COMBINED EFFECT OF NaCl AND IBA ON MS MEDIA ON GROWTH OF RICE MUTANT STRAIN IN VITRO

PRIYANTI[1], AZRI KUSUMA DEWI[2], EFRILIA NURJANAH[1]

[1, 1] UIN Syarif Hidayatullah Jakarta, [2] BATAN
Alamat: priyanti_uin@yahoo.com

ABSTRACT

The Influence of NaCl and IBA at the MS media for the growth of rice mutant in vitro was conducted in the tissue culture laboratory and green house plant's breeding group (PATIR BATAN). The aims of this research was to know the concentrate variation of NaCl, IBA, and MS media combination for growing percentage, height and root length in planlet Diah Suci variety and three rice mutant lines. The experiment was arranged in Completely Randomized Design, with three factors, fourty eight treatments and five replications. The first factor was four concentration of NaCl (0%, 0.5%, 1% and 1.5%). The second factor was three concentration of IBA (0%, 2.5% and 5%). The third factor was Diah Suci variety as plant control and three rice mutant lines (Obs 1700, Obs 1701, and Obs 1704. The result of four weeks observation was show that the added of NaCl up to 1.5%, so the growth percentage, height and root length of plantlet would be to descend. The concentration of IBA until 5% influenced to the growth percentage, height and root length of planlet. The combination of three parameter refered to Obs 1704 better than control and the others (Obs 1700 and Obs 1701) with the growth percentage was 80%, planlet height 19.84 cm and root length 5.62 cm in combination of NaCl 1% and IBA 5% on four weeks observation.

Key words: *in vitro, mutant lines, rice, NaCl and IBA*

BACKGROUND

Rice is the staple food of Indonesian people. The need is also increasing every year but the production tends to decline. One factor is the cause is a narrowing of fertile agricultural land and the transfer function of the land to non-agricultural activities such as industry, housing, roads, and others. The farmers find alternative to large areas of land used as rice cultivation, the vast land around the beach area. Problems that arise are not all rice varieties resistant to soil with high salinity.

Salinity is either an accumulation of dissolved salts in the soil and become one of the problems often faced in agriculture in the lowlands. Salinity stress on food crops can cause plants can not grow. One of the efforts undertaken to overcome salinity stress is through tissue culture. Through the selection of tissue culture can be improved salinity tolerance, drought, temperature, and disease in plant breeding programs (Nahlony et al., 1997).

Variety Holy Diah (DS) is the rice varieties released by Nasinal Atomic Energy Agency (BATAN) as a result of mutation breeding with gamma rays. Varieties of this Holy Diah as high yielding varieties have been released nationally by the Ministry of Agriculture in 2003. The advantages possessed by the Holy Diah varieties is the potential for dry grain production high gilingnya namely 9.4 tons / ha, the area distribution and planting a larger area compared with other neutron output of rice varieties, rice fluffier texture, can adapt in Rice fields in the plains lower to a height of 650 m above sea level atasa. Phenotypes of rice varieties Holy Diah still high enough to encourage pemulianya to restore these varieties without changing other properties by re-irradiating with gamma rays to obtain new and better varieties. The result of re-radiation of the Holy Diah rice varieties with gamma rays is a mutant strain of observation (Obs) 1700, Obs Obs 1701 and 1704 at this time have entered the stage of yield trial. Salinity test in vitro the mutant strains of rice varieties Holy Diah is expected to provide additional information about mutant strains which are tolerant to abiotic stresses such as salinity. Mutant lines are expected to be planted in areas with high salinity and can adapt well to other environmental stress factors (ICRP, 2003).

RESEARCH METHOD

Materials and Equipment

The main material used is rice varieties explants Holy Diah sebgai mutannya control and three lines of Obs 1700, Obs 17 001, and Obs 1704. Other materials are

bacto agar, sugar, aquades, MS culture media, NaCl, IBA, chlorox, Tween (for Saponification in sterilization of explants), spirits, and alcohol 96%.

The instrument used is an autoclave, laminar air flow cabinet (LAFC), hot plate and magnetic stirrer + spin bar, oven, microwave oven, analytical balance, pH meter digitas, fridge, bottle plant, aluminum foil, Erlenmeyer, rubber bracelets, glass Backer , tweezers, pipette volume, measuring flask, rack culture, the culture bottles, dissection tools, Petri dishes, Bunsen, plastic buckets, markers, paper labels, stroller, and equipment cleaning glass tools.

Procedure

1. **Sterilization Equipment**

   Sterilization equipment in laboratory tissue culture BATAN there are two kinds of concave and sterilization sterilization wet. Dry sterilization using the oven, the tools are sterilized dry dissection tools, petri dish, Erlenmeyer and culture bottles. Wet sterilization using an autoclave, the tools are sterilized moist culture media that have been made and contaminated culture bottles.

2. **Preparation of Culture Media**

   Media dasar yang digunakan adalah media MS untuk memudahkan pembuatan media maka Basic media used were MS media to facilitate media preparation is necessary first made stock solution of 1 to 6. 1-3 stock solution of 10 ml, 4-6 stock solution of 5 ml. NaCl concentration 0%, 0.5%, 1%, 1.5% and 0% IBA, 2.5%, 5% added to 1000 ml glass Backer then added white sugar by 20 g and diluted with aquades as much as 1000 ml and stirred with a magnetic stirrer + spin After the bar so homogeneous and homogeneous medium pH measurements were taken using a digital pH meter, pH of media 5.8. The next step is to enter into the media above 250 ml Erlenmeyer containing the bacto for as much as 2 g. Erlenmeyer then covered with aluminum foil and tied with rubber. The media was then sterilized using an autoclave for 15 minutes, 1 atm pressure, and temperature of 121o C. Media that has been sterilized and then stored in the culture room.

3. Induction Rice

Induction of rice conducted in LAFC. Each explant Holy Diah rice varieties as a control and three mutant lines of rice (Obs 1700, 1701, 1704 Obs) peeled gabahnya chlorox then sterilized in a solution of tween 40% and the solution is homogenized for 25 minutes. Rice seed is cut into two parts, namely endosperm and embryo using dissection tools. Part endosperm removed, bagia rice embryos grown in culture medium MS with variakombinasi NaCl and IBA, in each culture bottle containing three embryos from the Holy Diah varieties and three embryos from mutant strains Obs 1700, Obs 1701, Obs 1704. The culture bottles were incubated in culture room with temperature 26-28o C. Observations were made at one week after planting (MST) to four MST.

Observation Parameter

Observations made at the age of one to four MST MST based on morphologic characters. Observations were conducted one to two times each week. Observations were

a. Percentage of plantlets growing power

Plantlets are alive when plantlets grow, experiencing the inhibition or cessation of growth but not dead. Plantlets growing starts counting from the beginning to the end of observation (four MST).

b. High plantlets

High plantlets were measured at the age of two to four MST. Measurement-old plantlets of two to three MST by using a ruler attached to the walls of culture bottles because of plantlets is not removed from the culture bottles. The measurement starts from the boundary until the surface of the upper main stem of the plant. Measurement of plantlets at the age of four MST planet way out of the bottle culture, plantlets were washed first with flowing ait then laid on a ruler and measured height from the main trunk line to the end of the longest plant.

c. Root length

Root length measurement perfomed at the age of four MST. Plants that have been removed from the culture bottle and were washed with running water and then

spread its roots to the ruler, measurement of boundary main stem up to the longest root hairs.

Experimental Design

The experimental design using a completely randomized design (CRD) with three factors of four concentrations of NaCl (0%, 0.5%, 1%, 1.5%), three concentrations of IBA (0%, 2.5%, 5%) and four explants of one variety of rice Holy Diah (as control) and three mutant lines of rice (Obs 1700, 1701, 1704 Obs.) The combination of these three factors resulted in 48 treatments. The experiment was repeated five times.

Data Analysis

Data obtained statistically tested using ANOVA test. If significantly different Duncan's test at 5% test level.

RESULTS AND DISCUSSION

1. Percentage of plantlets growing power

Data percentage of power from plants grown plantlets of control and three mutant strains age of four MST shown in table 1.

Table 1. Percentage of Age Four Plantlets Growing Power MST

NaCl (%)	Tanaman Kontrol dan Galur Mutan											
	C_0 (DS)			C_1 (Obs.1700)			C_2 (Obs.1701)			C_3 (Obs.1704)		
	IBA (%)			IBA (%)			IBA (%)			IBA (%)		
	0	2,5	5	0	2,5	5	0	2,5	5	0	2,5	5
	%T	%T	%T	%T	%T	%T	%T	%T	%T	%T	%T	%T
0	80	70	80	100	100	80	100	70	90	90	80	80
0,5	80	70	80	100	100	90	100	90	100	90	100	80
1	80	90	90	100	70	100	80	70	80	90	70	80
1,5	10	0	30	30	30	20	20	10	20	0	10	0

At NaCl concentrations of 0% and 0% IBA, the mutant strains grew Obs Obs 1700 and 1701 reached 100%. The percentage of mutant strains Obs Obs 1700 and 1701 higher than the DS (control) and Obs 1704. At NaCl concentration of 1.5% and

0% IBA mutant lines Obs 1700 percentage growth of the power is still far better than the DS (control), Obs Obs 1701 and 1704.

At NaCl concentrations of 0% and 5% IBA, the percentage of power grows between the DS (control), Obs 1700, Obs 1701, and Obs 1704 showed no significant differences ranged between 80-90%. At NaCl concentration of 1% and 5% IBA, 1700 Obs mutant strain has an ability to grow better (100%) compared with DS (control) that is 90% and Obs Obs 1701 and 1704 (80%). This indicates that the mutant lines Obs hidaup 1700 has a better ability than the control plants and other mutant lines (Obs Obs 1701 and 1704).

2. High Planlets

At the age of two MST with NaCl concentrations of 0% and 5% IBA, high plantlet Obs mutant strains 1700 (11.98 cm) and Obs 1701 (11:48 cm) is much more baih compared with control plants and mutant lines Obs 1704. In granting IBA NaCl 1% and 5% only mutant strains that have a high 1701 Obs better plantlets of 5.3 cm compared with control plants and the two other mutant lines. At NaCl concentration of 1.5% and 5% higher IBA plantlets best seen in the mutant lines Obs 1701 (1.00 cm) while the control plant height, Obs Obs 1700 and 1704 is less than 0.6 cm (Table 2).

Table 2. Interaction NaCl, IBA, High Against Mutant Lines Plantlets (cm) Rice In vitro In the Age of Two MST

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_0 (DS)			C_1 (1700)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0%	14.12^{a}	13.15^{ab}	9.55^{abcdef}	11.61^{abc}	11.11^{abc}	11.97^{abc}
0,5%	10.44^{abcd}	9.59^{abcdef}	$9.07^{abcdefg}$	$9.26^{abcdefg}$	$8.88^{abcdefg}$	9.53^{abcdef}
1%	$5.67^{defghij}$	4.29^{fghijk}	4.31^{fghijk}	$5.00^{efghijk}$	4.20^{ghijk}	4.49^{fghijk}
1,5%	0.73^{jk}	0.30^{k}	0.24^{k}	0.50^{jk}	1.15^{jk}	0.57^{jk}

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_2 (1701)			C_3 (1704)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0%	11.94^{abc}	11.35^{abc}	11.48^{abc}	10.41^{abcd}	9.56^{abcdef}	10.26^{abcde}
0,5%	10.14^{abcde}	8.66^{bcdef}	$8.19^{bcdefgh}$	$9.11^{abcdefg}$	7.80^{cdefgh}	8.65^{bcdefg}
1%	$6.67^{cdefghi}$	3.98^{ghijk}	$5.30^{defghijk}$	4.62^{fghijk}	3.17^{hijk}	4.14^{ghijk}
1,5%	2.60^{ijk}	0.92^{jk}	1.00^{jk}	0.30^{k}	0.30^{k}	0.10^{k}

Description: Value averaging is followed by the same letter in same column indicates no significant difference ($\alpha = 5\%$)

Provision of NaCl concentration 0% and 5% in three IBA MST showed that plantlets of both plant height control and mutant lines are not much different, high plantlets ranged from 18.76-21.17 cm. At NaCl concentration of 1% and 5% higher IBA plantlets between control plants with mutant strains Obs 1701 is not too much different from the 16:06 and 16:15 cm. High Obs plantlets in 1700 (14.74 cm) and Obs in 1704 (10.71 cm) is much lower compared with control plants and mutant lines Obs 170. Granting of NaCl 1.5% and 5% IBA addition, there are still high plantlet. High plantlets in 1700 Obs mutant lines (1.70 cm) and Obs 1701 (1:22 cm) was still higher than the control plant height (0.60 cm) and mutant strains Obs 1704 (12:17 cm) (Table 3).

At the age of four MST, NaCl concentration of 0% and 5% IBA showed no significant difference between plant height control with a strain of mutant Obs 1700, Obs 1701, and 1704 Obs ranging from 27,25-28.64 cm. Giving NaCl 1% and 5% showed high IBA best plantlet shown by control plants (23:40 cm). High plantlets Obs mutant strains 1701 (21.92) is still far better than the other mutant strains of high (<20 cm). At NaCl concentration of 1.5% and 5% IBA, there is no height at the age of four plantlets of MST (Table 4).

Table 3. Interaction NaCl, IBA, High Against Mutant Lines Plantlets (cm) Rice In vitro In the Age of Three MST

\

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_0 (DS)			C_1 (1700)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0%	21.39^{a}	20.18^{ab}	18.76^{abcd}	19.18^{abcd}	20.55^{ab}	21.17^{a}
0,5%	17.90^{abcd}	20.18^{ab}	20.06^{ab}	19.06^{abcd}	17.64^{abcde}	20.20^{ab}
1%	13.44^{cdefg}	10.45^{fgh}	16.06^{abcdef}	14.21^{bcdefg}	10.99^{fgh}	$14.74^{abcdefg}$
1,5%	2.39^{i}	0.73^{i}	0.59^{i}	1.59^{i}	1.93^{i}	1.70^{i}

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_2 (1701)			C_3 (1704)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0,5%	18.48^{abcd}	20.27^{ab}	19.58^{abc}	18.50^{abcd}	18.96^{abcd}	18.80^{abcd}
1%	$14.98^{abcdefg}$	11.64^{efg}	16.15^{abcdef}	13.01^{defg}	9.01^{gh}	10.71^{fgh}
1,5%	5.15^{hi}	2.13^{i}	1.21^{i}	0.24^{i}	1.31^{i}	0.17^{i}

Description: Value averaging is followed by the same letter in same column indicates no significant difference ($\alpha = 5\%$)

Table 4. Interaction NaCl, IBA, High Against Mutant Lines Plantlets (cm) Rice In vitro At Age Four MST

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_0 (DS)			C_1 (1700)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0%	29.86^{abc}	26.68^{abcde}	27.64^{abcde}	23.63^{abcdefgh}	27.01^{abcde}	28.63^{abcd}
0,5%	27.91^{abcde}	30.07^{ab}	32.87^{a}	24.18^{abcdefgh}	25.17^{abcdefg}	27.11^{abcde}
1%	19.73^{defghi}	15.37^{hi}	23.40^{abcdefgh}	20.51^{bcdefghi}	16.29^{ghi}	19.66^{defghi}
1,5%	3.06^{k}	0.00^{k}	1.34^{k}	3.31^{k}	3.68^{k}	3.22^{k}

NaCl (%)	Tanaman Kontrol dan Galur Mutan					
	C_2 (1701)			C_3 (1704)		
	IBA (%)			IBA (%)		
	0%	2,5%	5%	0%	2,5%	5%
0%	24.60^{abcdefgh}	20.38^{cdefghi}	27.25^{abcde}	25.80^{abcdef}	27.84^{abcde}	28.52^{abcd}
0,5%	24.24^{abcdefgh}	25.95^{abcdef}	27.82^{abcde}	25.57^{abcdef}	28.17^{abcde}	28.60^{abcd}
1%	19.34^{defghi}	16.61^{fghi}	21.92^{bcdefgh}	18.80^{efghi}	12.00^{ij}	19.84^{defghi}
1,5%	6.05^{jk}	2.14^{k}	3.14^{k}	0.00^{k}	1.00^{k}	0.00^{k}

Description: Value averaging is followed by the same letter in same column indicates no significant difference ($\alpha = 5\%$)

According Suwarno (1985) that administration of NaCl at different concentrations can increase leaf damage, reduce the number of tillers, plant height, dry weight of canopy, roots and total plants. Damage to plant roots will cause difficulty in absorbing the nutrient elements of the media and damage to the leaves causing the leaves are not able to produce food through photosynthesis. The higher the salinity, the concentration of Na, Mg, Ca and Mn in growing media while the solubility of P will decrease. NaCl concentration of 5% can suppress the growth of plantlets of rice because of nutrient imbalance absorption elements, elements kekahatan P, disruption of protein synthesis in plants and poisoning Na and Cl (Dinata, 1985).

3.Root Length

At NaCl concentrations of IBA concentration 0% and 5% root length Obs 1700 mutant lines have a better appearance than control plants, mutant strains Obs Obs 1701 and 1704 is 4:50 cm. Provision of NaCl concentration of 1% and 5% IBA mutant lines Obs 1704 has a better root length compared with control plants and the two other mutant strains of 5.62 cm. At the age of four MST, 1701 Obs root mutant lines still have

a long accretion of about 0.70 cm compared with control plants and the two other mutant lines. At NaCl concentration of 1.5% and 5% IBA color and texture more grim roots of fragile roots and root hairs are few in number.

The results in line with the results of the research performed by Ishak (1994) on rice roots Pelita I / I are given 1% NaCl concentration in MS media has lowered the growth of roots and leaves as much as 30%, about 47% of control while the long growth Atomita rice root length-2 root growth decreased by approximately 9% and leaf drop about 38% of the control and rice roots Atomita-1 approximately 19% and 37% leaf. The use of 1.5% NaCl concentration causes a decrease in rice root length growth padab Pelita I / I almost 69% and 68% leaf, in rice Atomita length-2 root and leaf growth decreased by 74% and 70%, while in Atomita-1 root length growth and leaves decreased by 77% and 67%.

The high concentration of NaCl in the medium of plant growth will result in growth suppression and disruption of root length on the formation of new roots that will reduce the cruising range to reach the roots in nutrient elements. The amount of IBA concentration is 5%, also can damage the plants that were injured (Sriwidodo, 1985 in Dinata, 1985).

ZPT is not too high given its concentration on root cuttings to encourage the growth of roots and shoots (Abidin, 1980). Plants already have a hormone in the body itself but because jumahnya little needs to be done so that the addition of synthetic hormone, plant growth is expected to be faster than before (Wudianto, 1989 in Harsanti and Mugiono, 2001).

CONCLUSION

• The High Concentration of NaCl (1.5%), the percentage of power grows, plantlet height and root length decreased.

• IBA concentration of 5% effect on the growth, plantlet height and root length.

• After 4 MST 1704 Obs mutant lines better than the control and the two other mutant lines grew 80% the percentage of power, plantlet height 19.84 cm, 5.62 cm long roots in NaCl concentration of 1% and 5% IBA

- The combination of IBA NaCl 1% and 5% the percentage of power grew, height and root length of plantlets was still good.

REFERENCES

Abidin, Z. 1980. Knowledge Base about the growth regulator substances. Angkasa. Bandung

BATAN. 2003. Superior Variety Rice Technology The combination of radiation and Marriage Cross Movement. Applications of Radiation in the field of Agriculture. BATAN. Jakarta.

Dinata, K.K. 1985. Effect of Salinity on Growth and Production of Rice Varieties Atomita II and IR 32. Thesis. IPB Bogor.

Ishak. 1994. Analysis of Amino Acid Content of Mutant Rice (Oryza sativa L.) CV Atomita Atomita-1 and-2 and Its Relation to the Tolerance to Salinity. *Zuriat* Vol.5 No.2:56-64.

Nahlohy, Karmana, Darsa, dan Widiyanto. 1997. Variations Somaklon Rice Callus induction and subculture results in Media with and without NaCl. *Zuriat 8 (2)*: 64-67

Suwarno. 1985. Dissertation Summary: Inheritance and Physiological Properties Against Salinity tolerance in Rice. IPB. Bogor