

PHOTOSYNTHESIS AND YIELDS OF GRASSES GROWN IN SALINE CONDITION

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Received November 19, 2009; Accepted February 23, 2010

ABSTRACT

The aim of this study was to know effects of saline condition to crop physiology, growth and forages yield. A factorial completed random design was used in this study. The first factor was type of grass, these were king grass (*Pennisetum hybrid*), napier grass (*Pennisetum purpureum*), panicum grass (*Panicum maximum*), setaria grass (*Setaria sphacelata*) and star grass (*Cynodon plectostachyus*). The second factor was salt solution (NaCl) with concentration 0, 100, 200 and 300 mM. Parameters of this experiment were the percentage of chlorophyll, rate of photosynthesis, number of tiller, biomass and dry matter yield. Data were analyzed by analysis of variance and followed by Duncan's multiple range test when there were significant effects of the treatment. Panicum grass had the highest chlorophyll content (1.85 mg/g of leaf). Photosynthesis rate of setaria grass was the lowest. The increasing of NaCl concentration up to 300 mM NaCl reduced chlorophyll content, rate of photosynthesis, tiller number, biomass yield and dry matter yield. Responses of leaf area, biomass and dry matter yield to salinity were linear for king, napier, panicum and setaria grasses. In tar grass, the response of leaf area and biomass were linear, but those of dry matter yield was quadratic. The response of tiller number to salinity was linear for all species.

Keywords : biomass, dry matter yield, grass, photosynthesis, saline.

INTRODUCTION

The increase in human population require more resources be devoted to food production systems, including marginal land such as saline soils. Soil salinity is one of the most serious stresses in agriculture. It has been estimated that about one billion hectares of the world's land was affected by salt, sixty percent of which was cultivated (Rain and Goyal, 2003). In Indonesia, salt problem in coastal area are becoming intrusion sea water occupation the land.

Salinity and low N soil availability are growth limiting factors for most plants. Soil salinity decreased plant relative growth rate up to first mature pod stage. Previous study indicated that over fertilization during early plant

development contributed to salinity and decreased pod yield in Chile pepper (*Capsicum annum L*) (Villa-Castorena *et al.*, 2003). Salinity reduced number of leaf stomata per unit area (stomatal frequency of leaf) at the critical salinities (12 mmhos/cm and above) of irrigation water (Kumar, 2005). Salinity reduced leaf chlorophyll, salinity also affected plant growth, nodulation and nitrogenase activity of chickpea (Garg and Singla, 2004). Growth reduction under salt stress could be attributed to excessive ion accumulation in the plant tissue and to water stress due to low external osmotic potential resulting from salinity. Selection for salt tolerant species and cultivars in the field is time consuming and difficult due to spatial and temporal variation of salinity level and salt composition (Eghball *et al.*, 2004). New

screening methods to identify genetic variation for increasing the salt tolerance of cereal crops need to be developed. Physiological mechanisms that underlie traits for salt tolerance could be used to identify new genetic sources of salt tolerance (Munns *et al.*, 2006). Screening for salt tolerant of plant can be done with varying concentration of salt (0,4,6,8 dS/m NaCl, Na₂SO₄,CaCl₂) (Garg and Singla, 2004), level 1,3 to 6 dS/m for chile pepper (Villa-Castorena *et al.*, 2003), salinity tolerant index equal to reduction of 50% in crop yield from that of the non-saline yield (Lee *et al.*, 2005). Objectives of the present study was to know the effects of salinity on tiller number, chlorophyll, rate of photosynthesis, content of dry matter and biomass yield of five grasses.

MATERIALS AND METHODS

Plant Culture

The experiment was conducted in the greenhouse using sand culture system. Grasses were planted on pot (diameter 20 cm with depth 25 cm) filled with sand media. Sixty pot were used in this experiment. Plant were grown under natural light. Briefly, pols of king (*Pennisetum hybrid*), napier (*Pennisetum purpureum*), panicum (*Panicum maximum*), setaria (*Setaria sphacelata*) and star (*Cynodon plectostachyus*) grasses were hand-washed to remove soil and then planted in sand culture. The grasses were fed by liquid fertilizer containing N 8 %, P₂O₅ 10 %, K₂O 34 %, MgO 2,5 %, Fe 0,1 %, B 0,02 %, Mn 0,1 %, Cu 0,01 %, Zn 0,01 %, Mo 0,02 Co 0,001 %, Se 0,0006 % and I 0,001 % with concentration 1,5 g/liter.

Salinity treatments were applied as saline condition in the drip irrigation system by adding NaCl solution of 100, 200 and 300mM. Nutrient solution without the addition of NaCl solution served as control. Grasses were exposed to final salinity treatment for a period of 4 weeks (tripping plant uniformity) and 6 weeks (biomass yield).

Data Collection

Parameters observed were tiller number, chlorophyll content, rate of photosynthesis, content of dry matter and biomass yield. Chlorophyll measured by taking sample 3 – 9 of leafs in underside, middle shares and tip of crop (young leaf). Chlorophyll was measured according to Suseno *et al.* (1974). One gram fresh leaf was grinded and 10 ml of 80 % acetone was added. After homogen, the solution was filtered.

The 80% acetone was added to make 50 ml solution. Then, 2.5 ml filtrate was put into a covet and 7.5 ml 80 % acetone was added to make 10 ml solution. The sample absorbance was read by spectrophotometer 652 nm wavelength. Chlorophyll content was calculated by equation :

$$\text{Chlorophyll total} = \frac{(\lambda_{652} - \lambda_0)}{34.5} \times 1000 \times \frac{50}{1000} \times \frac{50}{1} \times \frac{1}{1} \text{ (mg chlorophyll / g leaf)}$$

where :

λ_{652} = sample wavelength for chlorophyll

λ_0 = liquid standard wavelength (pure chlorophyll)

34.5 = coefficient of wavelength absorbance 652 nm

Measurement procedure of photosynthesis rate was done by measuring CO₂ yielded by crop during 30 minutes; the grass was placed in plastic chamber (512000 ml volume) and put under sun light. After 30 minutes, CO₂ was taken from chamber by using spuit (50 ml volume) then injected into 5 ml NaOH. Furthermore, titration by HCL 0,1 N until red colour disappeared.

$$\text{PR} = \frac{10240 \times (\sum \text{CO}_2 \text{ start} - \sum \text{CO}_2 \text{ final})}{\text{leaf area} / 30}$$

mg CO₂ /dm²/minute

where:

PR = photosynthesis rate

Measurement dry matter yield started by preparing 100 g sample from each type of grasses. Sample was taken and dried at temperature of 105 ° C during 24 hours. Dry matter percentage was calculated using formula:

$$\text{dry matter} = \frac{B_o}{B_s} \times 100\%$$

$$\text{Dry matter yield (g)} = \% \text{ DM} \times \text{FY}$$

where:

B_o = the weight of sampel after being dried

B_s = the weight of fresh sample (100 g)

DM = dry matter

FY = forage yield

Data Analysis

The effects of salinity, species and their interaction were determined by analysis of

variance (ANOVA) according to Steel and Torrie (1990). The differences between treatment means were determined using Duncan's Multiple Range Test. Regression analysis were performed to define linear or quadratic relationships between each variable and the salinity level (Minitab Release 13.1).

RESULTS AND DISCUSSION

With the increasing of salinity, all species exhibited reduction leaf area though leaf area decreased, more rapidly in napier than king, panicum, setaria and star grasses (Table 2). At all salinity treatment king grass exhibited lower leaf area than elephant, panicum, setaria and star grasses.

The type of grass and the concentration of NaCl affected chlorophyll content and the rate of photosynthesis. However, there was no interaction between the 2 factors (Tables 1). The highest chlorophyll content was shown by panicum (1.85 mg/g of leaf) while the lowest content of chlorophyll was shown by king grass (0.92 mg/g of leaf) (Figure 1A). The ncreasing concentration of NaCl up to 300 mM decreased content of chlorophyll 37 % from control (Figure 1B).

Photosynthesis rate of panicum grass (1.51 mg CO₂/ dm²/minute) was greater than all other grasses. Setaria grass showed the lowest photosynthesis rate (0.91mg CO₂/dm²/minute (Figure 2 A). The increasing concentration of NaCl up to 300 mM decreased photosynthesis rate from 1.7 mg CO₂/ dm²/minute to 1.0 mg CO₂/ dm²/minute for salt 300 mM. (Figure 2 B). The decrease was about 39% of control. The inhibition of growth could be through the

reduction of leaf cell growth. Furthermore, concentration of NaCl decreased leaf area, as a result the rate of photosynthesis decreased too. Plant of C4 in general have rate of photosynthesis in comparison with plant of C3. Plant crop of C4 have rate of photosynthesis 120 until 180 mg CO₂/ hour (Lakitan, 2000). According to Qian *et al.* (2004), under salinity stress a greater degree of stomatal closure decreased photosyntentic capacity contributing to the declines in growth and turf quality.

Salinity was growth limiting factor for most plants. In micro-plot study of 6 cultivar of Indian mustard (*Brassica juncea* L) it was observed that germination of seed per plot decreased linearly with the increasing of salinity levels. Compared to the cultivars of the sensitive group, the tolerant ones exhibited a higher magnitude of reduction in number of leaf stomata per unit area at the critical salinities (12 mmhos/cm and above) of irrigation water, whereas the decrease in leaf water potential was not so marked at the same salinity level (Kumar, 2005). Salinity limits water uptake by plants by reducing the osmotic potential and thus the total soil water potential. Salinity may also cause specific ion toxicity or upset the nutritional balace. The salt composition of the soil water influences the composition of cations on the exchange complex of soil particles which influences soil permeability and tilt depending on salinity level and exchangable cation composition (Corwin and Lesch, 2003).

The analysis of variance showed significant effects of salinity, species and their interaction in the number of tiller (Table 1). With the increasing of salinity, king, setaria and star grasses showed the decrease number of tiller. However, the

Table 1. Analysis of Variance with Mean Squares and Treatment Significant Levels

Parameters	Salt	Cultivar	Salt x cultivar
Leaf area	550320,9**	25562,5**	27360,3**
Chlorophyll content	1,64**	1,07**	0,03 ns
Photosynthesis rate	2,35**	0,63*	0,15 ns
Plant height	1163,2**	3769,6**	141,24ns
Number of tiller	39,4**	60,47**	13,55 **
Dry matter percentage	5,47ns	70,2**	10,48ns
Biomass	1102349,3**	155184,19**	49444,01**
DM yield	28690,15**	7606,53**	1660,35**

* significant at the 0,05 level

** significant at the 0,01 level

ns : not significant

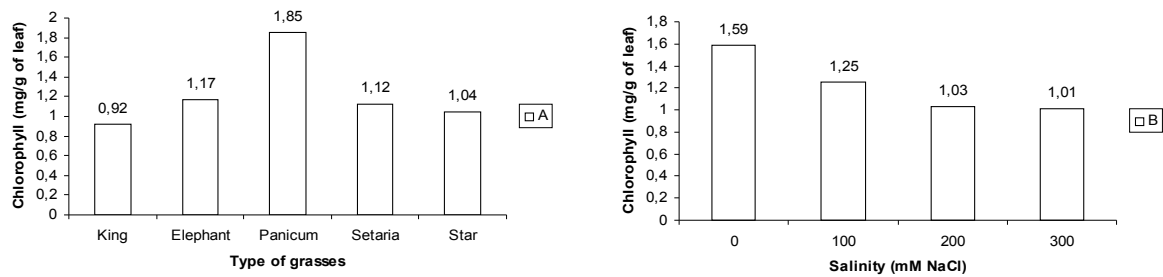


Figure 1. Chlorophyll Content of Five Forage Grasses (A), Effect of Salinity on Chlorophyll Content (B)

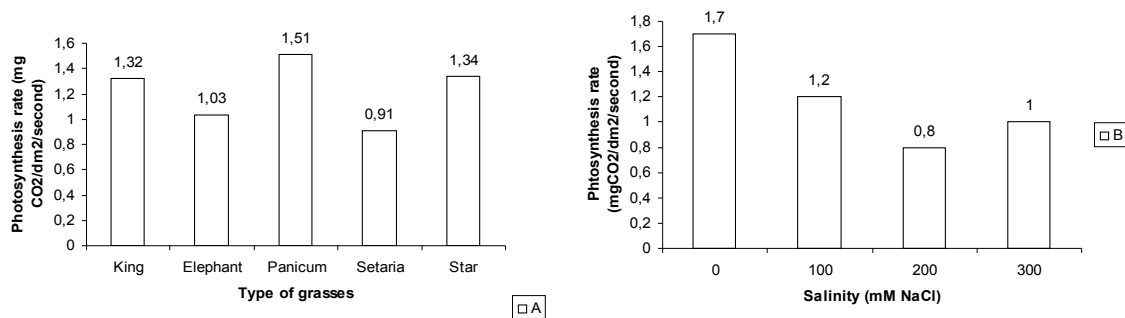


Figure 2. Photosynthesis Rate of Five Forage Grasses (A), Effect of Salinity on Photosynthesis Rate (B)

number of tiller of napier and panicum grasses increased in the concentration of 100 mM NaCl then decreased in the concentration of 200 mM NaCl and above. Response number of tiller to salinity was quadratic for all species (Table 2). The lowest of tiller number was king grass (2,3) incurred by the concentration of 300 mM NaCl.

Sodium is a beneficial element required by grass. The addition of salt (in this case element of Na) will increase growth at certain condition. This phenomenon was shown by napier and panicum grasses where tiller number increased at 100 mM NaCl. However, tiller number decreased at higher salinity. Experiment of Malinowski *et al.* (2003) reported initial of tiller number was influenced by water availability, intensity of defoliation, time of defoliation, suggested the importance of a rapid re-establishment of photosynthesis rather than root reserves as the main source of C for regrowing shoots.

Biomass was influenced by salinity, species and their interaction (Table 1). Mean biomass yield decreased linearly with the increasing of salinity for all species (Table 2). Under none saline condition king grass has the same biomass yield as napier and panicum. Growth of crop on

saline condition will cease due to the effect of high salt concentration (high Na content). The increasing of salt content could cause plasmolysis. Plasmolysis of this study was shown by deterioration of root. Deterioration of root will reduce nutrient uptake and growth. Increasing salinity up to 300 mM reduced biomass weight by 79.8, 87.6, 58.4, 56.0, 59.8% respectively for king, napier, panicum, setaria and star grasses compared to control.

Dry matter percentage was influenced by species, but saline condition and their interaction had no significant effect (Table 1). Dry matter content influenced by crop ability in accumulating dry matter in crop. Dry matter yield was influenced by salinity, species and their interaction (Table 1). Dry matter yield decreased linearly with the increasing salinity for four species (Table 2) with coefficient determination (R^2) were 60.7, 92.0, 95.9 and 97.2% for setaria, napier, king and panicum grasses. Star grass showed a quadratic with R^2 99.16%. High concentration of NaCl caused a reduction in assimilating so that the grasses formed smaller leaves with a fewer number of leaves, as a result, the grasses showed higher stem:leaves ratios,

Table 2. Effect of salinity on leaf area, tiller number, biomass and dry matter yield of five type grasses

Grass	Salinity (mM NaCl)				Regression	R ²
	0	100	200	300		
Leaf area (cm ²)						
King	835.6 ^a	612.8 ^{bc}	290.5 ^{fg}	168.2 ^{gh}	Y = 825 – 2.32 X	97.5
Elephant	801.3 ^a	502.0 ^{cde}	402.0 ^{ef}	187.6 ^{gh}	Y = 764 – 1.94 X	96.6
Panicum	846.1 ^a	644.7 ^b	582.3 ^{bcd}	405.1 ^{ef}	Y = 827 – 1.39 x	96.6
Setaria	610.6 ^{bc}	458.1 ^{de}	312.7 ^{fg}	268.7 ^{fgh}	Y = 588 – 1.17 X	95.3
Star	304.0 ^{fg}	231.4 ^{gh}	206.4 ^{gh}	126.1 ^h	Y = 301 – 0.559 X	96.6
Tiller number						
King	7.3 ^{bcd}	4.0 ^{defg}	6.3 ^{bcd}	2.3 ^g	Y = 6.7 - 0.0075 X - 0.000017X ²	53,6
Elephant	3.6 ^{defg}	5.0 ^{cdefg}	3.0 ^{fg}	2.6 ^{fg}	Y = 3.85 + 0.0085X - 0.000045X ²	62,2
Panicum	7.6 ^{bcd}	13,6 ^a	8.0 ^{bcd}	3.0 ^{fg}	Y = 8.21 + 0.0631X - 0.000275X ²	86.8
Setaria	11.0 ^{ab}	9.6 ^{abc}	7.6 ^{bcd}	5.6 ^{cdefg}	Y = 11 - 0.0137X - 0.000015X ²	99.9
Star	5.0 ^{defg}	3.3 ^{efg}	4.6 ^{defg}	4.3 ^{defg}	Y = 4.77 - 0.0113X + 0.000035X ²	33
Biomass (g/pot)						
King	1050.67 ^a	545.67 ^{cde}	315.00 ^{ghi}	213.33 ^{ij}	Y = 943 - 2.74X	90
Elephant	1060.67 ^a	582.67 ^{bcd}	418.67 ^{efgh}	131.67 ^j	Y = 991 - 2.95X	95.9
Panicum	1061.67 ^a	697.00 ^b	529.67 ^{edef}	445.33 ^{defg}	Y = 986 - 2,02X	90.9
Setaria	651.67 ^{bc}	330.00 ^{ghi}	228.33 ^{ij}	286.67 ^{hi}	Y = 554 - 1.2X	66.4
Star	603.33 ^{bc}	620.00 ^{bc}	401.67 ^{fgh}	242.33 ^{ij}	Y = 662 - 1.3X	87.5
Dry matter yield (g/pot)						
King	13.590 ^a	10.350 ^{bcd}	7.933 ^{efgh}	6.790 ^{ghi}	Y = 13,1 - 0,0228X	95.9
Elephant	12.313 ^{ab}	10.410 ^{bcd}	9.340 ^{cdef}	5.026 ⁱ	Y = 12,7 - 0,0229X	92
Panicum	14.106 ^a	11.980 ^{ab}	10.523 ^{bcd}	9.546 ^{cde}	Y = 13,8 - 0,0152X	97.2
Setaria	10.700 ^{bcd}	7.350 ^{fgh}	7.190 ^{fgh}	6.430 ^{hi}	Y = 9,63 - 0,0114X	60.7
Star	11.056 ^{bc}	12.410 ^{ab}	11.260 ^{bc}	8.706 ^{defg}	Y = 8,023 + 4,065X - 0,977X ²	99.1

Y=variable estimated, X=salinity

except setaria grass. Salinity depress growth of crop with effects on decreasing cell division, protein synthesis and biomass accumulation. Crop experiencing of salt stress generally does not show direct damage but growth is depressed (Sipayung, 2003). Abou-Hadid (2003) reported that plants affected by salinity are generally stunted. Leaves was smaller, though they may be thicker than those of normal plants. Osmotically stressed plants may show no distinctive symptoms, however, so that only comparison with normal plants from the same growing environment reveals the extent of salt inhibition.

Salinity will alter crop morphology structure

to influence dry matter accumulation rate of crop. The increasing of salinity will decrease the number of leaf and the size of leaf. The increasing of salinity will also cause the formation a thick wax coat and leaf cuticle and earlier lignification. Under low salinity, crop will be able to grow and accumulate dry matter. According to study of Bennett and Kush (2003) on paddy crop, dry matter production was not affected when the crop grown under NaCl concentrations of 0, 20, 35, 50 mM.

Furthermore, crop grown under saline condition will have difficulty on nutrient absorption especially nitrogen, K, Mn, Fe and Zn.

As a result, plant production will decrease. Tuna *et al.* (2007) showed that the plants grown under salt stress (75mM NaCl) produced lower dry matter, fruit weight and relative water content than those grown in standart nutrient solution. Qian *et al.* (2004) reported that Kentucky blue grass grown under salinity up to 4,9 dS/m reduced yield of northstar and moonlight cultivar by 25%.

CONCLUSION

The increasing of NaCl concentration from 0 to 300 mM NaCl reduced chlorophyll content, rate of photosynthesis, tiller number, biomass yield and dry matter yield. Response of leaf area, biomass and dry matter yield to salinity were linear for king, napier , panicum and setaria grasses. In star grass, the response of leaf area and biomass was linear but dry matter yield was quadratic. The response of tiller number to salinity was linear for all species.

ACKNOWLEDGMENT

The author acknowledge to the Scholarship of Post Graduate Program Fund of the Directorate General of the Higher Education Department of National Education for gift of fund years 2005-2008.

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