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# Effect of plant growth regulators on shoot elongation and root growth parameters of micro-shoots derived through androgenesis in marigold (*Tagetes* spp.)

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**Abstract**
In the present investigation, an effort was directed to maintain haploid/di-haploid lines derived through androgenesis in African marigold genotype "Af/w-4" and French marigold genotype "Fr/R-5-2" under *in-vitro* condition. For successful maintenance it is essential for the shoots and roots to be of optimum quality. Thus, the effect of $GA_3$ on shoot elongation was studied and recorded to have positive influence on shoot growth. In "Af/w-4", maximum shoot length increase occurred in MS media+ $GA_3$ @ 1.00 mg/L both after 15 days (190.62%) and 30 days (312.00%) of shoot inoculation. In "Fr/R-5-2", GA3 @ 0.50 mg/L recorded maximum shoot length increase (72.88%) after 15 days. For various rooting parameters, IBA was a better as compared to NAA. Half MS media+ IBA @ 1.50 mg/L proved to better for all the rooting attributes in both "Af/w-4" and "Fr/R-5-2" genotypes *viz*., rooting percentage number of roots per shoot, and root length compared with all the treatment combinations. Marigold micro-shoots did not have difficulty in rooting even in control (media devoid of plant growth regulators) however it was of inferior quality.

**Keywords:** Marigold, *in-vitro*, androgenesis, shoot elongation, rooting parameters

## 1. Introduction
Marigold (*Tagetes* spp.) is a hardy herbaceous annual plant commonly grown as loose flowers, garden plants, landscape flower and pot plants amongst all. It is definitely one of the most widely cultivated loose flower crops in India due to enormous demand in socio-religious functions such as mandap and stage decorations in marriage, wedding anniversary parties, other official functions, festivals such as Diwali, Ganesh Chaturthi, etc. (Sadhukhan *et al*., 2014) [16]. Its demand as a cut flower is also increasing day by day (Kumar *et al*., 2018) [12]. Besides just using as an ornamental plant, its brightly colored flowers are an important raw material for different pharmaceutical and nutraceutical industries. It has been widely suggested as an alternative medicine for many skin ailments like eczema, sunburns, bruises, inflammation. Culinary delight includes adding a dash of color to foods and mild flavor to soups and drinks or as such as floral tea. Flower petals being rich in components like lutein (oxygenated carotenoid xanthophyll) are used in food and feed industries (Chauhan *et al*., 2022) [6]. Flower contains phytochemicals and nutraceuticals which can be used to cure eye-related diseases like cataracts and age-related macular degeneration (AMD), and even cardiovascular diseases, cancer, etc. (Dwyer *et al*., 2001, Chauhan *et al*., 2022) [7, 6].
Androgenesis is a successful *in-vitro* method to induce haploid plants through male gametophytes in various crop species. The haploidy in plants is due to arrest of normal development of pollen cells into sexual cells and force the development into plants. The resultant haploid plant can be directly used as a variety or as a parental line in further breeding process (Murovec and Bohanec, 2012) [14]. These haploid plants can be doubled with the help of chromosome doubling agents and maintained *in-vitro* or *ex-vitro* to obtain Doubled haploids. *In-vitro* maintenance of haploid or doubled haploids lines consists of many steps including stem elongation and rooting which can be manipulated using different types, sources and concentrations of plant growth regulators in the culture medium. Efficient maintenance of haploids and doubled haploids is dependent on rapid shoot development and elongation. In this research, effect of gibberellic acid ($GA_3$) at various doses was examined for efficient shoot elongation. For rooting of micro-shoots, effect of two different types of auxins *viz*., NAA and IBA was investigated. The goal of this research was to find suitable dosage of gibberellic acid

($GA_3$) for shoot elongation and suitable auxin type and concentration for rooting of androgenesis derived micro-shoots.

## 2. Materials and Methods

The current investigation was carried out during the year 2022-23 at Central Tissue Culture Laboratory, National Institute of Plant Breeding (CTCL-NIPB), Lal Bahadur Shastri Building, New Delhi- 110012. African marigold genotype "Af/w-4" and French marigold genotype "Fr/R-5-2" was selected for the investigation based on their better response to the preliminary anther culture initiation experiments. The flower buds of appropriate length having suitable florets were taken as explants from disease free plants grown in net house for another culture initiation. The components of the media required for each experiment such as MS media, Sucrose, $AgNO_3$, Adenosine sulphate, Agar and plant growth regulators were measured and poured inside clean beaker. The volume was made up with double distilled water. pH was adjusted between 5.75-5.78 and solidified with 8g/L agar. Around 50 ml of the media were poured into jam bottles and were sterilized in autoclave at 121ºC at 15 $lbsinch^{-2}$ pressure for 21 minutes. For *in-vitro* response of growth regulators in shoot elongation, micro shoots were inoculated into culture medium comprising of Murashige and Skoog including macro-salts, micro-salts and vitamins (Duchefa, Netherlands) supplemented with 2.5 ml/L $AgNO_3$ (Himedia, India), 3% sucrose (Himedia, India), 16 mg/L Adenosine sulphate (Himedia, India) and different concentration of gibberellic acid (GA3; Himedia, India). For *in-vitro* response of growth hormones in rooting of shoots, the elongated shoots were used for rooting experimentation. Well-developed shoots of appropriate length were inoculated into culture medium containing half strength Murashige and Skoog inclusive of macro-salts, micro-salts and vitamins supplemented with 2.5 ml/L $AgNO_3$, 6% sucrose, different concentration of auxins *viz*., 1-Naphthaleneacetic acid (NAA; Himedia, India) and Indole-3-butyric acid (IBA; Himedia, India). The cultures were maintained at 25± 1ºC temperature and 16:8 hours photoperiod of light and dark cycles under fluorescent white light (47 µmol/$m^2$/s). Data pertaining to different parameters with respect to each experiment was taken at appropriate days. The experimentation was laid out in Factorial Completely Randomized Design (FCRD) with three replications each. The mean data pooled in each replication are of experiments repeated twice so as to minimize error as much as possible. Two factorial analysis was done using R-statistical software and its significance is calculated based on one-way Analysis of Variance (ANOVA). The data as and when required were transformed using Arc sine transformation to stabilize the variation and the transformed data is presented under parenthesis along with the original mean data.

## 3. Results and Discussion

### 3.1 Percent shoot length increase after 15 days

Data pertaining to percentage increase in shoot length after 15 days of inoculation in medium containing different concentrations of $GA_3$ is presented in Table no. 1. It is evident from the data that Gibberellic acid ($GA_3$) at various concentrations influenced the shoot length significantly in both the genotypes of "Af/w-4" and "Fr/R-5-2". Significant increase in shoot length after 15 days was observed when MS medium was supplemented and $GA_3$ @ 1.00 mg/L (190.62% increase) in "Af/w-4" genotype and $GA_3$ @ 0.50 mg/L (72.88% increase) in "Fr/R-5-2" genotype. The lower concentration of Gibberellic acid ($GA_3$) didn't result in significant increase of shoot length after 15 days when compared with control (media devoid of plant growth regulators).

### 3.2 Percent shoot length increase after 30 days

Perusal of data presented in Table no.1 below with respect to percentage increase in shoot length after 30 days of shoot inoculation in media containing varying concentration of $GA_3$ indicates differential response of hormones when compared with control. Maximum shoot elongation in "Af/w-4" was observed when medium was supplemented with $GA_3$ @ 1.00 mg/L (312.00% increase) followed by $GA_3$ @ 0.50 mg/L (265.57% increase) when compared with control (146.99% increase). In "Fr/R-5-2" genotype, elongation was highest in $GA_3$ @ 0.20 mg/L (112.80**%** increase) followed by $GA_3$ @ 0.50 mg/L (107.75% increase) however, effect of both the concentration was statistically at par.

The effect of $GA_3$ at different concentrations showed differential response with respect to shoot elongation in the both the genotypes of marigold at different intervals. In general, there was increased shoot elongation when supplemented with higher concentration of $GA_3$. Nevertheless, shoot elongation was also achieved when medium was devoid of any shoot elongation growth regulator. The result is in consistent with previous findings of Kumar *et al*. (2010) [13] and Badge *et al*. (2013) [3] in marigold. Growth regulators have been known to influence the length of shoots in crop species (Kacar *et al*., 2005) [10]. Cultures treated with Gibberellic acid have produced elongated shoots in *Prunus institita* L. (Reeves *et al*., 1985) [15]. Sarkar *et al*. (2018) [17] and Acharya *et al*. (2021) [1] also found similar results where plant height increased with an increased concentrations of $GA_3$ supplementation. The resultant increase in shoot length when supplemented with higher concentration of $GA_3$ application must be due to increase in the intermodal length. Cell enlargement takes place which leads to increase in plant growth. Also, it increases auxin content in the plant which further enhances the apical dominance indirectly (Acharya *et al*., 2021) [1]. Sekioka and Tanaka (1981) [18] opined that $GA_3$ could behave as stand-in for auxin in shoot induction, thus cytokinin: $GA_3$ ratio may decide differentiation in certain plant tissues. Shoot elongation of marigold in media without any growth regulator supplementation may be due presence of high level of endogenous auxin as suggested by Kumar *et al*. (2017) [11].

### 3.3 Rooting percentage

Percentage of rooting was investigated by supplementing medium with two different types of auxins *viz*. IBA and NAA and the data thereof has been presented in Table no. 2. There were significant differences in rooting percentage between both the auxin sources where it was observed that rooting percentage was higher when medium was supplemented with IBA as compared with NAA. The effect of auxins was similar in both the genotypes. Maximum rooting was observed in MS medium fortified with IBA @ 1.50 mg/L (95.37%) which was at par with control (95.06%) in "Af/w-4" genotype. In "Fr/R-5-2" genotype also, rooting percentage was maximum in IBA @ 1.50 mg/L (95.37%) which was at par with control (94.44%). Least rooting percentage was recorded in MS media+ NAA @ 2.00 mg/L i.e., 46.29% and 50.93% in "Af/w-4"and "Fr/R-5-2" respectively. Interestingly, overall rooting percentage was high also in control (media devoid of plant growth regulators).

### 3.4 Days taken for initiation of roots

Data pertaining to days taken for root initiation presented in Table no. 2 implies that there was significant differences between two different types of auxin supplemented. Maximum days taken for root initiation was recorded in MS media+ NAA @ 2.00 mg/L i.e., 9.73 days and 9.44 days in "Af/w-4" and "Fr/R-5-2" genotypes respectively. Minimum number of days taken for root initiation in "Af/w-4" genotype was recorded in MS media+ IBA @ 1.00 mg/L (5.11days) which was found to be at par with control (5.50 days). In "Fr/R-5-2" genotype, earliest rooting was observed in MS media+ IBA @ 1.00 mg/L (5.22 days) which was at par with control (5.50 days). IBA and NAA both at lower concentration induced roots earlier as compared to higher doses.

### 3.5 Number of roots per shoot

As observed from the data presented in Table no. 2 with regard to number of roots per shoot, it was found that MS media+ IBA @ 1.50 mg/L induced maximum number of roots per shoot in "Af/w-4" genotype (59.95) as well as "Fr/R-5-2" genotype (52.78). Minimum number of roots was recorded in MS media+ NAA @ 2.00 mg/L in "Af/w-4" genotype (12.16) as well as "Fr/R-5-2" genotype (9.22). Medium devoid of plant growth regulators (control) also recorded more number of roots (41.06) than NAA supplemented medium in general. With increasing concentration of NAA, number of roots declined in both the genotypes.

### 3.6 Root length (cm)

Root length data as presented in Table no. 2 suggests that the effect of two different types of auxin *viz.* IBA and NAA was different from each other. The genotypes also differed from each other with respect to root length. Longest root length was observed in MS media+ IBA @ 1.50 mg/L (4.41 cm) while shortest root length was observed in MS media+ NAA @ 2.00 mg/L (1.84 cm) in "Af/w-4" genotype. In "Fr/R-5-2" genotype, root length was maximum in MS media+ IBA @ 1.50 mg/L (6.64 cm) and minimum in MS media+ NAA @ 2.00 mg/L (1.53 cm). Control i.e., medium devoid of plant growth regulators performed better than the one supplemented with NAA at various concentrations in both "Af/w-4" and "Fr/R-5-2" genotypes. Even though IBA performed better, just increasing its concentration did not result in increased length of roots while in case of NAA, increase in dosage lead to reduced length of roots.

For most of the *in-vitro* rooting parameters, IBA proved to be better than NAA. Similar results were found when the African marigold cuttings were rooted with the help of IBA and NAA by Aparna *et al*., 2021 [2]. Gupta *et al*. (2013) [8] also suggested that IBA is the better auxin for rooting in marigold as compared to NAA based on their studies. Increased number of roots, rooting percentage, early root induction in medium supplemented with IBA might be possibly due to optimum hormonal effect that promoted rapid hydrolysis of carbohydrate substances facilitating their downward movement for rapid cell division activity. The results were in concordance with findings of Aparna *et al*. (2021) [2] in marigold, Bharmal *et al*. (2005) [4] in chrysanthemum. Longest root length when supplemented with IBA @ 1.50 mg/L might be an effect of early root initiation thereby resulting in rapid utilization of nutrients which possibly contributed to the endosmosis of water as well as cell expansion in roots (Jadhav, 2007) [9]. Singh *et al*. (2003) [19] opined that IBA could have hydrolysed the carbohydrates and nitrogenous compounds which upon translocation to the base of micro-shoots resulted in accelerated cell division and cell elongation.

Quality of roots was better with root hairs uniformly distributed throughout in IBA supplemented medium whereas NAA showed poor quality off roots which were stunted and brittle. The findings corroborate well with that obtained by Bhatia (2007) [5] in gerbera and Kumar *et al*. (2017) [11] in marigold. MS medium devoid of any plant growth regulators (control) also produced roots. Length of roots and root initiation was better than hormone supplemented medium. However, the quality of the roots were inferior than that of MS medium supplemented with IBA at different doses as they were thin with thread like appearance with lesser root hairs. The rooting of shoots even without hormone supplementation may be due to higher level of endogenous auxin in marigold tissues. Similar finding was reported by Kumar *et al*. (2017) [11] in African and French Marigold.

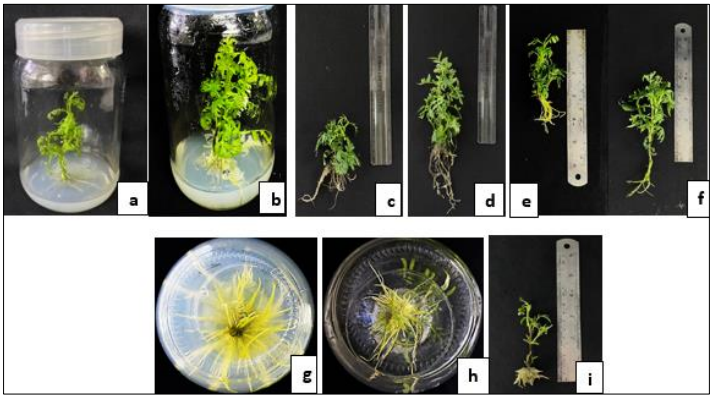


**Fig 1:** *In- vitro* regeneration of plants through androgenesis in African marigold genotype "Af/W-4" and French marigold genotype "Fr/r-5-2".

a) Shoots of "Fr/r-5-2" genotype in elongation medium containing $GA_3$ @ 0.50 mg/L (after 15 days)
b) Shoots of "Af/W-4" genotype in elongation medium containing $GA_3$ @ 1.00 mg/L (after 15 days)
c) Shoots of "Fr/r-5-2" genotype in elongation medium devoid of growth regulators (after 30 days)
d) Shoots of "Fr/r-5-2" genotype in elongation medium supplemented with $GA_3$ @ 0.20 mg/L (after 30 days)
e) Shoots of "Af/W-4" genotype in elongation medium devoid of growth regulators (after 30 days)
f) Shoots of "Af/W-4" genotype in elongation medium supplemented with $GA_3$ @ 1.00 mg/L (after 30 days)
g) Rooting of "Af/W-4" genotype shoots in medium supplemented with IBA @ 1.50 mg/L
h) Rooting of "Fr/r-5-2" genotype shoots in medium supplemented with IBA @ 1.50 mg/L
i) Rooted plants

**Table 1:** Effect of $GA_3$ on percent shoot increase after 15 days and 30 days of shoot inoculation on African and French marigold genotypes

| Treatments | Initial shoot length | | Shoot length after 15 days | | Percent shoot length increase after 15 days | | Shoot length after 30 days | | Percent shoot length increase after 30 days | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 |
| MS media devoid of plant growth regulators | 2.40 cd | 2.68 bcd | 4.77 c | 3.69 e | 99.14 bc | 40.70 f | 5.90 de | 4.87 e | 146.99 cd | 81.71e |
| MS media+ GA3 @ 0.10 mg/L | 3.55 a | 2.59 cd | 5.77 b | 3.79 de | 62.40 def | 49.26 ef | 10.54 ab | 5.17 e | 197.05 b | 99.03 de |
| MS media+ GA3 @ 0.20 mg/L | 2.61 cd | 2.78 bcd | 4.27 cde | 4.58 cd | 65.16 def | 64.38 def | 6.65 cd | 5.94 de | 157.15 bc | 112.80 cde |
| MS media+ GA3 @ 0.50 mg/L | 3.13 ab | 2.89 bc | 7.13 a | 4.63 c | 127.56 b | 72.88 cde | 11.46 a | 5.86 de | 265.57 a | 107.75 de |
| MS media+ GA3 @ 0.75 mg/L | 2.44 cd | 2.79 bcd | 4.42 cde | 4.06 cde | 81.40 cd | 60.51 def | 7.32 c | 5.37 e | 200.72 b | 92.13 e |
| MS media+ GA3 @ 1.00 mg/L | 2.37 d | 2.76 bcd | 6.86 a | 4.06 cde | 190.62 a | 43.05 ef | 9.73 b | 5.39 de | 312.00 a | 96.31 e |
| S.Em± (Genotypes) | 0.07 | | 0.12 | | 4.42 | | 0.18 | | 6.76 | |
| S.Em± (Treatments) | 0.12 | | 0.20 | | 7.66 | | 0.31 | | 11.71 | |
| S.Em± (Gen*Trt) | 0.18 | | 0.29 | | 10.83 | | 0.43 | | 16.56 | |
| CD (Genotypes) | 0.21 | | 0.34 | | 12.91 | | 0.52 | | 19.73 | |
| CD (Treatments) | 0.36 | | 0.59 | | 22.36 | | 0.90 | | 34.17 | |
| CD (Gen*Trt) | 0.51 | | 0.84 | | 31.62 | | 1.27 | | 48.33 | |

Means followed by the same letter are not significantly different at the 5% level according to LSD multiple comparison test

**Table 2:** Effect of IBA and NAA on various rooting parameters of African and French marigold genotypes

| Treatments | Rooting percentage | | Days taken for initiation of roots | | Number of roots per shoot | | Root length (cm) | |
|---|---|---|---|---|---|---|---|---|
| | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 | Af/w-4 | Fr/R-5-2 |
| MS media devoid of plant growth regulators | 95.06 (77.33) a | 94.44 (76.66) ab | 5.50 ef | 5.50 ef | 41.06 d | 38.17 e | 3.05 e | 5.89 b |
| MS media+ IBA @ 1.00 mg/L | 90.12 (71.75) c | 91.67 (73.37) bc | 5.11 f | 5.22 f | 27.89 g | 52.00 b | 2.34 f | 4.24 d |
| MS media+ IBA @ 1.50 mg/L | 95.37 (77.71) a | 95.37 (77.71) a | 6.09 e | 5.94 e | 59.95 a | 52.78 b | 4.41 cd | 6.64 a |
| MS media+ IBA @ 2.00 mg/L | 80.56 (63.87) d | 81.48 (64.53) d | 7.11 d | 6.89 d | 28.94 g | 48.83 c | 3.09 e | 4.54 c |
| MS media+ NAA @ 1.00 mg/L | 59.26 (50.34) f | 65.74 (54.18) e | 8.44 c | 8.89 bc | 18.06 h | 37.94 e | 2.99 e | 2.19 fg |
| MS media+ NAA @ 1.50 mg/L | 54.63 (47.66) fg | 53.70 (47.12) fg | 9.50 ab | 9.06 bc | 15.89 h | 35.22 f | 1.92 gh | 1.90 gh |
| MS media+ NAA @ 2.00 mg/L | 46.29 (42.87) h | 50.93 (45.53) gh | 9.73 a | 9.44 ab | 12.16 i | 9.22 j | 1.84 h | 1.53 i |
| SEM± (Genotypes) | 0.44 | | 0.08 | | 0.32 | | 0.04 | |
| SEM± (Treatments) | 0.83 | | 0.16 | | 0.59 | | 0.07 | |
| SEM± (Gen*Trt) | 1.17 | | 0.22 | | 0.84 | | 0.10 | |
| CD (Genotypes) | 1.28 | | 0.25 | | 0.92 | | 0.11 | |
| CD (Treatments) | 2.39 | | 0.46 | | 1.72 | | 0.21 | |
| CD (Gen*Trt) | 3.39 | | 0.65 | | 2.43 | | 0.30 | |

Means followed by the same letter are not significantly different at the 5% level according to LSD multiple comparison test

## 4. Conclusion

From the present investigation, it can be concluded that $GA_3$ application was found to be beneficial for shoot elongation when optimum hormone level inside plant tissue is reached. Concentrations of $GA_3$ application was also important as it can been seen that lower concentration did not influence the shoot elongation much. For most of the rooting parameters, IBA proved to better than NAA. Medium when supplied with IBA @ 1.50 mg/L was best with respect to rooting percentage, number of roots per shoot and root length. Even though rooting was observed in media without any hormones, the quality of roots were inferior to that of IBA supplemented medium.

## 5. Future scope

For *in-vitro* and *ex-vitro* maintenance of androgenesis derived plants, it is necessary to have well elongated and rooted shoots. Well rooted plant is of utmost importance for its *ex-vitro* plant survival and growth. Plants without well-developed root system fails to sustain itself in the long run. The proper rooted shoots are must to further subject plants to different *in-vitro* hardening techniques so that the plants can be transferred outside the laboratory condition. These plants can be established in the field for using it as parental line in breeding programmes or as a cultivar directly if found to have better plant or flower attributes.

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