Tuberose (*Polianthes tuberose* L.) Shoots Multiplying and Callus Induction by Benzyladenine, Naphthaline Acetic Acid and Oryzaline

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Abstract

Explants from axillary buds of *Polianthes tuberose* L. were used in this experiment. They were cultured on Murashige and Skoog (MS) medium supplemented with various combination of Benzyladenine (BA) (0, 0.1, 0.5, 1.0 and 2.0 mg/l) and Napthaline acetic acid (NAA) (0, 0.1, 0.5, 1.0 and 2.0 mg/l). Explants developed into callus after culturing for 4 weeks and increasing on MS medium supplemented with all concentration of BA. Callus transferred to MS medium supplemented with various combination of BA (0, 0.1, 0.5, 1.0 and 2.0mg/l) and NAA (0, 0.1, 0.5, 1.0 and 2.0 mg/l), and subcultured every 4 weeks into the same medium. It was found that MS medium supplemented with 0.5 mg/l BA and 2.0 mg/l NAA was sufficient for shoots induction and multiplication roots regeneration from callus within 12 weeks. Callus turned brown and had low survival rate (%) when soaked them with high concentration of oryzalin. (more than 1.0 and 2.0 mg/l).

Keywords: axillary bud; BA; NAA; oryzalin; *Polianthes tuberose*.

1. Introduction

Tuberose (*Polianthes tuberose* L.) is a perennial plant related to the agaves and become an economically important ornamental bulb crop [15]. Tuberose is belonging to family Amaryllidaceae. In Thailand, tuberose grew for perfume processing and cut flower production. The conventional method of propagation through bulbs is rather slow and not enough to meet a growing demand [1].

Oryzalin is a selective preemergence herbicide for controlling annual grasses and broadleaf weeds [2]. Oryzalin disrupts mitosis by inhibiting the formation of microtubules [13]. It poses low toxicity to humans and is preferable to colchicine [20]. Oryzalin has been used to induce polyploidy in many ornamental plant such as *Lilium longiflorum* [19] and Gerbera [18].

Micropropagation has been proven to be an extremely useful technique for clonal propagation of many species, especially ornamental plants [4]. Multiplication shoot regeneration through tissue culture is useful for rapid and large-scale multiplication. This paper was carried out to investigate the possibility of micropropagation tuberose for mass production.

2. Materials and Methods

2.1 Establish of aseptic explants

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Axillary bud of tuberose were used as explants, surface cleaned by washing with tepol detergent, rinsed with tap water and dipped in 70% alcohol for 20 minutes. Surface sterilized by soaking with 20 % (v/v) clorox for 15 minutes, followed by 5% (v/v) clorox for 10 minutes, then rinsed three times with sterile distilled water for 2 minutes each. Explants were cultured on solid MS medium (Murashige and Skoog,1962) supplemented with various combination of BA (0, 0.1, 0.5, 1.0 and 2.0 mg/l), NAA (0, 0.1, 0.5, 1.0 and 2.0 mg/l) and 3% sucrose. Cultures were incubated at 25± 2 ^{o} C under 16 photoperiod with illumination provided at 60 µmolm^{-2} sec^{-1} (TLD 18 w/18 lm Phillips, Holland). The callus obtained after 4 weeks of cultures were then used as the source of explants for the subsequent experiments.

2.2 Multiple shoots induction

Callus were cultured on MS medium supplemented with various combination of BA (0, 0.1, 0.5, 1 .0 and 2.0 mg/l), NAA (0, 0.1, 0.5, 1.0 and 2.0 mg/l) and 3 %sucrose. Cultures Cultures were incubated at 25± 2 ^{o} C under 16 photoperiod with illumination provided at 60 µmolm^{-2} sec^{-1} (TLD 18 w/18 lm Phillips, Holland). The cultures were subcultured every 3 weeks into the same medium for 3 times.

2.3 Effect of oryzalin

Callus were cultured on MS medium supplemented with various concentration of oryzalin (0, 0.1, 0.5, 1.0, 2.0 and 3.0 mg/l) with different duration (3, 4, 5, and 6 days) and 3% sucrose.

All cultures were incubated at 25± 2 ^{o}C under 16 photoperiod with illumination provided at 60 µmolm^{-2} sec^{-1} (TLD 18 w/18 lm Phillips Holland). The cultures were subcultured every 3 weeks into the same medium for 4 times.

2.4 Data Analysis

This experimental was completely randomized design with 10 replications per treatment. The new shoot length and numbers of new shoots formed and the diameters of callus were recorded. Data were analyzed with Analysis of Variance (ANOVA) and comparison of mean performed with Duncan's Multiple Range Test at $p \leq 0.05$.

3. Results and Discussion

After one week, explants from axillary buds were swollen then callus were formed at the surface of explants which cultured on MS medium supplemented with various combination of BA(0, 0.1, 0.5, 1.0 and 2.0 mg/l), NAA (0, 0.1, 0.5, 1.0 and 2.0 mg/l) and 3% sucrose within 4 weeks . However, there were significant different on callus formation ($p \leq 0.05$) as in Table 1 . They suggested that the highest average weight of callus (7.00-7.20 mg/explants) depended on the concentration of BA (0.5-2 mg/l). The explants from axillary buds cultured on basal MS medium without growth regulator gave small amount of callus (3.0 mg), this result was similar to[14] and[16]. [5] reported that callus of gladiolus was formed in MS medium supplemented with 0.5 mg/l BA.

More multiple shoots were induced when subcultured callus in MS medium supplemented with low concentration of BA (0.5 – 1.0 mg/l), NAA 2.0 mg/l and 3%sucrose and this medium gave the highest growth rate of callus (Table 1). However, [11]reported that new shoot could proliferated only in buds derived callus and the highest number of shoots were regenerated from the callus which cultured on MS medium supplemented with 3.0 mg/l BAP. However, the number of new shoots formed in each combination of BA and NAA were significant different (($p \leq 0.05$). It was found that MS medium supplemented with 0.5 mg/l BA and 2.0 mg/l NAA was suitable for multiplying shoots induction of

tuberose and an average of 5.2 shoots were formed in this medium within 12 weeks. [3] and [9] reported that BA was found to be the most effective for induction of multiple shoot when BA lower than 1.0 mg/l.

When individual shoots were separated and cultured on the same medium, it could induce 5.2 new shoots and increase number of multiple shoots with complete root system. The result was similarily observed by [14] and [7] with tuberose but they used 1.5 mg/l BAP and 0.5mg/l IAA and the highest number of shoots.

Table1. The average weight of callus per medium and number of shoots per bud of *Polianthes tuberose* which cultured on solid MS medium supplemented with various concentration of BA and NAA for 8 weeks.

BA(mg/l)	NAA (mg/l)	Weight of callus (mg)*	Number of shoot /bud*
0	0	3.00 ± 3.32c	2.60 ±0.24e
0.1		6.20 ± 0.37a	2.80 ± 0.20de
0.5		7.20 ± 0.20a	2.60 ± 0.24 e
1.0		7.20 ± 0.20a	2.80 ± 0.20de
2.0		7.00 ± 0.32 a	3.00 ±0.00cde
0.1	0.1	4.20 ± 0.20 c	3.20 ±0.20bcde
	0.5	4.00 ± 0.32c	3.20 ±0.20bcde
	1.0	4.00 ± 0.32c	3.40 ±0.24bcde
	2.0	4.00 ± 0.45c	3.80 ±0.37bc
0.5	0.1	3.40 ± 0.51c	4.00 ±0.45bc
	0.5	3.80 ± 0.49c	4.00 ±0.45bc
	1.0	4.20 ± 0.37c	3.60 ±0.45bcd
	2.0	3.60 ± 0.24c	5.20 ±0.24a
1.0	0.1	4.20 ± 0.37c	4.20 ±0.20b
	0.5	4.20 ± 0.37c	4.00 ±0.37bc
	1.0	4.00 ± 0.32c	3.80 ±0.49bc
	2.0	3.80 ± 0.37c	4.20 ±0.37b
2.0	0.1	4.00 ± 0.32c	3.40 ±0.24bcde
	0.5	3.80 ± 0.20c	3.40 ±0.24bcde
	1.0	3.80 ± 0.37c	3.80 ±0.20bc
	2.0	4.40 ± 0.24c	3.60 ±0.24bcd

*Mean within the same column followed by the same alphabet are not significantly different using DMRT, $p \leq 0.05$

When transferred callus in liquid MS medium supplemented with various concentration of oryzalin (0, 0.1, 0.5, 1.0, 2.0 and 3.0 mg/l) and different duration period (3, 4, 5 and 6 days) for soaking. It was found that the average color of callus was significant difference ($p \leq 0.01$).

Table 2. Average color and percentage of survival rate of Tuberose callus after soaked with different concentrations of oryzalin concentration and duration times.

Duration time for soaking(day)	Oryzalin conc. (mg/l)	Average color of callus **	survival rate (%)
3	0	4± 0.21c	100
	0.1	3.6± 0.37c	90
	0.5	3.4± 0.35c	85
	1	3.2± 0.48c	80
	2	3.4± 0.25c	85
	3	2.4± 0.37c	60
4	0	4.0± 0.26c	100
	0.1	4.0± 0.45c	100
	0.5	3.6± 0.23c	90
	1	2.2± 0.46c	55
	2	2.6± 0.37c	65
	3	2.0± 0.45c	50
5	0	3.8± 0.37c	95
	0.1	3.8± 0.26c	95
	0.5	3.6± 0.33c	90
	1	2.2± 0.39c	55
	2	2.2± 0.41c	55
	3	0.8± 0.39c	0
6	0	3.8± 0.37c	95
	0.1	1± 0.27c	25
	0.5	0.6± 0.37c	15
	1	0± 0.00c	0
	2	0.4± 0.19c	10
	3	0± 0.00c	0

** significant different ($P \leq 0.01$) a,b,c,d Means followed by the same letters are not significantly different ($p < 0.05$) using DMRT test.
0 = brown, 1 = brown more than green, 2 = light green mixed with brown, 3 = light green, 4 = green

After callus were treated with various concentration of oryzalin and soaked with different duration times. It was found that high average survival rates (%) of callus (90-100 %) were obtained from low concentrations of oryzalin (0, 0.1 and 0.5 mg/l) and short period in soaking(3, 4 and 5 days). When callus soaked longer period with higher concentration of oryzalin, callus turned brown. When soaked callus in 0.1 and 0.5 mg/l oryzalin for 3 -5 days, all callus were green. Callus soaked for longer period all callus were turned brown and died. This result was the same trend as [12] did oriental hybrid lily, potato [13], and wheat [6]. [5] studied on tuberose. They found that low concentrations of oryzalin (0.5 – 1 μM) gave the best efficiency to

induce callus to regenerate new plantlets and their callus color were green.

4. Conclusion

Axillary buds explants of tuberose were cultured on solid Murashige and Skoog (MS) medium supplemented with 0.5 mg/l benzyladenine (BA) and 3% sucrose could developed into callus after culturing for 4 weeks. Callus which cultured on MS medium supplemented with 0.5 mg/l BA and 2.0 mg/l NAA was sufficient for inducing and multiplying shoots and roots within 12 weeks. Callus turned brown and low survival rate (%) when soaked them in oryzalin with high concentrations (more than 1.0 and 2.0 mg/l) and long periods (4,5 and 6 days) .

5. References

[1] Arora, J.S., Sing, K., Grewal, H.S., Gosal, S.S. and Chanana, Y.R., 1996, *In Vitro* Cormel Production from Nodal Buds and Cormel Tips in *Gladiolus*, pp. 50-53, In Islam, A.S. (Ed)., Plant Tissue Culture, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

[2] Atland, J.E., Gilliam, C.H. and Wehtje, G., 2003, Weed control in field nurseries, Hort. Technol. 13: 9-14.

[3] Bose, T.K., Jana, B.K. and Moulik, S., 1987, A note of micropropagation of tuberose from scale stem section, Ind. J. Hort. 44: 100-101.

[4] Edwards, M., 2006, Fragrances of the World, Crescent House publishing.

[5] Gi, H.S. and Tsay, H.S., 1989, Anther culture and somaclonal variation of tuberose (*Polianthes tuberose* L.), J. Agri. Res. China 38: 346-352.

[6] Hassawi, D.S. and Liang, G.H., 1991, Antimitotic agents: Effects of double haploid production in wheat, Crop Sci. 31: 723-726.

[7] Hutchinson, M.J., Onamu, R. and Obukosia, S., 2004, Effect of thidiazuron, benzylaminopurine and naphthalene acetic acid on *in vitro* propagation of tuberose (*Polianthes tuberosa* L.) from shoot tip explants, J. Agri. Sci. Technol. 6: 48-59.

[8] Jala, A., 2013, Potential of benzyl adenine, naphthalene acetic acid and sucrose concentration on growth, development, and regeneration of new shoot and cormel on Gladiolus, Ame. Trans. Eng. Appl. Sci. 2: 277-285.

[9] Kumar, S., Awasthi, V. and Kanwar, J.K., 2007, Influence of growth regulator and nitrogenous compounds on *in vitro* bulbil formation and growth in oriental lily, Hort. Sci. (Prague) 34: 77-83.

[10] Murashige, T. and Skoog, F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant. 15: 473-497.

[11] Nazneen, S., Jabeen, M. and Ilahi, I., 2003, Micropropagation of *Polianthes tuberose* (Tuberose) through callus formation, Pak. J. Bot. 35: 17-25.

[12] Nhut, T., Van, B., Silva, J., 2002, Change of shoot regeneration potential of oriental hybrid dormancy, Sci. Hort. 105: 483-490.

[13] Ramulu, K., Verhoeven, H.A. and Dijkhuis, P., 1991, Mitotic blocking, micronucleation and chromosome doudling by oryzalin, amiprofosmethyl and colchicines in potato, Protoplasma 160: 65-71.

[14] Sangavai, C. and Chellapandi, P. 2008. *In vitro* propagation of a tuberose plant (*Polianthes tuberosa* L.), E. J. Biol. 4: 98-101.

[15] Shen, T.M., Huang, T.S., Du, B.S. and Huang, K.L., 1993, Breeding of tuberose for cut flower, J. Chinese Soc. Hort. Sci. 39: 23-29.

[16] Shweta, S. and Nagar, P.K., 2005, Alterations in endogenous polyamines in bulb of tuberose (*Polianthes tuberosa* L.) during dormancy, Sci. Hort. 63: 77-82.

[17] Sood, S. and Nagar, P.K., 2005, Alteration in endogenous polyamines

in bulbous of tuberose (*Polianthes tuberosa* L.) during dormancy, Sci. Hort. 105: 483-490.

[18] Tosca, A., Pandolfi, R., Citterio, S. and Sgorbati, S., 1995, Determination by flow cytometry of the chromosome doubling capacity of colchicine and oryzalin in gynogenic haploids of Gebera, Plant Cell Rep. 14: 455-458.

[19] van Tuyl, J.M., Meijer, B. and van Dien, M.P., 1992, The useof oryzalin as an alternative for colchicine *in vitro* chromosome doubling of Lilium and Nerine, Acta Hort. 325: 625-630.

[20] Wan, Y., Duncan, D.R., Rayburn, A.L., Petolino, J.R. and Widholm, J.M., 1991, The use of antimicrotubule herbicides for the production of doubled haploid plants from anther-derived maize callus, Theor. Appl. Genet. 81: 205-211.