Somatic embryogenesis on irradiated callus of garlic (*Allium Sativum* L.)

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Abstract. The research was done at the Tissue Culture Laboratory of Center for Application of Isotope and Radiation Technology, Jakarta. Callus derived from shoot tip of garlic cloves was exposed to gamma rays, then cut into sized of 1 mm$^3$ and cultured in Gamborg medium enriched with plant growth regulators (PGRs) depends on the treatment. The PGRs applied are. The experiment was arranged in a randomized factorial design with 2 factors and 3 replications. The first factor was the doses of gamma rays (0, 10 and 20 Gy) and the second factor was different concentrations of PGRs (untreated, 1 and 2 ppm of thidiazuron (TDZ) and Zeatin). The result indicated that the application of 2 ppm TDZ or Zeatin promoted development on irradiated callus at the dose 10 Gy. The highest number of the shoot and the longest shoot were obtained from non-irradiated callus grown on medium enriched with 2 ppm TDZ, followed by 1 and 2 ppm Zeatin, 1 ppm TDZ and without PGRs. Irradiated callus at the dose 10 Gy grown on medium with 2 ppm TDZ was able to form a shoot, while other irradiated callus at the dose 29 Gy were not able to form the shoot.

1. Introduction
Garlic (*Allium sativum* L.) is one of the important horticultural commodities and used daily as spices in Indonesian culinary because of its ability to improve the taste of food. Besides, garlic can be used as medicine since its biological activities that include antitumor and anticancer [1]; [2]; [3], cholesterol-lowering [4] and able to inhibit fungi and bacterial growth [5].

Generally, garlic was grown at the elevation of 700 – 1200 m above sea level (ASL). Limited area for garlic in the high land area is an obstacle for garlic plantation in Indonesia. Recently well-adapted garlic at the low land area is not met the satisfying yield. Garlic production grown below 700 m ASL decreased 5 – 10 % on every lowering 100 m elevation [6]. Kateng, imported garlic from China was not able to produce cloves either at highland (Cipanas, 1000 m altitude) or lowland area (Bogor, 250 m altitude). It seems that Kateng variety needs a lower temperature to form cloves [7]. Genetic improvement of garlic varieties through sexual hybridization is very difficult to be done in Indonesia. Garlic was not able to bloom in tropical climates since low temperatures (-2 to 2$^\circ$ C) and short photoperiod are known to promote flowering in garlic and to obtain viable seeds [8]; [9]. Commercial varieties of garlic are sexually sterile and should be propagated vegetatively [10]. This confers low propagative rate in the field, limited genetic improvement and severe sanitary constraints since the varieties of garlic are clones.
Garlic (*Allium sativum* L.) is propagated vegetatively using cloves as plant material since most of the garlic flowers are sterile, therefore variety improvement of garlic through conventional breeding by means hybridization is very difficult to be done, moreover genetic variability of garlic is narrow [11]. Genetic improvement of garlic through conventional breeding is very difficult due to the sterile nature of its flower. Hence, an alternative system is desirable to induce genetic variation [12]. Genetic variability on garlic can be increased by applying mutation techniques [13]; [14]; [15] and [16]. Choudary and Dyansagar (1982) [17] reported that 16 mutant lines on shallot were obtained by exposing the bulbs to gamma (γ) rays. Marchesi et al (1982) [18] exposed garlic cloves to γ rays at the doses 1 – 4 Gy. Sumiyarsih and Aliudin (1990) [19] stated that exposing garlic cloves to γ rays at the doses 20 – 120 Gy influenced plant height and number of stomata.

Vegetative propagation through *in-vitro* culture in garlic is a technique to support mutation breeding since *in-vitro* culture may increase vegetative propagation rapidly [20], therefore methods for high multiplication rate are developed by applying clonal propagation through *in-vitro* culture for rapid production embryonic callus. Somatic embryogenesis (SE) is a means by which plants can regenerate bipolar structures from a somatic cell [21]. Several works on organogenesis or embryogenesis in garlic tissue culture have been reported, such as using shoot-tips as source of explants for callus culture and plant regeneration [22]; [23]; [24] and [25].

Efforts in increasing genetic variability on garlic were done by exposing callus to γ rays combined with *in-vitro* culture techniques. These techniques are expected to develop promising mutant lines with the desirable agronomic characters in a shorter period of time compared to conventional breeding through hybridization. The long term objective of this experiment is to select promising mutant lines of garlic that can be well adapted at the medium elevation, while the short term objective is to determine the appropriate medium for *the in-vitro* culture of garlic.

2. Materials and Methods

The research was conducted at the tissue culture laboratory of Center for Isotopes and Radiation Technology, National Nuclear Energy Agency, Jakarta. Garlic callus derived from the best medium for callus formation [26] was exposed to gamma rays at the doses 0, 10 and 20 Gy. Irradiated callus was cut in the sized of 1 mm$^3$, cultured in the jars filled with the 25 ml of solid Gamborg medium enriched with the different PGRs concentrations and maintained in the growth room at 24 ± 2°C under 16 hours photoperiod. The experiment was arranged in a factorial randomized block design with 2 factors and 3 replications, the first factor was the doses of gamma rays (untreated, 10 and 20 Gy) and the second factor was different PGRs concentration (untreated, Thidiazuron 1 and 2 ppm, and Zeatin 1 and 2 ppm).

The medium used in callus formation and shoot regeneration was Gamborg solidified by 8 % agar. Before autoclaving, media were adjusted to pH 5.8 by adding HCl 0.1 N or NaOH 0.1 N. Naphthalene Acetic Acid (NAA) was added for shoot formation medium. Each unit of the experiment consisted of five jars with one explant in every jar. After the explants were planted, the culture jars were sealed by transparent cellophane tapes sized 5 cm and tight with cellophane tape sized 1.25 cm. Data of diameter and score of irradiated callus were analyzed by Least Significant Different (LSD) test in order to test the difference between the treatments [27]. Score of callus or plantlet was observed based on the colour (0 – 1 = black, 1 – 2 = brown, 2 – 3 = yellow, 3 – 4 = pale green, 4 – 5 = green). The data of a number of green spots and shoots and shoot length were not analyzed since the growth and regeneration of callus were not spread evenly.

3. Results and Discussion

Non-irradiated callus showed better growth of callus compared to irradiated callus. All of the callus grew well but some of them especially irradiated at the dose 20 Gy were not able to form the shoot. The widest callus diameter was obtained from non-irradiated callus grown on the media enriched with 2 ppm Zeatin followed by those irradiated calli at the doses 10 Gy and significantly different
compared to untreated, non-irradiated and irradiated callus grown on the media enriched with 1 and 2 ppm TDZ (Table 1).

**Table 1.** Diameter (mm) of irradiated callus grown on Gamborg media enriched with TDZ and Zeatin (5 months after cultured)

| Irradiation doses (Gy) | Plant growth regulator concentration | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
|------------------------|-------------------------------------|-----------|-----------|--------------|--------------|
| 0                      | Untreated                           | 4,50 d    | 4,50 d    | 7,58 bc      | 11,58 a      |
| 10                     |                                     | 4,89 d    | 5,45 d    | 5,67 c       | 8,42 b       |
| 20                     |                                     | 3,82 d    | 4,27 d    | 4,18 d       | 3,83 d       |

Remark: Numbers followed by the same letters are not significantly different based on LSD 0.05.

Better growth quality of callus was indicated by the color with the score color ranging from 2.0 – 5.0 or yellow to green. Application of 2 ppm TDZ or 2 ppm Zeatin showed better callus color, (pale green) on irradiated callus at the dose 10 Gy (Table 2). The higher score color of the callus may promote the number of green spots.

**Table 2.** A score of color of irradiated callus grown on Gamborg media enriched with TDZ and Zeatin (5 months after cultured)

| Irradiation doses (Gy) | Plant growth regulator concentration | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
|------------------------|-------------------------------------|-----------|-----------|--------------|--------------|
| 0                      | Untreated                           | 1,80 e    | 2,30 e    | 2,08 e       | 2,92 bc      |
| 10                     |                                     | 2,78 d    | 3,09 ab   | 2,22 e       | 3,25 a       |
| 20                     |                                     | 2,45 de   | 1,91 e    | 1,82 e       | 1,92 e       |

Remark: 1. Score of callus or plantlet colour (0 – 1 = black, 1.0 – 1.9 = brown, 2.0 – 2.9 = yellow, 3.0 – 3.9 = pale green, 4 – 5 = green), 2. Numbers followed by the same letters are not significantly different based on LSD 0.05.

The highest number of green spot was demonstrated on non-irradiated callus grown on medium enriched with 2 ppm Zeatin, followed by those irradiated callus at the dose 10 Gy cultured on the medium 1 ppm Zeatin, non-irradiated callus without PGRs, irradiated callus at the dose 10 Gy with 2 ppm Zeatin, and 2 ppm TDZ. Irradiated callus at the dose 20 Gy did not show any green spot. (Table 3).

**Table 3.** Number of green spots appeared on irradiated callus grew on Gamborg media enriched with TDZ and Zeatin (5 months after cultured)

| Irradiation doses (Gy) | Plant growth regulator concentration | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
|------------------------|-------------------------------------|-----------|-----------|--------------|--------------|
| 0                      | Untreated                           | 1,50      | 5,14      | 10,10        |
| 10                     |                                     | 0,00      | 10,00     | 5,50         |
| 20                     |                                     | 0,00      | 0,00      | 0,00         |

The highest number of the shoot (Table 4) and the longest shoot (Table 5) were obtained from non-irradiated callus grown on medium enriched with 2 ppm TDZ, followed by 1 and 2 ppm Zeatin, 1
ppm TDZ and without PGRs. Irradiated callus at the dose 10 Gy grown on medium with 2 ppm TDZ was able to form shoots, while other irradiated callus were not able to form any shoot.

**Table 4.** Number of the shoot on irradiated callus grown on Gamborg media enriched with TDZ and Zeatin (5 months after cultured)

| Irradiation doses (Gy) | Plant growth regulator concentration |
|------------------------|--------------------------------------|
|                        | Untreated   | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
| 0                      | 3,67        | 4,50      | 13,00     | 12,50        | 6,50         |
| 10                     | 0,00        | 0,00      | 1,00      | 0,00         | 0,00         |
| 20                     | 0,00        | 0,00      | 0,00      | 0,00         | 0,00         |

**Table 5.** Shoot length (mm) on irradiated callus grew on Gamborg media enriched with TDZ and Zeatin (5 months after cultured)

| Irradiation doses (Gy) | Plant growth regulator concentration |
|------------------------|--------------------------------------|
|                        | Untreated   | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
| 0                      | 12,33       | 42,50     | 70,00     | 86,25        | 52,50        |
| 10                     | 0,00        | 0,00      | 5,00      | 0,00         | 0,00         |
| 20                     | 0,00        | 0,00      | 0,00      | 0,00         | 0,00         |

Non-irradiated callus and irradiated callus at the dose 10 Gy grown on Gamborg media enriched with Zeatin demonstrated better growth compared to those enriched with TDZ, while callus cultured on media without PGRs did grow and showed brown color. Irradiated callus at the dose 20 Gy cultured without PGRs also indicated browning symptoms (Table 6).

**Table 6.** The growth of irradiated garlic callus on Gamborg medium enriched with TDZ and Zeatin (5 months after cultured).

| Irradiation doses (Gy) | Plant growth regulator concentration |
|------------------------|--------------------------------------|
|                        | Untreated | TDZ 1 ppm | TDZ 2 ppm | Zeatin 1 ppm | Zeatin 2 ppm |
| 0                      |          |        |          |          |            |
| 10                     |          |        |          |          |            |
| 20                     |          |        |          |          |            |
4. Conclusion
It was concluded that all of the callus cultured on media enriched with PGRs grew well but some of
them especially irradiated at the dose 20 Gy were not able to form the shoot. Callus cultured on media
without PGRs did not grow and showed brown color. Irradiated callus at the dose 20 Gy cultured
without PGRs also indicated browning symptoms. Shoot formation on irradiated garlic callus at the
dose 10 Gy can be promoted by applying 2 ppm TDZ or 2 ppm Zeatin.

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References
[1] Campbell, J.H., J.L. Efendy, N.J. Smith, and G.R. Campbell. 2001. Molecular basis in which
garlic suppresses artherosclerosis. J. Nutr 131(3):1006-1009
[2] Milner, J.A. 2001. A historical perspective on garlic and cancer. J Nutr. 131(3):1027-1031
[3] Gao C, Jiang X, Wang H, Zhao Z and Wang W. 2013. Drug Metabolism and Pharmacokinetics
of Organosulfur Compounds from Garlic. J Drug Metab Toxicol. 4:5
[4] Utami M.M.D, Pantaya D, Agus A. 2018. Addition of Garlic Extract in Ration to Reduce
Cholesterol Level of Broiler. The 2nd International Joint Conference on Science and
Technology (IJCST) 2017 IOP Publishing IOP Conf. Series: J.Physics: Conf. Series 953 (2018)
012124 doi :10.1088/1742-6596/953/1/012124
[5] Gebreyohannes and Gebreselema. 2017. Medicinal values of garlic: Review. Vol.5 401-408
[6] Grubben, G. J. H. 1994. Constraints for shallot, garlic and Welsh onion in Indonesia: A case
study on the evolution of Allium crops in the equatorial tropics. Acta Hort. 358: 333 - 339.
[7] Sutarto, I., K. Dewi, and Arwin. 2000. Increasing genetic variance of garlic and shallot. Progress
Report. CRDIRT. 9p.
[8] Research Institute for Vegetables. 1999. Garlic. Center for R & D of Horticulture. Jakarta.
156p.
[9] Kaur Y., Dhall RK. 2017. Effect of vernalization on flowering and true seed production
behaviour of garlic (Allium sativum) under North Indian Plains. Indian J. Agr Sci. Vol. 87
:1554-1558.
[10] Zheng S., Kamenetsky R., Fereol L., Barandiaran X, Rabinowitch H., Chovelon V., Kik C.
2007. Garlic breeding system innovations. Medicinal and Aromatic Plant Science and
Biotechnology 1. https://www.researchgate.net/publication/282319561
[11] Al-Zahim, M. A., B. V. Ford Lloyd, H. J. Newbury. 1999. Detection of somaclonal variation in
garlic ( Allium sativum L.) using RAPD. Plant Cell Report. 18 : 473 - 477.
[12] Hassan1 M.N., Haque M.S., and Hassan M.M. 2014. An efficient protocol for somatic
embryogenesis of garlic (Allium sativum L.) using root tip as explant. J. Bangladesh Agril.
Univ. 12(1): 1–6.
[13] Mc. Collum, G. D. 1976. Onions and Allies. In: N.W. Simmonds (Ed.). Evolution of crop plants.
Longman. London. P. 186 – 190.
[14] Koul, A. K., R. N. Gohil, A. Langer. 1979. Prospect of breeding improved garlic in the light of
its genetics and breeding systems. Euphytica. 28 : 457 – 454.
[15] Selvaraj, N., S. Natarajan, B. Ramaraj. 2001. Studies on induced mutations in garlic. Mutation
Breeding Newsletter. Issue No. 45. p. 40 - 42.
[16] Shalaby T.A., T., Taher D, Kansouh A and Hamoud M. 2012. In vitro induction of mutation in
garlic through gamma radiation and callus culture. J. Agric.Res. Vol. 37 p.1191-1202.
[17] Choudary, A. and V. R. Dyansagar. 1982. Morphological mutants of garlic. J. Indian Bot. Soc.
61 : 85 – 92.
[18] Marchesi, G., A. Fouci, R. Colombi. 1982. The response of three garlic biotypes to treatments with mutagens. *Sementi Ellete*. 28: 17 – 20.

[19] Sumiyarsih, S., Aliudin. 1990. Pengaruh sinar γ Co 60 terhadap pertumbuhan dan struktur anatomi daun pada bawang putih. Bul. Penel. Hort. 19: 4.

[20] Novak, F. J. 1980. Phenotype and cytological status of plants regenerated from callus cultures of *Allium sativum* L. Z. Pflanzenzucht. 84: 250 – 260.

[21] Méndez-H.H.A., Ledezma-RM, Avilez. M.R.N., Juárez G.Y. L., Skeete A., Avilez M. J., De-la-Peña C., Loyola V.V.M. 2019. Signaling Overview of Plant Somatic Embryogenesis. *Frontiers in Plant Science*. Vol. 10. 77 p.

[22] Kehr. A.E., and G.W. Schaeffer. 1976. Tissue culture and differentiation of garlic. *Hortic Sci* 11:422-423

[23] Novak, F.J. 1981. Chromosomal characteristic of long term callus culture of *Allium sativum* L. *Cytologia* 46:371-379

[24] Nagakubo, T., A. Nagasawa, and H. Ohkawa. 1993. Micropropagation of garlic through in-vitro bulblet formation. *Plant Cell Tissue Organ Cult* 32:175-183

[25] Myers, J.M. and P.W. Simon. 1998. Continuous callus production and regeneration of garlic (*Allium sativum* L.) using root segment from shoot tip-derived plants. *Plant Cell Rep* 17:726-730

[26] Sutarto, I and M. Yuniaawati. 2009. Somatic embryogenesis through callus formation and olantlet regeneration on garlic (*Allium sativum* L.). *SABRAO J. Breeding and Genetics*. Vo. 41, Special Supplement. 8 p.

[27] Gomez, K. A. and A. A. Gomez. 1984. Statistical procedure for agricultural research. John Wiley and Sons Inc. Singapore.