Tolerance Plantlets Falcataria Moluccana On Aluminum In In-Vitro

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Abstract: This study aimed to test the tolerance of callus cells of the Al and get Falcataria moluccana Al tolerant plantlets in vitro. The study used a completely randomized design (CRD) 2 factors. To determine the effect of treatment, the use of variance . To determine the level of treatment that significantly by Tukey's test used SPSS v.22.0. The results showed that callus cells F. moluccana tolerant of Al to the level of 180 ppm, while media treatment with 2,4-D 1 ppm favor callus cell tolerance to Al. F. moluccana tolerant Al plantlets until the Al content of 180 ppm which has a high average of 3.15±0.52 cm and 9.02±0.60 cm, number of roots, 2.40±0.70 to 9.10 and root length averaging 1.33 ± 8.23 cm . Tolerance plantlets F. moluccana against Al indicated by the PAR of 81.68 % on the Al content of 135 ppm , while at 180 ppm Al is somewhat tolerant .

Key words: Plantlet, Falcataria moluccana, tolerance, aluminium.

1. INTRODUCTION

Crop tolerance to aluminum (Al) is an important factor for adaptation to acid soils. Barriers to growth on acid soils is the result of the low solubility of essential nutrients, causing deficiency (Takita et al ., 1999). Criteria tolerant crops Al is able to reduce Al so that root growth is not disturbed (Marschner, 1991), preventing a decrease in the absorption of nutrients, accumulate Al a little more so the toxicity of Al is relatively small and induce pH rhizosfir higher near the pH optimum for plant growth (Degenhard et al., 1998). The influence of Al toxicity mainly limit the depth and root branching, so it will inhibit the absorption of other nutrients to the plant. The roots are injured typically seen to be heavy and fragile. Shoots root and lateral roots become thick and turn brown. The root system as a whole looks clustered , with many lateral roots are fattening but without branch/root hairs, so it is not effective in the absorption of nutrients (Foy, 1988). Plants that Al toxicity is inhibited the development of roots that disrupts the growth of the top of the plant. Disruption of the top plant growth caused by deficiency of nutrients such as P and imbalance of hormones (Hairiah, 1992, Hanafiah, 2005 and Yunita , 2009). The aluminum ion can bind phosphorus in surface roots and reduce root respiration, cell division and retrieval and utilization of Ca, Mg, P, K and H_2O (Fitter and Hay, 1991). Due to the lack of phosphorus would cause the plant roots become very less and undeveloped. Al toxicity inhibits root growth and primer extension and prevents the formation of lateral roots and root hairs (Rao, 1995). F. moluccana including the types of plants that are developed nationally to meet the needs of timber. F. moluccana timber is a timber versatile (Soerianegara and Lemmens, 1993). Thus, the necessary provision of seeds in large quantities, growth is uniform and has the properties as desired. Seedlings were required for post-mining land coal is resistant to soils with high Al content. Techniques of in vitro culture via callus culture is one method to get a plant that is expected to obtain plants tolerant to high Al content. This method refers to heritable changes that accumulate in meristematic callus from explants and expressed in offspring in the regeneration of in vitro derived from callus. During this time F. moluccana research that has been done has not been directed at the assembly plants that are tolerant to high Al solubility. Therefore, to help the supply of seeds in an effort to land reclamation of coal mines with high Al content, need to be examined F. moluccana tolerance to Al in vitro. Benefits of in vitro culture of one of them can lead to significant changes in accordance with the desired properties. Young cotyledons selected as explants for the network is a network that is still young (juvenile), the cells are still actively dividing. Every part of the plant can be used as an explant, but cells that have undergone differentiation is more difficult grown-up compared with cells that still meristematis (Gunawan, 1995).

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Totipotensi condition of planting material from one plant to another plant that is very different, even differences may also occur at a similar plant. Differences in the manner, timing and decision-season planting material is also an impact on the success of in vitro culture (Santoso and Nursandi, 2004). Breeding plants through in vitro cultures helpful in stimulating the genetic diversity and maintain genetic stability. Wattimena et al. (1992) stated, genetic diversity in tissue culture can be achieved through non-differentiated phase (phase callus and cell-free) were relatively longer. To obtain the genetic stability of the tissue culture techniques, can be done in the shortest possible way to induce the growth phase is not differentiated. According Skirvin et al. (1993) somaclonal variation in tissue culture occur due to the use of growth regulators and the level of concentration, long callus growth phase, the type of culture used (cell protoplasm, callus tissue), as well as whether or not the media used in vitro selection. Growth regulator auxin group of 2,4-D and 2,4,5-T usually can lead to somaclonal variation. Mattjik (2005) states, in the in vitro propagation, what happens is somatic mutation. When mutated cell divides to form a set of different cells with the cell of origin. Plants derived from the mutated cells will form a plant that may be a new clone that is different from the parent.

2. MATERIAL AND METHODES

Materials research is F. moluccana seed from the region Sumedang, West Java, Murashige and Skoog (MS) medium, 6-Benzyl aminopurine (BAP) and thidiazuron (TDZ), 2,4-dichlorophenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA); aluminum sulphate ($Al_2(SO_4)_3$); sterile distilled water, alcohol 70 % and 95 %, Na-hypochlorite, NaOH 0.1N and 0.1N HCl, tisue paper,

transparent plastic 0.3 mm, abel paper, rubber bands . Tools used an analytical balance, beakerglass, erlenmeyer, scalpel, measure pipette, a pipette, magnetic stirrers, spatulas, petridish glass, bottle culture, Bunsen lamp, autoclave, laminar air flow cabinet (LAFC), hot plate stirrer, pH meter .

Procedure:

2.1. Sterilization seed

a. The seeds were washed with soap water, rinsed with water, then the seeds were soaked in a solution of fungicide active ingredient mankozeb 80% 1g/l for 1 hour, then rinsed with running water. Next soaked in hot water ± 80° C for 24 hours until cool.

b .LAFC seeds are soaked in the solution of Na-hypochlorite 30%, 20% and 10% respectively 5 minutes later rinsed sterile distilled water three times. Then drained seeds and seeds ready for planting.

2.2. Seed Germination

Sterile seeds germinated in vitro on medium ½ MS . After germination, the age of 10 days was taken cotyledons as explants .

2.3. Induction of Callus

Cotyledon explants grown on MS medium added with BAP and 2,4-D concentrations correspond combined treatment (BAP 0; 0.5; 1.0; 1.5; 2 ppm and 2,4-D 0; 0.5; 1,0; 1,5; 2 ppm). Then placed in incubation chamber at a temperature of 25±1^{o}C, with a 40 watt fluorescent lamp irradiation within ± 20 cm above the culture bottles, until it forms a callus for 12 weeks.

2.4. Tolerance Test against Al Callus Cells

(embryogenic callus induction)

Subculture of callus mass media MS + 2,4-D (a concentration of 0.5; 1.0; 1.5; 2 ppm) + $Al_2(SO4)_3$ (concentration of 0; 45; 90; 135; 180; and 225 ppm), to form the embryogenic callus for 12 weeks.

2.5. Induction of shoot

Embryogenic callus mass media subculture MS + TDZ (concentrations of 0.2, 0.4 and 0.6 ppm) + Al_2 $(SO_4)_3$ (concentration of 0; 45; 90; 135 and 180 ppm), for 12 weeks.

2.6. Elongation/High and Rooting

Bud height 0.5 cm further in the subculture into new media for the process of elongation and rooting for 12 weeks and to form a tolerant plantlets Al. Media treatment used is the medium MS + NAA (concentration of 0, 0.05, 0.1 and 0.15 ppm) + $Al_2(SO_4)_3$ (concentration of 0; 45; 90; 135 and 180 ppm), for 12 weeks.

3. RESULTS AND DISCUSSION

3.1. Percentage of Callus

The average percentage of callus on a 12 week high of 98 % in the treatment of 0.5 ppm BAP + 2,4-D 1.0 ppm, while on treatment without growth regulator (control) and with BAP without 2,4-D explants of cotyledons all do not form a callus. The treatment combination of BAP and 2,4-D can cotyledon explants formed callus with different percentages (Table 1).

Table 1. Percentage of Callus (%) on Media MS + BAP + 2,4-D at 12 Weeks

BAP (ppm)	Concentration 2,4-D (ppm)					Mean
	0	0,5	1,0	1,5	2,0	
0	0,0±0,0 f	34,00±9,66 e	42,00±14,76 de	52,00±10,33 cd	62,00±6,32 c	38,00± 23,30 d
0,5	0,0±0,0 f	94,00±9,66 a b	98,00±6,32 a	96,00±8,43 a b	92,00±10,33 ab	76,0± 39,18 a
1,0	0,0±0,0 f	80,00±9,43 b	94,00±9,66 a b	92,00±10,33 ab	92,00±10,33 ab	71,6± 37,49 ab
1,5	0,0±0,0 f	76,00±8,43 b c	84,00±12,65 ab	86,00±13,50 ab	90,00±10,54 ab	67,20± 35,63 b
2,0	0,0±0,0 f	70,00±10,54 bc	74,00±9,66 b c	76,00±8,43 b c	88,00±10,33 ab	61,60± 32,79 c
Mean	0,0±0,0 d	70,80±22,21 c	78,40±22,80 b	80,40±18,73 ab	84,80±14,88 a	

Description : number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 (BAP = 2,4D = 5.41, BAP X 2,4D = 16.14) .

MS medium supplemented with 2,4-D with a concentration equal to or higher than the concentration of BAP can be induced to form callus tissue, whereas without the use of BAP and without 2,4-D, or with BAP alone is not capable of stimulating the formation of callus. The combination of auxin concentration is higher than the cytokines will produce callus (Hartman and Kester, 1986). According Wattimena (1988), auxin and cytokinin sufficient and balanced required in vitro because of auxin 2,4-D can increase the activity in promoting cell division. Continuous cell division without being followed by the enlargement and elongation of cells will lead to the formation of callus. Provision of low concentrations of cytokines in MS medium containing auxin can help callus. Auxin affect initiation of DNA replication, while cytokinins useful for controlling the start of mitosis and cytokinesis (Gaspar et al., 1996). Thus, the level of auxin and cytokinin in culture must be balanced and controlled carefully.

3.2. The percentage of embryogenic callus

Callus cell tolerance to Al in treatment $Al_2(SO_4)_3$ and 2,4-D is not all callus cells tolerant to Al concentration. Callus cells that are tolerant to Al can form embryogenic callus that is, until the concentration of $Al_2(SO_4)_3$ 180 ppm, while intolerant brown callus and embryogenic callus formed, namely the treatment of $Al_2(SO_4)_3$ 225 ppm (Table 2) .

Table 2. Percentage of embryogenic callus (%) on the treatment concentration of $Al_2(SO_4)_3$ and 2,4-D at 12 Weeks

$Al_2(SO_4)_3$ (ppm)	Consentration 2,4-D (ppm)				Mean
	0,5	1,0	1,5	2,0	
0	56,00±8,94^{ab}	76,00±8,94^{a}	64,00±8,94^{ab}	48,00±10,95^{b}	61,00±13,73^{a}
45	36,00±8,94^{bc}	64,00±8,94^{a}	52,00±10,95^{ab}	36,00±8,94^{bc}	47,00±14,90^{b}
90	28,00±10,95^{bc}	56,00±8,94^{ab}	44,00±8,94^{b}	32,00±10,94^{bc}	40,00±14,51^{b}
135	24,00±8,94^{bc}	40,00±0,00^{b}	32,00±10,95^{bc}	20,00±14,14^{c}	29,00±12,10^{c}
180	12,00±10,95^{c}	24,00±8,94^{bc}	24,00±8,94^{bc}	16,00±8,94^{c}	19,00±12,10^{d}
225	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{e}
Mean	26,00±19,76^{c}	43,33±26,82^{a}	36,00±22,53^{b}	25,33±18,89^{c}	

Description : number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 ($Al_2(SO_4)_3$ = 8.58 ; 2,4-D = 6.31, $Al_2(SO_4)_3$ X 2,4-D = 21.86).

The mean percentage of embryogenic callus formation as the highest, 76.0±8.94% on media treatment without $Al_2(SO_4)_3$ + 2,4-D 1.0 ppm. The higher the concentration of $Al_2(SO_4)_3$ given declining percentage of embryogenic callus formed. It is the possibility of Al goes into callus cells is toxic, so that only the callus cells tolerant to Al capable of forming embryogenic callus. According to Finer (1988) will be formed from the embryo cell division in the embryo cell surface for long. With the cell division there will be an increase in mass of cells resulting in the growth of embryogenic callus. The results showed that the development of callus obtained from induction process has a mixed response to the treatment medium. In the process of callus proliferation, ie globular callus callus callus surface forming shiny spheres, or callus non globular callus surface that is flat or finely toothed (Figure 1).

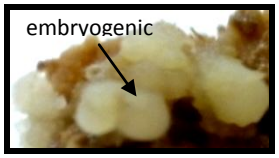

Figure 1. Age 12 Weeks Embryogenic Callus on MS + Al 90 ppm 2,4-D 1 ppm .

Callus granulated texture and translucent white color is a period of embryogenic cells. Embryogenic occurred on callus culture medium might be due to nutritional needs can be met with the use of MS medium with 2,4-D concentration of 0.5-2 ppm . The experimental results Sulistiani (1997) shows the incidence rate of embryogenic callus highest in nearly mature embryos are at a concentration of 2,4-D 1-3 mg/l kinetin and 0.5 mg/l reaches 80-100%, while the percentage of mature embryo the highest concentrations of 2,4-D 2-3 mg/l without kinetin reached 60%. According to Sujatha and Prabakaran (2001) from the group of growth regulators auxin 2,4- D is important for callus induction .

3.3. The number of shoots

The results showed that the treatment of $Al_2(SO_4)_3$180 ppm with Thidiazuron (TDZ) 0.4 ppm average number of buds formed shoots as much as 2.10 ± 0.57, while in treatment $Al_2(SO_4)_3$ 0 ppm TDZ 0.4 ppm shoots as much as 5.60±0.84 (Table 3 and Figure 2) .

Table 3. Mean Number of Shoots on MS Medium + $Al_2(SO_4)_3$ + TDZ at 12 Weeks

Consentration Al_2SO_4	Consentration TDZ			Mean
	0,2 ppm	0,4 ppm	0,6 ppm	
0 ppm	4,50±0,85	5,60±0,84	5,10±0,57	5,07±0,87^{a}
45 ppm	3,30±0,95	5,00±0,47	4,20±0,63	4,17±0,99^{b}
90 ppm	2,20±0,79	4,20±0,42	3,20±0,79	3,20±1,06^{c}
135 ppm	1,60±0,70	3,10±0,57	2,40±0,52	2,37±0,85^{d}
180 ppm	1,10±0,32	2,10±0,57	1,70±0,48	1,63±0,61^{e}
Mean	2,54±1,43^{b}	4,00±1,40^{a}	3,32±1,36^{a}	

Description: number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 ($Al_2(SO_4)_3$ = 0.45, TDZ = 0.69).

Treatment $Al_2(SO_4)_3$ 45-180 ppm with TDZ 0.2-0.6 ppm embryogenic callus capable of forming buds averaged 1.1±0.32 to 3.3±0.95 shoots. Thus embryogenic callus cells tolerant to aluminum up at a concentration of 180 ppm. The combination is able to induce the cells to bypass the process of somatic embryogenesis as globular shape, torpedo and heart shape that eventually form the cotyledons and the shoot primordia. Organogenesis bud formation is influenced by two factors, namely limiting the induction of somatic embryos and plant regeneration. Both require the right conditions including media composition and growth regulators. Callus tissue composed by meristematis that can be directed to the formation of organs, if supported by nutrients, combinations of plant growth regulators and environmental factors as appropriate. TDZ given different concentrations affect the number of shoots produced. TDZ 0.4 ppm given increasing the number of shoots produced. George and Sherrington (1984) stated that the administration of cytokines to the culture medium in higher concentrations can spur growth and reduce apical axillary buds on the main shoot of plants into pieces two cultures. Jiang et al. (2005) suggested that the effects of TDZ is very important for the process of morphogenesis in vitro or somatic embryogenesis because of its potential as bioregulan. Park et al. (2002) and Jiang et al. (2005), suggests that in general TDZ which is believed to be more active phenylurea derivatives stimulate bud formation than somatic embryogenesis.

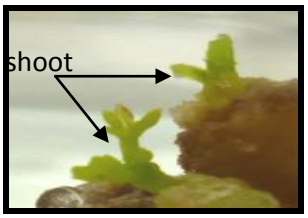

Figure 2. Shoots in Media with $Al_2(SO_4)_3$180 ppm + 0.4 ppm TDZ

3.4. Elongation (High plantlets)

The results showed that the treatment of $Al_2(SO_4)_3$ and NAA produce different heights, which ranged from 3.15±0.52 to 9.02±0.60 cm. High plantlets were highest on the media's treatment with that without $Al_2(SO_4)_3$ and NAA 0.10 ppm, which is 9.02±0.60 cm. In the treatment of $Al_2(SO_4)_3$180 ppm to 0.15 ppm NAA + high yielding 5.99±0.48 cm (Table 4).

Table 4. High plantlets (cm) on media MS + $Al_2(SO_4)_3$ + NAA at 12 Weeks

Consentration $Al_2(SO_4)_3$	Consentration NAA				Mean
	0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
0 ppm	4,13±0,53^{e}	6,10±0,45^{c}	9,02±0,60^{a}	8,06±0,88^{b}	6,83±2,00^{a}
45 ppm	3,99±0,42^{e}	5,68±0,18^{d}	8,53±0,57^{ab}	7,83±0,78^{b}	6,51±1,89^{b}
90 ppm	3,64±0,39^{e}	5,13±0,25^{d}	7,82±0,29^{b}	7,56±0,73^{b}	6,04±1,81^{c}
135 ppm	3,40±0,35^{e}	4,26±0,27^{e}	6,25±0,42^{c}	6,91±0,58^{c}	5,21±1,50^{d}
180 ppm	3,15±0,52^{e}	3,94±0,21^{e}	5,59±0,53^{d}	5,99±0,48^{d}	4,67±1,26^{e}
Mean	3,66±0,56^{c}	5,02±0,87^{b}	7,44±1,41^{a}	7,27±1,01^{a}	

Description: number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 ($Al_2(SO_4)_3$ = 0.31 ; NAA = 0.26 , $Al_2(SO_4)_3$ X NAA = 0,80) .

The addition of auxin NAA in the culture medium can increase the elongation of the cell, so as to increase the height of plantlets. Hormone auxin in the plant body produced by the tops of the stems, buds of branches and twigs were spread into all parts of the plant. According Pierik (1987) the function of which auxin to stimulate cell elongation. NAA is a synthetic auxin groups that are stable, because it is not easily broken down by enzymes released by cells or by heating the sterilization process.

2.5. The number of roots

Highest number of roots that are at the media's treatment without $Al_2(SO_4)_3$ + NAA 0.10 ppm, ie 9.1±0.74 strands, while the media's treatment with $Al_2(SO_4)_3$180 ppm + NAA 0.1 ppm ie 5,6±0.52 strands (Table 5).

Table 5. Number of Roots on Media MS + $Al_2(SO_4)_3$ + NAA at 12 Weeks

Consentration $Al_2(SO_4)_3$	Consentration NAA				Mean
	0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
0 ppm	3,90±0,32^{e}	6,10±0,57^{c}	9,10±0,74^{a}	6,75±2,11^{b}	6,75±2,11^{a}
45 ppm	3,60±0,52^{e}	5,70±0,48^{cd}	8,60±0,84^{ab}	7,40±1,17^{b}	6,33±2,06^{a}
90 ppm	3,40±0,52^{e}	5,00±0,47^{cd}	7,50±0,53^{b}	6,60±0,97^{bc}	5,63±1,70^{b}
135 ppm	2,60±0,70^{ef}	4,20±0,42^{de}	6,40±0,52^{bc}	6,00±0,82^{cd}	4,80±1,65^{c}
180 ppm	2,40±0,70^{ef}	3,60±0,52^{e}	5,60±0,52^{cd}	5,30±0,95^{d}	4,22±1,48^{d}
Mean	3,18±1,05^{d}	4,92±1,28^{c}	7,44±1,46^{a}	6,64±1,35^{b}	

Description: number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 ($Al_2(SO_4)_3$ = 0.43; NAA = 0.36, $Al_2(SO_4)_3$ + NAA = 1, 12).

In the treatment of $Al_2(SO_4)_3$180 ppm without NAA is able to take root even though few in number at an average of 2.40±0.70. It is alleged in the body of the plant are endogenous hormones auxin to stimulate root growth. At

0.10 ppm NAA treatment with $Al_2(SO_4)_3$ concentration of 45-180 ppm produces an average number of roots the most compared to NAA concentration of 0.05 ppm and 0.15 ppm, which ranged from 5.60±0.52 to 8,6±0.84 strands. The higher the concentration of Al is given declining number of roots. The possibility of this Al concentration of 180 ppm resulted in inhibition of cell division, so that the number of roots formed decreased. This means that is treated with $Al_2(SO_4)_3$ tolerant to aluminum at concentrations up to 180 ppm are capable of forming roots. Thus in $Al_2(SO_4)_3$180 ppm to increase the number of roots with 0.1 ppm NAA administration can support the number of roots formed. According Wattimena et al. (1992), auxin NAA is a plant growth regulator that is often used in in vitro culture, especially for root growth. Pierik (1987), suggests that in general auxin increases cell elongation, cell division, and the formation of adventitious roots. Yusnita (2003) suggested that auxin type are often used in the formation of roots in the bud is the NAA, due to the high effectiveness.

2.6. Long of roots

The results showed that the combination treatment of $Al_2(SO_4)_3$ and NAA resulted in a different roots, which ranged from 1.33±0.22 to 7.81±0.23 cm (Table 6) .

Table 6. The Mean Length of Roots (cm) on Media MS + $Al_2(SO_4)_3$ + NAA at 12 Weeks

Consentration $Al_2(SO_4)_3$	Consentration NAA				Mean
	0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
0 ppm	1,71±0,22^{j}	2,61±0,12^{h}	8,23±0,21^{a}	6,46±0,66^{de}	4,75±2,74^{a}
45 ppm	1,59±0,15^{j}	2,45±0,12^{hi}	7,81±0,23^{ab}	6,09±0,74^{e}	4,48±2,62^{b}
90 ppm	1,37±0,13^{j}	2,24±0,13^{hi}	7,42±0,29^{bc}	5,75±0,72^{ef}	4,19±2,54^{c}
135 ppm	1,40±0,21^{j}	1,91±0,31^{ij}	7,06±0,22^{cd}	5,16±0,70^{fg}	3,88±2,40^{d}
180 ppm	1,33±0,22^{j}	1,49±0,28^{j}	6,53±0,25^{de}	4,67±0,64^{g}	3,44±2,32^{e}
Rataan	1,06±0,08^{d}	2,14±0,45^{c}	7,41±0,64^{a}	5,63±0,93^{b}	

Description: number followed by the same letter in the same column and row are not significantly different at Tukey test α 0.05 ($Al_2(SO_4)_3$ = 0.23; NAA = 0.21, $Al_2(SO_4)_3$ X NAA = 0.62).

In the combination treatment of $Al_2(SO_4)_3$ 45 ppm + NAA 0.1 ppm longest root length is 7.81 ± 0.23 cm, while in treatment $Al_2(SO_4)_3$ 180 ppm + NAA 0.10 ppm ie 6.53±0.25 cm. This shows that the higher the concentration of Al is given to the media, it can decrease the length of the roots, because $Al_2(SO_4)_3$ at high concentrations can be toxic to plants. Ikeda and Tadano (1993) suggests that inhibition of the extension of the roots due to high Al can cause thickening of the cell walls and the accumulation of bubbles (vacuolization) small around the Golgi apparatus. Delhaize and Ryan (1995) megemukakan, Al pectin molecules bind to the cell wall or cell wall components which negatively charged cells of the root epidermis and cortex. Free carboxyl groups of pectin molecules that bind negatively charged ions Al causes the cell wall stiffening to be inhibited root elongation. According Kataoka et al. (1997) and Sasaki et al. (1997) high concentrations of Al besides causing damage and loss of viability roots, due to Al fastened at the end of the root epidermis, followed by cell death. Clune and Copeland (1999) suggests the influence of high Al causes the formation of covering layers of the epidermis at the root end of the plant. In the combination treatment of $Al_2(SO_4)_3$ 45 ppm + NAA 0.1 ppm longest root length is 7.81 ± 0.23 cm, while in treatment $Al_2(SO_4)_3$180 ppm NAA + 0.10ppm ie 6.53±0.25 cm. This shows that the higher the concentration of Al is given to the media, it can decrease the length of the roots, because $Al_2(SO_4)_3$ at high concentrations can be toxic to plants. Ikeda and Tadano (1993) suggests that inhibition of the extension of the roots due to high Al can cause thickening of the cell walls and the accumulation of bubbles (vacuolization) small around the Golgi apparatus. Delhaize and Ryan (1995) megemukakan, Al pectin molecules bind to the cell wall or cell wall components which negatively charged cells of the root epidermis and cortex. Free carboxyl groups of pectin molecules that bind negatively charged ions Al causes the cell wall stiffening to be inhibited root elongation. According Kataoka et al. (1997) and Sasaki et al. (1997) high concentrations of Al besides causing damage and loss of viability roots, due to Al fastened at the end of the root epidermis, followed by cell death. Clune and Copeland (1999) suggests the influence of high Al causes the formation of covering layers of the epidermis at the root end of the plant. F. moluccana tolerance to Al in vitro based on the scores of the criteria belong to the very tolerant to moderately tolerant to the concentration of Al 45 ppm to 180 ppm. Based on Table 6, in the treatment of Al 45 ppm has value PAR 94.31% means very tolerant, in the treatment of Al 90 ppm and 135 ppm has value PAR 88.21% and 81.68% means tolerant, whereas the Al 180 ppm treatment had PAR value of 72.42% means rather tolerant. Tolerance shown by F. moluccana is highly influenced by the pH. At pH>5.5 are generally the dominant $Al_2(SO_4)_3$ + and is not poison the trees roots. However, at high concentrations (180 ppm) Al $(OH)^{2+}$ can also affect tolerance in plants. Marschner (1995) suggested that at pH <5.0 very high solubility of Al contained in the form of Al^{3+} and Al $(OH)_2^{+}$ is highly toxic to plants, in addition, there are also other forms, namely Al(OH) $^{2+}$ at pH> 5.5 influence ugly of Al^{3+} can be ignored. Kochian et al. (2004) states that if the planting medium pH below 5, then Al3 + will dissolve in solution and cause poisoning planting medium for rooting.

4. CONCLUSION

1. F. moluccana callus cells tolerant to Al up at a concentration of 180 ppm , while media treatment with 2,4 - D 1 ppm favor callus cell tolerance to Al .
2. F. moluccana plantlet Al tolerant to the concentration of 135 ppm Al . This is indicated by the PAR value of 81.68

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