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# Screening six varieties of rice (*Oryzasativa*) for salinity tolerance

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**Abstract**

Salinity is a constraint to the sustainable agricultural production. Increasing salinity is found to inhibit growth, height, and total of leaf area ofrice from vegetative to generative stages. Electrophysiologically, increasing salinity and exposure duration reduce the PD of the plant leaves. The screening focuses on the response of six varieties of rice (*Oryza sativa*) grown in a glasshouse subjected to five different salinity levels [0mM; 50mM; 100mM; 150mM and 200mM of NaCl]. Death occurred at 200mM of NaCl though all six varieties were tolerant to 100 mM of NaCl during 4 weeks of application, and four varieties of rice were found to be sensitive to salinity around 150mM of NaCl during 3 weeks of application. In turn, this study will help increase the security of the major food demand consumed by around 90% of Indonesian and protect the livelihood sustainability of around 67% Indonesian farmers, and help reduce starvation risk of a community due to salinity effects.



*Keywords:*screening rice; salinity; electrophysiology; sustainable agricultural

## 1. Introduction

**Nomenclature**

| | |
|---|---|
| USA | United States of America |
| IPCC | Intergovernmental Panel on Climate Change |

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| | |
|---|---|
| PD | Potential Differences |
| NaCl | Sodium Chloride |
| LA | Leaf Area |
| s.e | Standard error |
| B.Asin | Banyu Asin |
| IPRA-3 | Inpara-3 |
| Anova | Analysis of Variance |

Upcoming climate change and its saline effects on growth and development of plant and agricultural productivity have become a major environmental issue[1,2,3] in the USA, Australia, and Asia including Indonesia. There are no exact data on how much salinity has affected agricultral lost, but the numbers could be billions[2]. The effect could be greater on developing countries such as Indonesia especially upon its rice production since around 50 % of irrigated paddy fields in the north coastal regions of Java are becoming saline due to the sea levels increasing [4].

Rice is one of Asia's largest food demands, consumed, and grown in around 90% of Asia. The stock is still inadequate to fullfil people's demand[5]. Salinity will be a constraint to sustainable rice production, therefore combating salinity is urgently needed to reduce the risk. Developing saline adaptive plants and better understanding in its mechanism[6,2,], especially rice, is urgently needed sincemere soil remediation management will be difficult in Indonesia. Unfortunately, the tolerant mechanism to salt stressis not fully understood. Problems in breeding techniques also show that it has lower reliability and time consuming, and it is costly; some criterias used in screening usually do not correlate to salinity[7].

The research aims to investigate the possibility of how rice growth may correlate tosalinity using the electrophysiology technique[8] to fill the gap, reducethe time, and save the money to support breeding programs and farmers. Developing saline adaptive plants by integrating several strategies on the screening plant including electro physiological measurement in Biophysics are needed to advance sustainable development and adaptive capacities. Increasing adaptive capacity e.g improving rice varieties and their wild relativecan build resilience[9]. It means that both resilience and adaptation could also be used as a means in reducing the stress and the change of the climate impact due to salinity. However, there is a possibility that adaptation and resilience may differ in response due to different plant species and rice varieties.This screening varieties of rice to its tolerance of salinity will assist farming communities to overcome the negative effects of salinity as fast as possible to enhance there silience and adaptation to climate change to sustain rice production; and avoidance of huge lost on agricultural. In turn, it will help increase the major food demand security consumed by around 90% of Indonesian and protect the livelihood sustainability of around 67% farmers of Indonesian, and help reduce starvation risk of communities due to salinity effects.

## 2. Methods

Homogeneus seeds from six varieties of rice (Mekongga, Banyuasin, Madura, Ciherang, Inpara-3 and Inpari 13) were selected and sowed in sterilized sand, germinated, and grown in the lab. The 21 days old healthy and similarly grown seeds were selected and moved into two plastics pots with different size containing soil medium, and were grown in the glasshouse between various concentration of 0mM of NaCl (control), 50 mM of NaCl, 100 mM of NaCl, 150 mM of NaCl and 200 mM of NaCl.

The growth response was then observed by measuring the total of leaf area per plant, height, and electrophysiology by measuring potential differences (PD's) of leaves on a weekly basis on two different stages. The results are then analysed statisticaly using Anova. Screening of the six varieties of rice was conducted by comparing the overall results on growth and electrophysiology measurement.

## 3. Result and Discussion

### *3.1. Growth Measurement*

There is no significant difference in reduction of plant growth observed under the application of 50 mM NaCl during early vegetative stages of the rice plants.On the other hand, the application of 200 mM NaCl caused the plant to diein less than 10 days. Similar effects were also found in the application of 200 mM NaCl during generative stages. We concluded that all rice varieties used in the experiment did not survive at 200 mM of NaCl salinity. While at 100 mM and 150 mM NaCl, rice plants response differently to their growth (Fig.1).

a b

Fig.1. (a) Madura rice variety grown in four different levels of salinity [0 mM, 50 mM, 100 mM , and 150 mM] of NaCl; (b) Adapatability of varieties of rice; Mekongga, Banyuasin, Madura, Inpari-13, Inpara-3and Ciherang under salinity of [100 mM] NaCl duringvegetative stage.

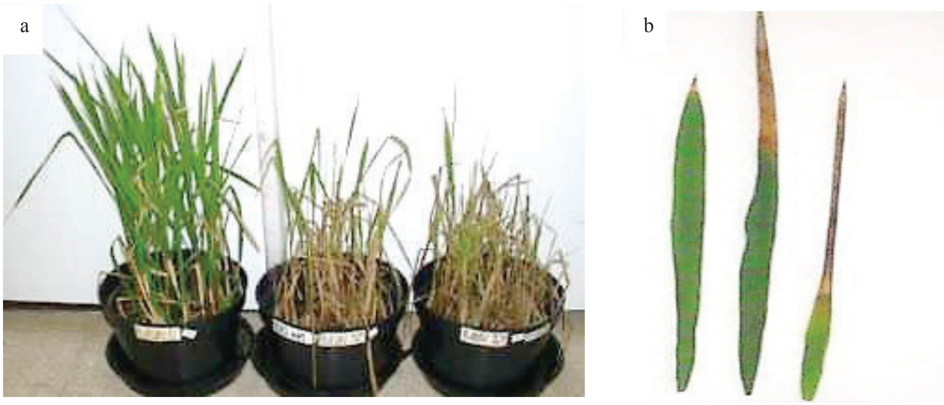


Fig. 2.(a) Rice variety of Banyuasin grown in three different levels of salinity [ 0mM, 100 mM , and 150 mM] of NaCl; (b) chlorosis in the tip of leaves of Banyuasin in round 60% compared to controlled plant in the application of NaCl during vegetative stage (each from left control, 100 mM and 150 mM NaCl

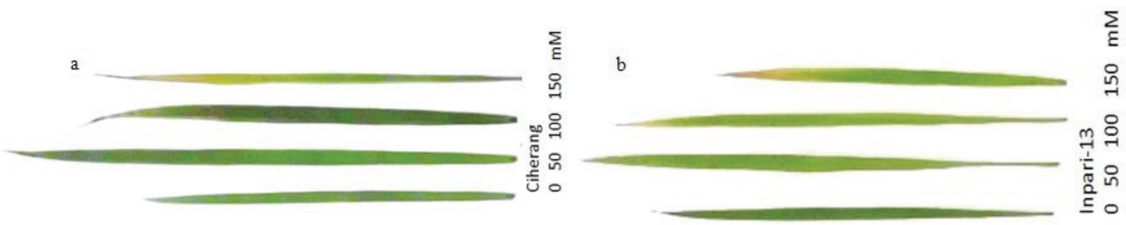


Fig.3. Early symptom of salinity stress at two varieties rice (a) Ciherang (left) and (b) (right) grown in four different levels of salinity, from the top each 150 mM, 100 mM, 50 mM and 0 mM of NaCl. Yellowing at the tips of leaves were observed at salinity of 100 mM and 150 mM NaCl during generative stages.

It had been reported that rice was more sensitive to salinity than what was previously thought in the guideline[10], but it seems that the threshold sensitivity differs among the varieties of rice and the duration of exposure. In this result, all six varieties of rice could survive at 100 mM NaCl in the third weeks of experiments (Fig 1b). However, some varieties are sensitive to this level of salinity on longer application as symptom stress of salinity. They were pronounced during vegetative (Fig 2) and generative stages (Fig 3). It appeared that higher salinity had adverse affects on the vegetative and generative growth of rice. The adverse effect of higher salinity could be seen in the tendencyoftotal ofleavesarea per plant reduced average in every rice varieties: Mekongga, Banyuasin, Madura, Ciherang, Inpara-3, and Inpari-13 at 150mM of NaCl starting atthe 2nd weekof measurement during the generative stage (Table1) and vegetative stages (Fig. 4).

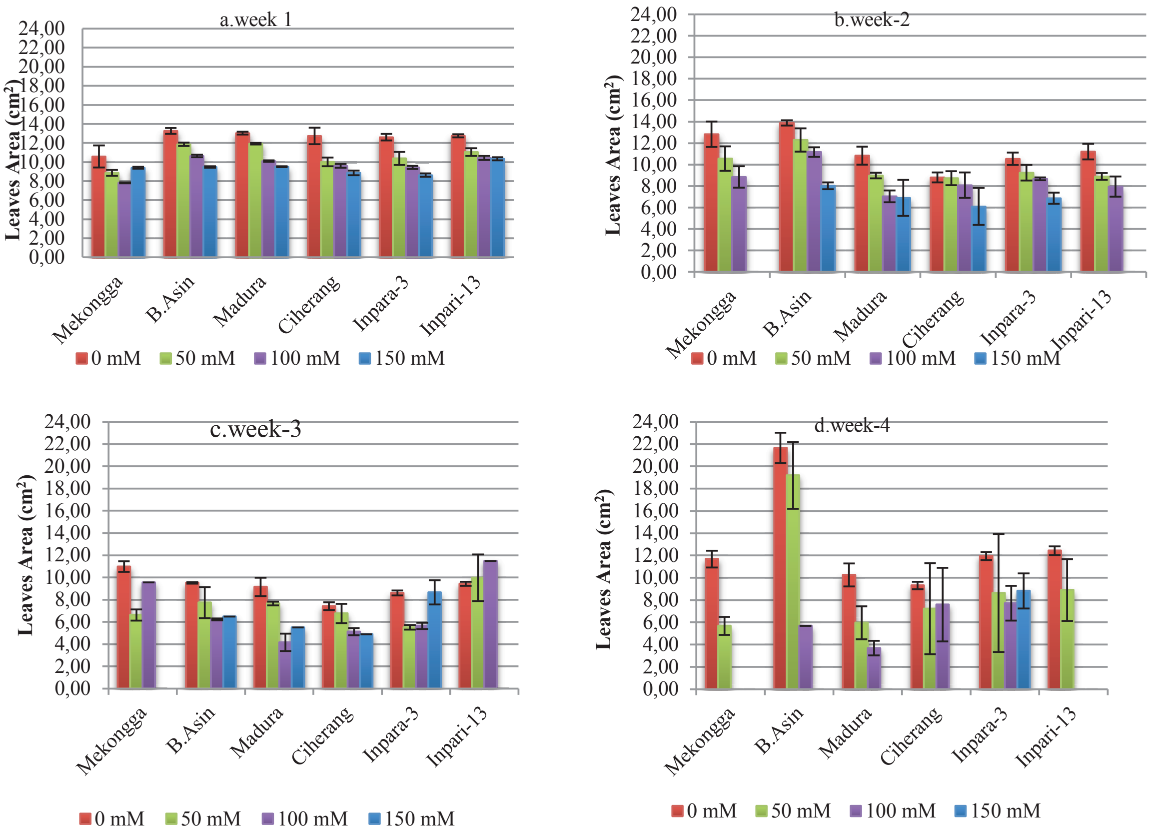


Fig 4. Average of leaf surface area per plant of six varieties of rice ( Mekongga, Banyuasin, Madura, Ciherang, Inpara-3 and Inpari-13) grown under different salinity level of 0 mM, 50 mM, 100 mM and 150 mM NaCl during week1, week2, week 3 and week4 (during vegetative stage).

The tendency of average of reduced leaves area per plant and average of height during the 2nd and 3rd week (Fig 5) during generative stages supported previous results that salinity inhibits plant growth from germination to maturity[11]. The growth inhibition in rice are reduction in seedling survival, tiller number perplant, and spikelet number per panicle[12;] yield and height of plant[13]. Salinity affects plant growth on different stages and different varieties[14]; and high accumulations of NaCl in the media growth of the rice plant inhibit it's growth[15]. The results showed that salinity inhibitsrice growth from vegetative to generative stages, and it seems that higher concentration of salinity has detrimental effect on growth and development ofrice as had been reported by previous researchers[6,17,18].

It had been reported that deeper water table in saline condition will have more effect on the number, length, and width of the leaves, and the leaves area of cabbage plant, in the growth media of 3.8 $dSm^{-1}$ during five weeks[19], but not their survival. From the results, it showed that different water depths (i.e. different size of pots) have influenced the leaves height and width of the plants, but the rice plants still can grow and produce rice normally. Reducing water table in rice did not influence the plant's survival in saline conditions despite of impeded plant growth.

Table1. Average of total leaf area (LA) per plant and standard error (s.e) in various concentration of NaCl for six different varieties of rice (Mekongga, Banyuasin, Madura, Ciherang, Inpara-3, and Inpari-13) every weekafter the application of NaCl during generative stage.

| | | LA ± S. e [$cm^2$] of treatments during generative stages | | | |
|---|---|---|---|---|---|
| Week | Varieties Rice | [0mM]NaCl | [50 mM] of NaCl | [100 mM] of NaCl | [150 mM] of NaCl |
| | Mekongga | | | | |
| 1 | | 18.75±2.50 | 17.89±1.75 | 21.29±2.08 | 23.92±3.96 |
| 2 | | 19.21±1.77 | 16.62±1.67 | 15.28±2.20 | |
| 3 | | 16.61±1.43 | 12.69±2.58 | 7.72±1.22 | |
| 4 | | 16.45±1.17 | 11.56±1.11 | | |
| | Inpari-13 | | | | |
| 1 | | 24.05±1.71 | 22.79± 2.06 | 19.68± 0.98 | 19.69± 1.51 |
| 2 | | 22.91±2.74 | 21.45± 2.51 | 15.61± 0.68 | |
| 3 | | 18.01±1.36 | 8.39± 1.47 | 8.19± 1.33 | |
| 4 | | 24.31±1.06 | 10.16± 0.54 | | |
| | Madura | | | | |
| 1 | | 26.53±0.15 | 21.84± 0.09 | 17.06± 0.07 | 18.76± 0.05 |
| 2 | | 16.95±1.29 | 14.37± 0.47 | 12.84± 0.48 | 12.57± 2.04 |
| 3 | | 12.83±0.59 | 13.13± 0.93 | 7.33± 1.05 | 6.14± 1.37 |
| 4 | | 11.74±0.61 | 9.59± 1.32 | 4.90± 0.98 | |
| | Banyuasin | | | | |
| 1 | | 30.67±2.36 | 26.22±1.99 | 24.55±1.92 | 21.45±0.79 |
| 2 | | 26.24±1.38 | 19.61±1.35 | 16.43±0.78 | 14.47±1.30 |
| 3 | | 26.07±1.80 | 14.21±1.98 | 10.01±1.51 | 6.70±1.02 |
| 4 | | 27.64±1.74 | 19.00±1.89 | 13.34±3.47 | |
| | Ciherang | | | | |
| 1 | | 22.89±2.52 | 19.24± 2.21 | 17.52± 0.92 | 15.36± 1.12 |
| 2 | | 16.55±1.64 | 16.00± 2.01 | 13.98± 1.96 | 6.33± 0.52 |
| 3 | | 14.35±1.14 | 12.38± 1.16 | 6.49± 0.99 | 5.07± 1.95 |
| 4 | | 13.30±0.70 | 9.95± 1.41 | 6.52± 2.11 | |
| | Inpara-3 | | | | |
| 1 | | 26.30±2.52 | 18.53± 1.29 | 17.47± 0.82 | 15.95± 1.27 |
| 2 | | 22.43±1.64 | 19.14± 1.51 | 16.06± 1.42 | 14.67± 2.01 |
| 3 | | 17.28±1.14 | 12.46± 0.52 | 12.98± 1.12 | 7.10± 1.13 |
| 4 | | 18.17±0.70 | 9.13± 1.41 | 8.50± 1.24 | 7.71± 1.58 |

Symptom of saline stress were observed on rice, i.e. chlorosis; drying leaves and shoot regardless the sizes of pots and different stages of application of $\geq$ 100mM NaCl (Fig. 1b, Fig.2 and Fig 3). Salinity also has an impact on rice height such as Mekongga, Madura, Ciherang and Banyuasin (Fig.5). Eventhough not much reduction in height of the plant during application of 100mM on generative stages, but the stress symptoms has risen at this level 100mM NaCl in Inpari-13, first in a smaller portions and then it becomes wider (Fig.3). It seems that rice has less adaptability to high saline compared to Quinoa[2].

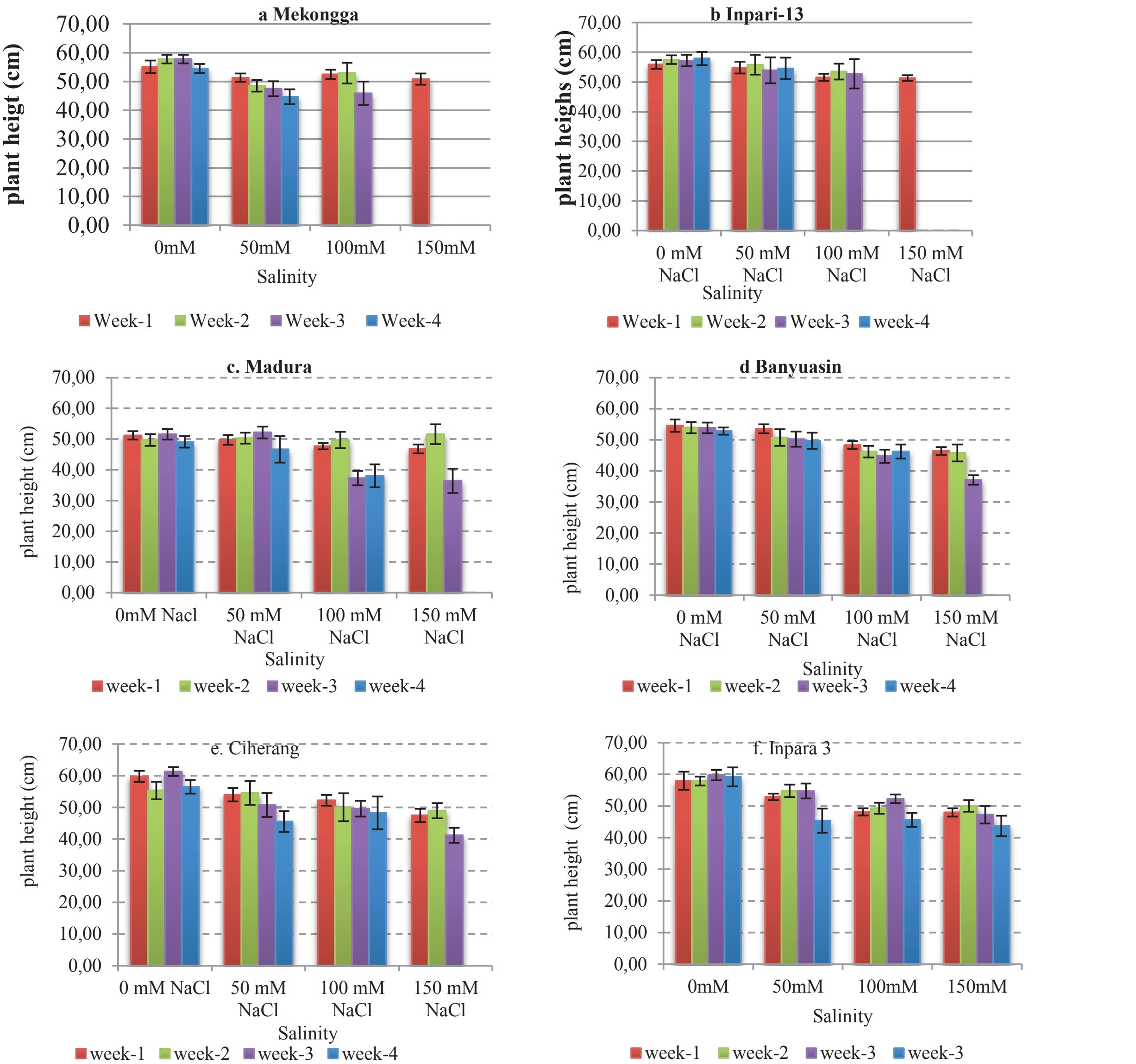


Fig 5. Average height of six varieties of rice plant grown under different level of salinity [0 mM, 50 mM,100 mM and 150 mM] NaCl during four weeks on the generative stages for each varieties (a) Mekongga; (b) Inpari-13; (c) Madura; (d) Banyuasin; (e) Ciherang; and (f) Inpara-3.

### *3.2 Electrophysiology Measurement*

The data presented in table 2 and its corresponding response behaviour (Fig.6 ) showed that different salinity levels affects differently on the electrical potential different (PD) on the leaves of the six rice varieties. It can be seen in Fig.6 that electrical PD was reduced if the salinity was increased. In Mekongga variety, the salinity reduced the value of electrical PD from their normal value ranged between (91.67±4.40) mV and (127.00±14.29) mVdown to (60.00±13.93) mV at 150 mM of NaCl (Table 2). In salinity level of 100mM NaCl, the electrical PD of Mekongga fell to 0 at week3. The value of electrical PD was smaller for rice grown in higher salinity level and longer duration of NaCl application on their growing media.

Table 2. Average electrical potential difference (PD) and standard error (s.e) in various concentration of NaCl for six different varieties of rice (Mekongga, Banyuasin, Madura, Ciherang, Inpara-3, and Inpari-13) every weekduring generative stage.

| | | **PD ± s.e [mV]** | | | |
|---|---|---|---|---|---|
| Week | Varieties Rice | [0mM]NaCl | [50 mM] of NaCl | [100 mM] of NaCl | [150 mM] of NaCl |
| | Mekongga | | | | |
| 1 | | 127.00±14.29 | 116.36±9.28 | 98.00±11.63 | 83.33±12.22 |
| 2 | | 102.50±20.87 | 80.00±16.79 | 64.17±21.93 | 60.00±13.93 |
| 3 | | 95.71±6.50 | 71.43±10.91 | 0.00±0.00 | 0.00±0.0 |
| 4 | | 91.67±4.40 | 63.33±8.79 | 0.00±0.00 | 0.0±0.0 |
| | Inpari-13 | | | | |
| 1 | | 120.00±0.0 | 120.0± 0.0 | 120.0± 0.0 | 73.3± 10.8 |
| 2 | | 106.30±11.0 | 96.7± 3.7 | 51.7± 1.8 | 48.3± 1.8 |
| 3 | | 87.10±13.3 | 66.0± 6.4 | 36.3± 6.5 | 0.0±0.0 |
| 4 | | 84.00±9.5 | 46.7± 5.4 | 0.0±0.0 | 0.0±0.0 |
| | Madura | | | | |
| 1 | | 96.7±11.9 | 81.7± 6.9 | 64.0± 7.5 | 60.0± 6.3 |
| 2 | | 108.3±10.7 | 83.3± 8.8 | 81.7± 12.8 | 28.3± 4.4 |
| 3 | | 100.0±13.6 | 66.7± 10.8 | 50.0± 12.3 | 25.8± 4.3 |
| 4 | | 93.3±9.2 | 56.7± 6.7 | 25.7± 4.3 | 0.0±0.0 |
| | Banyuasin | | | | |
| 1 | | 95.0±8.8 | 80.0±6.9 | 78.3±5.2 | 80.0±5.7 |
| 2 | | 90.0±9.4 | 80.0±9.4 | 51.7±7.2 | 45.0±9.3 |
| 3 | | 117.1±13.8 | 86.9±7.6 | 52.9±15.9 | 67.1±16.3 |
| 4 | | 70.0±7.7 | 43.3±11.1 | 35.0±3.7 | 0.0±0.0 |
| | Ciherang | | | | |
| 1 | | 116.7±6.7 | 86.7± 12.2 | 68.3± 14.0 | 50.0± 4.9 |
| 2 | | 113.3±20.3 | 80.0± 11.3 | 66.7± 7.8 | 47.5± 5.0 |
| 3 | | 115.0±19.5 | 81.7± 5.2 | 65.8± 7.3 | 44.2± 1.7 |
| 4 | | 96.7±24.3 | 76.7± 8.8 | 52.5± 5.6 | 0.0±0.0 |
| | Inpara-3 | | | | |
| 1 | | 98.0±5.8 | 74.0± 6.0 | 64.0± 4.0 | 60.0± 0.0 |
| 2 | | 86.7±9.2 | 75.5± 7.9 | 62.5± 5.2 | 51.7± 4.4 |
| 3 | | 85.0±3.7 | 65.0± 5.5 | 53.3± 3.4 | 38.5± 2.3 |
| 4 | | 86.7±3.7 | 45.0± 9.7 | 34.2± 3.0 | 28.3± 8.2 |

In Inpari-13 it seems that even the plant could survive inthe 3rd week at a salinity level of 100 mM NaCl, but the plant did not survive electrophysiologically at salinity level of 150mM early in the 3rd week where the value of PD was zero. The value of electrical PD of Inpari-13 was in range of (84.0±9.5) mV up to (120.0±0.0) mV ata normal growth and reducedup to (36.3± 6.50) mV due to increased salinity at 100mM in 3 weeks. Four varieties of rice i.e. Madura, Banyuasin, Ciherang, and Inpara-3 were tolerant to salinity level of 100 mM until four weeks, and tolerant to salinity level of 150 mM NaClduring the third week of experiment. The exception was Inpara-3 that might still survive at 150 mM for four weeks but only in limited numbers. It was implied that four candidates of rice tolerant to salinity were Inpara-3, Banyuasin, Ciherang, and Madura.

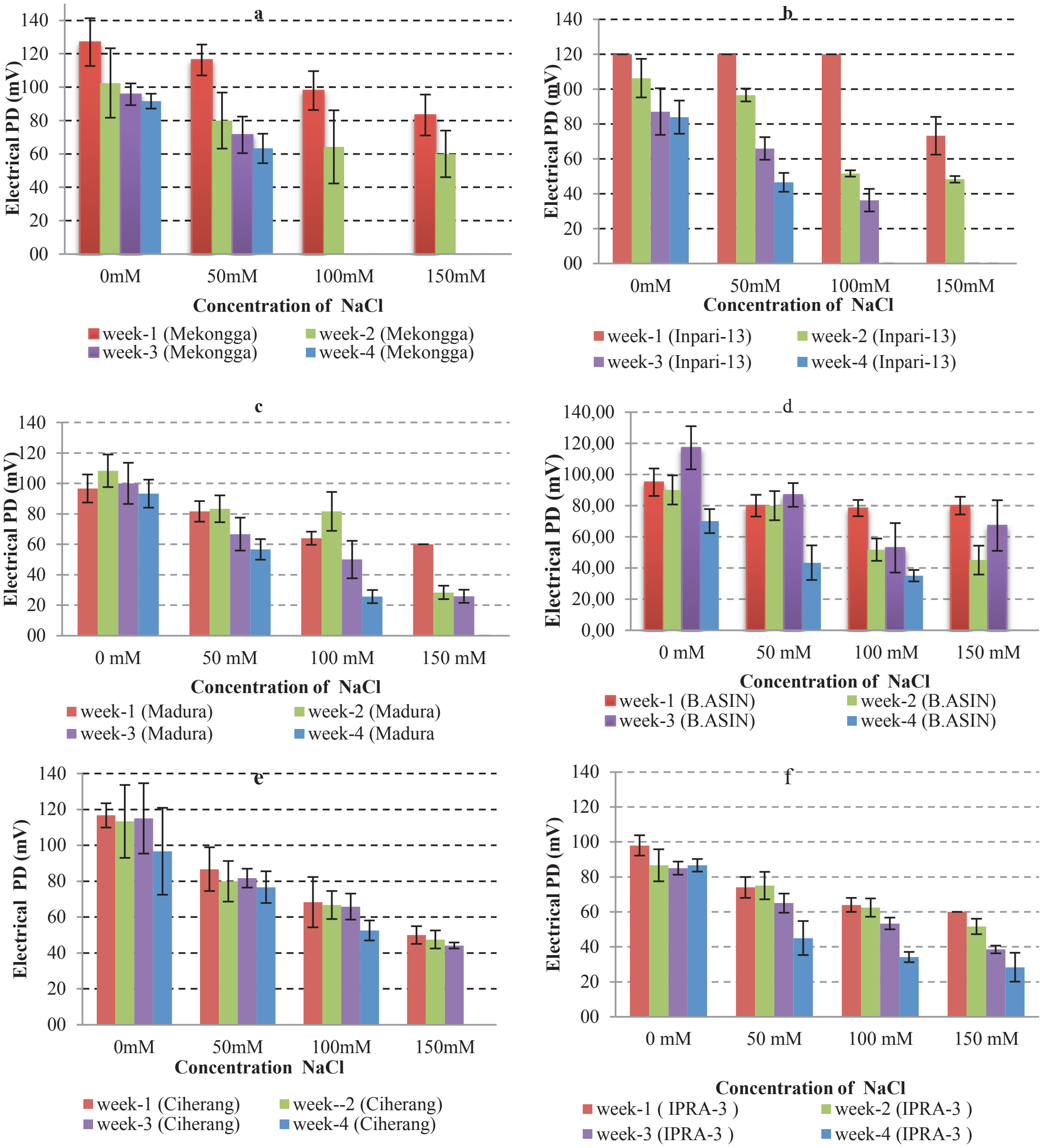


Fig.6 Effect of different concentration of salinity to leaves surface's electrical PD of six rice varietas during four weeks experiment in the generative stages; (a) Mekongga, (b) Inpari-13; (c) Madura (d)Banyuasin; (e) Ciherang and (f) Inpara-3

The different response to salinity in plant possibly depends on the concentration and composition of the ions in the solution and genotype ofthe plant. Salinity influencing plant growth by ionic and osmotic ways sometimes differs between one and another and sometimes overlaps.It is clearly found that rice cannot tolerate high salinity levels of 150 mMNaCl, which is lower than quinoa at 300 mM NaCl or halophyte at 400 mMNaCl, but the results are very important for further research development with respect to the greater diversity in Indonesia.

The ability of the plant to respond to salinity was relatedto it's ability to regulate $K^+$ and exclude the $Na^+$toxicity [20,21]. In salinitylevel of 150 mMNaCl, there might bean imbalance of nutrition in the cell, osmotic stress or $Na^+$toxicity[22,23,24]. Higher ratio between $K^+$: $Na^+$ will also increase the plant's resistance to salinity[25].

The result showed that salt stress reduces growth of rice and productivity by reduction in photosynthetic capability[26]. The experiment showed that the electrical PD on rice treatment was reduced to the PD of control plant. It appeared that electrical PD of the rice was depolarized due to salinity stress[27,21,2]. Salinity caused significant reduction in germination, root, and shoot lengths, and fresh root and shoot weight[28]. Reduction in growth of rice from germination to maturity may be due to the increase of osmotic pressure of the root medium and or by the specific ion effects orboth[11]. Plant may adapt to survival and growth in the existence of environmental stress[29]. Mechanism of resistance involved homeostasis ion regulation between tissue and intrasellular compartmentalized $Na^+$and $Cl^-$ in some plants[30]. It is clear that electrophysiology measurement is important in stress[8]. The result showed that the addition of electro physiological indicator by measuring leaves surface electrical PD on rice gave more reliable criterion in measuring the effects of salinity on the plant. The tendency of declining electrical PD on all treatment (Fig 6), even at a low levels at 50 mM of NaCl compared to the normal one in early observation showed that the difference of electrical PD on each variety of the rice in different levels of salinity can be used as a screening tool for rice salinity tolerance.

## 4. Conclusion

Increasing salinity inhibits growth of the six rice varieties from vegetative to generative stages. Height and area of leaf per plant were reduced. Electro physiologically, increasing salinity reducedthe electrical PD of plant leaves. Different salinity affects differently on electrical plant leaf PD of the six varieties of rice. The difference of electrical PD on each variety of the rice in different levels of salinity can be used as a screening tool for rice salinity tolerance.

Death occurred at 200 mM of NaCl; though five of six varieties were tolerant to 100 mM of NaCl during 4 weeks experiment, but according to electrophysiology measurement only four varieties were tolerant to this level during four weeks experiment, i.e. Inpara3, Banyuasin, Ciherang, and Madura. The four varieties are possible candidates for rice salinity tolerant of around 100 mM NaCl levels, but further research is needed due to the growth limitation at150 mM NaCl in the four week duration during generative stages. It was concluded that salinity of 150 mM was used as a salinity threshold tolerance for rice, at least for the six varieties of rice used in the experiment.

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