The Pharma Innovation Journal 2021; 10(9): 1971-1973

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(9): 1971-1973

www.thepharmajournal.com
Received: 10-07-2021
Accepted: 23-08-2021

Hemant Wagh
Swami Vivekanand College, Udgir, Latur, Swami Ramanand Tirth Marathwada University, Nanded, Maharashtra, India

BM Kareppa
Dnyanopasak Shikshan Mandal's College, Parbhani, Maharashtra, India

Ravindra Satbhai
Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Sandesh Bangar
Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Maharashtra, India

Corresponding Author:
Hemant Wagh
Swami Vivekanand College, Udgir, Latur, Swami Ramanand Tirth Marathwada University, Nanded, Maharashtra, India

The effect of different concentrations of NaCl on the callus induction in safflower

Hemant Wagh, BM Kareppa, Ravindra Satbhai, Sandesh Bangar

Abstract
In present investigation the effect of salt induced (NaCl) stress on growth and development of callus induction of safflower variety AKS-207 was evaluated. The different concentration treatment of NaCl (50mM, 100mM, 150mM, and 200mM) with 2, 4-D (2.5mg/l) + Kinetin (1.0 mg/l) on MS media were used to screen the callus induction response. Among the four combinations of salt stress concentration 150mM NaCl with 2, 4-D (2.5mg/l) + Kinetin (1.0 mg/l) (T_4) recorded 65% growth of callus and showed hard texture and brown colour compare to other salt treatments. The control treatment (T_1) recorded white, soft and 75% callus induction response. The study revealed that the 150mM NaCl concentration was sustain the callus induction in safflower.

Keywords: Safflower, callus, kinetin, 2, 4-D, NaCl

Introduction
Safflower (*Carthamus tinctorius* L.) has been traditionally grown for its flower and oilseed to produce cooking oil, fabric dyes, food colouring, and animal and bird feeds. It has also found medicinal and industrial applications such as biofuel (Weiss 2000; Nimbkar 2008) [12, 7]. Salinity is the major abiotic stress factor restricts the crop productivity in arid and semi-arid region of the world wide (Hasegwa, 2013). Crop grown under higher salt levels in soil adversely affect the plant activity at physiological, biochemical and molecular level which hamper on final crop yield (Arzani and Ashraf, 2016) [1]. Plant breeding programme were commonly used for development of high yielding genotypes but it is difficult to introgressed traits related to abiotic stress. Plant tissue culture techniques prominent aspect screening salt tolerant genotype and for development of verity (Rai *et al*., 2011) [8]. *In vitro* culture of plant cell or tissue developed more interest over recent years because it provides the means to study plant physiological and genetic processes under different abiotic stresses such as cold hardiness, salt tolerance and drought tolerance in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability (Karp *et al*. 1987; Gawande *et al*. 2005) [6, 2]. *In vitro* selection of salt tolerant cell lines has been reported for several species (Tal 1994) [10]. Previously, callus induction reported under drought stress in sugarcane (Mahmood *et al*. 2012). This technique is based on the *in vitro* culture of plant cells, tissues or organs on a medium supplemented with selective salts and only the regenerated plantlets capable of sustaining such environments are selected to obtain desirable characteristics. Soil salinity is the major abiotic constraint which affects the production and productivity of oilseed, cereals, pulses and fibre crop in Marathwada and Viderbha region in view of that the effect of different concentration of salt stress (NaCl) with 2.4. D and Kinetin has been assessed on MS media in safflower genotype AKS-207 for their callus induction response.

Material and Method
Seed germination, callus induction through hypocotyls sand salt tress treatment
The present investigation was carried out at Plant tissue culture laboratory, Vasantrao Naik College of Agricultural Biotechnology, Yavatmal. Seeds of safflower AKS-207 were procured from Oilseed Research Station of Dr. PDKV, Akola, Maharashtra. Sterile seeds were inoculated on half basal MS media (Murashige and Skoog, 1962) [5] in plant tissue culture (PTC) bottle. Then PTC bottle are incubated in culture room at 25°C ± 2°C for 16 hrs under light (3000 lux) and 8 hr in dark condition. The condition was maintained throughout the experiment period. The grownd hypocotyls were excised from 9 to 10 days old seedling and cut into 0.5-1cm segments.

Hypocotyls were inoculated on MS media fortified with different concentration of 2, 4-D + 0.5 mg/lit of kinetin. In each PTC bottles, three hypocotyls were inoculated and incubated at 25±2°C under 24 hour dark conditions. After 4 week induced calli were placed on medium supplemented with NaCl (50mM, 100mM, 150mM and 200mM). MS Medium without NaCl salt used as a control (T_1). Calli inoculated in PTC bottle were incubated at 25 ± 2°C under 24 hour dark conditions. Calli showing growth/induction under salt condition was recorded on 7, 14, 21 & 28 days after inoculation table no.1 and fig.1.

Table 1: Callus subculture on NaCl containing nutrient medium

Treatment	MS+2,4-D (mg/L) + 0.5 mg/l Kinetin	NaCl (mM)	No. of bottle containing subcultured callus	Callus response	% of response	Texture of callus	Color of callus
T_1	2.5 +0.5	-	20	15	75	Soft	White, green
T_2	2.5 + 0.5	50	20	12	60	Hard	Brown
T_3	2.5 + 0.5	100	20	12	60	Hard	Brown
T_4	2.5 + 0.5	150	20	13	65	Hard	Brown
T_5	2.5 + 0.5	200	20	10	50	Hard	Brown

Results and Discussion

In present investigation callus induction was taken on media supplemented with 2, 4-D (2.5mg/l). Fully grown callus were sub cultured on MS media supplemented with NaCl salt. Total four combinations (T2 to T5) were tested for callus subculture on NaCl containing media for safflower genotypes AKS-207. Observations were recorded on 7, 14, 21 & 28 day. Out of four combinations MS media containing 150 mM NaCl with 2.5mg/l of 2,4D and 0.5 mg/l of Kinetin showed best response in AKS-207 genotype compare to other combinations (Table 1 and Fig 1). In this study, two major factors such as 2, 4-D and NaCl salt stress were used for subculturing of callus. The variation in the NaCl concentrations had remarkable response recorded in callus response percentage from 50 to 65%. The calli which were formed on salt-free medium (T_1-Control) became soft and callus from salt stress condition were hard (T2 to T5). Maximum callus response was obtained on salt free medium (75%) whereas minimum percent (50%) was noticed in media supplemented with 200mM NaCl. It was also observed a distinct impact of salt on callus texture and its colour. *In vitro* callus culture in MS media supplemented with NaCl provide efficient way to regenerate salt tolerance plantlets because it is difficult to detect salt tolerant plant under field condition (Richards, 1996). The application of NaCl salt in media induced adverse effect on callus growth and development was recorded previously in safflower by Soheilikhah *et al.*, (2013) [9] and our observation in the present study was in accordance with their results. MS media supplemented with 2.5mg/l 2, 4-D (Auxin) in our study showed good callus response suggesting auxin played an important role in callus induction. Similar observation has been recorded previously in safflower by Ghasempour *et al*. (2014) [3] and in wheat under different salt condition by Benderradji *et al*. (2011). Vaziri (2004) [11] observed that different treatments with different concentrations of NaCl in soybean showed that the growth of calli decreased when salinity was high.

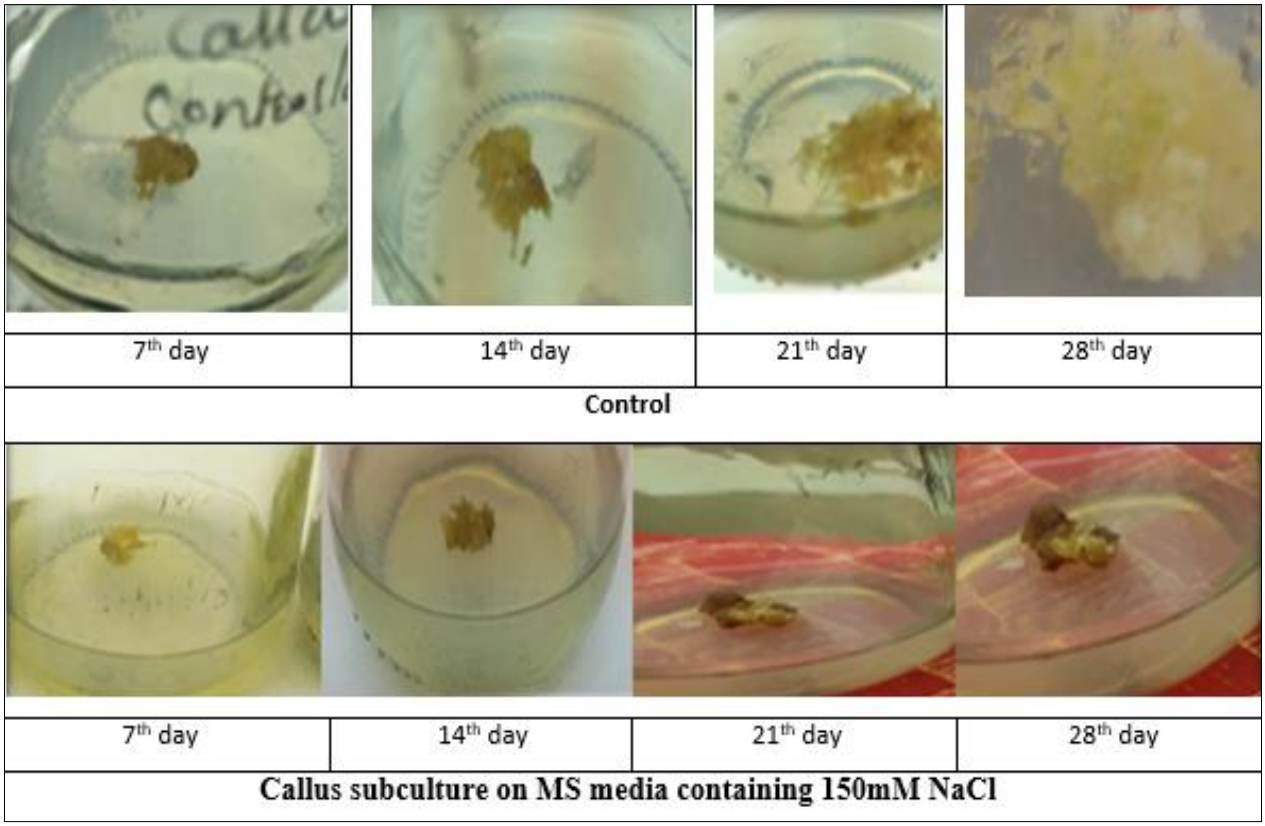

Fig 1: Comparison of callus induction between control and Callus induction on MS media supplemented with NaCl

Conclusion

The present study revealed that in *vitro* culture is the effective tool to study effect of salinity on callus growth and development in tissue culture. Among the four combinations of salt stress concentration 150mM NaCl with 2, 4-D (2.5mg/l) + Kinetin (1.0 mg/l) (T_4) recorded 65% growth of callus and showed hard texture and brown colour compare to other salt treatments.

Acknowledge

We are thankful to associated dean of Vasantrao Naik College of Agricultural Biotechnology, Yavatmal, Dr. PDKV, Akola providing laboratory facility for smooth conduct of experiment.

References

1. Arzani A, Ashraf M. Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Crit. Rev. Plant Sci 2016;35:146-189.
2. Gawande ND, Mahurkar DG, Rathod TH, Jahagidar SW, Shinde M. *In vitro* screening of wheat genotypes for drought tolerance. Ann. Plant Physiol 2005;19:162- 168.
3. Ghasempour HR, Zh Soheilikhah AR, Zebarjadi Ghasempour S, Karimi N. *In vitro* micro propagation, callus induction and shoot regeneration in safflower L. cv. Lesaf. Iranian Journal of Plant Physiology 2014;4(2):999-1004.
4. Hasegawa P, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annu Rev Plant Mol Biol 2000;51:463-499.
5. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with to bacco tissue cultures. Plant Physiol 1962;15:473-497. Doi: 10.1111/j.1399-3054.1962.tb08052.x
6. Karp A, Steel SH, Parmar S, Jones MGK, Shewry PR, Breiman A. Relative stability among barley plants regenerated from cultured immature embryos. Genome 1987;29(3):405-412.
7. Nimbkar N. Issues in safflower production in India. In: Knights S. E. and Potter T. D. (Eds) Safflower: Unexploited potential and world adaptability. Proceedings 7th International Safflower Conference, Wagga Wagga, New South Wales, Australia (3-7 November, 2008) 2008.
8. Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK. Developing stress tolerant plants through *in vitro* selection – an overview of the recent progress. Environ. Exp. Bot 2011;71(1):89-98.
9. Soheilikhah Z, Karimi N, Ghasmpour HR, Zebarjadi AR. Effects of saline and mannitol induced stress on some biochemical and physiological parameters of Carthamus tinctorius L. varieties callus cultures. Aust. J Crop Sci 2013;7:1866-1874.
10. Tal M. *In vitro* selection for salt tolerance in crop plants: theoretical and practical considerations. *In vitro* Cell Dev. Biol 1994;30(4):175-180.
11. Vaziri A, Ahmadi AR, Hamid F, Franceva B, Shaker H. The Effect of Different Concentrations of NaCl on the Callus Induction and Lipids of Soybean, Plant Tissue Cult 2004;14(21):75-77.
12. Weiss EA. Oil seed Crops. 2nd ed. Blackwell Science, Oxford 2000.