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Effect of different concentration of 2, 4-D on callus induction in safflower

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Abstract
In present investigation *in vitro* callus induction response of hypocotyls was studied in safflower cultivar AKS- 207. Callus induction response was evaluated with different four levels of 2, 4-D (1.0-2.5mg/l) and Kinetin (1mg/l). Among four combinations (Treatments) MS + 2,4D + Kinetin (2.5 + 1.0 mg/l) (T_4) showed more significant growth (90%) of callus as compared to other three combinations *viz*. (T_1) MS + 2,4D + Kinetin (1.00 + 1.00 mg/l) (50%), (T2) MS + 2,4D + Kinetin (1.5+1.00mg/l) (60%) and (T_3) MS + 2,4D + Kinetin (2.00 +1.00 mg/l) (60%). Study revealed that as concentration of 2,4D increases then growth of callus also increased.

Keywords: Callus, genotype, hypocotyls, NAA

Introduction
Safflower (*Carthamus tinctorius* L. 2n = 24), is a member of the family Composite or Asteraceae. It is mainly grown for florets, oil and orange red dye (Carthamin) extracted from plant and seed. It is known for its medicinal value used in traditional crude drug for promoting blood circulation and blood stasis in China. It has been used clinically in the prevention and treatment of cardio-vascular and thrombotic diseases (Bie, 2003; Jiang *et al*., 2005) [2, 5].
For genetic improvement in safflower through the exploitation of plant tissue culture technique offers a tremendous scope. Attila Feher (2019) has been reported plant cell and tissue culture methods are efficient means to preserve and propagate genotypes with superior germplasm as well as to increase genetic variability for breading. Callus culture is most important type of culture in plant tissue culture methods. Manipulation of growth regulators concentration in nutrient medium can lead to organogenesis and from which whole plant subsequently will be produced (Tariq *et al*., 2008) [9]. Callus culture can be use for secondary metabolite production (Janarthanam *et al*, 2010) [4] and producing whole plant containing genetic variability. The present study was aimed to study effect of different concentration of 2, 4D on callus induction for cultivar AKS-207.

Material and Method
The present investigation was carried out in Plant tissue culture laboratory, Vasantrao Naik College of Agricultural Biotechnology, Yavatmal. The Seeds of safflower AKS-207 were procured from Oilseed research unit of Dr. PDKV, Akola, Maharashtra.

Culture media
To prepare 1 lit of MS medium, stock solutions and other components were added as per the requirement in 800 ml double distilled water. Desired concentration of growth regulators were added from stock solution according to the culture media combinations. The medium was continuously stirred on magnetic stirrer and the pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCl and final volume was made to 1 lit. Agar was added in the medium prior to sterilization in an autoclave for 20 min at 121 ºC and 15 psi. Warm culture medium, still in liquid state was poured into pre-sterilized plant tissue culture bottle (30-35 ml/PTC bottle) under laminar air flow cabinet. Media was pre-incubated at 25 ºC in light for four day for screening of contamination.

Seed Sterilization
Seeds were washed with few drops of Tween-20 for 5 min. followed by four times washing with distilled water. Then seeds were surface sterilized with 70% ethanol for 30 sec and rinsed with sterile distilled water for thrice.

To reduce fungal contamination seeds were again treated with bavistin (0.8%) to reduce the fungal contamination and rinse with sterile distilled water thrice. The washed seeds were again treated with 0.1% (w/v) $HgCl_2$ for 5 min followed by rinsing with sterile distilled water for 4-5 min. Washed seed soaked in petri plate with help of sterile tissue paper for prior to germination.

***In vitro* seed germination**

Seeds were inoculated on half basal MS medium in PTC bottles. Then PTC bottles were incubated in culture room at 25 ± 2 for 16 hrs light (3000 lux) and 8 hrs dark condition was maintained.

Callus induction through different levels of MS media

Hypocotyls were excised from 9 to 10 days old grow seedling and cut into 0.5-1cm segments. Hypocotyls were inoculated on MS medium fortified with different concentration of 2, 4-D + 0.5 mg/l of kinetin (Table no.1). In each PTC bottle three hypocotyls were inoculated and incubated at 25±2 °C under 24 hour dark conditions. Observations were recorded on 7,14,21 and 28 days after inoculation.

Results and Discussion

Callus induction in safflower (*Carthamus tinctorius* L.) genotype AKS- 207 was optimized using hypocotyls. Callus induction was carried out on different concentration of 2, 4-D and kinetin. Four different combinations i.e. 1mg/l 2, 4-D + 1.0 mg/l Kinetin (T_1), 1.5 mg/l 2,4D + 1mg/l Kinetin (T_2), 2mg/l 2,4-D + 1mg/l Kinetin (T_3), 2.5mg/l 2,4-D + 1mg/l kinetin (T_4) were used for callus induction in safflower genotypes AKS-207. Twenty plant tissue culture (PTC) bottles of each combination were prepared for inoculation of explants.

The cultures incubated in a dark culture room at 24 °C for 4 week. Out of four combinations (Treatments), MS media supplemented with 2.5 mg/ l 2, 4-D + 1 mg/l Kinetin (T_4) showed white and green embryogenic callus response and high rate of callus induction (90%) depicted in table 1 and fig 1.Treatment (2) and (3) showed 60% callus growth on the surface of explants and Treatment (1) showed 50% callus growth on the surface of explants showed in table no.1.

Table 1: Effect of 2, 4D and Kinetin on callus induction in safflower

Sr. No.	Treatments	MS composition	Hormone conc. (mg/L)	No. of explants responded/Cultured explants	Callusing Percentage %	Nature of callus
1	T_1	MS + 2,4D+Kinetin	1.00 + 1.0	10/20	50	Callus response
2	T_2	MS + 2,4D+Kinetin	1.50 + 1.0	12/20	60	Good callus response
3	T_3	MS + 2,4D+Kinetin	2.00 + 1.0	12/20	60	Good callus response
4	T_4	MS + 2,4D+Kinetin	2.50 + 1.0	18/20	90	High rate of callus induction

The present results showed similar finding accordance to Thomas and Massena (2006), as concentration of 2,4D decrease or increase results growth of callus also varied. Similar results were obtained by Rani *et al* (2003) [8] and Hassen *et al*. (2009) [3] they observed that as concentration of 2,4D increase in cultured media then callus response also increased. The result revealed that auxin plays an important role in callus induction (Baskaran *et al*., 2006) [1].

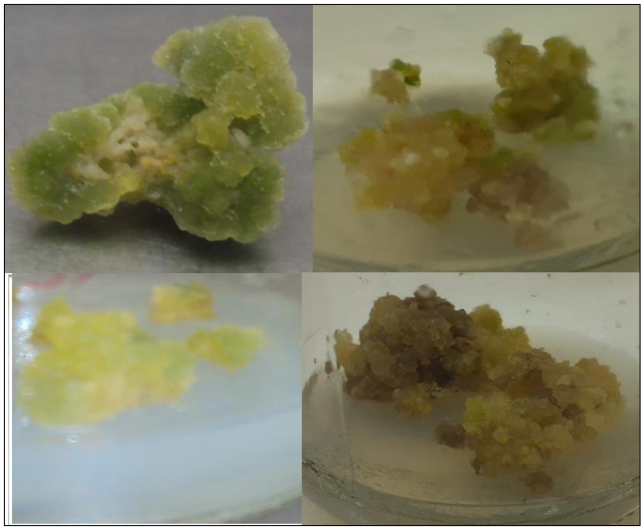

Fig 1: Callus induction on MS media supplemented with 2.5 mg/l 2, 4-D and 0.5 mg/l kinetin

Conclusion

We are concluded that callus induction was efficiently influence by optimum concentration of growth regulators auxin and cytokinin. When 2, 4-D concentration increased then growth of calli also increased.

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