International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Callus Induction and Shoot Regeneration of *Lycopersiconesculentum* L.

Niroj Paudel[1], Lila Pati Paudel[2], P. D. Rai[3]

[1,2]Department of Botany, P.G. Campus, T.U., Biratnagar, Nepal

[3]Centralal Department of Botany, T.U., Kathmandu, Nepal

Abstract: ***The benefit of callogenesis and subsequent shoot regeneration using differentlengths (3 mm, 4 mm, 5 mm, 6 mm, and >6 mm) of flower buds of tomato were investigated in another culture.Calli were induced on MS media Supplemented with different combinations of growth regulators (2 ppm indole-3-acetic acid (IAA) plus 1 ppm N^6- (2-isopentenyl) adenine (2ip), 2 ppm IAA plus 3 ppm 6-benzylaminopurine (BA), and 2ppm IAA plus 0.25 ppm zeatin riboside). Thedeveloped calli were transferred to the MS medium containing 2 ppm IAA plus 1 ppm2ip for organogenesis and developed shoot buds were transferred to the samemedium containing 2 ppm IAA plus 0.25 ppm zeatin riboside for shoot regeneration. The flower bud length ranging from 3 mm to >6 mm produced calli. Flower buds of 4mm produced the highest number of calli and shoots (63% and 29% respectively) followed by that of 5 mm (57% and 14% respectively). Number of calli produced inthe MS medium containing 2 ppm IAA plus 1 ppm 2ip were highest (71%) followed by2 ppm IAA plus 3 ppm BA (65%). This finding would be an efficient technique for callogenesis and sho129, 942,416 Tm in 2007) and cultivated area (4,643,957 Ha in2007) (Seguı´-to regeneration in tomato anther culture***

Keywords: Anther, callus,*Lycopersiconesculentum*,MS medium,Shoot regeneration

1. Introduction

Lycopersicon esculentum L., 2n = 2x = 24 is a member of a Solanaceae. It is the first vegetable crop worldwide, both in terms of production 129,942,416 Tm in 2007) and cultivated area (4,643,957 Ha in 2007) (Seguı´-Simarro*et. al.*, 2011). In the field and especially in the greenhouses, tomato is mainly produced via seeds of F1 cultivars possessing genes of interest for characteristics such as high yield, resistance to diseases, ability to thrive and the production of fruits under unfavorable conditions or in out-of-season production schemes in greenhouses (Atherton and Rudich, 1986, Bal and Abak, 2007). Developing F1 cultivar is expensive and time consuming but another culture can aid to reduce the time required for getting almost homozygous inbred lines with the potential of developing completely homozygous lines in short time. Haploid production would facilitate the fundamental studies of tomato and the breeding process (Zagorska *et. al.*, 1998). Despite the enormous economic importance of *Lycopersiconesculentum* all over the world, doubled-haploid technology is still far from being routinely applied in tomato breeding programs, mostly due to the lack of knowledge about androgenesis induction in this species (Seguı´-Simarro and Nuez, 2007). There has been some progress in the field of haploid in *Lycopersicon esculentum* from anthers and. Seguı´-Simarro *et.al* (2006) confirmed that meiotic metaphase I to telophase II is the most responsive stage during microspore development for callus induction in tomato (*Solanum lycopersicum*) anther cultures. Later, on 2007, the same author suggested another window at uninucleate stage for direct embryogenesis in tomato via pollen culture (Seguı´-Simarro and Nuez (2007).

2. Material and Methods

Solanum Lycopercium F1 seeds were collected forexperiment, Plants were grown in the glasshouse of at around 26°C under natural light from May to August 2015.Fordetermination of pollen grain development stages in buds of particular length, and theflower buds of 2 to 8 mm were collected. The anthers from particular bud were excised, and deposited on a glass slide. A drop of acetocaramine was added and the anthers were crushed after placing cover slide on the top by pressing gently. The slides were observed under a Nikon E400 microscope. To determine the prevalence of particular development stage of pollen grains, at least 10 pictures of 10 random fields were taken at x600.Flower buds of 3, 4, 5, and 6 mm were collected in the morning and subjected to cold pre-treatment of 4ºC under dark condition for 72 h .Buds were sterilized with 94% ethanol (for 3 min.) then with sodium hypo chloride (for 10 min.), anthers were excised out of bud and plated in MS medium with following treatments–1: Control, 2: IAA 2 ppm + BA 3 ppm, 3: IAA 2 ppm + Zeatin riboside 0.25 ppm, 4: IAA 2 ppm + 2ip 1 ppm. The anthers plated in the medium were incubated at 26±6 °C in dark for one month. Thereafter, developing calli was transferred to fresh medium with same hormone composition and incubated under 16/8 hrs. Photoperiod for.another month. Then they were sub-cultured on MS medium supplemented with 20 $g.l^{-1}$sucrose, 2.50 $mg.l^{-1}$phytagel, 2 $mg.l^{-1}$ IAA and 1 $mg.l^{-1}$2ip.After one month, the regenerated shoots wereexcised and transferred to regeneration medium supplemented with 20 g.l-1sucrose, 2.50 $mg.l^{-1}$phytogel, and 0.25 $mg.l^{-1}$ Zeatin riboside. For shoot elongation, the developing shoot was again transferred to second regeneration medium supplemented with 20 g.l-1 sucrose, 2.50 $mg.l^{-1}$phytagel, and 0.50 $mg.l^{-1}$GA3.For Rooting, the well-developed individual shoots were transferred to MS medium supplemented with 10 $g.l^{-1}$sucrose, and 2.50 mg.l-1phytagel (Segui et. al., 2006).Completely regenerated plants is to be acclimated and transferred to pots under glasshouse condition

3. Result and Discussion

The anthers from bud with particular length (3 to 10 mm) were excised, squashed with acetocarmine and observed under microscope to assess the prevalent development stage of the microspores lying inside (fig. 1). Buds of 3 mm prevalently contained anthers with pre-meiotic microspore mother cells (MMC) (Fig. 1). Likewise, buds of 4 mm contained anthers with microspores at the development stage of

Metaphase I, that of 5 mm Contained buds with microspores at tetrad stage, that of 6 mm contained anthers with young microspores, and other buds of>6 mm length contained anthers exclusively with mature microspores. The response of anthers from particular bud length to callus induction and shoot regeneration was studied (Table 1). Anthers from buds of all length studied gave callus, but the ones giving shoots effectively were the anthers from buds of 3-5 mm. Still, the efficient ones were the buds of 4 and 5 mm only as 13% shoots in 4mm and 8% shoots in 5mm were survived after regeneration from callus (Table 1).

Table 1: Callus induction and shoot regeneration from flower buds of specific lengths

Flower bud size	*No. of anther cultured*	*No. of responsive anthers*	*No. of callus giving shoots*	*No. of shoot survived*
3mm	100	35	4	2
4mm	100	67	31	14
5mm	100	54	16	9
6mm	100	27	3	0
>6mm	100	21	0	-

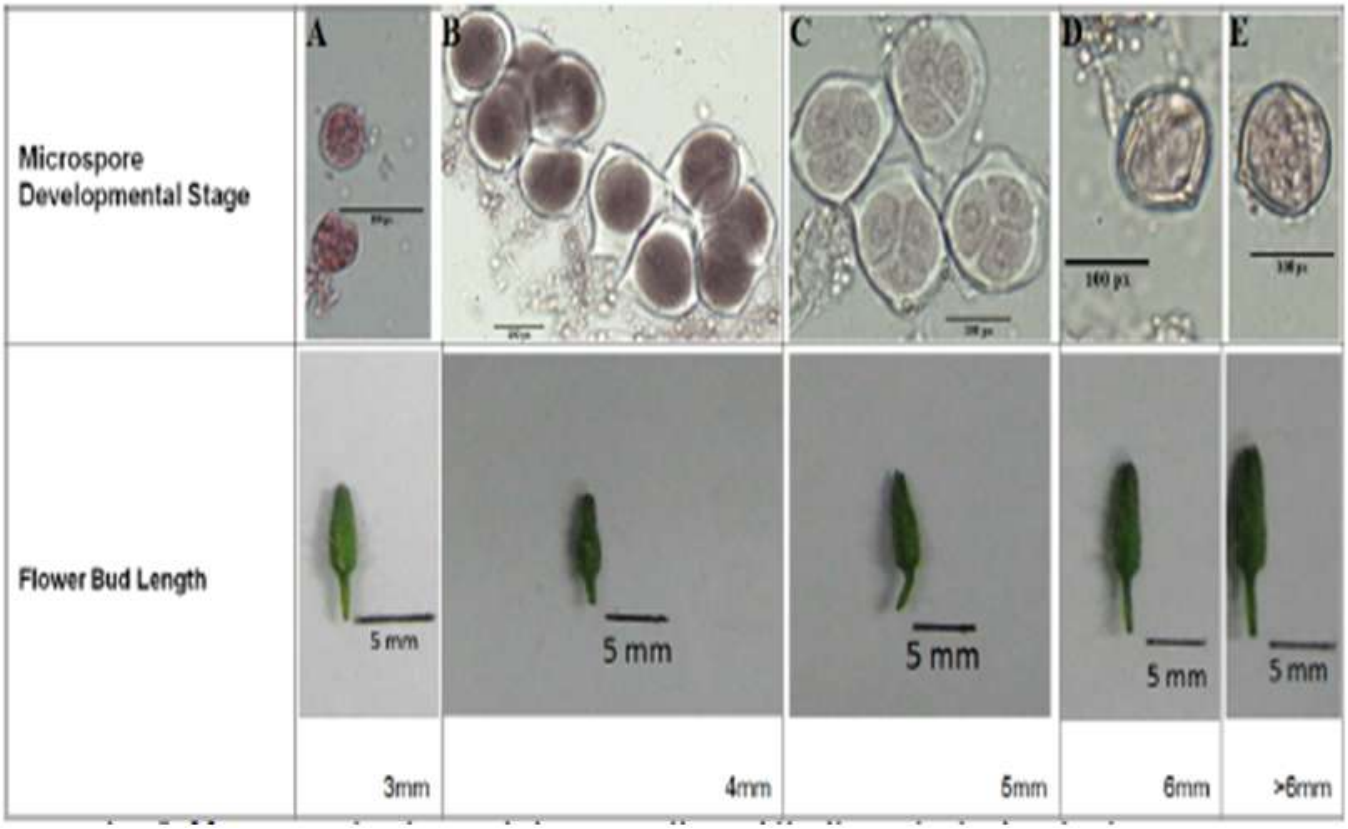

Figure 1: Microspore development stage on anthers of the flower buds of particular. A. Pre-meiotic stage, B. Metaphas- I, C. Tetrad, D. young microspore, mature microspore

Table 2: Callus induction and Shoot regeneration responses to the combination of different hormone treatment

	No. of Anther planted	No. of responsive Anthers	No. of callus giving shoots	No. of shoot survived
Control	100	0	-	-
IAA 2 ppm+BA 3ppm	100	63	24	7
IAA 2 ppm+Zeatin riboside 0.25 ppm	100	49	11	2
IAA 2ppm+2ip 1 ppm	100	69	25	10

Regarding the response of anthers to different combinations of hormones in MS induction media (Table 2), MS media without any hormone could not induce callus but all other studied hormone combinations were able to induce callus. Media with IAA 2 ppm + 2ip 1ppm was found to be the best combination for both callus induction (71%) and shoot regeneration (29%) followed by IAA 2 ppm + BA 3 ppm which gave 65% callus induction and 26% shoot regeneration. Hormone combination of IAA 2 ppm + zeatin riboside 0.25 ppm in MS media was found to be the poorly effective on Callus induction (53%) and also on shoot regeneration (12%). Callus induction and shoot regeneration process has been illustrated in fig. 2

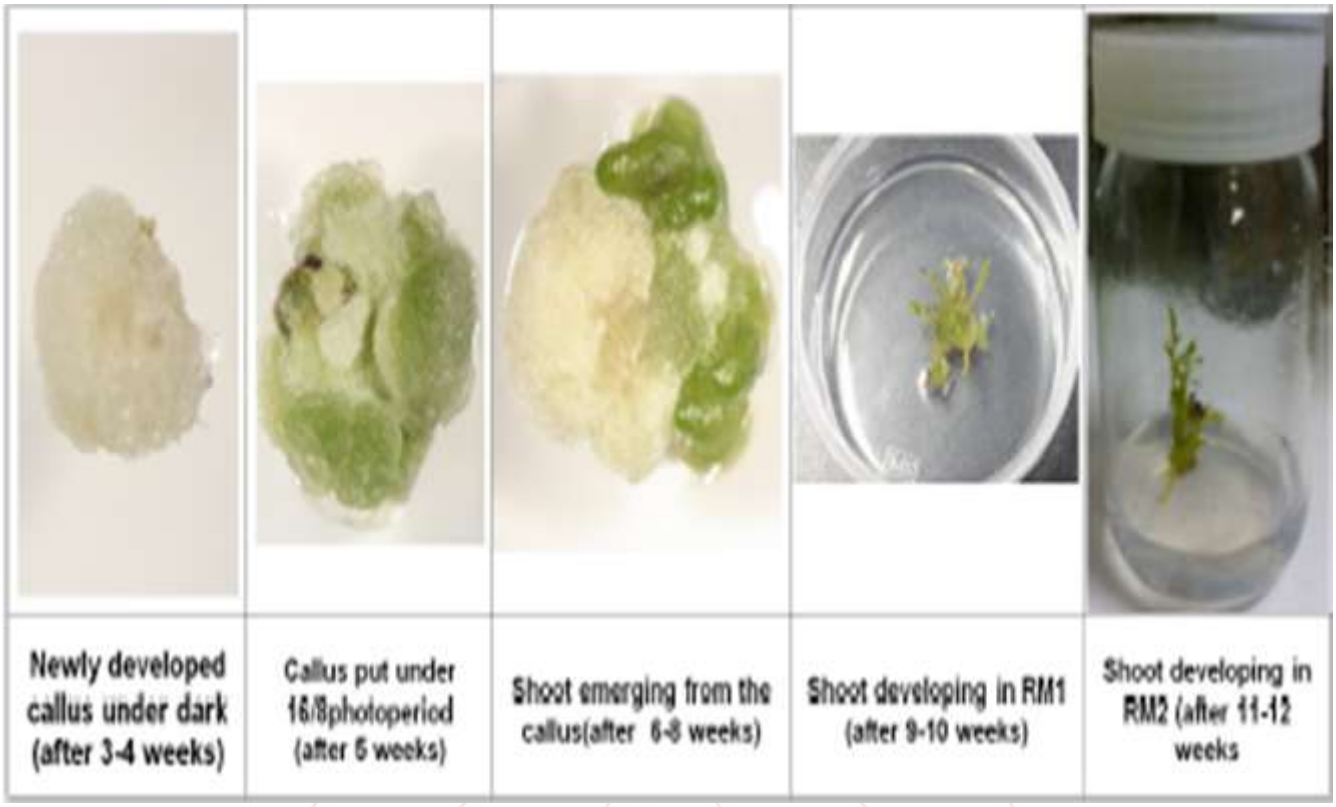

Figure 2: Callus induction and shoot regeneration from anther

4. Acknowledgements

The authors are grateful to the Central Department of Botany, T.U. KirtipurKathmandu Nepal for providing the laboratory facilities.

References

[1] Atherton, J.G. and J. Rudich eds.1986. The tomato crop. A scientific basis for improvement. Chapman Hall, London
[2] Bal, U. and K. Abak. 2007. Haploidy in tomato (*Lycopersicon esculentum* Mill.): a critical review. Euphytica, 158: 19.
[3] Cabral GB, Carneiro VT, Dusi DM, Martinelli ,2016*;* Somatic Embryogenesis and Plant Regeneration of Brachiaria brizantha.*Methods Mol Biol. 2016; 1359:395-402.*
[4] Geierf, P.M. and C.H. Doy. 1972. Development and difference of haploid *Lycopersicon esculentum* (Tomato), Planta 107:161–170. Grice, S. 20040713. That's Campari. National Post (CanWest Global Communications Corp.
[5] Grice, S. 2004 .That's Campari. National Post (CanWest GlobalCommunications Corp)
[6] *Obert B, et.al 2009;* Doubled haploid production in Flax (*Linum usitatissimum* L.). *Adv. Jul-Aug; 27(4):371-5. Epub 2009 Feb 20.*
[7] Parra-Vega V, Seguí-Simarro JM 2016*;* Anther Culture in Pepper (*Capsicum annuum* L.)..*Methods Mol Biol; 1359:467-74*
[8] Seguı´-Simarro.2006. Nuclear bodies' domain changes with microspore reprogramming to embryogenesis, European Journal of Histochemistry 2006; vol. 50 issue 1 (Jan-Mar): 35-44
[9] Seguı´-Simarro and Nuez,2007.Embryogenesis induction, callogenesis, and plant regeneration by *in vitro* culture of tomato isolated microspores and whole anthers ;Journal of Experimental Botany, Vol. 58, No. 5, pp. 1119–1132.
[10] *Supena ED, Suharsono S, Jacobsen E, 2006:*Successful development of a shed-microspore culture protocol for doubled haploid production in Indonesian hot pepper (*Capsicum annuum* L.).
[11] *Custers JB.Plant Cell Rep.; 25(1):1-10. Epub 2005 Sep 20.*
[12] Zagorska N.A., A. Shtereva, B.D. Dimitrov, M.M. Kruleva. 1998. Induced androgenesis in tomato (*Lycopersicon esculentum* Mill.)I. Influence ofgenotype on androgenetic ability. Plant Cell Rep. 17: 968973.