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SELECTION OF EFFICIENT AM FUNGI TO ENHANCE THE XANTHOPHYLL CONTENT OF MARIGOLD (*Tagets erecta* L.)

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Abstract

Xanthophyll are the typical yellow pigment leaves that play major role in the metabolism of plants. The different Arbuscular mycorrhizal (AM) fungi were screened for their mutualistics effect with marigold (*Tagets erecta* L.) var. Salem lemon yellow. The present investigation was carried out in Pot culture house, Department of Microbiology, Annamalai University. Five AM fungal species were used *Glomus fasciculatum*, *Glomus mossae*, *Gigaspora margarita*, *Glomus versiforme* and *Acaulospora laevis.* All the inoculated seedlings showed significant results over control after 30, 60 and 90 days after transplanting. *Tagets erecta* L. (Var. Salem lemon yellow) seedlings raised in the presence of Arbuscular mycorrhizal fungi showed highest root colonization percentage, spore number, acid phosphatase activity, alkaline phosphatase activity, relative mycorrhizal dependency and xanthophyll content compared to uninoculated control plants. It was observed that *Glomus fasciculatum* the best AM fungal Symbiont for marigold plants compared to the others.

Key words: AM fungi, *Glomus fasciulatum*, Xanthophylls and Marigold.

1. Introduction

Marigold is one of the famous flowers belongs to "compositae" family and cultivated throughout India all around the year. There flowers are used for many purpose like religions, party, most of the festivals. This flower grows on wide range of soils. However, fertile sandy loam soils. Marigold flower are used to make garlands they are used to decorate the religious places. The leaves of its flowers are used as salads. Yellow dye has also been extracted from the flower. The pigments in the marigold are sometimes extracted and used as the food coloring for humans and live stocks.

Arbuscular mycorrhizal (AM) is a symbiotic association between plant roots and certain fungi which play a key role in natural cycling of various nutrients in ecosystem (Smith and Read, 2008). The mutually beneficial relationship between feeder roots of plants and fungi is called Mycorrhiza. The symbiotic association increases the supply of mineral nutrients to the plant, particularly those whose ionic forms have a poor mobility rate or those which are present in low concentration in the soil and thus promote plant growth (Erco-lin and Reinhardt, 2011). Arbuscular mycorrhizal fungi are obligate symbionts that colonize the roots of most cultivated plant species (Cavagnara *et al.*, 2006; Singh et *al.*, 2008; Lakshman, 2009, 2012).

Mycorrhizal symbiosis can be found in nearly all types of ecological situations and most plant species are able to form this symbiosis

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naturally (Smith and Read, 2008; Lakshman and Kadam, 2011). The purpose of this present research was screening for selection of efficient stains of AM fungi inoculating with marigold (*Tagets erecta* L.) var. Salem lemon yellow to enhance the highest root colonization percentage, AM fungal spore number, acid phosphatase activity, alkaline phosphatase activity, relative mycorrhizal dependency and xanthophyll content. Xanthophyll are type of pigments that play a role in the metabolism of plants, there are yellow pigment of leaves. Since, as protection of excessive amount of sunlight to prevent further damage in the plants. Presently, the use of AMF application as a biofertilizer has been recommended with the aim of increasing xanthophyll content and flower yield by reducing chemical fertilizer.

2. Materials and Methods

Purchase of seeds

The physical and chemical characteristics of soil were estimated by Jackson (1973). The seeds of marigold (*Tagets erecta* L.) var. Salem lemon yellow were collected from Department of Horticulture, Annamalai University, Annamalai Nagar. Seeds were surface sterilized by treating with 1 % sodium hypo chloride for 2 - 3 min before sowing.

Inoculation of AM fungi

The five AM fungal species were collected from twenty five locations of Cuddalore district Tamil Nadu. The survey was done to ascertain the colonization ability of AM fungi in the roots of marigold. For the mass multiplication of mycorrhizal inoculum in the earthen pots of 30 cm diameter filled with sterilized sand soil (1:1) fumigated by using 2 per cent formaldehyde solution. About 50 gram of *Glomus faciculatum* was added to each pot containing the substrate. Sorghum seeds at 10 seeds per pot were sown. After examining the roots for AM fungal colonization 30 Days after sowing the roots were cut into small bits and mixed with the soil. The sorghum seeds were sown in AM fungi applied pots for multiplication. After 40 days, the roots and soil were tested for the colonization of AM fungi and spore number. This was called as "Mother culture - 1".

The root pieces from the mother culture -1 as well as soil were incorporated again into earthen pots containing the similar soil mixture and sown with the seeds of sorghum. After 40 days, the roots and soil were tested for mycorrhizal colonization and a number of spores. The soils along with root pieces were taken. "This soil based root inoculum" containing more than 15 spores per gram of soil was used as inoculum in the pot culture studies. The soil based root inoculum was stored at 5°C. The following observation were recorded on 30, 60, 90 days after showing.

1) Relatively effective colonization in the marigold as evidenced by percent root infection.
2) Number of spores present in the 100g of rhizosphere soil of marigold.
3) The acid and alkaline phosphatase enzyme activities of marigold (Morton, 1952).

Mycorrhizal percent root colonization in marigold

The percent root colonization was evaluated microscopically followed by clearing of roots in 10 % KOH, neutralized in 2% HCl and stained with 0.05 % trypan blue in lactophenol according to method described by Phillips and Hayman (1970) and mycorrhizal root colonization was calculated as mentioned below.

$$\text{Percentage of root colonization} = \frac{\text{Number of root bits with infection}}{\text{Total number of root bits examined}} \times 100$$

AM fungal spores population

The AM fungal spores population was counted in 50 g of soil by Wet sieving and decanding method (Gerdmann and Nicolson, 1963).

Enzyme activity of marigold roots

The enzyme phosphatase hydrolyzed para-nitrophenyl phosphate. The released p-nitrophenol

was yellow in colour in alkaline medium and was measured at 725 nm. The optimum pH for acid phosphatase was 4.5 and for alkaline phosphatase were 8.5

Enzyme extract

AM fungal inoculated 10g of marigold roots inoculated with AM fungal culture were ground thoroughly with acid washed sand in a pre-chilled pertle and mortar in grinding medium containing 20 ml of 0.2 M acetate buffer (pH 4.5) for acid phosphate or 0.2 M acetate buffer (pH 8.5) for alkaline phosphate. The homogenate was passed through four layers of cheese cloth and filtrate was centrifuged at 3000 rpm for five minutes. Supernatant was used as enzyme source.

The substrate P-Nitrophenyl phosphatase of 10 g was dissolved in 100 ml of distilled water. One ml of substrate was pipette out into a test tube and two ml of enzyme extract and five ml of 0.2 M acetate buffer (pH 4.5) were added. This was incubated for 24 hour and one drop of 10 per cent TCA was added and centrifuged from this one ml of clear supernatant, 1 ml of folin cio calteau reagent and 2 ml of 20 per cent sodium carbonate were added and boiled for one minute at 1000ºC. Then the test tube was cooled and volume was made up to 10 ml with distilled water. The color intensity was read at 725 nm, standard curve using P-Nitrophenol was drawn and from this activity was calculated.

Estimation of alkaline phosphatase

Alkaline phosphatase activity was measured by adopting the procedure described for acid phosphatase. Except that here the borate buffer (0.2 M pH 8.5) was used instead of acid buffer.

Estimation of acid phosphatase activity

The phosphatase activity was measured in the roots as described by Morton (1952).

Estimation of xanthophyll content

Xanthophyll content was estimated by AOAC method (AOAC, 1960). The dried petals are collected and then it is finely powdered, then it is weighed to 0.05 g and taken in the volumetric flask, about 30 ml of the extracting (10 parts of hexane + 7 parts of acetone + 6 parts of absolute alcohol + 7 parts toluene) was added into the flask and it is shaken well.

Two ml of 40 % methanolic KOH was pipette into the flask is refluxed in water bath at 56 °C. Air condenser was also attached to prevent the loss of solvent by evaporation, then the sample is cooled and kept in dark for one hour and 30 ml hexane was added into the flask after shaking for one minute the volume was make up to 10 % sodium solution after vigorous shaking it was placed in dark for one hour, the upper phase was collected in 50 ml volumetric flask. Three ml of the upper phase was pipette into a 100 ml volumetric flask and the volume was made up with hexane and the absorbance was measured at 474 nm.

The total xanthophyll content in the sample was calculated by using the formula

$$\text{Total xanthophyll}\left(\frac{\text{g}}{\text{kg}}\ \text{petal meal}\right) = \frac{\text{A}_{474} \times \text{D}}{\text{W} \times 236}$$

Where,

$$\text{A}_{474} = \text{Absorbance at 474 nm}$$

W = weight of the sample (petal meal) in g

$$\text{D} = \text{Final dilution} = \frac{50 \times 100}{3}$$

236 = translation specific absorptivity for 1 gm/litre

Xanthophyll yield (kg ha^{-1})

After estimating the xanthophyll content from one kilogram of petal meal it was multiplied by the total petal meal yield/ha and expressed as kg ha^{-1}.

$$\text{Xanthophyll yield kg ha}^{-1} = \text{Total xanthophyll (g/kg/petal/meal)} \times \text{Petal meal (yield/ha)}$$

Treatments and experimental details

The experiment was conducted by completely Randomized block design with three

replication of each treatment and uninoculated control without inoculum was maintained. The treatments schedule was as follows.

T_1 : Control

T_2 : 100% NPK

T_3 : *G. fasciculatum*

T_4 : *Bacillus megaterium*

T_5 : 100% NPK + *G. fasciculatum*

T_6 : 100% NPK + *Bacillus megaterium*

T_7 : 100% NPK + *G. fasciculatum* + *Bacillus megaterium*

T_8 : 75% P + 100% N and K + *G. fasciculatum*

T_9 : 75% P+ 100% N and K + *Bacillus megaterium*

T_{10} : 75% P +100% N and K+ *G. fasciculatum* + *Bacillus megaterium*

T_{11} : 50% P+ 100% N and K + *G. fasciculatum*

T_{12} : 50% P+100% N and K + *Bacillus megaterium*

T_{13} : 50% P+ 100% N and K + *G. fasciculatum* + *Bacillus megaterium*

3. Results and Discussion

The isolated spores *viz*., *G. fasciculatum, G. mossae, Gi. margarita, A. leavis* and *G. versiforme* were screened to select on efficient strain for further studies. Pot culture experiment was conducted to screen the five AM fungal isolates *viz., G. fasciculatum, G. mossae, Gi. margarita, A. laevis* and *G. versiforme* for the root colonization percentage, spore number (per 100 g of rhizosphere soil), acid and alkaline phosphatase activity in marigold plant var Salem lemon yellow (Table - 1). The per cent root colonization, spore number ($100g^{-1}$ of rhizhosphere soil), acid and alkaline phosphatase enzyme activities were found to be higher in *G. fasciculatum* inoculated marigold plants compared to other isolates *G. mossae*, *Gi. margarita, A. laevis* and *G. versiforme* inoculated plants.

The highest root colonization (79.23 %) and spore number (184.43/100 g of rhizosphere soil) were recorded in *G. fasciculatum* inoculated plants followed by *G. mossae* (65.20 % and 179.23/100 g rhizosphere soil), *Gi. margarita* (55.16 % and 169.68/100 g rhizosphere soil), *A. laevis* (59.16 % and 173.83/100 g rhizosphere soil) and *G. versiforme* (50.20 % and 167.45/100 g rhizosphere soil) on 90 DAS.

The highest acid and alkaline phosphatase activities were recorded in *G. fasciculatum* inoculated roots as 31.73 and 31.36 µg 24 h^{-1} 10 g of root) followed by *G. mossae* (31.53 and 29.55 µg 24 h^{-1} 10 g of root). The other isolates were able to exhibit between 28.43 - 31.53 and 25.20 – 29.55 µg 24 h^{-1} 10 g of root respectively for acid and alkaline phosphatase activity. In general, the acid phosphatase activity was more when compared to alkaline phosphatase activity. The inoculation effect of five AM fungal isolates *viz., G. mossae, G. fasciculatum, G. versiforme, A. laevis* and *Gi. margarita* on relative mycorrhizal dependency (RMD) and mycorrhizal inoculation effect (MIE) were studied and the results are presented in Table - 2.

The results clearly revealed that the unsterilized soil (normal) supported the growth of marigold than sterilized soil. It was observed that the plant dry weight was able to increased 17.94 % in normal soil (unsterile soil) over sterilized soil. When AM fungal isolates were inoculated with the plant dry weight was increased from 1.91 % to 73.07 % levels. The maximum plant dry weight of 28.56 g/plant was observed in *G. fasciculatum* inoculated plants and the minimum 15.26 g/plant was observed in *G. versiforme*.

Table -1: Screening on AM fungal isolates for colonization spore population and phosphatase activity in Marigold

S. No.	AM fungal isolate	Root colonization (%)			AM fungal spore number (100 g^{-1} of rhizosphere soil)			Acid phosphatase activity (µg/ 24 hrs^{-1} $10g^{-1}$ of root)			Alkaline phosphatase activity (µg/ 24 hrs^{-1} $10g^{-1}$ of root)		
		30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
1	*Glomus mosseae*	42.23	58.43	65.20	146.20	168.90	179.23	28.56	29.60	31.53	27.30	28.59	29.55
2	*Glomus fasciculatum*	54.20	65.26	79.23	155.17	172.42	184.43	29.51	30.73	31.73	28.70	30.03	31.36
3	*Glomus versiforme*	28.27	40.23	50.20	129.73	160.03	167.45	25.43	27.20	28.43	24.17	24.86	25.20
4	*Acaulospora laevis*	39.50	52.30	59.16	141.46	165.86	173.83	27.30	28.40	30.23	26.06	27.10	27.46
5	*Gigaspora margarita*	32.23	45.36	55.16	134.43	160.93	169.68	26.46	28.01	29.21	24.56	25.63	25.65
	SE	1.59	1.13	1.03	1.19	0.76	1.25	0.36	0.23	0.41	0.49	0.45	0.69
	CD (p = 0.05)	3.19	2.25	2.06	2.35	1.53	2.49	0.76	0.45	0.83	0.96	0.93	1.39

Table 2: Screening of AM fungal isolates for relative mycorrhizal dependency and mycorrhizal inoculation effect of Marigold

S.No.	Treatments	Dry matter production (g/plant)
1	Sterilized soil	13.20
2	Unsterilized soil	15.03
3	Unsterilized soil + *G. mosseae*	23.82
4	Unsterilized soil + *G. fasciculatum*	28.56
5	Unsterilized soil + *G. versiforme*	15.26
6	Unsterilized soil + *A. laevis*	21.58
7	Unsterilized soil + *Gi. Margarita*	17.28
	SE	0.56
	CD (p = 0.05)	1.20

A	Relative mycorrhizal dependency	31.80%
B	Mycorrhizal inoculation effect of *G. mosseae*	18.88%
C	Mycorrhizal inoculation effect of *G. fasciculatum*	20.58%
D	Mycorrhizal inoculation effect of *G. versiforme*	14.59%
E	Mycorrhizal inoculation effect of *A. laevis*	17.70%
F	Mycorrhizal inoculation effect of*Gi. Margarita*	16.46%

Table 3: Effect of AM fungi (*G. fasciculatum*) and phosphobacteria (*Bacillus megaterium var phosphaticum*) with graded levels of inorganic phosphorus on the xanthophyll content of Marigold at harvest stage

Treatments		Xanthophyll content	
		gkg^{-1}	$kgha^{-1}$
T_1	Control	33.43	12.73
T_2	100% NPK alone	33.00	14.60
T_3	*G. fasciculatum* alone	35.10	15.50
T_4	*Bacillus megaterium* alone	35.00	15.43
T_5	100% NPK + *G. fasciculatum*	37.95	17.80
T_6	100% NPK + *Bacillus megaterium*	37.60	17.60
T_7	100% NPK + *G. fasciculatum* + *Bacillus megaterium*	41.01	20.80
T_8	75% P and 100% N&K + *G. fasciculatum*	39.95	19.56
T_9	75% P and 100% N&K + *Bacillus megaterium*	38.05	18.40
T_{10}	75% P and 100% N&K + *G. fasciculatum* + *Bacillus megaterium*	43.15	22.66
T_{11}	50% P and 100% N&K + *G. fasciculatum*	36.99	15.80
T_{12}	50% P and 100% N&K + *Bacillus megaterium*	36.46	15.60
T_{13}	50% P and 100% N&K + *G. fasciculatum* + *Bacillus megaterium*	39.03	19.60
	SE	1.05	0.90
	CD (p = 0.05)	2.13	1.83

The marigold plant var Salem lemon yellow was considered as AM fungi moderately dependent as it RMD was 31.80 % on 90 DAS.

All the five AM fungal isolates inoculated in marigold significantly increased the plant dry weight over uninoculated soil. The mycorrhizal inoculation effect (MIE) of *G. fasciculatum, G. mossae, A. laevis, Gi. margarita* and *G. versiforme* were 28.56 %, 23.82 %, 21.58 %, 17.28 % and 15.26 % respectively. The inoculation of *G. fasciculatum* recorded the highest MIE among the five AM fungal cultures tested.

Based on the root colonization percentage, spore number and alkaline phosphatase activities, RMD and MIE parameters *G. fasciculatum* was found to be efficient for the marigold crop and selected for further studies.

The inoculation effect of *G. fasciculatum* and *Bacillus megaterium* along with graded levels of inorganic P and recommended dose of N and K on the xanthophyll content of marigold were recorded and presented in Table - 3.

The xanthophyll content was measured at the time of harvest stage of marigold. The significant effect of *G. fasciculatum* and *Bacillus megaterium* along with graded levels of inorganic P and recommended dose of N & K fertilizers over control was observed. The maximum xanthophyll content was found in (T_{10}) 75 % P and 100 % N & K + *G. fasciculatum* + *Bacillus megaterium* (43.15 g kg^{-1} and 22.66 kg ha^{-1}) which was followed by T_7 (41.01 g kg^{-1} and 20.80 kg ha^{-1}). The minimum amount of xanthophyll content was recorded in control (33.43 g kg^{-1} and 12.73 kg ha^{-1}).

Marigold is a recognized as a mycotrophic plant (Bharathiraja and Tholkappian, 2011a) the present study mycorrhizal parameters, such as percent root colonization and spores, were considerably higher in all the inoculated treatments compared to the uninoculated control treatment. The extent of colonization and the spore count varied with different AM fungi. However, (Declerck *et al*., 1995), working with several banana cultivars and Arbuscular mycorrhizal fungi, observed different growth promotional effects defending on the banana cultivar and the *Glomus* strain the quality of inoculums also is important. (Ortas *et al*., 2002; Ortas, 2008, 2009).

4. Conclusion

Based on the above findings, it's clear that the use of AM fungi can able to promote growth and xanthophyll content of marigold *Tagets erecta* L. with the minimized use of chemical fertilizers and leads to reduction of 50 % cost of chemical fertilizers and as well as reduction of pollution to some extent.

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