step during haploid production. Marigold shoots are known to root without any extraneous growth regulators (Kumar *et al.*, 2017). However, functional quality and percent rooting are known to improve significantly with the use of auxins particularly IBA and NAA. In this study, we have observed that with the increase in NAA concentration, roots became shorter and thicker and their number increased significantly, while in the case of IBA long, fiber-like tender roots with well-distributed root hairs appeared (Fig. 2). The rooting on half strength MS is much effective than full MS (Yousssef *et al*., 2010; Saklani *et al*., 2015; Shyama *et al*., 2017). ½ MS media supplemented with different auxins (NAA and IBA) significantly reduced the number of days required for root emergence compared with media devoid of hormones. Significant early rooting (4.33 days) from the gynogenic shoots was recorded in the treatment comprising of ½ MS medium supplemented with 0.5 mgl^{-1} NAA than medium enriched with 1.0 mgl^{-1} IBA (5.67 days) (Fig. 2b). Lower concentrations of auxins were found to be more effective for early root emergence, while higher doses of auxins promoted callus induction and hence significantly delayed the process of root emergence. While comparing the efficacy of two auxins, *i.e*., NAA and IBA, for early rooting, it was observed that NAA was found to be more effective than IBA for early root emergence. When comparing the two genotypes, DAMH-24 gives roots statistically earlier (6.90 days) than DAMH-55 (7.76 days). The interaction between genotypes and treatments were non-significant for days in rooting of micro-shoots. The highest rooting percent (100.0%) was recorded in the treatment comprising of ½ MS medium supplemented with 0.5 mgl^{-1} NAA, which was higher than the treatment devoid of growth regulator (83.33%) and medium supplemented with 0.5 mgl^{-1} IBA (80.0%). The NAA supplemented medium had a significantly higher rooting percentage than the IBA. Among both genotypes of African marigold, the maximum rooting (82.86%) was recorded in the DAMH-24, which was higher than the percent rooting induced in the DAMH-55 (67.62%). Genotypes x treatment interactions were found non-significant for per cent rooting. Similarly, Kumar (2002) used a marigold male sterile line and found that half-strength MS media supplemented with 0.5 mgl^{-1} IBA and 0.5 mgl^{-1} NAA resulted in the highest rooting frequency (98.82 %). The root length was significantly reduced with different auxins concentrations, whereas the number of roots and their strength improved. The longest roots (10.18 cm) were observed on medium having ½ MS devoid of any auxins, which were longer than medium supplemented with 0.50 mgl^{-1} NAA (5.53 cm), but those longer shoots are pale green in colour and so less in number (Fig. 2). The results are in agreement with the findings of Misra and Datta (2000) that roots grown in the absence of any growth regulator did not support plant establishment in soil in White marigold. Root length was found to be better on half-strength MS medium supplemented with NAA than the IBA. While assessing the root length in both genotypes, the maximum root length was recorded in DAMH-24 (5.12 cm), which was found to be longer than the roots induced in genotype DAMH-55 (4.05 cm). The

genotypes × treatments interactions were found non-significant for the length of roots in gynogenic shoots. The highest score for the root growth character (4.0) was assigned to a treatment comprising ½ MS medium supplemented with 0.5 mgl^{-1} NAA (Fig. 2b), which was at par (3.5) with treatment supplemented with 1.5 mgl^{-1} IBA. The score for the root growth character was found to be significantly better in the NAA supplemented medium than the IBA. The better score for root growth character (2.67) was assigned to DAMH-24 among both genotypes of African marigold, which was significantly higher than the root growth character rating in DAMH-55 (1.95). Genotypes × treatment interactions were found non-significant for root growth character. The highest increase in number of roots per shoot (36.33) was noted in MS media enrich with 0.5 mgl^{-1} NAA which was non-significantly higher than the treatment with 1.5 mgl^{-1} IBA (30.17). While, comparing the two genotypes it was observed that DAMH-24 produced a non-significantly higher number of roots per micro-shoot (25.48) than DAMH-55 (19.71). Genotype × treatment interactions for the number of roots induced from the gynogenically developed micro-shoots was found to be significant. However, the maximum number of roots was noted in DAMH-24 (46.67) when cultured on MS medium enriched with 0.5 mgl^{-1} of NAA followed by genotype DAMH-55 (30.33) on MS medium enriched with 1.5 mgl^{-1} of IBA (Table 2). This observation is in accordance with the report of Majumder *et al.* (2014), when they cultured the shoots on half strength MS media supplemented with 0.5 mgl^{-1} IBA and 0.5 mgl^{-1} NAA, highest rooting (87.33%), maximum number of roots/shoot (20.67) and earliest rooting (13.15 days) were observed. Similar observations on rooting parameters have earlier been reported by Kumar (2002); Gupta *et al.* (2013); Kumar *et al.* (2017) in African marigold, Qi *et al.* (2011) in *Tagetes patula*, Sadhu *et al.* (2021) in *Callistephus chinensis* and Satyajit *et al.* (2012).

Table 2: *In vitro* rooting in gynogenically developed shoots.

Treatment			Days to root emergence			Percent rooting			Root length (cm)			Root growth character			No. of roots per explant		
	NAA	IBA	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean	DAMH-24	DAMD-55	Mean
T0	0	0	11.67	14.00	12.83	93.33	73.33	83.33	12.51	7.85	10.18	1.00	1.00	1.00	3.00	3.33	3.17
T1	0.5		3.33	5.33	4.33	100.00	100.00	100.00	5.69	5.36	5.53	4.67	3.33	4.00	46.67	26.00	36.33
T2	1		6.00	6.00	6.00	86.67	66.67	76.67	3.46	3.91	3.69	2.67	1.67	2.17	27.33	20.67	24.00
T3	1.5		8.67	6.67	7.67	73.33	46.67	60.00	2.48	2.29	2.38	2.00	1.33	1.67	33.33	18.00	25.67
T4		0.5	6.33	6.33	6.33	86.67	73.33	80.00	3.13	2.33	2.73	1.67	1.33	1.50	12.00	19.67	15.83
T5		1	8.00	9.00	8.50	66.67	60.00	63.33	3.47	3.17	3.32	2.67	2.00	2.33	26.00	20.00	23.00
T6		1.5	4.33	7.00	5.67	73.33	53.33	63.33	5.09	3.47	4.28	4.00	3.00	3.50	30.00	30.33	30.17
Mean			6.90	7.76		82.86	67.62		5.12	4.05		2.67	1.95		25.48	19.71	
			C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±	C D (p=0.05)		SEm±
Genotype			N/A		0.57	0.667		0.23	N/A		0.4	0.528		0.18	2.033		0.7
Treatment			3.11		1.06	1.247		0.43	2.181		0.75	0.988		0.34	3.804		1.31
Genotype × Treatment			N/A		1.51	N/A		0.61	N/A		1.06	N/A		0.48	5.379		1.85

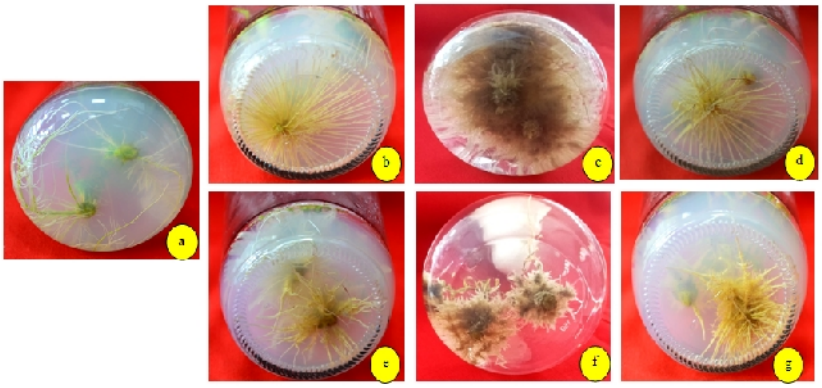

Fig. 2. *In vitro* rooting in gynogenically developed shoots. Control-0.0 mgl^{-1} NAA + 0.0 mgl^{-1} IBA (a); DAMH-24 { 0.5 mgl^{-1} of NAA (b), 0.5mgl^{-1} of IBA (c), 1.5 mgl^{-1} of NAA (d)}; DAMH-55 { 0.5 mgl^{-1} of NAA (e), 0.5 mgl^{-1} of IBA (f), 1.5 mgl^{-1} of NAA (g)}.

CONCLUSION

It can be concluded that the modified MS medium supplemented with KIN (0.25 mgl^{-1}) resulted in the maximum increase in the number of micro-shoots from gynogenically induced shoots and thus can be successfully utilized for mass proliferation. While the ½ strength MS medium supplemented with NAA (0.5 mgl^{-1}) can be successfully utilized for early root induction, highest rooting percent, better root growth character, and induction of highest number of roots per micro-shoots. The developed protocol can be effective for *in vitro* maintenance of gynogenically induced haploid. Among both the varieties 'DAMH-24' performs best *viz*. days required for shoot emergence, increase in fresh weight of cultured shoots, shoot length, number of leaf pairs per branch, days to rooting,

rooting percent, root growth character and no. of roots per micro-shoots. On the other hand 'DAMH-55', has less efficiency towards *in vitro* proliferation and rooting of gynogenically induced shoots. The standardized protocols can, also be effectively utilized for improving the vigor and large-scale mass multiplication of gynogenically induced shoots.

FUTURE SCOPE

Nowadays, the F_1 hybrids of African marigold are gaining tremendous popularity in India and other countries owing to their early and uniform flowering habit. Most of the marigold F_1 hybrid seeds are being imported from other countries like China, Thailand, and Germany. This is mainly because of the non-availability of homozygous inbred lines in this crop. *In vitro*, gynogenesis/ androgenesis approaches are widely used for the rapid fixation of homozygosity through doubled haploid production. This study will be highly effective forthe mass proliferation of haploid and double haploid lines induced through gynogenesis. The standardized protocols will be highly useful in improving the vigor and large-scale mass multiplication of gynogenically induced shoots. In the long run, this study will be effective in the rapid multiplication of homozygous lines generated through *in vitro* gynogenesis/ androgenesis approaches. The rapid multiplication and large-scale availability of gynogenically induced homozygous lines will facilitate the hybrid breeding research in the marigold crop. The *in vitro* proliferated plants can be utilized as parental lines in hybrid breeding, and for basic and strategic research in marigold.

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Conflict of Interest. None.

REFERENCES

Anonymous (2017). Indian Horticulture data base. (Online) Available at http://nhb.gov.in.

Anonymous (2022). Welcome to OPSTAT

Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P. and Shircore, A. (2001). Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angels atherosclerosis study. *Circulation*, *103*, 2922-2927.

Gupta, Y. C., Vaidya, P., Dhiman, S. R. and Sharma, P. (2013). *In vitro* propagation and maintenance of genetic male sterility in marigold. *Progressive Horticulture*, *45*(1), 152-159.

Kumar, A. (2002). *In vitro* production of male sterile lines of African marigold (*Tagetes erecta* L.) for F1 hybrid seed production. Ph.D. thesis submitted to Indian Agricultural Research Institute, New Delhi-12.

Kumar, K. R., Singh, K. P., Raju, D. V. S., Bhatia, R., and Panwar, S. (2020). Maternal haploid induction in African marigold (*Tagetes erecta* L.) through *in-vitro* culture of un-fertilized ovules. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *143*(3), 549-564.

Kumar, K. R., Singh, K. P., Raju, D. V. S., Panwar, S., Bhatia, R. and Jain, P. K. (2017). Standardization of rapid multiplication protocol in petaloid male sterile lines of African marigold (*Tagetes erecta*) through *in-vitro* culture. *Indian Journal of Agricultural Sciences*, *87*(10), 1295-1302.

Kumar, K. R., Singh, K. P., Raju, D. V. S., Panwar, S., Bhatia, R., Kumar, S., & Verma, P. K. (2018). Standardization of *in-vitro* Culture Establishment and Proliferation of Micro-Shoots in African and French Marigold Genotypes. *International Journal of Current Microbiology and Applied Sciences*, *7(1*), 2768-2781.

Majumder, J., Kanwar, P. S., Singh, S. K., Prasad, K. V. and Verma, M. (2014). *In vitro* morphogenesis in marigold using shoot tip as explant. *Indian Journal of Horticulture*, *71*(1), 82-86.

Misra, P. and Datta, S. K. (2000). *In vitro* maintenance of F1 hybrid. *Current Science*, *78*, 383-387.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. *Plantarum*, *15*, 473-497.

Qi, Y., Ye, Y. and Bao M. (2011). Establishment of plant regeneration system from anther culture of *Tagetes patula*. *African Journal of Biotechnology*, *10*(75), 17332-17338.

Sadhu, D. S., Nishani, S., Patil, B. C., Shiragur, M., Lakshmidevamma, T. N. and Bhat, A. S. (2021). Callus induction and plant regeneration through unpollinated ovary culture in China aster (*Callistephus chinensis* L. Nees).

Saklani, K., Pant, H. and Rawat, V. (2015). Micropropagation of rose cultivars: biotechnological application to floriculture. *Journal of Environmental Research and Development*, *10*, 40- 46.

Satyajit, K., Chinmay, P., Santi, L. S. and Rajani, K. S. (2012). Role of auxins in the *in vitro* rooting and micropropagation of *Holarrhena antidysenterica* Wall., a woody aromatic medicinal plant, through nodal explants from mature trees. *J. Med. Plant Res.*, *6*, 4660-4666.

Shyama Kumari, Singh, K. P., Raju, D. V. S., Panwar, S. and Sarkhel, S. (2017). Establishment of *in vitro* micropropagation protocol for Rose (Rose × hybrid) cv. Raktgandha. *Indian Journal of Ecology*, *44*(4), 166-170.

Yousssef, M., El-Helw M. R.., Taghian A. S. and El-Aref, H. M. (2010). Improvement of *Psidium guajava* L. using Micropropagation. *Acta Horticulturae*, *84*, 223-230.

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