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RESPONSE OF MARIGOLD VARIETIES FOR CALLUS DEVELOPMENT

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ABSTRACT

In vitro **development of callus from leaf explants of marigold (*Tagetes erecta*) was tried with the objective to study the response of different flower colour varieties and culture media composition for induction of callus during the year 2015-16 in FCRD replicated thrice at College of Agriculture, Nagpur. The leaf explants of 0.25 cm^2 of three flower colour marigold varieties *i.e* yellow, orange and white were inoculated in the MS medium supplemented with nine different combinations of 2, 4-dichlorophenoxyacetic acid (2, 4-D 4.5, 9.0,13.5 µM) and BAP (BAP: 2.2, 4.4, 8.8 µM). Observations on response to callus induction (%), number of days for callus initiation, number of days for full grown callus, weight of full grown callus and colour of callus were recorded. Significant variation for varieties and media composition were observed for all the characters related to callus formation. Interaction effect of variety X media composition was significant only for days for full grown callus and weight of full grown callus. In all the aspects related to callus formation yellow flower variety V1 performed better for early callus formation (5.25 days), highest percentage of callus formation (67.38%), early formation of full grown callus (16.81 days) and highest weight of full grown callus (6.915 g). The highest percentage of callus induction was 67.38% , when explants were cultured in MS medium supplemented with 9.0 µM 2,4-D and 8.8 µM BAP (T6). Early callus formation (5.00 days), early full growth of callus (17.44 days) and highest weight of full grown callus (7.03 g) was also observed in T6. None of the interaction effect showed consistent superiority for all the callus formation related charactes studied. However, yellow flower variety V1 in (T6) MS + 9.0 µM 2,4-D + 8.8 µM BAP performed better for early growth of full grown callus (15.00 days) and highest weight of full grown callus (8.53 g). It inferred from this study that yellow flower variety were suitable for callus production in media composition T6 (MS + 9.0 µM 2,4-D + 8.8 µM BAP), hence identified as suitable for callus formation on large scale.**

(*Key* words: Marigold, callus, media composition)

INTRODUCTION

Marigold is one of the commercially exploited flower crop that belongs to the family *Asteraceae* and genus *Tagetus*. Marigold has Indian origin although it appears that its natural origin is Mexico compared to other flowering annuals.

Total area under marigold crop in India during the year 2016-2017 was 56.04 thousand ha with the production of 497.59 thousand metric tonnes of loose flowers and 4.28 lakhs number of cut flowers (Anonymous, 2017). Major marigold growing states are Karnataka, Maharashtra, Andhra Pradesh and West Bengal. The major centers of flower marketing are metropolitan cities like Mumbai, Kolkatta, Chennai, Bangalore, Delhi in India and Pune, Mumbai, Nasik, Ahmednagar, Sangali, Kolhapur, Thane, Satara and Nagpur in Maharashtra.

Marigold is widely cultivated as bedding plant in landscape design. Beside the pristine uses as loose flower and the bedding plants, marigold occupies anthelmintic, analgesic, anti-inflammatory, aromatic, bronchodilatory, digestive, diuretic, emmenagogue, sedative and stomachic properties. It is also widely used as perfumes, herbal, gulal, organic manure, anticarcinogenic agent, antioxidant in retinotherapy and for oil extraction. The floral extract of marigold is used for treating eye diseases and ulcers. Flowers are important for their economic use as well as aesthetic value. Among the flowers grown by farmers, marigold has its own importance. It has gained popularity among flower growers because of its easy cultivation and wide adaptability. The growers are attracted towards marigold flower as it has a habit of free flowering, short duration to produce marketable flowers of attractive colours having good keeping quality.

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Large scale plant tissue culture has been demonstrated as being an alternative approach to traditional crops as it offers controlled supply of metabolites, independent of plant availability (Raut *et al,* 2006). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations, producing useful compounds under controlled conditions independent of climatic changes or soil status, free of microbes and insects (Siahsar*et al.,* 2011). In this sense, plant tissue culture has been used for production of several secondary metabolites. This study offers a great scope of continuous system of cell proliferation as callus culture which may be a useful tool for the production of secondary metabolites. With this idea in mind this work was carried out with the objective to assess the effect of marigold varieties and culture media for callus development.

MATERIALS AND METHODS

The research work on response of marigold varieties for callus culture development was conducted in the year 2015-16 in the tissue culture laboratory of Agril. Botany Section, College of Agriculture, Nagpur. Seeds of three pigment varieties (yellow, orange and white flowers) of *Tagetes erecta* were used as source of explant. Three week old plantlets were used as a source explants for callus induction. Young leaves were collected from the mother plant at the active stage of growth.Young leaves were cut in portions of about 0.25 cm^2 and the adaxial face were placed on the MS medium supplemented with all combinations of 2,4D (4.5, 9.0, 13.5 µM),and BAP (2.2, 4.4, 8.8 µM). The media composition used in the experiment are presented in table 1. The surface sterilized and cut explants were inoculated on callus induction media by working in the laminar air flow cabinet. Cultures were incubated in the dark at 25 ± 2^0C for three weeks of culture. Obtained calluses were continuously subcultured every 15 days to fresh MS medium, supplemented with 2,4-D/BAP concentrations to reduce the browning of callus. The experiment was conducted in three replication in FCRD.Observations related to callus obtained from three different flower colour varieties, different media composition and different varieties x media interaction such as number of days for callus initiation (days), response to callus induction (%), number of days for full grown callus (days) and weight of full grown callus (g), colour of callus and texture were recorded. The data related to callus culture were analysed in FCRD. The aliquots having one explants of each treatment were used for recording different observations. For the analysis of variance the mean values of five aliquots were used in duplex. Per cent data were subjected to arsine transformation before statistical analysis. The statistical analysis in FCRD were carried out as described by Panse and Sukatme (1954).

RESULTS AND DISCUSSION

The callus development in three different flower colour varieties of marigold were tried by culturing leaf explants on MS medium containing different concentrations of 2,4-D and BAP and results obtained on different traits related to callus development are presented in table 1 and plate 1.

Number of days for callus initiation

The overall mean number of days required for callus initiation observed in the three flower colour varieties of marigold ranged from 5.25 days in yellow flower variety to 7.70 days in white flower variety. Minimum days for callus initiation (5.25 days) was observed in yellow flower variety (V1) and was significantly superior over other two varieties followed by orange flower varieties (6.74 days).Treatment (T_6) MS+9.0 µM 2,4-D + 8.8 µM BAP (5.00 days) and (T_7) MS + 13.5 µM 2,4-D + 2.2 µM BAP (5.33 days) recorded the minimum number of days for callus initiation and were at par with each other and significantly superior over all other treatments. This was followed by treatment (T_8) MS+13.5 µM 2,4-D + 4.4 µM BAP (5.78 days), treatment (T_5) MS+9.0 µM 2,4-D + 4.4 µM BAP (6.11 days) and treatment (T_4) MS+9.0 µM 2,4-D + 2.2 µM BAP (6.33 days) and were found to be at par with each other. Treatment (T_1) MS+4.5 µM 2,4-D + 2.2 µM BAP (8.33 days) and treatment (T_9) MS+13.5 µM 2,4-D + 8.8 µM BAP (8.44 days) recorded maximum number of days for callus initiation.The interaction between the variety and media composition for days required for callus initiation were found to be non significant.

Response to callus induction (%)

Percentage response for callus induction was observed to be maximum in yellow flower marigold variety V1 (67.38 %) and significantly superior over other varieties. Minimum response for callus induction was observed in white marigold variety V3 (51.83 %). Maximum percentage response for callus induction (64.43%) was observed in treatment MS+9.0 µM 2,4-D + 8.8 µM BAP (T_6) and treatments MS + 13.5 µM 2,4-D + 2.2 µM BAP (T_7) 63.74% and MS+13.5 µM 2,4-D + 4.4 µM BAP (T_8) 63.74% and were found to be at par with each other and significantly superior over other treatments. Minimum per cent response for callus induction were observed in treatments MS+4.5 µM 2,4-D + 2.2 µM BAP (T_1) 56.96% and MS+4.5 µM 2,4-D + 8.8 µM BAP (T_3) 58.41%. The interaction effect between varieties and media composition were found to be non significant.

Number of days for full grown callus

Minimum days of 16.81 for full grown callus was observed in yellow flower marigold variety (V1) and was significantly superior over other varieties followed by 21.96 days in orange flower marigold variety (V2) and maximum days of 24.04 days in white flower marigold variety (V3).Minimum days of 17.44 days and 18.22 days for full grown callus were observed in treatment (T_6) MS+9.0 µM 2,4-D + 8.8 µM BAP and (T_7) MS+13.5 µM 2,4-D + 2.2 µM

Table 1. Response of marigold varieties for callus development

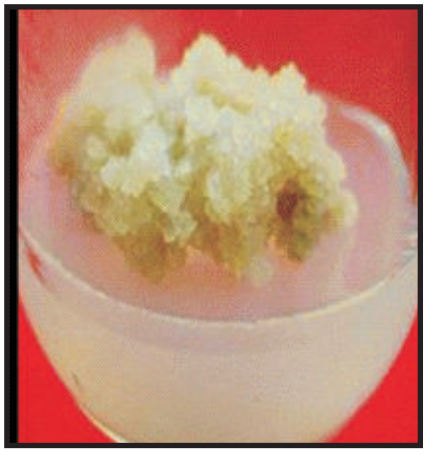
Treatments (V)	Days for callus initiation	Per cent response for callus	Days for full grown callus	Weight of full grown callus (g)
V1 (Yellow)	5.25	67.38	16.81	6.91
V2 (Orange)	6.74	60.74	21.96	6.14
V3 (White)	7.70	51.83	24.04	4.97
SE m (±)	**0.15**	**0.52**	**0.18**	**0.10**
CD (5%)	**0.45**	**1.56**	**0.56**	**0.31**
Media composition (T)				
T1(MS +4.5 µM 2,4-D+ 2.2 µM BAP)	8.33	56.96	24.78	5.12
T2(MS +4.5 µM 2,4-D+ 4.4 µM BAP)	7.22	58.98	22.33	5.81
T3(MS +4.5 µM 2,4-D+ 8.8 µM BAP)	6.56	58.41	21.00	5.38
T4(MS +9.0 µM 2,4-D+ 2.2 µM BAP)	6.33	59.18	20.56	6.30
T5(MS +9.0 µM 2,4-D+ 4.4 µM BAP)	6.11	59.59	20.11	5.98
T6(MS +9.0 µM 2,4-D+ 8.8 µM BAP)	5.00	64.43	17.44	7.03
T7 (MS+13.5 µM 2,4-D+2.2 µM BAP)	5.33	63.74	18.22	6.80
T8 (MS+13.5 µM 2,4-D+4.4 µM BAP)	5.78	63.74	19.00	6.44
T9 (MS+13.5 µM 2,4-D+8.8 µM BAP)	8.44	61.18	25.00	5.25
SE m (±)	**0.26**	**0.91**	0.32	**0.18**
CD (5%)	**0.78**	**2.73**	0.97	**0.54**
Interaction (V x T)				
V1T1	—	—	20.67	5.41
V1T2	—	—	18.00	6.45
V1T3	—	—	16.33	5.8
V1T4	—	—	16.00	8.14
V1T5	—	—	15.67	6.81
V1T6	—	—	15.00	8.53
V1T7	—	—	15.33	8.18
V1T8	—	—	15.67	7.49
V1T9	—	—	18.67	5.43
V2T1	—	—	25.33	4.99
V2T2	—	—	23.33	6.02
V2T3	—	—	23.00	5.69
V2T4	—	—	21.67	5.76
V2T5	—	—	21.00	6.11
V2T6	—	—	19.00	7.48
V2T7	—	—	18.67	7.13
V2T8	—	—	19.67	6.79
V2T9	—	—	26.00	5.34
V3T1	—	—	28.33	4.96
V3T2	—	—	25.67	4.97
V3T3	—	—	23.67	4.66
V3T4	—	—	24.00	5.00
V3T5	—	—	23.67	5.02
V3T6	—	—	18.33	5.09
V3T7	—	—	20.67	5.07
V3T8	—	—	21.67	5.04
V3T9	—	—	30.33	4.97
SE m (±)	**0.53**	**1.93**	**0.69**	**0.38**
CD (5%)	**—**	**—**	**2.07**	**1.16**
Mean	**6.57**	**59.99**	**20.04**	**6.01**

V1(Yellow)

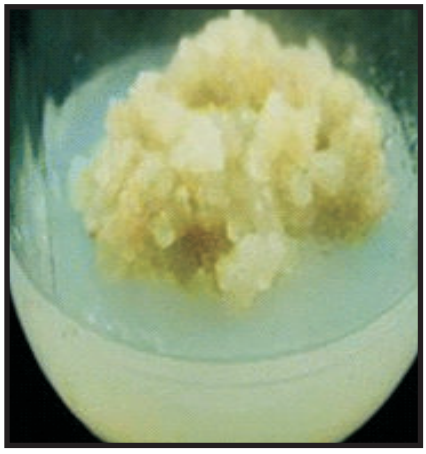
V2 (Orange)

V2 (Orange)

Leaf inoculated for callus induction



V1(Yellow)



V2 (Orange)

V2 (Orange)

Response of marigold varieties and media composition for callus induction

V1(Yellow)

V2 (Orange)

V2 (Orange)

Response of marigold variety and media composition for colour and texture of callus

BAP respectively and significantly superior over other treatments. This was followed by treatment MS+13.5 µM 2,4-D + 4.4 µM BAP(T_8) significantly superior over other treatments which took 19 days for full grown callus. Maximum days of 25.00 days and 24.78 days for full grown callus were recorded in treatment MS+13.5 µM 2,4-D + 8.8 µM BAP (T_9) and MS+4.5 µM 2,4-D + 2.2 µM BAP (T_1) respectively.Interaction effect between variety and media composition were significant for days required for full grown callus.The combination of V1T6 (15.00 d),V1T7 (15.33 d), V1T5 (15.67 d), V1T8 (15.67), V1T4 (16 d), V1T3(16.33 d) recorded less days for full grown callus and were at par with each other and significantly superior over each other. This was followed by combinations of V1T2 (18.00 d), V3T6 (18.33 d), V1T9 (18.67 d) , V2T7 (18.67 d), V2T6 (19.00 d), V2T8 (19.67 d) and were observed to be at par with each other. Maximum days for full grown callus were observed in combination of V3T1 (28.33 d) and V3T9 (30.33 d).

Weight of full grown callus

Maximum weight of full grown callus (6.91 g) was observed in yellow flower variety (V1) followed by (6.14 g) in orange flower variety (V2) and minimum weight for full grown callus (4.97 g) was observed in white flower variety (V3). These three varieties were found to be significantly superior over each other.Maximum weight 7.03 g and 6.80 g for full grown callus were observed in treatment(T6) MS + 9.0 µM 2,4-D + 8.8 µM BAP, (T_7) MS + 13.5 µM 2,4-D + 2.2 µM BAP respectively and were at par with each other and significantly superior over other treatments. This was followed by (T_4)MS+ 9.0 µM 2,4-D +2.2 µM BAP(6.30 g) , (T_5)MS+ 9.0 µM 2,4-D + 4.4 µM BAP(5.98 g) and (T_2)MS+4.5 µM 2,4-D + 4.4 µM BAP(5.81 g) which were found to be at par with each other and significantly superior over other treatments. Minimum weight of full grown callus were recorded in treatments (T_3) MS+4.5 µM 2,4-D + 8.8 µM BAP (5.38 g), (T_9)MS+ 13.5 µM 2,4-D + 8.8 µM BAP(5.25 g) and (T_1) MS+ 4.5 µM 2,4-D + 2.2 µM BAP(5.12 g).Interaction effect of variety and media composition for maximum weight of full grown callus were observed in combination of V1T6 (8.53 g), V1T7 (8.14), V1T4 (8.14 g), V1T8 (7.49 g), V2T6 (7.48 g) Minimum weight of full grown callus was observed in combination of V3T3 (4.66 g), V1T9 (5.43 g) , V2T9 (5.34 g), V3T4 (5.00 g), V2T1 (4.99 g) andV3T3 (4.66 g).

Colour and texture of callus

Colour of callus was observed to be pale green colour (Plate 1) for callus obtained from all the three flower colour varieties and all the media composition. Similar to this result Hussain and Latif (2012) obtained green callus in *Tagetes erecta*. Callus obtained from all the three flower colour varieties and all the media composition were observed to be friable in texture (Plate 1). Similar to this result Garcia *et al.* (2014) obtained friable callus on MS media supplemented with combination of BAP and 2,4-D.

From the overall studies it was observed that yellow flower variety and orange flower variety performed better for early callus formation (5.25 days , 7.70 days),highest percentage of callus formation (67.38 % , 60.74%), early formation of full grown callus (16.81 days, 21.96 %) and highest weight of full grown callus (6.91 g, 6.14 g). Among the different media composition T_6 (MS + 9.0 µM 2,4-D + 8.8 µM BAP) exhibited better performance in all the traits related to callus formation.

Thus, it is inferred from this study that yellow flower variety were suitable for callus production in media composition T6 (MS + 9.0 µM 2,4-D + 8.8 µM BAP), hence identified as suitable for callus formation on large scale.

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