Inducing potato tuber formation at low elevation of tropical region by foliar spray of PGR mixtures at different application times

U.K.J. Suharjo[1*], *T.* Pamekas[2], *P.* Harsono[3], and *A.M.* Silalahi[1]

[1]Bengkulu University, Department of Agronomy, Bengkulu, Indonesia
[2]Bengkulu University, Department of Plant Procetion, Bengkulu, Indonesia
[3]Sebelas Maret University, Department of Agronomy, Surakarta, Indonesia

Abstract. Growing potato crops at low elevation in the tropical region may face many problems related to high temperature, such as an increase in endogen GA_3 levels, causing the inhibition of tuber formation. Suppressing GA_3 biosynthesis, by applying a mixture of plant growth regulators (PGRs), is of important. The objectives of this experiment were to study the best PGR mixtures, PGR application time, and their interaction in promoting tuber formation at low altitude of tropical areas in Indonesia. The experiment used a completely randomized design arranged in factorial (2 factors, 3 replications). The first factor was a mix of plant growth regulator solution (M), consisting of 4 levels, which were: M_0 = No PGR; M_1 = 10 ppm 2,4-D + 100 ppm Daminozide, M_2 = M_1 +10 ppm BAP, M3 = M_1 + 25 ppm COU, and M_4 = M_1+ 500 ppm PBZ. The second factor was application time of the solutions (T), consisting of 3 levels, which were: T_1 = 3, 4, 5, 6 weeks after emergence (WAE), T_2 = 3, 4, 5 WAE, and T_3 = 3, 4 WAE. The results showed that all of the PGR mixtures promoted tuber formation with 10 ppm 2,4-D + 100 ppm Daminozide + 500 ppm Paclobutrazol was found as the best PGR mixture. Moreover, the best time to spray the mixture was 3,4,5,6 WAE. Finally, the best interaction between PGR and application time was10 ppm 2,4-D + 100 ppm Daminozide + 10 ppm BAP applied at 3,4,5 WAE.

1 Introduction

Low altitude of tropical areas, like Indonesia, is known to be warm and wet. The average daily temperature is about 29 ± 2 °C during the day, which is quite warm for potato crops whose optimum temperature is 17 – 20 °C [1]. Warm temperature has been reported to increase gibberellic acid (GA) biosynthesis in the bud of potato crops [2], promoting haulm growth [3], but preventing tuber formation [4].

The negative effects of GA at high temperatures might be overcome by application of growth retardants, such as Coumarin (COU), Paclobutrazol (PBZ), acting to inhibit GA biosynthesis in the plant. Adding Coumarin (25 ppm) or Paclobutrazol (500 ppm) to MS media supplemented with 10 ppm 2,4-D + 100 ppm Daminozide promoted tuber formation

* Corresponding author: usmankris@unib.ac.id

in vitro at 29 ± 2 °C [4]. Previous works demonstrated that Paclobutrazol promoted tuber formation when potato crops grown at medium and low altitude [5, 6]. In an *in vitro* experiment, paclobutrazol, daminozide, and ancymidol had been reported to promote tuber formation in *in vitro* experiment [7]. They also reported that retardant could be used to induce tuber formation in a high temperature and long photoperiod [7,8]. Furthermore, paclobutrazol inhibits GA3 biosynthesis by blocking the oxidative kaurenic acid and kauren as wel las by oxidation of ent-kauren [8].

The positive effects of cytokinin on potato tuberization has also been reported by [9] and [10]. Furthermore, *in vitro* experiment showed that applying a single plant growth regulator (PGR), has been reported to be less effective in inducing tuber formation than applying the combination of PGR and retardant, especially at high temperature [11]. The authors expected the same results when applying PGR mixture for potato crop grown in the glass house at low altitude. Owing to these, an experiment to study the effect of PGR mixtures on crop growth and tuber formation of potato crop grown at low altitude need to carry out.

The right application time of growth retardant is another issue needs to be addressed. *In vitro* experiments showed that application of growth retardant at planting time, inhibited plantlet growth and failed to produce microtuber [11]. On the other hand, application of retardants when the plantlets were already 6 weeks old significantly promoted tuber production [11]. However, potato crops grown in the glass house may have different growth habits from those grown in *in vitro* and it therefore need a specific time for applying growth retardant to promote tuber formation. When grown in the glass house, potato crops demonstrate the following growth pattern: seed germination (0-9 days after planting, DAP), leaf development (10-19 DAP), shoot development (20-39 DAP), tuber development (40-49 DAP), and tuber maturation above 79 DAP [12]. Moreover, a field experiment demonstrated that application of paclobutrazol (29.9% active ingredient) at t6 DAP inhibited crop growth, reduced tuber number, and reduced sugar content of potato tuber, but increased starch content of the tuber [13].

In this respect, application of growth retardant was expected to induce tuber formation and promote tuber growth, leading to the high number of tuber production. It was therefore an experiment to study the effect of a repeated application of growth retardant on tuber induction is very important. The objectives of this experiment were to study the effect of plant growth regulator (PGR) mixtures, the effect of application time of PGR mixture, and the combination of both on potato growth and tuber formation at low altitude.

2 Materials and methods

The experiment was carried out at the Department of Agronomy Experimental Station, College of Agriculture, Bengkulu University (10 m above sea level), from January to October 2018. The experimental design used in this experiment was completely randomized design, arranged in factorial (2 factors, 5 replications). The first factor was a mix of plant growth regulator solution (M), consisting of 4 levels, which were: M_0 = No PGR; M_1 = 10 ppm 2,4-D + 100 ppm Daminozide, $M_2 = M_1$ +10 ppm BAP, M3 = M_1 + 25 ppm COU, and $M_4 = M_1$+ 500 ppm PBZ. The second factor was application time of the solutions (T), consisting of 3 levels, which were: T_1 = 3, 4, 5, 6 weeks after emergence (WAE), T_2 = 3, 4, 5 WAE, and T_3 = 3, 4 WAE.

Media used for the experiment was a sterile mixture of top soil and cow manures (4:1; v/v). Media sterilization was done by spraying formalin solution (4%) thoroughly to the media until all part of it got wet, covering the media tightly with plastic sheet for 2 weeks, and shovelling the media to get rid of the leftover of formalin gas.

Potato seeds c.v. Granola, 30 gram each, was planted in the media, fertilized with Urea (400 kg ha^{-1}), KCl (150 kg ha^{-1}), and TSP (300 kg ha^{-1}). A half of the fertilizers were given at planting time and the rest were given when the crops were 3-week-old. Disease control was done by application of Mankozeb 80% (2 g L^{-1}) and streptomycin sulphate 30% (1 g L^{-1}), pest by Deltametrin (2 cc L^{-1}), and nematode and soil insect by Carbofuran 3%. PGR mixes application was done accordingly by spraying the crops until the haulm got wet.

Variables measured included stem number plant height, leaf number, tuber number per hill, tuber weight per hill, weight of the biggest tuber, weight of the smallest tuber, and tuber diameter. While plant height and leaf number were measured when the crops were 7-week-old, the rest of the variables were measured at harvesting time. All data were statistically analyzed with analysis of variance followed by Duncan's multiple range test at 5%.

3 Results and discussions

3.1 PGR mixtures

PGR Mixtures significantly affected crop growth, except for leaf number. This mixture reduced plant growth, in which the shortest crops was found in M1+ 500 ppm PBZ (Table 1). In average, these crops were much shorter than those of grown at low altitude without retardant application, reaching up to 50.8 cm [8]. It was likely that the reduction in plant height was attributed to the retardants contained in the mixture. In fact, all of those mixtures contained 100 ppm Daminozide, a retardant that inhibits Gibberellic acid action [14], resulting in growth inhibition [15]. In addition to Daminozide, other mixtures also contained Coumarin (25 ppm COU) or Paclobutrazol (500 ppm PBZ) because of which reduced crop growth. This reduction in plant growth indicated that the retardants used in these experiments were effective and therefore were expected to promote potato tuber formation at low altitude. These confirmed the previous research reporting that Paclobutrazol significantly reduced stem length about 50% [16]. Similar results were reported by Florez-Lopez *et al.* [8], in which paclobutrazol reduced plant height, increased tuber number, and increased starch content in microtubes.

Table 1. Effects of PGRs mixture on crops growth.

PGR Mixtures	Plant Height (cm)	Leaf Number
Mo = No PGR	25.31 a	8.4 a
M1 =10 ppm 2,4-D + 100 ppm Daminozide	20.03 ab	9.5 a
M2 = 10 ppm 2,4-D + 100 ppm Daminozide + 10 ppm BAP	20.96 ab	10.8 a
M3= 10 ppm 2,4-D + 100 ppm Daminozide + 25 ppm COU	20.26 ab	10.4 a
M4 = 10 ppm 2,4-D + 100 ppm Daminozide + 500 ppm PBZ	17.23 b	8.9 a

Note: number at the same column followed by different letter were significantly not different.

Application of PGR Mixture significantly increased tuber number. Without PGR, the crops produced only 2.5 tubers. On the other hand, application of PGR Mixture resulted in 6.6 to 16.3 tubers, in which the highest number was found in M_1 + 500 ppm PBZ and the lowest in M_1 + 25 ppm COU (Figure 1). Plant hormones are reported to play important roles in the tuberization process of potato. While cytokinin and auxin are known to be promotive factors [17], gibberellin is a suppressive factor [18]. However, the negative effect of gibberellin could be minimized by application of retardant, such as paclobutrazol, coumarin, daminozide, or ancymidol [14]. In this experiment, we did not measure the content of endogen gibberellin. Yet, adding cytokinin and retardants (PBZ, DMZ, or COU) to the PGR mixture could promote tuber formation as reported previously [11, 17, 18].

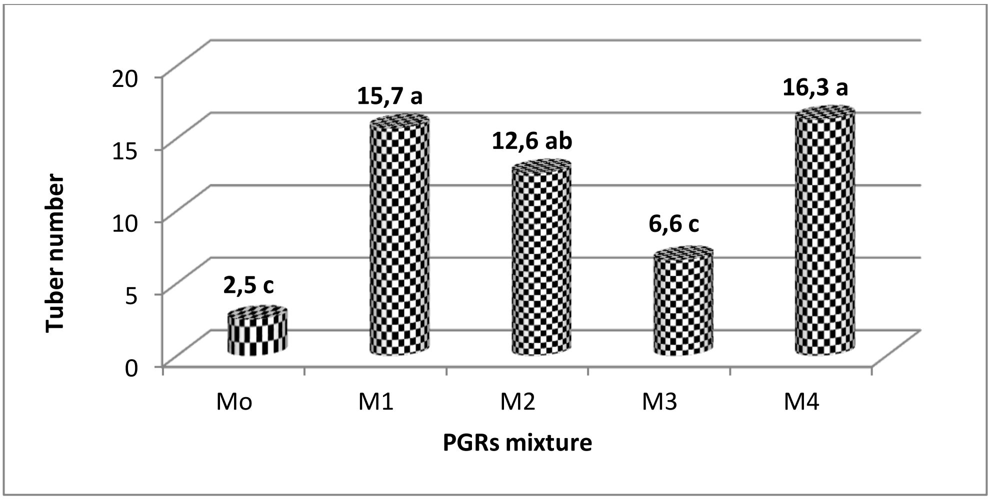

Fig. 1. The effect of PGRs mixture on tuber number. M0: without PGR, M1= 10 ppm 2,4-D + 100 ppm Daminozide, M2 = M1 +10 ppm BAP, M1 + 25 ppm COU, and M4 = M1 + 500 ppm PBZ.

Tuber qualities, expressed in tuber diameter and tuber fresh weight, were significantly affected by the application of PGR Mixtures. Without PGR mixture (Mo), the crops produced the smallest tuber diameter (Fig. 2) and the smallest tuber fresh weight (Fig. 3). These findings suggested that the PGR Mixture, containing retardant and cytokinin could suppress the negative effect of GA at high temperature, as reported previously [4,13,19]. In fact, exogenous application of cytokinin was reported to promote tuber growth [20] while that of GA_3 inhibited tuber initiation [21].

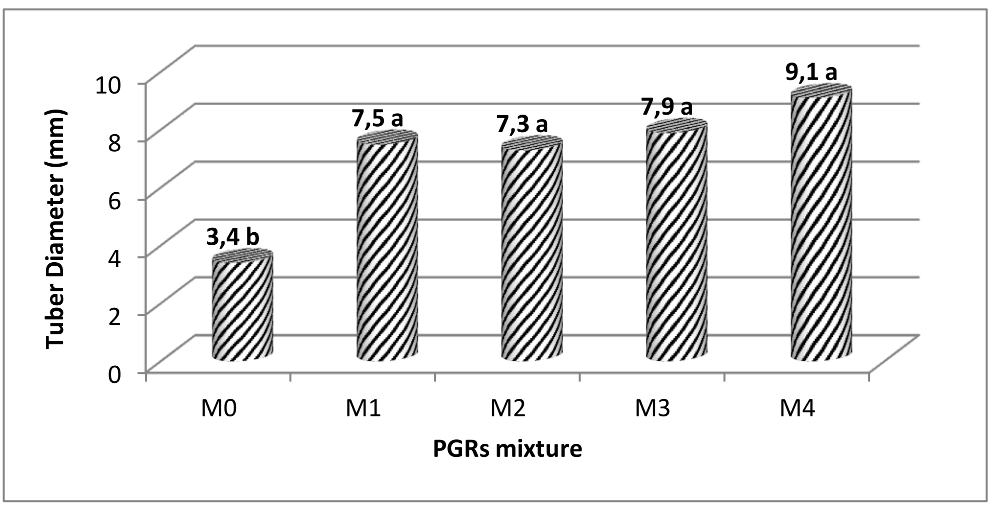

Fig. 2. The effect of PGRs mixture on tuber diameter. Note: M_0: without PGR, M_1= 10 ppm 2,4-D + 100 ppm Daminozide, M_2 = M_1 +10 ppm BAP, M_1 + 25 ppm COU, and M_4 = M1 + 500ppm PBZ.

 https://doi.org/10.1051/e3sconf/202337303027

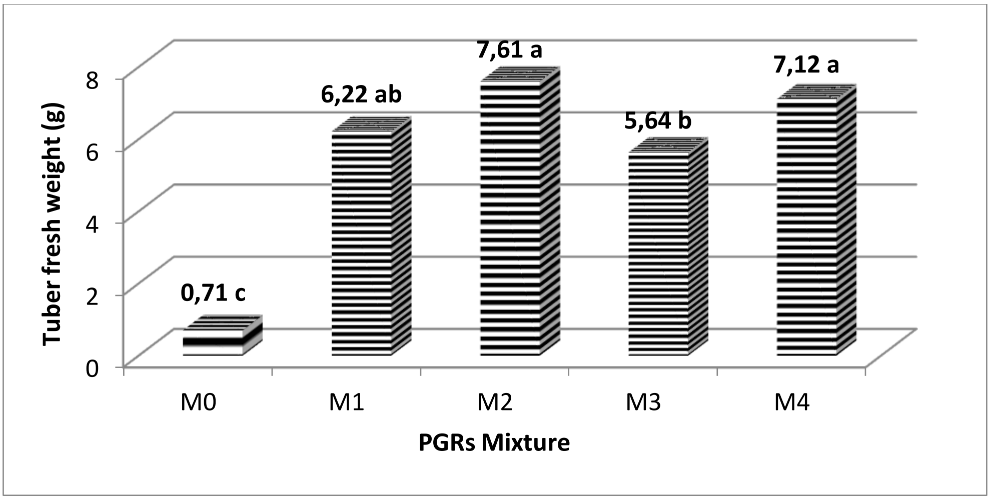

Fig. 3. Effects of PGR Mixtures on tuber fresh weight (g). Note: M0: without PGR, M1= 10 ppm 2,4-D + 100 ppm Daminozide, M2 = M1 +10 ppm BAP, M1 + 25 ppm COU, and M4 = M1 + 500ppm PBZ.

3.2 Application time

Application time of PGR Mixture did not significantly affect plant height, leaf number, and tuber diameter. On the other hand, it significantly affected tuber number, tuber fresh weight, and weight of the biggest tuber. The less frequent PGR mixture was applied, the smaller number of tubers were produced and the lighter the tuber would be (Table 2; Figure 4). In this experiment, the application of PGR mixtures was started in week 3 after plant emergence (WAE) and repeated in the following week for 1, 2, and/or 3 weeks afterward, which were 3, 4, 5, 6 WAE (T_1), 3,4,5 WAE (T_2), and 3,4 WAE (T_3). It was likely that spraying the crop at 3,4,5,6 WAE satisfied the need of growth retardant to initiate tuber formation and to promote tuber growth, as tuber initiation starts early in the crop growth phase and tuber formation completes in 40-49 days [12].

Applying PGR mixture more frequently also resulted in larger tuber size (Figure 5), more tuber weight (Figure 6), suggesting that the retardant worked well in preventing GA biosynthesis or GA activity, resulting in less starch hydrolysis [22], leading to more starch accumulation in the tuber [13]. These findings have recently been confirmed by Nuraini [20] reporting that GA_3 biosynthesis was reduced up to 60%, resulting in an increase in tuber formation by 40% when the potato crops were sprayed with a mixture of paclobutrazol and cytokini. An increase in tuber formation was also reported by Kołodziejczyk M & Gwóźdź [23] when potato crops were sprayed with plant growth regulator.

Table 2. Effect of application time on plant height, leaf number and biggest tuber fresh weight.

Application Time	Plant Height (cm)	Leaf Number	Biggest tuber fresh weight (g)
T_1 = 3,4,5,6 WAE	20.41 a	10.4 a	15.92 a
T_2 = 3,4,5 WAE	19.65 a	9.8 a	14.02 a
T_3 = 3,4 WAE	18.22 a	9.8 a	8.76 b

Note: number at the same column followed by the same letter were significantly not different. WAE = week after emergence.

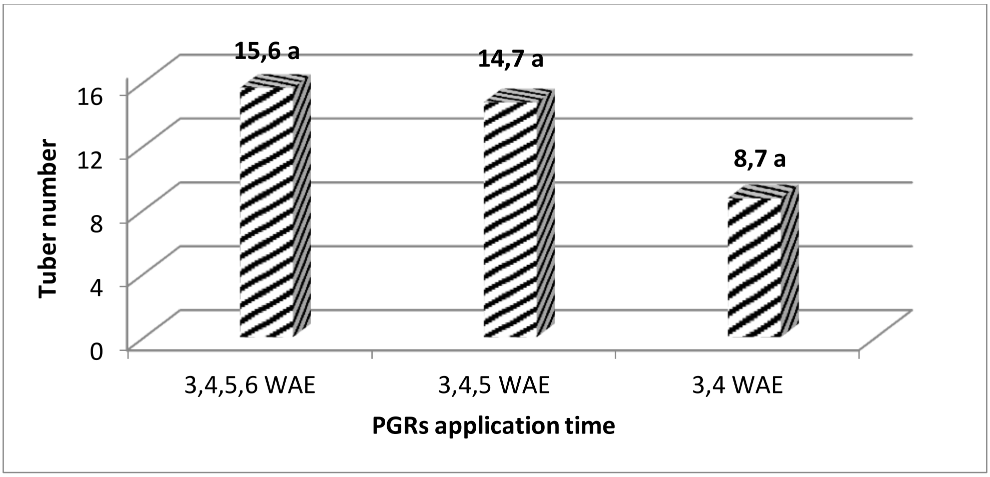

Fig. 4. Effects of PGRs application time on tuber number; WAE = weeks after emergence.

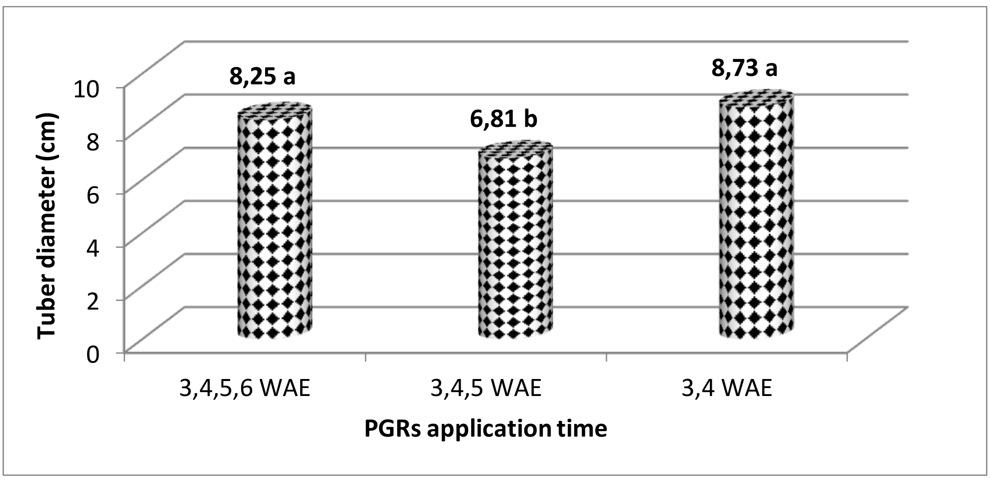

Fig. 5. Effects of PGRs application time on tuber diameter; WAE = weeks after emergence.

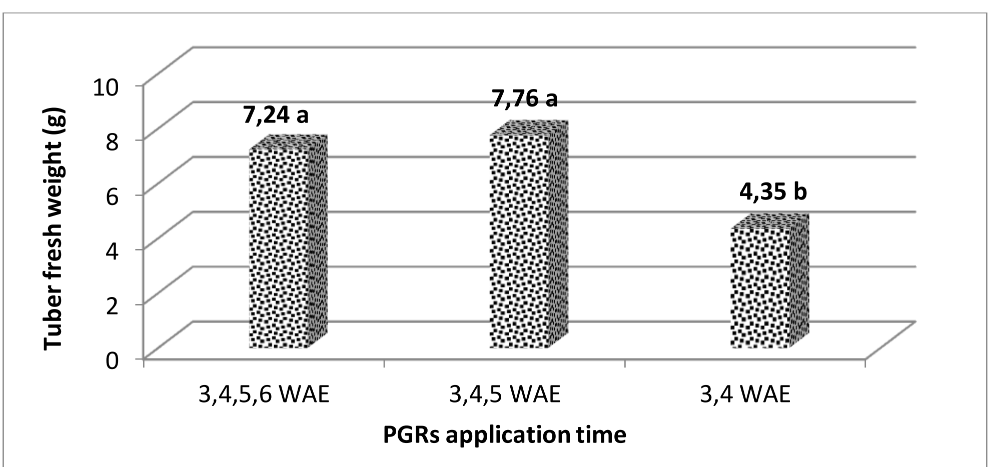

Fig. 6. Effects of PGRs application time on tuber weight. WAE = weeks after emergence.

3.3 Interaction of PGR mixture and application time

PGR Mixture did not affect plant height, leaf number, and tuber diameter regardless of the application times (Table 3), suggesting that the effects of PGR Mixture on plant growth were independent from the Application Time. However, this interaction significantly affected tuber number (Table 4) and tuber fresh weight (Table 5).

The highest number of tubers per plant (11.2 tubers) was found when the crops were sprayed with 10ppm 2,4-D + 100 ppm DMZ +10 ppm BAP (M2) at 3,4,5 WAE while the lowest number (3.2 tubers) was found when the crops were sprayed with M1 + 100 ppm COU at 3,4 WAE (Table 5). It seemed that daminozide and BAP worked very well in promoting tuber formation and tuber growth. In this respect. Daminozide inhibited gibberellic acid biosynthesis, hence, promoted tuber formation while BAP promoted starch synthesis which also promoted tuber formation and tuber growth [24].

Table 3. Effects of PGR mixture and application time on crop growth and tuber formation.

PGR Mixtures[1)]	**Application Time[2)]**	**Plant Height (cm)**	**Leaf number**	**Tuber diameter (mm)**
M_1 = 10 ppm 2,4-D + 100 ppm DMZ	T_1 = 3,4,5,6 WAE	20.11 a	10.8 a	6.11 a
	T_2 = 3,4,5 WAE	21.60 a	8.5 a	6.72 a
	T_3 = 3,4 WAE	18.51 a	10.2 a	9.71 a
M_2= 10 ppm 2,4-D + 100 ppm DMZ + 10 ppm BAP	T_1 = 3,4,5,6 WAE	23.21 a	11.8 a	8.23 a
	T_2 = 3,4,5 WAE	18.21 a	10.2 a	4.84 a
	T_3 = 3,4 WAE	18.10 a	10.4 a	8.86 a
M_3 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm COU	T_1 = 3,4,5,6 WAE	22.61 a	10.4 a	10.81 a
	T_2 = 3,4,5 WAE	19.21 a	12.3 a	6.45 a
	T_3 = 3,4 WAE	18.63 a	8.7 a	6.67 a
M_4 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm PBZ	T_1 = 3,4,5,6 WAE	15.72 a	8.2 a	7.98 a
	T_2 = 3,4,5 WAE	18.31 a	8.4 a	9.32 a
	T_3 = 3,4 WAE	17.73 a	9.2 a	9.97 a

Note: 1) DMZ = daminozide, BAP = benzyl amino purine, COU= coumarin, PBZ = paclobutrazol; 2) WAE = week after emergence. The number at the same column followed by the same letter were significantly not different (DMRT α:5%).

The results of analysis variance showed that the effects of PGR mixture on tuber number was dependent on the application time of the solutions. In this respect, each mixture had its own best time to apply. For example, M_1 was best applied at 2,4 WAE while M_3 was at 2,3,4,5 WAE and M_4 could be applied anytime (Table 4). Likewise, the effect of PGR Mixtures was o tuber fresh weight was depending on their Application Time (Table 5). It might be attributed to the growth pattern of potato crops [12], leading to the differences their response to growth retardants contained in the PGR Mixtures.

Table 4. Two-ways table on the effect of PGR mixture and application time on tuber number.

PGR mixtures	Application time of PGRs		
	3,4,5,6 WAE (T_1)	**3,4,5 WAE (T [12$_2$)**	**3,4 WAE (T_3)**
M_1 = 10 ppm 2,4-D + 100 ppm DMZ	6.6 b	7.8 ab	9.1 a
	B	AB	A
M_2 = 10 ppm 2,4-D + 100 ppm DMZ + 10 ppm BAP	7.4 ab	11.2 a	4.2 b
	AB	A	B
M_3 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm COU	8.2 a	5.6 ab	3.2 b
	A	B	B
M_4 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm PBZ	7.4 a	7.8 a	6.0 a
	AB	AB	AB

Note: the number at the same column followed by the same capital letter were significantly not different; the same number at the same row followed by the same small letter were significantly not different

Table 5. Two-ways table on the effect of Tuber Promoter Solutions and application time on tuber fresh weight.

PGR mixtures	Application time of PGRs		
	3,4,5,6 WAE (T_1)	**3,4,5 WAE (T_2)**	**3,4 WAE (T_3)**
M_1 = 10 ppm 2,4-D + 100 ppm DMZ	7.05 b	9.17 ab	12.67 a
	C	AB	A
M_2 = 10 ppm 2,4-D + 100 ppm DMZ + 10 ppm BAP	30.37 a	16.41 b	5.56 b
	A	A	B
M_3 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm COU	9.13 a	5.28 ab	3.20 b
	BC	BC	C
M_4 = 10 ppm 2,4-D + 100 ppm DMZ + 1000 ppm PBZ	12.54 a	3.34 b	8.64 ab
	BC	C	AB

Note: the number at the same column followed by the same capital letter were significantly not different; the same number at the same row followed by the same small letter were significantly different

An increase in application frequency increased the number of tubers, even though the highest tuber number was found in 3,4,5 WAE. Furthermore, the highest tuber weight was found when PGR mixture was applied in 3,4,5,6 WAE. These suggested that PGR mixture needs only to be applied in 3,4,5 WAE to promote tuber formation, but it needed another week (3,4,5,6 WAE) to promote tuber growth to yield the highest tuber weight (Table 3). It suggested that an additional application of PGR mixture (daminozide and BAP) in week 6 was very effective, because week 6 is the time when potato tuber starts to accumulate starch [12]. However, this research was focused on promoting tuber formation, expressed in tuber number. Therefore, treatment M1+10 ppm applied in 3,4,5 WAE was more interested than when that applied in 3,4,5,6 WAE.

4 Conclusion

The best PGR mixture applied to promote tuber formation was 10 ppm 2,4-D + 100 ppm Daminozide+ 500 ppm Paclobutrazol. When applied alone, the best time to apply PGR mixture to promote tuber formation was in 3,4,5,6 WAE. And the best interaction of PGR mixture and application time was 10 ppm 2,4-D+ 100 ppm Daminozide + 10 ppm BAP applied at 3,4,5 WAE.

This research was partly funded by the Minister of Research and Higher Education of the Republic of Indonesia through Applied Research Scheme for year 2018. The author offered their gratitude to the Government of the Republic of Indonesia.

References

1. J. C. Stark, M. Thornton, and P. Nolte, *Potato Production Systems* (Springer Nature, 2020).
2. C. M. Menzel, Ann. Bot. **52**, 697 (1983).
3. N. Wicaksana, Zuriat **12**, (2001).
4. U. K. J. Suharjo, B. G. Murcitro, T. Pamekas, and H. Haryuni, Biog. J. Ilm. Biol. **5**, 61 (2017).
5. A. A. Alexopoulos, K. A. Akoumianakis, and H. C. Passam, Can. J. Plant Sci. **86**, 1217 (2006).
6. T. Handayani, P. Basunanda, R. H. Murti, and E. Sofiari, (2013).
7. B. M. R. Harvey, S. H. Crothers, N. E. Evans, and C. Selby, Plant Cell. Tissue Organ Cult. **27**, 59 (1991).
8. R. Flores-López, R. Martínez-Gutiérrez, H. A. López-Delgado, and M. Marín-Casimiro, Rev. Mex. Ciencias Agrícolas **7**, 1143 (2016).
9. J. A. García-García, J. B. Azofeifa-Bolaños, F. Solano-Campos, and R. Orozco-Rodríguez, Uniciencia **33**, 1 (2019).
10. S. N. Lomin, Y. A. Myakushina, O. O. Kolachevskaya, I. A. Getman, E. M. Savelieva, D. V Arkhipov, S. V Deigraf, and G. A. Romanov, Front. Plant Sci. **11**, 613624 (2020).
11. U. Suharjo, Fahrurrozie, and S. Sudjatmiko, in *Pros. Semin. Pekan Kentang Nas.* (Bandung, 2008).
12. D. Djuariah, T. Handayani, and E. Sofiari, J. Hortik. (2017).
13. O. Mabvongwe, B. T. Manenji, M. Gwazane, and M. Chandiposha, Adv. Agric. **2016**, (2016).
14. U. K. J. Suharjo, in *Proceeding Semin. 2nd Int. Conf. Biosci. Biotechnol.* (2010).
15. L. Taiz and E. Zeiger, Plant Physiol **9**, 172 (2002).
16. T. Tekalign and P. S. Hammes, Plant Growth Regul. **45**, 37 (2005).
17. D. J. Hannapel, P. Sharma, T. Lin, and A. K. Banerjee, Plant Physiol. **174**, 845 (2017).
18. O. O. Kolachevskaya, Y. A. Myakushina, I. A. Getman, S. N. Lomin, I. V Deyneko, S. V Deigraf, and G. A. Romanov, Int. J. Mol. Sci. **22**, 8207 (2021).
19. P. Bhattarai, SAARC J. Agric. **15**, 149 (2017).
20. A. Nuraini, P. S. Nugroho, W. Sutari, S. Mubarok, and J. S. Hamdani, Agric. Nat. Resour. **55**, 171 (2021).
21. A. Saidi and Z. Hajibarat, J. Genet. Eng. Biotechnol. **19**, 1 (2021).

22. F. B. Salisbury and C. W. Ross, *Plant Physiology* (Wadsworth Inc, 1991).
23. M. Kołodziejczyk and K. Gwóźdź, Plant, Soil Environ. **68**, 375 (2022).