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Full Length Article

Improvement of Micropropagation through Combination of Plant Growth Regulators in Indonesian Sorghum Hybrid Cultivar

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Abstract

Sorghum is a tropical grass used for various purposes including food, animal feed, and biofuels source, but its *in vitro* propagation still found an obstacle due to tissue browning. This study aims to propagate and regenerate callus in the Indonesian sorghum hybrid, named Numbu cultivar from seed and meristematic leaf whorl explants. The combined effect of plant growth regulators (PGRs), namely 2,4-D and proline as well as NAA (naphthalene acetic acid) and BAP (6-benzyl amino purine) was investigated on embryogenic callus induction and regeneration, respectively. The results showed that callus induction was significantly faster in the seed explants compared to leaf whorl. The optimal medium used to obtain a large induction in the seed explants was MS salt containing 3 mg L^{-1} 2,4-D and 5000 mg L^{-1} proline, which gave a 68% yield. Furthermore, the best medium used to obtain a high plant regeneration frequency was the MS medium containing 0.1 mg L^{-1} NAA and 3 mg L^{-1} BAP, which regenerated 78% of the callus into plantlet. The addition of 560 mg L^{-1} proline to the combination medium increased doubled the regeneration into the planlet. Based on the results, PGRs combinations are required to improve embryogenic callus induction and regeneration into planlet in sorghum. © 2022 Friends Science Publishers

Keywords: Sorghum; Plant growth regulator; Somatic embryogenesis; Embryogenic callus; Regeneration

Introduction

Sorghum (*Sorghum bicolor*) is a tropical grass with high adaptation and productivity in hot and dry climate regions. One of the Indonesian superior hybrid Numbu cv, is a sweet sorghum hybrid from the SADC (South African Development Community) IS23509 strain. The sorghum hybrid Numbu cv is drought and heat tolerant, and rust disease and leaf spot resistant. Furthermore, it has an average height of 135 cm, immature panicle closed by leaf and opened when mature, harvesting age of 100–110 days and a productivity of 7.695 ton/ha. Sorghum is an important plant used for the production of food, feed, renewable energy, and national food diversification (Pabendon *et al.* 2012). Therefore, the biotechnology is an important technique for improving sorghum cultivars that requires development of the tissue culture. However, tissue culture of sorghum is found an obstacle due to tissue browning caused by a high level of phenolic compound production and recalcitrant traits.

Somatic embryogenesis (SE) in tissue culture is an essential process for micropropagation starting with the formation of an embryo from a somatic cell, which then develops into a complete plant (Ikeuchi *et al.* 2013)**.** There are several advantages such as bipolar structure for shoot and root, plant propagation improvement, as well as facilitation of physiological and biochemical plant study. The SE process consists of 3 stages, namely callus induction, proliferation, and regeneration. Several studies explored the embryogenic callus induction of sorghum using different explant sources or plant growth regulators (PGRs). Furthermore, some of the explants used for the induction include leaf whorl (Silva *et al.* 2020), immature embryo (Flinn *et al.* 2020), mature embryo (Avci 2019) and leaf (Amali *et al.* 2014), but they all have a low regeneration rate. Combining PGRs is expected to overcome this problem as well as to improve the induction of embryogenic callus and its regeneration into sorghum plantlets.

The PGRs have an essential role in the physiological and genetic process for the growth and development of plants. Recently, 1–6 mg L^{-1} of 2,4-D was used for embryogenic callus induction in sorghum. The 2,4-D is an auxin that is often used for callus induction from explant

tissue in various plants (Hu *et al*. 2000; Ikeuchi *et al.* 2013). The callus induction was achieved by adding of 2,4-D at different concentration and combination with kinetin or supplements, such as proline, glutamine, or casein hydrolysate (Pola *et al.* 2009; Wu *et al*. 2014). The combination of proline (as the nitrogen source) and auxin improved sorghum callus induction and generated a large amount of callus (Assem *et al.* 2014). The concentration of proline at 2 mg L^{-1} increased the number of embryogenic callus for *Ferula jaeschkeana* (Sharma and Arun 2020) and Malaysian wild rice at 3 g L^{-1} (Paramasivam and Harikrishna 2020). However, increasing the induction yield by combining PGRs for sorghum has not been reported, specifically somatic embryogenesis of Indonesian Numbu cv.

Another problem for the micropropagation of sorghum is an ability of embryogenic callus to regenerate into whole plants due to browning caused by phenolic compound. Phenolic compound is produced as a result of oxidation stress that interfere enzyme activities, poison to the plant tissues and inhibit cellular growth (Feng *et al.* 2007). It also causes a low number or unsuccessful callus regeneration into shoot and root. Several cytokinins have been used to regenerate sorghum callus into plantlet, such as kinetin (Assem *et al*. 2014), BAP (Dreger *et al*. 2019), Zeatin (Chou *et al*. 2020), and TDZ (Karumba 2021). Furthermore, when the cytokinin concentration is higher than auxin, it triggers cell division as well as tissues or organ development in sorghum (Liu *et al*. 2015; Flinn *et al*. 2020). Previous study showed that supplementing an MS medium with 1 mg L^{-1} BAP and 1 g L^{-1} IAA can regenerate 70% callus of sorghum variety, namely Tx430 (Kanani and Sayadat 2020). Karumba (2021) also reported a high regeneration rate when 4 mg L^{-1} BAP was added to the medium. However, the toxic effect of browning or phenolic compound production on explants during regeneration cannot be avoided. The addition of L-proline to the culture medium reduces the production of the toxic pigment and also helps to obtain a more friable embryogenic callus. Proline acts as an antioxidant to retard the browning of explants caused by phenolic oxidation (Blistrubienė *et al.* 2020).

This study aims to improve propagation of sorghum Numbu cv. using the explant seed and meristematic leaf whorl. The 2,4-D was combined with proline to induce embryogenic callus. The combination of NAA and BAP was further used for regeneration, while proline was added as a phenolic retardant to accelerate the process. The result showed that the combination of PGRs is an important treatment, which helps induce embryogenic callus development and its regeneration into plantlets.

Materials and Methods

Plant material and sterilization

Sorghum Numbu cv seeds were provided by the Indonesian Cereals Research Institute (Balitserealia) and two types of explants were used for the callus induction, namely the seed and meristematic leaf whorl of a two-month old plant. The seeds were sterilized by washing twice with 90% alcohol for 10 min followed by immersion in commercial bleach containing 0.5% natrium hypochlorite for 30 min. The seeds then were rinsed three times with sterile distilled water and then imbibed by soaking in distilled water for 24 h. The leaf whorl was sterilized by spraying the outer layer with 70% alcohol followed by burning for 3 sec. The middle meristematic of leaf whrol was isolated using a scalpel and sliced from the apical meristem into a 3 mm thick disk.

Callus induction

Callus induction for the seed explant was initiated by culturing the imbibed sterile seed on a solid MS medium that was supplemented with 30 g L^{-1} sucrose and 0.5 mg L^{-1} kinetin. Combination of various concentrations of 2,4-D (2, 3, 4 and 5 mg L^{-1}) and proline (3000, 4000 and 5000 mg L^{-1}) were added thereafter into the medium. Meanwhile, the meristematic leaf whorl was cultured on an MS medium supplemented with 30 g L^{-1} sucrose, 500 mg L^{-1} casein hydrolysate and various concentrations of 2,4-D (2, 3, 4 and 5 mg L^{-1}). The medium's pH was then adjusted to 6.2 and 11 g L^{-1} agar was added as the gelling agent. The cultures were incubated in a dark condition for callus induction and proliferation at 24˚C ± 2˚C for two weeks. Subsequently, the induced callus was subcultured with the same medium and the percentage of callus formation and induction time were recorded.

Plantlet regeneration and phenolic control

The embryogenic callus regeneration was carried out using an MS solid medium containing 30 g L^{-1} sucrose, 100 mg L^{-1} glutamine, 500 mg L^{-1} casein hydrolysate and combination of PGRs. The NAA concentrations at 0.1 and 0.3 mg L^{-1} were combined with BAP at 2 and 3 mg L^{-1} and applied to induce callus regeneration into plantlet. The callus was cultured in the combination medium and placed in a dark room at 24±1ºC for 2 weeks. It was then transferred into a regeneration room where it was exposed to 16 h light at an intensity of 1500–1600 lux as well as 8 h in a dark condition. Proline concentrations at 150, 300, 450 and 560 mg L^{-1} were added to the combination as the phenolic retardation agent. The percentage of greenspot callus, planlet and browning callus were then recorded once a week.

Microscopy observation and histological analysis

The development of callus was monitored using LEICA EZ4 W stereomicroscope. The callus was placed on a sterile petri dish, sealed and then viewed under the microscope. The observation was performed throughout somatic embryogenesis stages at globular, scutellar and coleoptillar phases.

The histological analysis was carried out to evaluate the development stage of somatic embryogenesis. The embryogenic calli were fixed for 24 h using FAA solution containing formaldehyde, acetic acid, and 100 mg L^{-1} ethanol in a ratio of 17:1:2. The fixed calli were washed with aquades, dehydrated with ethanol, and then saturated with the toluene-paraffin mixture and pure paraffin. They were sliced into 7–8 μm sections using a microtome and then molded with paraffin. The sections were glued to the preparate glass slides using a toluene-paraffin solvent (Sass 1951). After removing paraffin, the preparate was stained with hematoxylin and eosin. Subsequently, they were observed under a light microscope (Olympus CX31 Japan) and then photographed.

Acclimatization

The healthy planlets completed with root and shoot were washed with sterile water and then soaked in fungicide (Dhytane) for 5 min. After drained, the planlets were adapted to grow in the bottles containing sterilized sand, which was watered with liquid nutrition (Growmore) and incubated in a growth chamber for 2-3 weeks. The adapted plants were then transferred to a polybag containing a mixture of sand and soil in a ratio of 1:1 and grown in green house. They were watered twice a week and fertilized once a month until harvesting.

Experimental design and statistical analysis

Complete randomized design (CRD) was applied to determine the importance of different culture media combinations. Each treatments had three replications. For each replication, around 10–11 seeds were plated on callus induction media and 7 clumps or embryoids were plated on regeneration media. All data were collected and presented as means ± standard error from three replicates. Statistical significance was then calculated using ANOVA and Tukey's test. A *p*-value of 0.05 ($p<0,05$) was considered for determining the significance.

Result

Induction of embryogenic callus by combining PGRs

Seeds and leaf whorl explants were used to examine the combined effects of 2,4-D and proline on embryogenic callus induction. The formation of embryogenic callus was observed after incubated for two weeks on the combination medium ranging from 10–68% (Table 1). The increase of both 2,4-D and proline led to an increase in the percentage of embryogenic callus. Combining 3 mg L^{-1} of 2,4-D and 5000 mg L^{-1} of proline (D2P3) had the highest percentage followed by 3 mg L^{-1} 2,4-D and 4000 mg L^{-1} proline (D2P2). Although increasing PGRs induced callus formation, the highest induction was observed after combining 3 mg L^{-1} 2,4-D with 5000 mg L^{-1} proline. The D2 level was assumed to be the optimal level for 2,4-D because a lower concentration decreased the callus formation, while higher levels did not accelerate the process. Increasing proline concentration induced embryogenic callus and P3 level showed the highest induction. Observation of the induction time showed that neither the increase of 2,4-D nor proline concentration resulted in a faster embryogenic callus development (Table 1). However, combining 3 mg L^{-1} 2,4-D with 3000 or 5000 mg L^{-1} proline significantly produced the quickest embryogenic formation with an induction time of approximately 3 weeks. These results indicate that the combination of 3 mg L^{-1} 2,4-D and 5000 mg L^{-1} proline is the effective concentration for producing a large number of embryogenic callus in sorghum.

Table 1: Effect of 2,4-D and proline concentrations on percentage of callus induction from seed explants. The percentage values are presented as means ± SD for three independent observations and the different lowercase denote significant differences (Tukey's test, $p \leq 0.05$)

2.4-D (mg L^{-1})	Proline (mg L^{-1})	Callus induction frequency (%)	Callus induction time (WAG)
2	3000 (P1)	10 ± 7d	8.3 ± 0.47a
	4000 (P2)	20 ± 7d	8.0 ± 0.80ab
	5000 (P3)	30 ± 7cd	3.3 ± 0.47cd
3	3000 (P1)	50 ± 7c	2.7 ± 0.47d
	4000 (P2)	60 ± 10.8ab	6.0 ± 0.80b
	5000 (P3)	68 ± 4a	3.0 ± 0.80d
4	3000 (P1)	45 ± 4cd	4.3 ± 0.47c
	4000 (P2)	60 ± 7ab	6.0 ± 0.80b
	5000 (P3)	60 ± 7bc	6.0 ± 0.80bc
5	3000 (P1)	45 ± 10.8cd	4.3 ± 1.20cd
	4000 (P2)	50 ± 7c	5.0 ± 0.80c
	5000 (P3)	63 ± 7ab	6.0 ± 0.47b

To compare embryogenic callus induction, the leaves whorl explant was incubated in a MS medium, which was supplied with the same PGR combination. The addition of proline produced a dark brown callus at the early stages of incubation (Supplement 1). Therefore, the leaves whorl explant was incubated in a medium that was only supplied with various concentrations of 2,4-D. Observation of the induction time showed that increasing 2,4 D concentration resulted in a longer development of embryogenic callus. As a consequence, the lowest 2,4-D with concentration of 2 mg L^{-1} produced the quickest incubation time of approximately 2–3 weeks (Table 2). Furthermore, the percentage of embryogenic callus formed was not affected by 2,4-D concentrations in the leaf whorl explant. Based on the induction time and percentage of the callus induced, lowest concentration of 2 mg L^{-1} might the effective level for callus induction using the leaf whorl explant.

Morphological of embryogenic callus

To observe the development of embryogenic callus, the morphological character of sorghum during incubation on the medium was viewed using stereomicroscope. The features of embryogenic callus were observed as dry friable, yellowish and a globular structure after two weeks on the appropriate

Table 2: Effect of 2,4-D concentration on percentage and callus induction time from leaf whrol explants. The values are presented as means ± SD for three independent observations and the different lowercase denote significant differences (Tukey's test, $p \leq 0.05$).

2,4-D (mg L^{-1})	Callus induction frequency (%)	Callus induction time (WAP)
2 (D1)	37±4.7bc	2.3±0.47b
3 (D2)	47±4.7a	3.3±0.94b
4 (D3)	40±4.7ab	4.3±0.47ab
5 (D4)	33±0.0b	5.7±0.47a

Table 3: Effect of 2,4-D and proline concentration on characteristic of embryogenic callus generated from seed and leaf whorl explants

Explants	2,4-D (mg L^{-1})	Proline (mg L^{-1})	Callus color	Surface structure
Seed	2 (D1)	3000 (P1)	Browning	Wet, no friable
		4000 (P2)	Browning	Wet, no friable
		5000 (P3)	White	Dry, friable
	3 (D2)	3000 (P1)	White	Dry, friable
		4000 (P2)	White	Dry, friable
		5000 (P3)	Yellowish	Dry, friable
	4 (D3)	3000 (P1)	Yellowish	Dry, friable
		4000 (P2)	Yellowish	Dry, friable
		5000 (P3)	Yellowish	Dry, friable
	5 (D4)	3000 (P1)	Yellowish	Dry, friable
		4000 (P2)	White	Wet, no friable
		5000 (P3)	White	Wet, no friable
Leaf whrol	2 (D1)		White	Wet, no friable
	3 (D2)		Yellowish	Dry, friable
	4 (D3)		Yellowish	Dry, friable
	5 (D4)		White	Wet, no friable

medium (Table 3). In the seed explant, all combinations of 2,4-D with proline induced callus, as shown in Table 1. However, morphological observation showed that the combination of PGRs at the lowest concentration produced a brown, wet, and not friable callus. Yellow, dry and friable callus was induced on the medium with increasing 2,4-D concentration in the addition of proline. These results are consistent with the works of Ramulifho *et al*. (2019) that the embryogenic callus was formed on the medium with increasing 2,4-D concentration. However, combining the highest 2,4-D and proline concentrations produced a white, wet, and not friable callus, which indicates that it was not an embryogenic callus. Similar results were also observed when the callus was induced from the leaf whorl explant (Table 3). The concentration of 2,4-D at 3 and 4 mg L^{-1} produced a yellow, dry and friable embryogenic callus, but at the lower and higher concentration, a wet and not friable callus was obtained.

To observe embryogenic callus development, the multi-step process starting from pre-embryo mass (PEM), globular, scutellar and coleoptillar stages were viewed under a stereomicroscope and then photographed. The seed explants were incubated for 7 days in a combination medium that required germination before the embryogenic callus development started (Fig. 1a). Furthermore, the globular stage was developed from the germinated seed coleoptile after 30 days incubation in the combination medium (Fig. 1e). It continued to the scutellar and coleoptillar phases after 45- and 60-days incubation, respectively (Fig. 1f and g).

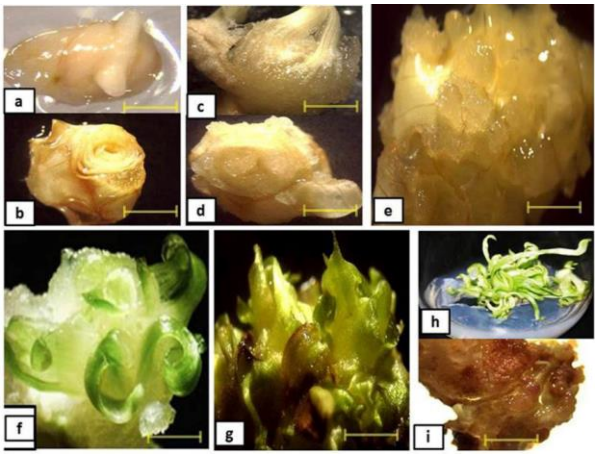

Fig. 1: Somatic embryogenesis (SE) phases of sorghum: (a, b) seeds and leaf whorl explants; (c, d) pre-embryo mass (PEM) from seeds and leaf whorl explants; (e) globular; (f) secutelar; (g) coleoptillar; (h, i) shoot regeneration from seed explant and shoot browning from leaf whorl explant. (⊢⊣= 1 mm)

Interestingly, the embryogenic callus produced from seed explants regenerated into a green whole plant (Fig. 1h).

The leaf whorl explants have similar the SE development stages, which consist of the PEM, globular, secutelar and colleoptillar phases. They showed swelling and developed into a pre-embryo mass (PEM) after 7- and 10-days incubation in the medium (Fig. 1b–d). Subsequently, PEM further developed into the globular stage on the 16th day (Fig. 1e). The next stage was the scutellar stage characterized by a heart shape structure, which was observed on the 41st day, and then followed by the choleoptillar stage, which produced a young greening shoot (Fig. 1f–g). The multi-step development of embryogenic callus from the explant leaf whorl was also observable in the four stages, but the explant was unable to regenerate into a whole plant due to browning (Fig. 1i).

The histological analysis confirmed the presence of a large parenchymal cell (PC) without a visible nucleus, which was surrounded by small meristematic cells during the initial stage of embryogenic callus development (Fig. 2a). Subsequently, the cell formed the globular callus that was characterized by a nodular shape (Fig. 2b) and then further developed into a hearth-shape or embryo structure due to protuberances of mitosis (Fig. 2c). Occurred embryo structure division led to the formation of cotyledon as well as initiation of vascular bundle, which in turn formed the shoot, young leaf, shoot apical meristem, bud primordial, and vascular tissue (Fig. 2d–e). This histological analysis supported SE callus development stage in sorghum.

Callus regeneration into plantlet

A preliminary study showed that callus was regenerated in an MS medium containing BAP and NAA. Regeneration was successfully performed from callus obtained from the seed

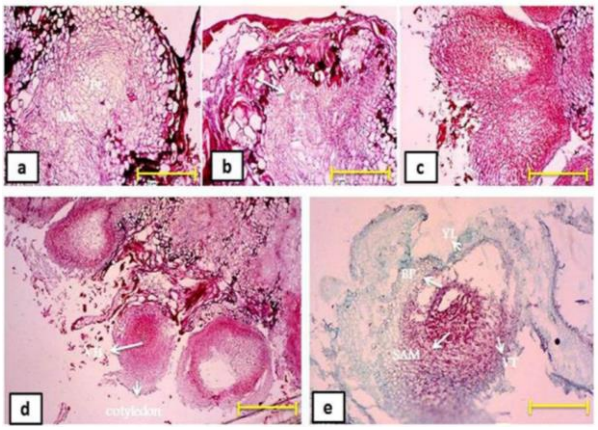

Fig. 2: Histological analysis of SE development phases in sorghum; (a)PEM; (b) globular; (c) scutellar with heart-like shape; (d) cotyledonary; (e) regenerated shoot. MC, meristematic cell; PC, parenchym cell; GC, globular cell; VB, vascular bundle; YL, young leaf; BP, bud primordial; SAM, shoot apical meristem; VT, vascular tissue (⊢——⊣200 µm)

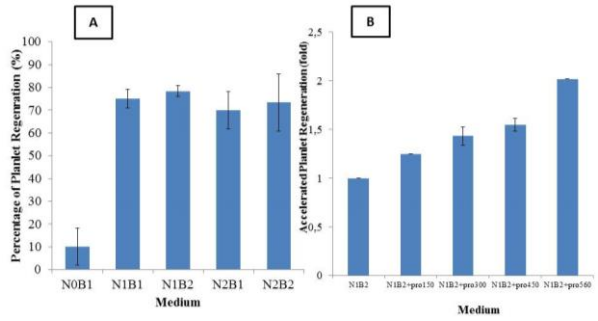

Fig. 3: Number of plantlets in the combining NAA and BAP medium (A) and acceleration of callus regeneration by proline (B). Number plantlet is calculated as percentage (%) of plantlets number per callus. Acceleration of callus regeneration are expressed as relative values of the number of regenerated plantlets in combining medium N1B2 without proline

explant, while leaf whorl did not regenerate into a plant, but only produced a green spot. To find the most appropriate combination of NAA and BAP concentrations for regeneration of seed callus, the PGRs concentration were varied. The combination of 0.1 mg L^{-1} NAA and 3 mg L^{-1} BAP (N1B2) had the highest green spot formation and regeneration percentage with 78% of the callus being converted into plantlet (Fig. 3a). The addition of proline to the regeneration medium containing N1B2 combination improved the regeneration rate of seed explant (Fig. 3b). The addition of proline reduced the browning and increased plantlet regeneration. Callus obtained from sorghum can have a characteristic brown color due to the presence of phenolic compound. However, proline can significantly reduce phenol levels and it also has antioxidant activity in stevia-plant callus (Blistrubienė *et al.* 2020). Adding approximately 560 mg L^{-1} proline into the N1B2 medium doubled the plantlet regeneration rate compared to the medium without the amino acid.

Acclimatization

A number of regenerated plantlets were successfully acclimatized to *ex vitro* conditions (Supplement 2). The plantlets regenerated from embryogenic callus were acclimated in a growth chamber for 21 days and then exposed to natural conditions in a greenhouse. The plant showed a remarkable growth performance during three months of observation. Morphologically, there were no differences between the growth, size, and leaf of the adapted *in vitro* plant and the plant obtained from the natural seed. The adapted plant had a height of approximately 2–3 meters and also produced normal seed that was harvested a month later.

Discussion

Sorghum Numbu cv is a superior hybrid sorghum in Indonesia and its tissue culture method needs to be improved because of prerequisites for genetic enhancement, transgenic production, and clonal propagation (Aregawi *et al*. 2021). It has been reported that cereals including sorghum are recalcitrant to *in vitro* propagation. This study used two types of explant, namely seed and leaf whorl to induce somatic embryogenesis callus and regeneration in sorghum. The results showed that the callus induced from the seed explant in a medium containing combination of 2,4-D and proline was friable, yellowish, and had a globular structure after two weeks (Table 1, 3). The embryogenic callus further developed into greening callus and finally into a plant after sub-cultured in a regeneration medium. In other hand, the addition of proline to the explant leaf whorl produced a dark brown callus, but was produced in an MS medium supplied with only 2,4-D. The results indicate that the seed explant quickly developed embryogenic callus, which has many advantages including its high availability and easy to prepare.

PGRs are important substances for callus induction, callus proliferation, and plant regeneration. The PGRs can also be combined with amino acids to increase the number of callus induction and regeneration. However, the concentration is very important for controlling the development of cereal somatic embryogenesis (Schnablová *et al*. 2006). The higher 2,4-D concentration increased the callus formation, and the concentration of 3 mg L^{-1} 2,4-D to be most effective concentration for the callus induction in both seed and leaf whorl explant. The addition of proline improved callus induction and the highest concentration at of 5000 mg L^{-1} proline produced 68% induced callus. The embryogenic callus produced in a medium containing 2,4-D and proline showed the characteristics crumble, yellowish, and not watery on callus surface (Table 3). Proline is an essential amino acid for supporting plant growth and acts as antioxidant to retard browning of explant caused by phenolic oxidation (Khokhar *et al*. 2021). Therefore, the combination of 2,4-D and proline is a determinant to produce a high

number of embryogenic callus in sorghum.

The efficiency of plant regeneration was improved by adding PGRs, such as NAA, BAP, and proline as the amino acid supplement. The combination of NAA and BAP had a significant effect on the callus regeneration. The optimal concentration was 0.1 mg L^{-1} NAA and 3 mg L^{-1} BAP, which was able to regenerate 78% callus into sorghum plantlet (Fig. 3a). The addition of 560 mg L^{-1} proline doubled the regeneration rate compared to the medium without proline (Fig. 3b). This result indicates that proline improve regeneration rate of callus, as reported in *Brassica napus* (Ahmadi and Mehran 2015) and in sugarcane (Kaur and Manish 2016). Amino acid proline has been postulated has a role to suppress enzymatic browning and improve plant regeneration.

Microscopic and histological observations confirmed that the embryogenic callus was proliferated using the combination of PGRs. Furthermore, combining 2,4-D with proline to proliferate and develop callus into the SE phases, namely globular, scutellar, colleoptillar, and shoot formation was observed (Fig. 1). The histological observation showed that there were significant changes in the cell structure, component, and shape at each phase (Fig. 2). The pre-embryo mass was characterized by large parenchymal cells and dense protoplasm, which then develop into a globular callus. The scutellar stage was characterized by cotyledon structure development as well as the formation of a heart-shaped structure by the embryo due to mitosis protuberances. Successful divisions of the embryo poles started the development of embryos and to be mature cotyledons. The cells differentiation also led to vascular bundles formation in the colleoptillar stage. All the SE development phases were confirmed by morphological and histological observation. The regenerated shoots that were completed with the root system were adapted to greenhouse condition and grew normally in the soil medium. These results indicate that the embryogenic callus induction and subsequent regeneration in sorghum of Numbu cv were successfully improved by combining PGRs with proline.

Conclusion

Embryogenic callus and plantlet regeneration were successfully developed using seed explant. The combination of 3 mg L^{-1} of 2,4-D and 5000 mg L^{-1} of proline produced a 68% embryogenic callus for 3 weeks. The combination of 0.1 mg L^{-1} NAA and 3 mg L^{-1} BAP regenerated the highest number of planlet, namely 78%, while the addition of 560 mg L^{-1} proline doubled the regeneration rate in sorghum.

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Author Contributions

WNN and BS planned the experiments, WNN, DPR and BS interpreted the results, DPR histological observation, WNN statistically analyzed the data and made illustration, WNN and BS writing original draft and editing, BS funding acquisition.

Conflict of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper

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