Auxin and Cytokinin Effect on In vitro Callus Induction of Maize (*Zea mays* L.) *Srikandi Putih*

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ABSTRACT
Maize plant (*Zea mays* L.) can be an alternative for sustainable forage because easy to obtain and can be cultivated in the dry season. Increasing plant biomass is needed to maximize maize plants as a forage crop through genetic mutations. Callus production by tissue culture is one of the planting materials used on plant mutagens. This study was done to identify the response of auxin and cytokinin hormone in callus induction of maize plant *Srikandi Putih* on root and stem explants. Kinetin and BAP (0.1 mg/L), and without cytokinin were combined with auxin 2,4-D (2,4-Dichlorophenoxyacetic acid) hormone, at two concentrations 1 and 2 mg/L. Stem explants from in vitro seedlings could produce callus 40-100%, while root explants from in vitro seedlings could produce callus 44-100%. MS medium containing 2,4-D 2 mg/L without cytokinin produced the highest callus both in roots and stems. The highest callus fresh weight (0.553g) was obtained in MS medium supplemented with 2,4-D 1 mg/L without cytokinin in the stem, while in the roots, 2,4-D 1 mg/L and Kinetin 0.1 mg/L produced the highest fresh weight (0085g).

Keywords: *Maize srikandi Putih, 2,4-Dichlorophenoxyacetic acid (2,4-D), kinetin, in vitro*

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide as human food, animal feed, and biofuel. Maize plant (*Zea mays* L.) can be a sustainable forage alternative in Indonesia because easy to obtain, high yield, high energy, and is cultivated in the dry season. Maize plant for forage is all parts of the plant, including stems, leaves, and young corn generally harvested at 45-65 days [1].

Local maize plants have the potential as a forage source with wide adaptability, can be developed on marginal land and fertile and dry land, the seeds can be used for several generations, some are early maturity, and the yield can be used relatively high. Factors that influence maize plant yield and quality are environmental, cultivation, and genetics. Plant breeding programs to increase plant biomass are needed to optimize maize plants as sustainable forage.

Genetic mutation is one method to carry out plant breeding programs. A mutation is not caused by genetic recombination or segregation but by a sudden heritable change in the DNA in a living cell [2]. Tissue culture is a technique used to provide planting material for plant genetic mutations [3] called callus. The tissue culture technique has been reported to be used for a genetic mutation in forages such as in dwarf cogongrass [4], dwarf napiergrass [5], and ruzi grass [6]. The callus is an irregular shape tissue that divides parenchyma cells continuously, then regenerates to be a complete plant. The most important factors for callus induction in tissue culture are the types of explants and the medium culture composition consisting of micronutrients and macronutrients, carbon source, nitrogen, and plant growth regulators. The most critical component for promoting callus initiation is the plant growth regulator, auxin and cytokinin [7]. The balance of auxin and cytokinin's combined concentration is not about the exogenous concentration of the hormones used but the balanced combination of endogenous and exogenous growth regulators.

Callus cultured in vitro are usually used for planting material in plant conservation and genetic mutation programs. Hence, this research aimed to identify the various types of explant and levels of auxin and cytokinin

combinations on callus induction in maize plant *srikandi putih*.

2. MATERIALS AND METHODS

2.1. Plant Materials and Seed Germination

The seeds of maize *srikandi putih* used for germination were the seeds collection from *Pusat Inovation Agroteknologi* (PIAT) of Universitas Gadjah Mada, Yogyakarta. The seeds were sterilized on the surface using ethanol 70% for 2 minutes, then 10% commercial bleaching that contains sodium hypochlorite for 20 minutes, and rinsed in sterile distilled water 3 times for 5 minutes each. Finally, seed germination was carried out using sterilized seeds in an agar medium.

2.2. Medium and Callus Induction

Medium for callus induction contained MS medium (Murashige and Skoog) supplemented with 30 g/L sucrose, 7 g/L agar, and various combination levels and types of plant growth regulators (Table 1.) that were auxin (2,4-Dichlorophenoxyacetic acid (2,4-D) 1 and 2 mg/L) and cytokinin (without cytokinin, Kinetin 0.1 mg/L, and BAP 0.1 mg/L). The explants used for callus induction were roots and stems of 7-day-old in vitro seedlings. The explants were cut approximately 0.5 cm long and cultured in a callus induction medium incubated without light for three weeks at 25 ± 2ºC. All experiments were repeated five times for each treatment.

Table 1. The various combination levels and types of plant growth regulators.

Treatment	Cytokinin 0 mg/L (C0)	Kinetin (kin) 0.1 mg/L	BAP 0.1 mg/L
2,4-D 1 mg/L	D1C0	D1Kin	D1BAP
2,4-D 2 mg/L	D2C0	D2Kin	D2BAP

2.3. Callus Growth Measurement

The measurements of callus growth for each treatment with a combination of plant growth regulators were determined by observing the percentage, fresh weight, and morphology of callus formed. The percentage of callus formed in every treatment was observed after 2 weeks of culture. The morphology and the fresh weight of callus formed were recorded in the third week. The morphology observed from the callus consisted of callus texture and colour. The quantitative data were analyzed using descriptive methods for describing the results of the experiment.

3. RESULTS AND DISCUSSION

The ability of callus induction depended on types of explant source and plant growth regulators both in type and level combination in culture medium. In this experiment, callus induction from the stem and root explants were cultured on MS medium [8] with different types and levels of the combination of plant growth regulators (auxin using 2,4-D 1 and 2 mg/L and cytokinins using Kinetin 0.1 mg/L, BAP 0.1 mg/L, and without cytokinin 0 mg/L).

In all treatments, callus induction began with the swelling explants in 1-2 days after culture. Furthermore, the first callus formed in 5 to 8 days at the tip of the explant in response to the injury caused by explant cutting and the presence of plant growth regulators. [9] Callus in the first formed consists of morphogenic cells growing around the injury area. These cells will cover the injured area as well as the entire explant. Furthermore, they will differentiate into whole plants. The Injury healing in explants occurs due to the strengthening of cell walls caused by biochemical activity that is a synthesis of phenolic compounds from induction of the phenylpropanoid pathway.

3.1. Percentage of Callus

The experiment results showed that the percentage of callus induction in stem explants ranged from 40 to 100% (**Fig.1.**), and in root explants ranged from 44 to 100% (**Fig.2.**). The highest percentage of callus formed in stem explants was found in the combination of plant growth regulators using 2,4-D 2 mg/L without cytokinin, while the highest of callus formed in root explants was found in the combination of plant growth regulators using 2,4-D 1 and 2 mg/L without cytokinin. The result indicated that adding cytokinin, both Kinetin and BAP 0.1 mg/L, did not affect the percentage of callus induction of maize *srikandi putih.*

Many studies used 2,4-D alone as auxin in the initiation and proliferation of primary and embryogenic callus of maize [10, 11, 12]. The previous studies conducted in which callus of maize can be grown on MS medium with 1 mg/L [10, 13] and 2 mg/L levels [14, 15]. Auxins were an essential factor to promote callus induction in maize. Establishing the auxin levels within plant cells during the induction phase of somatic embryogenesis was essential for initiating dedifferentiation and cell division. In this case, the exogenously supplied auxin played an essential role in forming the endogenous auxin levels. [16]. The combination of 2,4-D and Kinetin showed that even though Kinetin's presence was essential, the level of auxin gave a significant effect on the callus induction of maize [17].

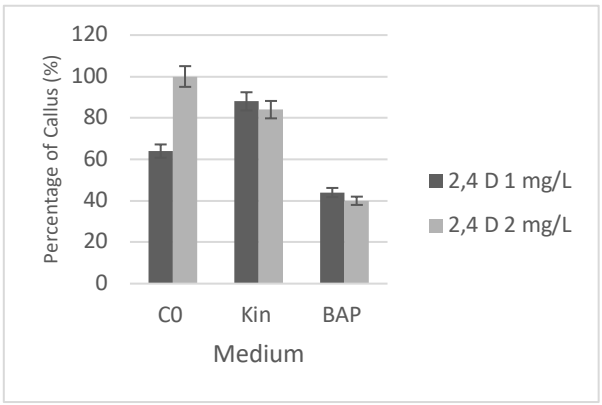

Fig. 1. Effect of the combination of level 2,4-D and types of 0.1 mg/L cytokinins on the percentage of callus formed in stem explants.

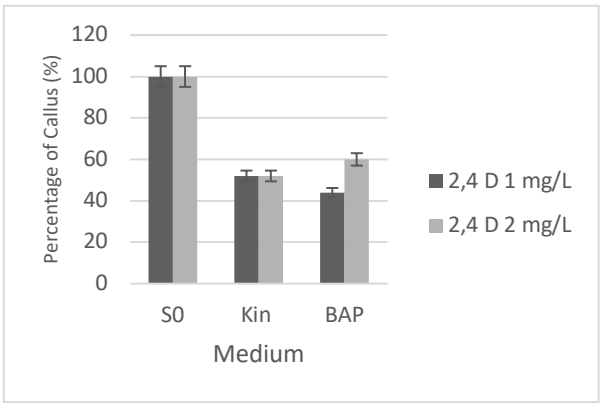

Fig. 2. Effect of the combination of level 2,4-D and types of 0.1 mg/L cytokinins on the percentage of callus formed in root explants.

3.2. Fresh Weight of Callus

The results showed that the fresh weight of callus induction in stem explants ranged from 0.085 to 0.553g (**Fig.3.**), and in the root, explants ranged from 0.033 to 0.085g (**Fig.4.**). The fresh weight was recorded in the third week. The highest fresh weight of callus formed in stem explants was found in the combination of 2,4-D 1 mg/L without cytokinin. In contrast, the highest fresh weight of callus induction in root explants resulted in the combination of growth regulators in 2,4-D 1 mg/L + Kinetin 0.1 mg/L.

Parameter to determine cell division is the fresh weight of callus, multiplication, and enlargement in callus biomass. The combination of growth regulators, endogenous and exogenous, was one of the factors affecting callus production. The balance of exogenous growth regulators between auxins and cytokinin in the monocotyledons required high auxin levels and low cytokinin [18]. In this experiment, callus induction in maize *srikandi putih* required low cytokinin, even the stem explants of maize *srikandi putih* could produce the highest fresh weight of callus without cytokinin. This result indicated that 2,4-D alone as an exogenous growth regulator could interact with an endogenous growth regulator owned by stem explants. Thus it could stimulate cell division. At the same time, root explants required Kinetin 0.1 mg/L to produce the highest fresh weight of callus. Endogenous growth regulators probably cause this in root explants required exogenous cytokinin to stimulate cell division. The appropriate balance of growth regulators could encourage cell division dan enlargement optimally.

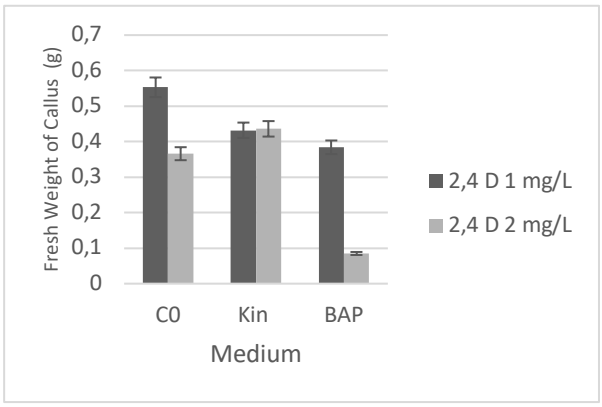

Fig. 3. Effect of the combination of level 2,4-D and types of 0.1 mg/L cytokinins on the fresh weight of callus formed in stem explants.

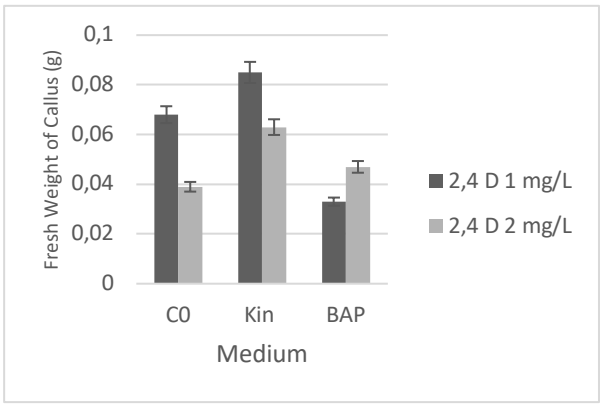

Fig. 4. Effect of the combination of level 2,4-D and types of 0.1 mg/L cytokinins on the fresh weight of callus formed in root explants.

3.3. Callus Morphology

The Morphology of callus consists of textures and colours. The texture and colour of callus formed were observed in the third week after culture. All treatments of growth regulators in stem explants resulted compact callus with nodular, while in root explants resulted two types of textures that were compact callus in medium containing 2,4-D 1 mg/L + Kinetin 0.1 mg/L, 2,4-D 1 mg/L + BAP 0.1 mg/L, and 2,4-D 2 mg/L + BAP 0.1 mg/L, and compact watery callus in medium containing 2,4-D 1 mg/L, 2,4-D 2 mg/L, and 2,4-D 2 mg/L + Kinetin 0.1 mg/L. The compact texture of callus in root explants showed that was not nodular texture. The compact and nodular texture of the callus indicated that the callus was embryogenic. The characteristic of embryogenic callus was a compact and nodular texture, cream or whitish-

yellow [19]. Callus formed watery in texture indicates that callus was not embryogenic callus [10, 14, 19].

Table 2. shows that the colors produced on callus induction in stem explants were white, whitish cream, cream, brownish, and brown. All treatments in stem explants produced dominant whitish cream and cream colours. Brownish and brown colours of callus were also produced in each treatment, while at 2,4-D 2 mg/L + BAP 0.1 mg/L produced the most brownish and brown colours. Table 3. shows that root explants in all treatments produced whitish cream, cream, brownish, and brown. Each treatment resulted in dominant whitish cream, cream, and brown colours. Brown colour was mostly found in 2,4-D 1 mg/L + BAP 0.1 mg/L. All explants cultured in a medium consisting of 2,4-D + BAP produced the most brownish and brown colours.

The callus colour can describe the visual appearance of the callus, which shows the level of cell division activity. White, whitish cream, and cream colors indicated that cell division was still actively occurring, while brown and brown indicated aging cells. The synthesis of phenolic substances on cells is the cause of the callus colour change [20]. The phenol substances in excessive concentration can be toxic to cells, thus inhibiting the growth of callus [21].

4. CONCLUSIONS

It can be concluded that all combinations of growth regulators could be used for callus induction in stem and root explant of maize *srikandi putih*. The most optimal treatment in inducing callus was in medium with 2,4-D 2 mg/L without cytokinin on the stem explants and 2,4-D 1 mg/L on root explants without cytokinin. Those treatments could induce 100% callus, although they did not produce the highest fresh weight. The addition of cytokinin, both Kinetin and BAP decreased callus induction. Thus cytokinin did not affect callus induction of maize *srikandi putih*.

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