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Propagation In Vitro of Sorgum in MS, VW and NT mediums

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ABSTRACT

Sorgum is one kind of plant from the family of grass in the Tropical and Subtropical regions. *Sorgum bicolor* is a species commonly used for molase, syrup and seeds productions. Furthermore, sorgum is also used as a biofuel material. The purpose of this research is to investigate which type of medium is best for the growth and development of sorgum explants. This research were implemented by using the Complete Random Design with 3 mediums treatment: M1 = Murashige and Skoog; M2 = Vacin and Went; and M3 = Nagata and Takabe. Repititions were done 9 times and each were using 10 test samples. Changes observed were of: qualities, quantities, and contents of phenol in the Sorgum calluses.The results of this research were:quantity of callus in general had shown real differences and most callus found were on the MS medium treatment;quality of callus formed had shown real differences in each mediums and tended to be compact callus, while in MS medium to become friable;NT medium tended to produce most contents of phenol, which was 0.25% .

Keywords: In Vitro, Medium, Phenol and Sorgum

1. INTRODUCTION

Plants are the main sources of chemical compounds genereally used for pharmaceutical industries, food additives, and fragrances. Most of those compounds are the products of secondary metabolism processes extracted from the plants species (Arijanti, Retna, Ribkahwati, 2006).

One plant of the Grass family from the Tropical and Subtropical regions is commonly planted and cultivated by mankind for the production of molase, seeds, biofuel, and more, which is Sorgum. Sorgum is the source of nutrients, because it contains Niacin, Riboflavin and Thiamine, and minerals such as Magnesium, Iron, copper, Calcium, Phosphor, and Kalium (Annonymous, 2016).

Sorgum seed has functional food nutrients such as anti-oxydant (polyphend), Fe, fibers, oligosaccharides, and B-glucose which is a type of non-starch carbohydrates polysaccharides. High content of Fe mineral is really helpful for the production of erythrocytes. Some benefits of sorgum for human health are: cancer deterrent, diabetes control, bones health, and increasing the circulation and production of erythocytes, as well as energy sources (Yuwono, 2015).

The agrobusiness development of sorgum commodities has a good prospect, because in the year of 2012, the Department of State-Owned Companies (BUMN) has committed to develop sorgums planting in state-owned plantations to reduce the imports of wheat and assigned targets to the Institute of National Science of Indonesia (LIPI), National Board of Nuclear and the Research Center of Sugarcane Plantation of Indonesia (Annonymous, 2015).

Based on the above, then as an effort to fulfil the needs of sorgum seeds, one of the most promising way is by using the tissue-cultures (in-vitro) technique, which is expected to be able to

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produce more quantities of seeds, better and cheaper, in short period of time and also to investigate the contents of its secondary metabolites (Ali, 2015).

Results of this research were hoped to gain informations of the reproduction of sorgum seeds through tissue-cultures technique in 3 (three) types of mediums (Murashige-Skooq, Vacin-Went and Nagata-Takabe) and to know the best contents of secondary metabolites produced by treatment of those 3 mediums.

2. METHOD OF RESEARCH

Research was conducted in the laboratory of tissue-cultures in the Department of Agriculture – Wijaya Kusuma University of Surabaya, through June to December 2017 time period. Materials and equipments used were Explants of Shoot Sorgum, basic Mediums of MS, VW and NT (with compositions as on Table 1, 2 and 3), growth regulator substances of NAA and BAP. Coconut water, Glucose, Alcohol 70% and 96%, Chlorox, Betadine, and laboratorium equipments.

In this research, the method used is complete random design with one factor, of 3 levels : M1 (Murashige and Skoog); M2 (Vacin and Went); and M3 (Nakata-Takabe). Each treatment were repeated 9 times with 10 samples.

2.1. Implementation

2.1.1. Sterilisation of Equipments

The equiments used were wrapped in brown-paper, then sterilised in the oven in temperature of 121^{o}C for 30 minutes. While the cultures tubes were sterilised in Autoclave 17psi for 30 minutes.

2.1.2. Making of Mediums

Mediums used were prepared as the treatments and modified by the addition of ZPT.

2.1.3. Planting

The sorgum explants were sterilised with Chlorox 25% + 1 drop of Tween for 5 minutes, Chlorox 15% + 1 drop of Tween for 10 minutes, and Chlorox 5% + 1 drop of Tween for 20 minutes, then rinsed with sterilised water. After sterilised, then the explants were cut into approximately 1 cm^2. Then planted in the cultures tubes in the mediums as treatments.

2.1.4. Incubation

After the planting, the cultures tubes were put in the incubation racks and observed for the forming of calluses.

2.2. Variables

The observation variables were as follows :

2.2.1. Quality of Calluses

Visually observed once in 1-week interval by scoring of :

1 = no calluses

2 = compact calluses

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3 = friable calluses

2.2.2. Quantity of Calluses

Visually observed once in 1-week interval by scoring of :

2 = no calluses

3 = low quantity of calluses (<1 of explant size)

4 = medium quantity of calluses (1-2 of explant size)

5 = high quantity of calluses (>2 of explant size)

2.2.3. Contents of Polyphenol on Calluses

Observed after the calluses were 8-weeks old, destructively through analysis by Spectrophotometer.

2.3. Data Analysis

Results were analised by using One-way Anova through SPSS and, if there were real differences, further tested with LSD 5%.

3. RESULTS AND DISCUSSIONS

3.1. Quality of Calluses

Results of type analysis had shown real differences in between the treatments of the mediums on the qualities of sorgum leaves' calluses on week 5 to 10.

Table 1. Results of the Observation on Quality of Callus in Sorgum Leaves

Treatment	Average / Week after planting									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
M1	1.00	1.00	1.00	1.19	1.41a	1.60a	1.76a	1.93a	2.07a	2.27a
M2	1.00	1.00	1.00	1.13	1.21b	1.26b	1.42b	1.66b	1.70b	1.98b
M3	1.00	1.00	1.00	1.13	1.11c	1.16c	1.21c	1.54c	1.62c	1.79c
LSD 5%	**NS**	**NS**	**NS**	**NS**	**0**	**0**	**0.02**	**0.4**	**0**	**0.4**

Table 1. had shown the effect of MS medium in forming the quality of callus tended to be compact to become friable, while VW and NT medium formed the callus to be compact. This has matched with the research by Prasetyo (2006). The callus were brownish-yellow and formed nodules which were the embryonic callus that have the ability of regeneration. The embryonic callus were callus that had grown and developed to form the structures to become embryo. While the organogenic callus were callus that their morphogenesis growth needed a medium with different concentration of auxin and cytokines in forming shoots and roots.

In medium by Murashige and Skoog (MS), there were enough macro and micro nutrients, as well as vitamins for plants growth. Explants of a plant needs to be compatible with their medium to be able to grow callus (Hendrayono and Wijayanti, 1994).

3.2. Quantity of Callus

Results of type analysis had shown real differences in between the treatments of the mediums on the qualities of sorgum leaves' callus on week 4 to 10.

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Table 2. Results of the Observation on Quantity of Callus in Sorgum Leaves

Treatment	Average / Week after planting									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
M1	1.00	1.00	1.00	1.26a	1.58a	1.69a	1.89a	2.39a	2.58a	2.76a
M2	1.00	1.00	1.00	1.21b	1.44b	1.54b	1.70b	1.91b	1.18b	1.37b
M3	1.00	1.00	1.00	1.17c	1.27c	1.36c	1.47c	1.54c	1.68c	1.86c
LSD 5%	**NS**	**NS**	**NS**	**0**	**0**	**0.03**	**0**	**0.03**	**0.04**	**0.04**

Table 2. had shown the effect of MS medium in producing the most quantities of callus than the other mediums. This has matched with the opinions of Hendrayono and Wijayani (1994), that medium by Murashige and Skoog (MS) has enough macro and micro nutrients as well as vitamins for a plant growth. Explants of a plant needs to be compatible with their medium to be able to grow callus.

The success of reproduction of tissue-cultures were affected by many factors, such as nutrients and explants (Ali, 2015). Additional nutrients into plant's mediums were very affecting to the growth and development of explants (Yusnita, 2003).

3.3. Contents of *Phenol*

Results of type analysis had shown real differences in between the treatments of the mediums on the contents of *Phenol* in sorgum leaves' callus on week 10.

Table 3. Results of the Contents of *Phenol* in Callus of Sorgum Leaves

NO	TREATMENT					
	MS Medium	% Phenol	VW Medium	% Phenol	NT Medium	% Phenol
1	M1U1S1	0.160	M2U2S3	0.195	M3U3S2	0.248
2	M1U2S3	0.158	M2U1S9	0.190	M3U5S6	0.156
3	M1U1S9	0.147	M2U3S4	0.198	M3U6S1	0.172
4	M1U3S4	0.155	M2U5S6	0.192	M3U9S5	0.188
5	M1U5S6	0.157	M2U4S5	0.201	M3U2S6	0.213
6	M1U6S9	0.149	M2U6S3	0.205	M3U1S7	0.231
7	M1U7S2	0.159	M2U5S1	0.196	M3U4S1	0.225
8	M1U4S5	0.162	M2U6S3	0.210	M3U1S6	0.213
9	M1U3S7	0.220	M2U8S5	0.188	M3U5S2	0.209
10	M1U8S5	0.181	M2U5S1	0.190	M3U5S3	0.242
11	M1U4S1	0.179	M2U3S7	0.206	M3U2S6	0.235
12	M1U6S3	0.185	M2U6S9	0.205	M3U2S7	0.242
13	M1U2S4	0.190	M2U7S2	0.213	M3U6S2	0.215
14	M1U5S1	0.185	M2U2S4	0.199	M3U9S5	0.223
15	M1U1S3	0.192	M2U5S6	0.197	M3U8S5	0.208
16	M1U3S2	0.205	M2U1S7	0.209	M3U4S1	0.250
17	M1U5S6	0.212	M2U5S2	0.197	M3U2S4	0.248
18	M1U3S1	0.198	M2U6S9	0.209	M3U4S5	0.240
19	M1U9S5	0.189	M2U3S3	0.190	M3U6S9	0.209
20	M1U6S3	0.205	M2U6S3	0.205	M3U1S9	0.203
21	M1U4S2	0.195	M2U5S6	0.185	M3U4S6	0.205
22	M1U2S6	0.178	M2U2S6	0.192	M3U3S4	0.202
23	M1U6S2	0.202	M2U9S5	0.199	M3U1S3	0.228
24	M1U3S3	0.215	M2U5S3	0.201	M3U8S5	0.232
25	M1U1S7	0.209	M2U6S2	0.193	M3U7S2	0.215
26	M1U5S2	0.186	M2U4S1	0.195	M3U1S1	0.247
27	M1U4S1	0.201	M2U2S6	0.202	M3U1S9	0.225
28	M1U6S9	1.809	M2U6S2	0.191	M3U8S5	0.240
29	M1U1S6	1.946	M2U3S2	0.191	M3U3S4	0.241
30	M1U5S3	0.186	M2U5S6	0.210	M3U7S2	0.250

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Treatment	Phenolic (%)
M1	0.16
M1	0.22
M2	0.19
M2	0.21
M3	0.25
M3	0.23

The highest contents of *Phenol* in callus were found in the condition of NT medium. This was because the sorgum plant were very responsive to the NT medium. While the highest phenolic contents, which was 0.25%, was found in the NT medium. Then, it was assumed that as NT medium has more simplified nutrients than MS medium, so the explants had stressings and this produced the highest contents of secondary metabolites.

This was matched with the opinions of Rahmawati (2006), that suggested that slow growth was needed for the cells to process the maximum secondary metabolites and also a balance of carbon nutrients in the plants' cells metabolism, where if the nutrients availibility for the plants were in excess than they will be used by the cells to process the secondary metabolites (Pratiwi, Ali, Setiawan, Budiyanto, & Sucahyo, 2017). But each commodity requires a different time of exposure to produce the secondary metabolites.

4. CONCLUSIONS AND SUGGESTIONS

3.4. Conclusions

1. Quantity of callus in general had shown real differences and most callus found were on the MS medium treatment.
2. Quality of callus formed had shown real differences in each mediums and tended to be compact callus, while in MS medium to become friable.
3. NT tended to produce most contents of phenol, which was 0,25% .

3.5. Suggestions

Further research were needed to be conducted to investigate the contents of *Phenol* with the addition of carbohydrates in NT medium.

ATTACHMENTS

Table 1. Compositions of Medium by Murashige and Skoog

Materials	Need (mg/L)
1. Macro nutrients :	
KNO_3	1.900
NH_4NO_3	1.650
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
2. Micro nutrients :	
$MnSO_4.7H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
Kl	0.83
$CuSO_4.5H_2O$	0.025
$NaMoO_4.2H_2O$	0.25

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$CaCl_2.6H_2O$	0.025
$FeSO_4.7H_2O$	27.8
$NaEDTA,2H_2O$	37.3
3. Macro nutrients :	
Myo-inositol	100
Thamin HCl	0.1
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Glycine	2
4. Energy source :	
Sucrose	30.000

Source: Arijanti et al. (2014)

Table 2. Compositions of Medium by Vacin and Went

Materials	Need (mg/L)
1. Macro nutrients :	
NH_4NO_3	200 mg/L
KNO_3	525 mg/L
KH_2PO_4	250 mg/L
$MgSO_4.7H_2O$	250 mg/L
$(NH_4)_2SO_4$	500 mg/L
2. Micro nutrients :	
Fe(EDTA)	37 mg/L
$FeSO_4.7H_2O$	28 mg/L
$MnSO_4.7H_2O$	7,5 mg/L
3. Energy source :	
Sucrose	20g/L

Source: Arijanti et al. (2014)

Table 3. Compositions of Medium by Nagata and Takabe

Materials	Need (mg/L)
1. Macro nutrients :	
KNO_3	950
NH_4NO_3	825
$CaCl_2.2H_2O$	220
$MgSO_4.7H_2O$	1.233
KH_2PO_4	680
2. Micro nutrients :	
$MnSO_4.7H_2O$	22.3
$ZnSO_4.7H_2O$	8.3
H_3BO_3	6.2
Kl	0.83
$CuSO_4.5H_2O$	0.025
$CoSO_4.7H_2O$	0.030
$FeSO_4.7H_2O$	27.8
$Na_2EDTA,2H_2O$	37.3
3. Organic substances :	
Myo-inositol	100
Thamin HCl	1
Sucrose	10.000
D Mannitol	12.700

Source: Arijanti et al. (2014)

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