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In vitro callus induction of safflower *(Carthimus tinctorius L.)*

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Abstract
In present study efforts were made to improve to study invitro callus induction of safflower. Safflower local genotype was used to study in vitro callus induction from leaf explant. Safflower seed were in vitro germinated on plane MS media. The leaf explants were excised from it aseptically cultured on MS media with different concentrations of 2, 4-D and incubated at 25 ± 2°C in light condition. Good callusing was observed on MS media containing 3.0, 3.5, 4.0, mg / l 2, 4-D within 3-4 week, yielding white embryonic and globular shaped calli. Profuse callusing was obtained on MS media containing 3.5 mg / l 2, 4-D.

Keywords: safflower, regeneration, 2, 4-D, Callus

Introduction
Safflower is one of humanity's oldest crops, but generally it has been grown on small plots for the grower's personal use and it remains a minor crop with world seed production around 80000 t per year (Gyulai, 1996) [1]. Oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now for its edible oil for cooking, margarine and salad oil. China has a significant area planted to safflower, but the florets are harvested for use in traditional medicines and the crop is not reported internationally.

Safflower offers a tremendous scope for genetic improvement through the exploitation of tissue culture techniques. The protocols defined are simple, rapid and highly efficient and it is possible to regenerate whole plantlets from somatic as well as gametic tissues. The regeneration frequencies reported are very high and regeneration is possible through embryogenic and organogenic pathways. Tissue culture techniques been developed for the Indian and American cultivars. In general, the mode of regeneration is through direct or indirect organogenesis (Tejovathi and Anwar, 1987; Orlikowska and Dyer, 1993; Sujatha and Suganya, 1996; Nikam and Shitole, 1999) [7, 6]. Direct somatic embryogenesis from cotyledonary leaves has been also reported (Mandal *et al*., 1995) [3]. Safflower tissues including roots are amenable to *in vitro* manipulations as evidenced by the simple media requirements and most of the experiments have employed Murashige and Skoog's, 1962 [4] salt as the basal medium. However, the fact that the studies are restricted to the use of seedling explants and rhizogenesis of *in vitro* regenerated/multiplied shoots has been consistently problematic thus reducing the overall efficiency of whole plant regeneration. There is a need to develop protocols of regeneration from mature plant parts, which would enable to propagate elite plants being selected based on phenotypic characters

Material and Methods
The safflower genotype used in this study is "Tara" was collected from the plot maintained at College Farm, pokharni, Nanded. Safflower seeds used as explants. Safflower seed was washed with clean water for 30min then put in a sterilized flask or universal bottle. First the explant was sterilized with Tween-20 for 5min. After shaking explants are rinsed with sterilized distilled water. Then explant were sterilized with fungicide Bavistin for 5min; washed with sterilized distilled water for 4-5 times. After explant sterilized with Sodium Hypochloride for 2-3 min. then washed with sterilized distilled water. The mercuric chloride ($HgCl_2$) at a concentration of 0.1% is used for surface sterilization of these explants. After 1min shaking with $HgCl_2$ explants were rinsed 5 - 7 times with sterilized distilled water in a laminar flow cabinet for the removal of any traces of $HgCl_2$. Completely sterilized leaf was used for culturing. Under sterilized condition of laminar flow cabinet these seeds were cultured in sterilized media test tubes near the burner. A spirit lamp was used for the sterilization of forceps. The cultured test tubes of explants are kept in a growth chamber at 25±2°C and 65-70% humidity with a photoperiod of 16/8 h. After growing explant take leaf of it transfer into the M.S media The basal MS (Murashige and Skoog, 1962) [4] media along with Gamborg B5 Vitamins were used.All the treatments were followed in all explants of the cultivar Tara to evaluate the response in different cultures.

Results and Discussion
Leaf explants were taken from 10-16 days old grown seedlings and cultured on the MS media with different concentrations of 2, 4-D for the callus induction. White, compact, embryogenic calli were produced from leaf explant within 15-25 days of inoculation on 2, 4-D supplemented media. Highest callusing was observed on MS media supplemented with 2, 4-D at 3.5 mg/l concentration (Table no.2 and Fig no.4).

The present findings are in accordance with Hassan et.al. (2009) [2] where in 0.5 ppm to 2.5 ppm 2, 4-D only callus was observed on the surface of the cultured explants and in 3.0mg/L to 4.0 mg/L

2, 4-D callus responded more. The present findings are in accordance with (Rani *et al*., (2003) [5]; Thomas and Masseena (2006) [8]. Slight decrease or increase in 2,4-D concentration gave varied results during present work.

Table 1: Effect of 2, 4-D on callus induction

MS composition	Hormone conc. (mg/L)	No of explant responded/cultured explants	Callusing ability of explants	Nature of callus
MS+2,4-D	3.0	06/10	+++	Good callus responded
MS+2,4-D	3.5	08/10	+++++	Extraordinary high rate of callus induction
MS+2,4-D	4.0	06/10	+++	Good callus responded

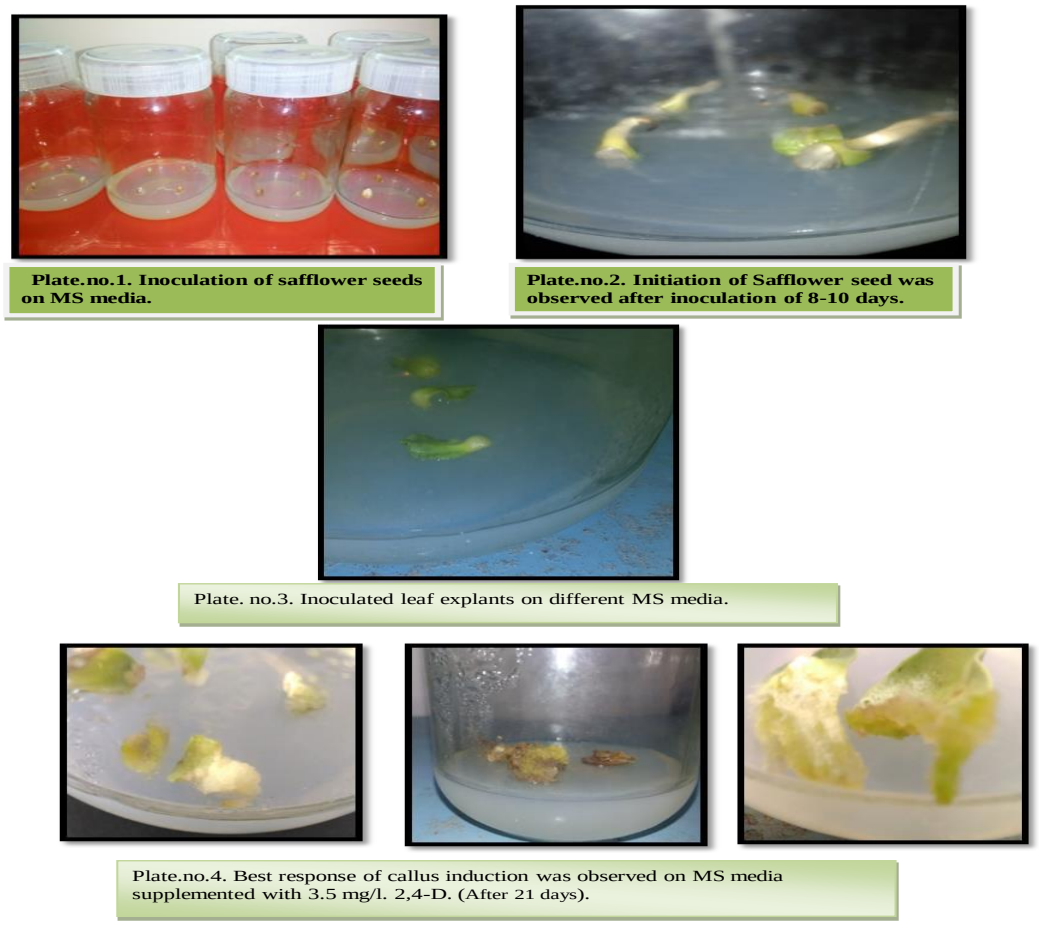

Plate.no.1. Inoculation of safflower seeds on MS media.

Plate.no.2. Initiation of Safflower seed was observed after inoculation of 8-10 days.

Plate. no.3. Inoculated leaf explants on different MS media.

Plate.no.4. Best response of callus induction was observed on MS media supplemented with 3.5 mg/l. 2,4-D. (After 21 days).

Fig 1

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