Research Article

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Growth and regeneration of rice (*Oryza sativa* L.) callus in salt medium.

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Effect of NaCl on the growth of rice callus depends on the concentration of NaCl and variety. Lethal concentration is one factor that shows the level of sensitivity of cells (callus) of the poison effect of NaCl. High concentration of NaCl can decrease growth of the callus; low concentrations stimulate the cells to form tolerance mechanisms in order to survive. Effect of NaCl measured based on the value of LC (lethal concentration). This study aims to determine the LC_{50} values of NaCl, studied its effect on the growth of rice callus and regeneration of the plantlets. The plant material used were mature embryo of rice Ciherang, Inpari 13, Inpara, Pokkali and IR 29. The treatment is concentrations of NaCl (0, 50, 100, 150 and 200 mM)]. Each treatment consisted of 10 replicates with 5 embryogenic calluses per bottle. The results showed the NaCl LC_{50} value Ciherang is 85, 78 , Inpari 13 is 90.91 and Inpara 3 is 91,77, IR29 70, 67. The increasing of NaCl concentration makes the percentage of callus browning increased, inhibited callus growth and decreased ability callus regenerate.

Key words: ***In vitro*** **selection, salt tolerant, NaCl LC_{50} value.**

Rice is a very important food crop and almost half of the world's population consumes rice (Khush and Virk, 2000). Based on the economic value of the global crops, rice has the highest value compared to other important food crops (maize, wheat, potato, cassava, and wheat), meanwhile based on the number of production, rice ranks second after maize (FAOSTAT, 2009). In Indonesia, rice commodities ranks first of the seven major food commodities in terms of both production and economic value (Statistics Indonesia, 2009). Rice demand continues to grow with increasing population. The Use of marginal lands such as saline soil is one way to increase rice production.

Salinity is abiotic stressor which affect the metabolic activity in plants. Ion deficiency in water and salt toxicity effects are some of the biotic stresses that reduce crop productivity. In salinity condition, rice production can decrease hingher than 50%. Salinity causes productions of crops reduced and plant demised. (Tan and Lang, 2003; Mahajan and Tutejan, 2005; Roychoudury, 2008; Nishimura *et al.* 2011; Rany *et al.* 2012).

Salinity can decrease plant growth through osmotic and toxic effects, osmotic effect can reduces the ability of plants to take of water and toxic effects can couses premature aging and reduces the photosynthetic leaf area of plants to a level that cannot sustain growth (Munns, 2002; Munns and Tester, 2008; Nemati *et al.* 2011; Horie *et al.* 2012). During long-term exposure to salinity, plants experience stress ions, which can lead to premature senescence of adult leaves (Shereen *et al*. 2005).

In vitro culture is a method that can be used to study the response of plants to salinity conditions and for the isolation and selection of plants tolerant (Vijayan *et al*. 2003). In vitro selection of salt callus and regeneration rice has been reported (Hwe *et al.* 2011; Zinnah *et al*. 2013) This methodology can be an alternative way for conventional breeding to genetic improvement, because it can screen germplasm enabling a precocious and quick. *In vitro* selection has use NaCl a approach to select salt-tolerant cells. The study of screening in vitro to obtain NaCl tolerant strains can use to explain the physiological aspects of tolerance. Types of explants (callus or cell suspension cultures), the concentrations of salt and the selection strategy a applied variation methode. NaCl effect to the growth of rice callus depends on the concentration of NaCl and variety.

The selective agent (salt) has been previously applied in many cases to the induction of callus or the initiation of the suspension culture. In some cases the selection system includes several explants of which callus, embryos or plantlets were induced to form directly in saline. (Sharry and Silva, 2006). The sensitivity of each variety callus to salt varies, callus growth on salt condition depend on the genotype and concentration of NaCl (Gandonou *et al.* 2005; Htwe *et al.* 2011)

Unfortunately to date information about the effect of NaCl on the growth and regeneration of rice callus variety of Ciherang, Inpari 13. Inpara 3, was not yet published. Therefore, the objectives of this study are to determine the NaCl LC_{50} values of rice callus, to study NaCl effect on the growth of rice callus and to study regeneration of plantlets.

MATERIALS AND METHODS

This project was conducted at Indonesian Center for Agricultural Biotechnology and Genetic Resources during January - December 2013. This experiment used five rice varieties Ciherang, Inpari 13, Inpara 3, Pokkali and IR29 were obtained from the Indonesian Center for Rice Research.

Effect of salt on callus browning and value of lethal concentration (LC_{50})

Callus inducted from mature embryo. Seeds were sterilized with 70% alcohol for 5 minutes followed by 15% of common bleach for 5 minutes then finally rinsed several times with the sterile distilled water before inoculation into the callus induction medium. The nutrient medium chosen for the study was Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 24-D (3 mg/l). The pH of the medium is 5,5 – 5,8 and 30 grams of sucrose (3%) was added per liter of medium as carbohydrate source and mixed well. Then 8 grams of agar (0.8%) was added per liter.

Primary callus induced from mature embryos were cultured in medium containing NaCl that consists of multiple level concentrations of 0, 50 mM, 100 mM, 150 mM, and 200 mM, each treatment consisted of 5 replicates, each replicate consisting 10 explants. Variables measured were the number of browning callus and callus growth remains a yellowish white color and weight change callus. Data were analyzed to determine the lethal concentration. Determination of lethal concentration 50 (LC_{50}), using the regression equation to find the equation of the best. The length of incubation callus in selection medium containing NaCl is 4 weeks.

Effect of Salt on Callus Regeneration

Selected callus was transferred to regeneration medium to see the ability to regenerate after selected callus. The treatments tested were 5 dose level of NaCl (0, 50 mM, 100 mM, 150 mM, and 200 mM). Each treatment consisted of 5 replicates; each replicate consisted of 10 explants. The regeneration was done with MS + BA 5 mg/l + Thidiazuron 0,5 mg/l. The observed variables were the percentage of the callus forming shoots and the number of shoots per callus.

RESULTS AND DISCUSSION

Effect of salt on callus browning and value of lethal concentration 50 (LC_{50})

The addition of salt to nutrient medium in all varieties can increase the percentage of callus browning. Increasing the concentration of salt given the increased percentage of callus browning Table1. It is clear from table 1 that increasing concentrations of NaCl also increased the percentage of callus browning. For callus rice of Ciherang , Inpari 13 and Inpara, Increasing 50 mM NaCl on nutrient medium 50 mM NaCl resulted above 40% callus browning. In Pokkali (resistant control) only 12% while the IR29 (sensitive control) 78%. Increasing concentrations of NaCl up to 150 mM make callus browning higher than 70%. In general the effect of NaCl on callus browning the rice varieties Ciherang, Inpari 13 and Inpara 3 were not different.

Table 1. Presentation of callus browning in MS medium with different concentration of (NaCl)

NaCl Concentration (mM)	varieties				
	Ciherang (%)	Inpari 13 (%)	Inpara 3 (%)	Pokalli (%)	IR 29 (%)
0	0	0	0	0	0
50	48	42	46	12	78
100	74	68	64	58	84
150	78	72	70	62	98
200	100	100	100	100	100

It showed the NaCl had an inhibitory effect on the growth of callus. With increasing NaCl concentrations indicate that the inability of plant cells and tissues to adjust with incremental increases of salt over sufficient time periods might be due to osmotic or ionic shock (Shanthi *et al*, 2010).

The results of program analysis best-fit curve analysis to determine the lethal concentration 50 (LC_{50}), the best obtained model equations are based on the number of browning callus can show in Table 2. Based on LC_{50} NaCl value can be seen each level of sensitivity to salt varieties.

Table 2. Lethal concentration 50 (LC_{50}) value

Variety	LC_{50} Value
Ciherang	85,79
Inpari 13	90,91
Inpara 3	91,77
Pokkali	103, 37
IR 29	70,67

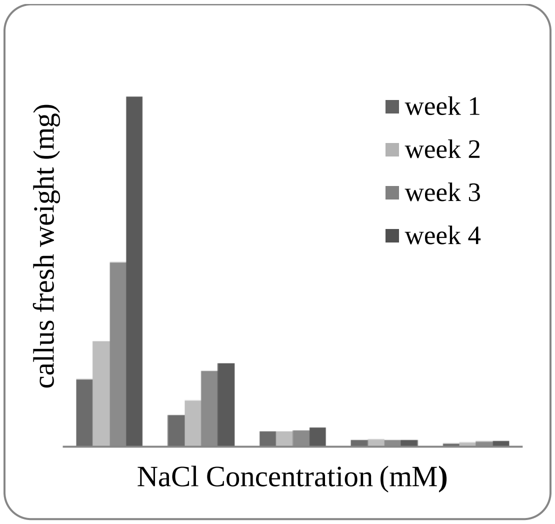
In Table 2 show each variety has a different sensitivity to NaCl. Pokkali varieties had the highest LC_{50} value followed by Ciherang (85,79), Inpari 13 (90,91), Inpara 3 (91,77) and IR29 (70.67). This shows pokkali varieties more tolerant to salt and IR 29 is more vulnerable. Therefore these varieties are used as a control tolerant and control sensitive. These results are relevant to research Shanthi *et al.* (2010) where callus from pokalli varieties can grow better in medium that had the salt of the varieties IR29.

Effect of Salt on weight of callus

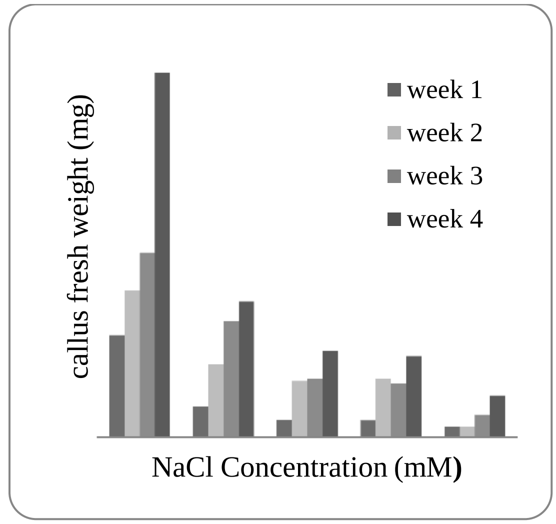
Increase of NaCl level concentration on callus medium influenced on callus fresh weight changes on a weekly basis. In figure 1 it can be observed that increasing the concentration of NaCl will decrease callus weight changes. This suggests that the addition of NaCl in the medium lowers the addition of fresh weight of callus.

Figure 1 shows that the variety Inpari 13, Inpara 3 and Pokkali giving as much as 50 mM NaCl resulted in a decrease in callus fresh weight changes. Increasing concentrations of NaCl into a 100-200 mM callus fresh weight for all three varieties are not too different. It relevant with Roan and Ratil (2012), However 200 mM NaCl tolerant callus obtained either by direct or indirect selection process did not sustained a regular growth on salt supplemented medium, these callus suffered with time a decrease in growth and all callus tissues died when the culture period was prolonged beyond one month A similar response was reported in salt tolerant potato (Queiros *et al.* 2007) and in *Chrysanthemum* sp. (Hossain *et al.* 2007) Reduction in the growth of callus is a common phenomenon in cultured cells grown on medium nutrient with NaCl (Venkataiah *et al*. 2004, Roa and Patil, 2012) and it has been interpreted that a certain amount of the total energy available for tissue metabolism is channeled to resist the stress.

In Ciherang and IR 29, adding 100 mM NaCl caused a very high weight change compared to the addition of 50 mM NaCl. There was a decrease change of callus on the higher NaCl concentration, due to the callus that contained in that medium experienced salinity stresses; such as imbalance of water and nutrient absorption, distrupted metabolism due to impaired inhibition of ion imbalance and the osmotic effects. All those factors cousce the callus require more energy for its metabolism and the growth decline.



A



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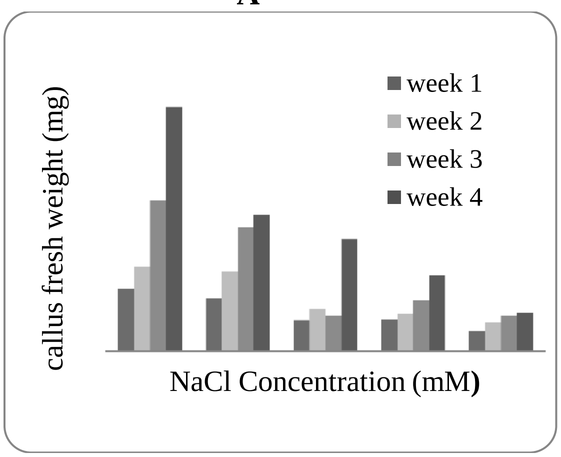

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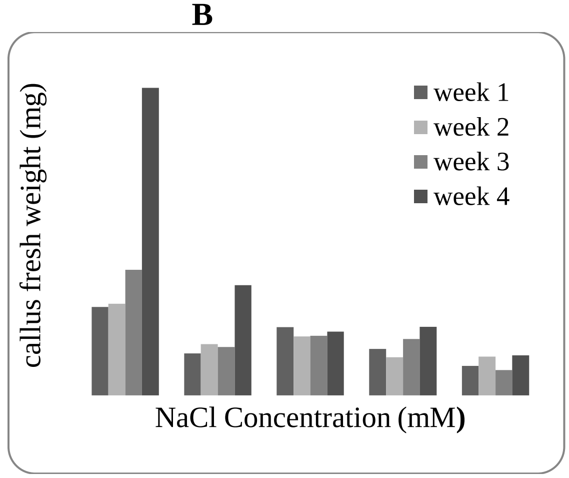

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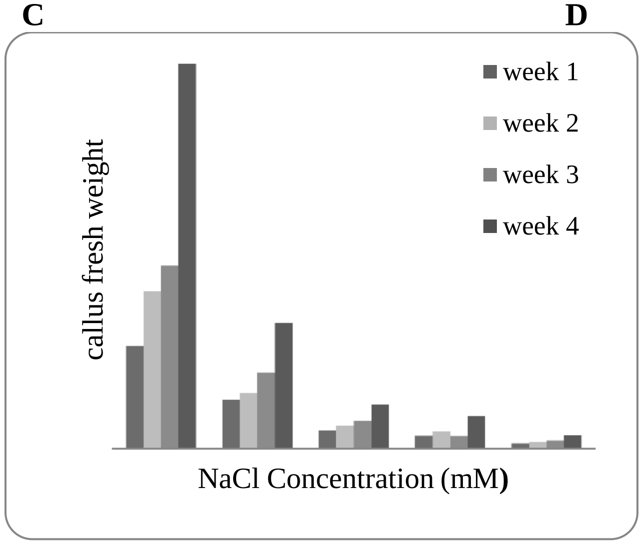

(E)

Figure 1. Changes in fresh weight of callus on Ciherang (A), Inpari 13 (B), Inpara 3 (C), Pokkali (D) and IR 29 (E) due to NaCl treatment

Table 3. Callus regeneration on MS medium supplemented with different concentration of NaCl

NaCl (mM)	percent callus forming shoots (PCFS)					number of shoots per callus (NSPC)				
	Ciherang	Inpari 13	Inpara 3	Pokkali	IR 29	Ciherang	Inpari 13	Inpara 3	Pokkali	IR 29
0	76.59	76.59	85.36	85.71	70	4.13	4.14	3.02	2.97	2.77
50	50	58	54	65.9	27	1.63	1.8	1.67	2.41	1.39
100	10	0	0	14,29	0	1	0	0	1	0
150	0	0	0	0.05	0	0	0	0	1	0
200	0	0	0	0	0	0	0	0	0	0

Babu *et al.(* 2007), states that the cells are exposed to salinity stress (NaCl) will spend more energy on metabolism compared to cell without salinity stress conditions (NaCl), therefor the energy generated are mostly used for osmotic adjustment and couse decreased in cell mass and impact on average reduction in cell mass at the higher NaCl concentration.

Effect of Salt on Callus Regeneration

The experiment of regeneration was performed to check the inherent capacity of callus which induced salt stress to regenerate on medium. There was increased NaCl concentration in medium decreased percent plant regeneration in all rice variety and normal plant regeneration in the no-stress medium (Table 3).

All the variety was performed better regeneration in control regeneration medium nutrient but it decreased with increased salt concentration percent callus forming shoots and number of shoots per callus was higher in Pokkali in all the three NaCl stress condition followed by Inpari 13, Inpara 3 and Ciherang whereas the variety IR 29 showed lowest. At 50 mM NaCl concentration Pokkali showed 85,71 of PCFS and 2,41 NSPC and the IR 29 showed the lowest PCFS 27 and NSPC 1.39. In the 100 mM NaCl concentration Pokkali showed PCFS 14 and NSPC 19. The Ciherang , Inpari 13 and Inpara 3 was not recorded PCFS and NSPC. It is relevant with Aditya and Backer, (2006) reported that the possibility for differentiation of salt susceptible lines was strongly inhibited in the presence of NaCl in the regeneration medium.

Conclusions

Based on the above data it can be concluded that the LC_{50} values f NaCl of rice callus for Ciherang varieties is 85.79, Inpari 13 is 90.91, Inpara 3 is 91.77 and , IR29 is 70.67. Increasing the concentration NaCl on rice callus medium resulted in increased the percentage of callus browning, decreased in rice callus fresh weight and decreased ability to regenerate.

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