# Ph.D. Thesis

## Agronomic Characteristics of *Sorghum* Plants Grown under Salt Stress

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# Chapter 1
# General Introduction

Several countries, particularly in the arid and semi-arid regions, face various problems associated with high population growths, deficiency in natural water sources, salt-affected areas, and shortage in food and feed supply (Ashour et al., 1997; Tomar et al., 2003). More than 800 million hectares of the world's total land areas are salt-affected (Munns, 2010). Due to the natural salinity and land clearing or irrigation system, cultivated agricultural land in recent times have a high risk of becoming saline that leads to increase high amount of salts in plant roots (Munns and Tester, 2008). Salt-affected areas normally are either abandoned or utilized for pasture but theirs fodder yield or produce is low, that is unstable and unfruitful (Tomar et al., 2003). Serious shortage of fodder when occurring can be overcome or solved by growing salt resistant plants or palatable forage grasses (Tomar et al., 2003). There are a range of plants that are capable of growing under conditions of saline soil and water and these plants represent a feed resource for livestock (Masters et al., 2007). Utilization of plants as a pasture or fodder for livestock under saline soils was reported in several countries (Ashour et al., 1997; Nedjimi, 2009; Khanum et al., 2010). Therefore, the utilization of salt-affected areas to cultivate forage plants and to produce enough fodder for animal production is the prospects for the good future.

There have been few attempts or trials to improve the feeding value of salt tolerant plants through selection or breeding, or to select livestock that are more capable of tolerating high salt intakes (Masters et al., 2007). *Sorghum* is the fifth most important cereal crop in the world because of its high productivity of dry matter yield and resistance to various environmental conditions. *Sorghum* has been considered moderately resistant to salinity and

as potential fodder crop for salt-affected areas (Maas et al., 1986; Almodares and Sharif, 2007; Krishnamurthy et al., 2007). There are several types of *Sorghum* such as grain sorghum, forage sorghum, sudangrass, and sorghum-sudangrass hybrids (Iptas and Brohi, 2003). For the past several years, there has been an interesting plant, namely sorghum-sudangrass hybrids (*Sorghum bicolor* × *S. sudanense*), which is cultivated annually. They are forage-grasses crossbreed between sorghum and sudangrass types, providing valuable forages for livestock consumption (Pedersen and Toy, 1997; Ketterings et al., 2005). These hybrids have some variability in growth characteristics which include rapid growth, heat and drought tolerance, and are capable of producing large amounts of dry matter (Pedersen and Toy, 1997).

The effects of salts on plant growth respond to salinity in two phases (Munns et al., 2006; Munns and Tester, 2008). Firstly, the osmotic or water-deficit effect of salinity inhibits growth or young leaves. Secondly, the salt-specific or ion-excess effect of salinity accelerates senescence of mature leaves. Soil salinity effects on plants thus include ion toxicity, osmotic stress, mineral imbalances, and physiological characteristics (Munns, 2002). Salt resistance is normally assessed as the percentage of biomass production in saline versus controlled conditions over a prolonged period of time (Munns, 2010). The presence of large genotypic variation for salinity resistance was found in *Sorghum* plants (Krishnamurthy et al., 2007). The impacts of salt stress on the growth of *Sorghum* plants have been investigated extensively and the results have shown that if salt stress exceeds a level of crop production, it resulted in the decrease in growth, yield, and quality. However, the levels of resistance to salinity vary by the genotypes and the growth stages. Some studies investigated the response of *Sorghum* plants during germination and seedling growth (Geressu and Gezaghegne, 2008). Others emphasized more on the seedlings after germination (Almodares et al., 2008).

In monocotyledon, the leaf consists of the blade and sheath and the mass of the photosynthesis and transpiration occurs in the leaf blade (James et al., 2006). Photosynthetic activity decreases subsequently, when plants are grown under salt stress, resulting in reduced growth and productivity (Netondo et al., 2004b; Abdeshahian et al., 2010). The presence of net photosynthesis is affected strongly under salinity conditions and it is greatly related to a decrease in stomatal conductance as well as to less intercellular $CO_2$ concentration (Ouerghi et al., 2000). In wheat, the high amount of $Na^+$ in the leaf can result the premature leaf senescence and loss of photosynthesis (James et al., 2002). To reduce the senescence process under salt stress, plants need to manage toxic ion concentration in different plant tissues. Therefore, the control of $Na^+$ transport in cereal crops and its effective exclusion from the mesophyll cells of leaves is considerable necessity for resistance to salt stress (James et al., 2011).

In term of forage, *Sorghum* has excellent potential to improve production systems for ruminants in several countries (Begdullayeya et al., 2007). Different *Sorghum* cultivars might vary in cell walls which differ in plant structures, differentiation, and rate and extent of degradation (Wilson, 1993). There is study reporting that sorghum silage types; grain sorghum, sweet sorghum, and bmr sorghum were not different in chemical composition except for lignin content, which was lower in sweet sorghum than in grain and bmr sorghum (Di Macro et al., 2009). The nutritive value of feed stuffs is determined by the concentrations of its chemical composition, as well as their rate and extent of digestion (Getachew et al., 2004). In last decade, the *in vitro* gas production has widely been used to investigate feed degradation (Huhtanen et al., 2008), and as the method which provided less expensive, rapid assessment, and initial screening of nutritional value in ruminant feeds (Hossain and Becker, 2002; Getachew et al., 2004). With increased utilization of *Sorghum*

plants, little studies have been conducted to evaluate its feeding value and ruminal fermentation characteristics among different *Sorghum* cultivars.

For the above mentioned, the agronomic characteristics of different *Sorghum* plants under salinity conditions can provide valuable information in physiological mechanisms associated with resistance to salt stress, which is important for *Sorghum* plant breeding programmes. Little is known about different features of *Sorghum* types; namely sorghum-sudangrass hybrids, sweet sorghum, grain sorghum, and sudangrass under salinity stress. For this reason, the seedlings of *Sorghum* plants were established in order to investigate the adaptation of whole plant and response to salt salinity. Therefore, it is needful to clarify the growth, ion distribution, and physiological features as comparing criteria in different cultivars of *Sorghum* under salt stress as well as the importance of *Sorghum* plants as forage crop in terms of the feeding value for livestock. The majority studies of thesis are as follows:

**First experiment: Comparison of Young Seedling Growth and Sodium Distribution among *Sorghum* Plants under Salt Stress,**

**Second experiment: Physiological Response, Sodium Distribution, and Essential Nutrient Absorption of *Sorghum* Plants under Salinity Stress,**

**Third experiment: Nutritional Evaluation and *In Vitro* Ruminal Fermentation of *Sorghum* Cultivars.**

# Chapter 2

# Comparison of Young Seedling Growth and Sodium Distribution among *Sorghum* Plants under Salt Stress

## I. Introduction

Soil salinity is an important constraint to plant growth, and is a limiting factor to crop production in arid and semi-arid regions around the world (Munns, 2002). Globally, a total land area of 831 million hectares is salt-affected (Asfaw, 2011). The yield of essential food and forage crops is limited by soil salinity in many regions of the world's land area, so genetic improvements to salt tolerance are essential to sustain global food production (Munns, 2010). Crop genotypes with increased salt tolerance are needed for stable cultivation, but the attempts to improve crop salt tolerance by conventional breeding programmes have been met with limited success. To achieve this goal by breeding and to select the desired traits in different genetic backgrounds, understanding of the complexities of the physiological and genetic mechanisms of salt tolerance is necessary (Munns et al., 2006).

Salt tolerance can be assessed in terms of yield, plant height, relative growth rate (RGR), and so on (Ashraf and Harris, 2004). It is usually assessed as the percentage of biomass production in saline versus control conditions (Munns, 2002). RGR has been considered to be a more suitable parameter for the comparison of growth among species or genotypes than absolute growth rate (Cramer et al., 1994) which was found in Gramineae plants in other studies such as wheat (El-Hendawy et al., 2005), maize (Azevedo Neto et al., 2004), and sorghum (Lacerda et al., 2005). Changes in RGR under salt stress could be attributed to enhanced or reduced salt effects on the net assimilation rate (NAR)

(physiological response) and/or leaf area ratio (LAR) (morphological response) depending on the variation of plant genotypes (Ishikawa et al., 1991; Bayuelo-Jiménez et al., 2003). Therefore, it would be possible to select salt tolerant plants based on these growth parameters.

The effect of salinity on plant growth varies with the plant genotype, ion toxicity, and growth environment. Plant growth responds to soil salinity in two contrasting phases (Läuchli and Grattan, 2007; Munns and Tester, 2008): First, in the rapid growth phase, which responds to the osmotic effect of salt, and secondly, in the slower growth phase, which responds to salt toxicity in the leaves. However, among all the effects of salinity, accumulation of $Na^+$ is the major cause of toxicity ion accumulation and damage in many cereal crops (Tester and Davenport, 2003).

*Sorghum* is a grain and fodder crop, that is moderately tolerant to salinity (Almodares and Sharif, 2007; Krishnamurthy et al., 2007). There are several types of *Sorghum* plants such as grain sorghum, forage or sweet sorghum, sudangrass, and sorghum-sudangrass hybrids (Iptas and Brohi, 2003), and they have been extensively used for forage production in salt-affected areas (Hedges et al., 1989; Begdullayeva et al., 2007; Khanum et al., 2010). The tolerance to high saline concentrations in *Sorghum* seems to vary with the genotype, and some studies revealed large genotypic differences in the tolerance to salinity of *Sorghum* (Maiti et al., 1994; Krishnamurthy et al., 2007). Several research studies have shown that salinity reduces the root and shoot growth of *Sorghum* seedlings (Lacerda et al., 2003; Netondo et al., 2004a; Shariat Jafari et al., 2009). Salt tolerances of *Sorghum* plants have also been associated with $Na^+$ concentrations in various plant tissues (Munns, 2002; Netondo et al., 2004a).

Yet, little is known about the differences in seedling growth characteristics with the *Sorghum* genotype under salinity. It is therefore necessary to investigate the salt tolerance of the seedlings of various *Sorghum* types, such as grain sorghum, sweet sorghum, sudangrass, and sorghum-sudangrass hybrids and also to investigate ion toxicity, mainly, $Na^+$ accumulation in *Sorghum* plant tissues. In this study, 22 *Sorghum* cultivars were cultivated by hydroponics with and without NaCl salinity to determine the plant growth, relative growth rate, and dry matter production and also to clarify the $Na^+$ distribution in different plant parts. The relationships among these responses were also examined for a more clear understanding.

## II. Materials and Methods

### 1) Plant materials and NaCl treatment

Twenty-two cultivars of *Sorghum* plants were used: 15 cultivars of sorghum-sudangrass hybrids, three of sweet sorghum, two grain sorghum [*Sorghum bicolor* (L.) Moench], and two sudangrass [*Sorghum sudanense* Stapf] which are shown in Table 2.1. The experiment was conducted in a greenhouse under natural light conditions at the Graduate School of Bioresources, Mie University, Japan, in June and July of 2010. The mean day and night temperatures during the experiment were 31°C and 23°C, respectively. Seeds were germinated on the surface of tap water in the plastic pots. Six days after germination (6 DAG), the seedlings at the second leaf age were transplanted into a hole in a styrene board placed on a 220L plastic container filled with a 100% strength of Kimura A culture solution containing (μM) 182 $(NH_4)_2SO_4$, 283 $K_2SO_4$, 365 $MgSO_4$, 548 $KNO_3$, 182 $KH_2PO_4$, 182 $Ca(NO_3)_2$, and 14 $FeO_3$ (Baba and Takahashi, 1958). At 13 DAG (fourth leaf age) salt treatment was started with 100 mM NaCl and at 21 DAG, the concentration of NaCl was increased to 150 mM, and the culture solution strength was adjusted to 150% nutrient

strength that is suitable and beneficial for growth. Then the plants were grown there until the end of the experiment (29 DAG). For the control group, the nutrient solution without NaCl was used. During the experiment, an air pump was used to supply enough air into the culture solution in both the control group and the treated plots. The culture solution was adjusted daily to pH6.5, using 1N $H_2SO_4$ or 1N KOH, and renewed every 3 days.

**2) Measurement of plant growth and growth analysis**

The plants were sampled two times: at 13 (before treatment), and 29 DAG. In each sampling, ten replicated plants for each cultivar in both the control and treatment groups were taken and carefully rinsed with distilled water. The plant samples were divided into three parts: leaf blade, stem (including the leaf sheath), and root. The leaf area was measured by using an automatic area meter (Hayashi Denko AAM-9, Japan). Dry weight was obtained after drying at 70°C for 72hr. Growth analysis was conducted according to Kevet et al. (1971) to determine RGR, NAR, LAR, and specific leaf area (SLA) at 13 and 29 DAG by the following equations:

$$RGR = (lnW_2 - lnW_1) / (t_2 - t_1)$$

$$NAR = [(W_2 - W_1) / (A_2 - A_1)] \times [(lnA_2 - lnA_1) / (t_2 - t_1)]$$

$$LAR = [(A_2 - A_1) / (InA_2 - InA_1)] \times [(lnW_2 - lnW_1) / (W_2 - W_1)]$$

$$SLA = [(A_2 - A_1) / (InA_2 - InA_1)] \times [(lnL_2 - lnL_1) / (L_2 - L_1)]$$

where $W_1$ and $W_2$ are the total dry weights in grams, $A_1$ and $A_2$ are the leaf area values, and $L_1$ and $L_2$ are the leaf dry weights in grams, obtained at times $t_1$ and $t_2$, respectively

**3) Nitrogen and sodium ion concentrations in different plant parts**

The dried samples ground into a powder were reduced to ash in a furnace (Yamato FO300, Japan), and then extracted with 1N $HNO_3$. In this extract, $Na^+$ concentration in each plant part was determined using ion chromatography with a conductivity detector (Shimadzu

CDD-6A, IC-C3, Japan). The amount of total nitrogen (N) was also analyzed by the semi-micro Kjeldahl method.

**4) Statistical analysis**

A statistical analysis was performed using Student's *t*-test: paired samples as means for all measurements. For the correlation among plant growth parameters, and $Na^+$ concentration, the correlation coefficients were determined for all pairs.

## III. Results

**1) Plant growth**

Fig. 2.1 shows the dry weight of *Sorghum* plants, both in control and treated groups at the end of the experiment (29 DAG). Salt stress significantly reduced the dry weight of plants in all cultivars ($P < 0.01$). Fig. 2.2 shows the relative value of the dry weight, which was calculated from the percentage of dry weight of the treated plants versus that of control; this is an indicator of salt tolerance. The relative value of the leaf dry weight was 67 – 26% showing a significant reduction ($P < 0.01$) under NaCl treatment. The stem dry weight was also reduced by NaCl treatment, but not as much as leaf dry weight (Fig. 2.2). The relative value of root dry weight exceeded 100% in 16 out of 22 cultivars, which shows that NaCl treatment increased the root dry weight in these cultivars. The relative value of the plant (whole plant) dry weight was lower than 100% in all cultivars showing weight reduction under salt stress. The relative value of the plant dry weight was less than 50% in HB5 and HB12, and highest in HB11 (Fig. 2.2). HB11 was the most salt-tolerant cultivar.

**2) Growth analysis**

Table 2.2 shows the results of multiple regression analysis between the relative value of the dry weight increment during NaCl treatment (from 13 to 29 DAG) of plant (whole

plant) (⊿W) and those of leaf blade (⊿LW), stem (⊿SW) and root (⊿RW). The partial regression coefficients, standard partial regression coefficients, and partial correlation coefficients of relationships between the relative values of ⊿W and those of ⊿LW, ⊿SW and ⊿RW are shown in this table. The standard partial regression coefficient was highest in ⊿SW (0.49), whereas, the partial regression coefficient was highest in ⊿LW (0.57). The partial correlation coefficient was significant in all plant parts. Fig. 2.3 shows the relationship between the relative value of root dry weight and that of shoot (stem and leaf blade) dry weight at 29 DAG, which was positively significant ($r = 0.64$, $P < 0.01$). The relative value of ⊿W in each cultivar is shown in Fig. 2.4. The mean of the relative value of ⊿W was 70%. Assuming that the cultivars showing a relative value higher than 70% are salt tolerant, nine cultivars: HB3 (Sudakkusu 316), HB7 (Brown toumitsu), HB8 (Lucky sorugo), HB10 (King sorugo), HB11 (Ryokuhiyou sorugo), SS17 (Supersugar sorghum), GS19 (Haiguren sorghum), GS20 (Mini sorghum), and SU22 (Oishii sudan), are tolerant to salt stress.

As shown in Fig. 2.5, the relative values of ⊿W significantly correlated with RGR ($r = 0.98$, $P < 0.01$). Fig. 2.6 shows the correlation of the relative value of RGR with that of NAR and LAR. The RGR significantly correlated with NAR ($r = 0.69$, $P < 0.01$), but not with LAR ($r = -0.22$, $P > 0.01$). There was a significant negative correlation between the relative value of NAR and that of specific leaf area (SLA) ($r = -0.65$, $P < 0.01$) as shown in Fig. 2.7A. In addition, there was a positive correlation between the relative value of NAR and that of nitrogen content per unit leaf area (NCLA) as shown in Fig. 2.7B ($r = 0.40$, $P < 0.10$).

**3) $Na^+$ concentration in different plant parts**

The $Na^+$ concentrations in different plant parts of *Sorghum* plants in the control and treated plants are shown in Table 2.3. A significant difference ($P < 0.01$) in $Na^+$

concentration (in whole plants) was observed between control and treated plants. The $Na^+$ concentration in almost all parts of the plant was higher in treated plants than in the control plants. The concentration was the highest in root followed by stem and leaf in both treated and control plants.

Fig. 2.8 shows the correlation of the relative values of dry weight of leaf, stem and root with the $Na^+$ concentration in leaf, stem and root, respectively, at the end of the treatment. The relative values of root dry weight was the highest followed by that of stem and leaf dry weight, but significant correlation with $Na^+$ content was not observed in all organs, even in root. Fig. 2.9 shows the correlation of the relative values of dry weight of leaf, stem and root with that of $Na^+$ concentrations in leaf, stem and root, respectively, at the end of the treatment. There was no correlation between the relative value of $Na^+$ concentration and dry weight of leaf and stem, but the relative value of the $Na^+$ concentration in root was positively correlated with that of root dry weight ($r = 0.44$, $P < 0.05$) (Fig. 2.9).

## IV. Discussion

The growth, growth rate and dry weight of *Sorghum* plants were obviously reduced under salt stress in the present experiment as in other studies (Lacerda et al., 2003; Shariat Jafari et al., 2009). In the present study using young seedlings of 22 cultivars of *Sorghum*, salt stress significantly inhibited plant growth in most of the cultivars (Fig. 2.1). The relative values of dry weight (% of dry weight under salt stress versus control condition, which represents salt tolerance) of whole plant, stem and leaf blade, especially leaf blade, were lower than 100%, but that of root dry weight was higher than 100% in more than half of the cultivars (Fig. 2.2). In other words, stem and leaf dry weights were decreased in all cultivars, but root dry weight of many cultivars was increased by salt stress (Fig. 2.2). However, there

was salt's effect on root growth under the NaCl treatment showing a decrease in root elongation in all cultivars compared with the control. This was in agreement with the report by Azevedo Neto et al. (2004) that the response of maize genotypes to salt stress was more substantial in the leaf than in the root. The pronounced effect of NaCl treatment on the leaf blade was also reported by Netondo et al. (2004a), in which the treatment with NaCl induced 67% greater decrease in dry weight in the young leaf blade than in the oldest leaf blade in grain sorghum varieties. Some studies showed that salt stress not only reduced the leaf elongation rate but also reduced the final leaf length and enhanced leaf senescence and injury in forage sorghum genotypes (Lacerda et al., 2003), and that reduction in shoot growth accounted for a reduction in leaf area and stunted shoot (Läuchli and Grattan, 2007).

Since the relative value of ⊿W relied on that of ⊿LW, ⊿SW and ⊿RW, the standard partial regression coefficients between relative values of ⊿W and that of each parameter were analyzed. As shown in Table 2.2, the standard partial regression coefficient of relationship between the relative value of ⊿W and that of ⊿SW (0.49) was the highest followed by that of ⊿LW and ⊿RW (r = 0.44 and 0.14, respectively). This implies that the effect of salt stress on ⊿SW was larger than that on ⊿LW and ⊿RW. Dry weight of *Sorghum* plants consists of the dry weight of stem (47%), leaf blade (31%) and root (21%), and was increased by cell division and enlargement at the growing point (Mccue and Hanson, 1990). However, the partial regression coefficient of relationships between the relative value of ⊿W and those of ⊿LW, ⊿SW and ⊿RW were the highest in ⊿LW (0.57) followed by ⊿SW (0.34) and ⊿RW (0.07). In other words, the dry weight of *Sorghum* plants decreased mainly due to the reduction in dry weight of leaf blade under the NaCl treatment. However, there was a strong relationship between ⊿LW and ⊿SW (data not shown), and the decrease in leaf dry weight may be attributed to that in stem dry weight.

The effect of NaCl treatment on the relationship between root dry weight and shoot dry weight may be important to elucidate the effect of salt stress on plant growth. In Fig. 2.3, the relative value of root dry weight positively correlated with that of shoot (stem and leaf) dry weight, and it was higher than 100% in 16 out of 22 *Sorghum* cultivars investigated. Therefore, the increase in root dry weight under salt stress can be considered due to the increase in shoot dry weight under NaCl treatment. This is inconsistent with the report by Läuchli and Epstein (1990) that salinity often reduces shoot growth more than root growth. On the other hand, NaCl stress reduced plant growth by a decrease in dry weight of both shoot and root in all maize genotypes although one cultivar was not affected by salinity (Azevedo Neto et al., 2004). The present results showed the same trend as that reported by Shariat Jafari et al. (2009), who concluded that the root/shoot ratio of *Sorghum* increased substantially under high salinity stress (at 240 mM NaCl), suggesting that most plants partition more assimilates to the roots rather than to the shoot under salt stress. Thus, it is assumed that the increase in root dry weight is one of the mechanisms for salt resistance among *Sorghum* plants.

The biomass reduction rate has been used as an index for salt tolerance (Shah et al., 1987) and the rate of biomass production normally correlates with yield (Munns, 2002). In addition, during the developmental stages of annual crops, the salt tolerance was usually based on relative growth reductions (Läuchli and Grattan, 2007). Thus, RGR was used to account for the change in total dry weight in the current experiment. In the present experiment, the relative of $\Delta W$ was positively correlated with that of RGR, meaning that the reduction in total dry weight is affected by the reduction of RGR in all *Sorghum* cultivars (Fig. 2.5).

In *Sorghum* plants, the relative value of RGR correlated with that of NAR, but not with that of LAR. It was therefore considered that RGR under salt stress of *Sorghum* plants is mainly correlated with NAR that is increased by the reduction of SLA (Figs. 2.6 and 2.7). According to Azevedo Neto and Tabosa (2000), RGR was one of the best parameters to express the salt stress effect on maize plants and also NAR and LAR were the best parameters to express the difference between cultivars in salt tolerance or salt sensitivity, suggesting that the NAR is a good physiological characteristic of salt tolerance in maize.

In the present experiment, there was a negative relationship between the relative values of NAR and SLA (Fig. 2.7A), that is, the leaf blade was thicker or had a lower SLA under the NaCl treatment. Cultivars that maintained comparatively higher NAR under salt stress had smaller SLA and were suggested to be salt-tolerant cultivars. Several studies showed that an increase in leaf thickness is associated with an increase in the ratio of mesophyll area available for the absorption of $CO_2$ to leaf area. In other words, the reduction in leaf area implies less assimilate production, and hence, the reduced plant growth (Burslem et al., 1996; Omami et al., 2006). The present results were consistent with those reported by Azevedo Neto et al. (2004) which showed that NAR of salt-tolerant maize was slightly higher than that of the sensitive genotype. Similarly, El-Hendawy et al. (2005) found that NaCl treatment reduced RGR and NAR, but did not affect LAR in wheat, and concluded that NAR was a more important factor than LAR in determining the salt tolerance of moderately tolerant and salt-sensitive genotypes. Some studies showed that NAR of rice was affected by leaf morphogenesis such as thinning of the leaf blade when nutrients were supplied sufficiently (Ehara et al., 1990; Ehara, 1993). According to Ehara (1993), two reactions in the photosynthesis occur when SLA affects NAR. First, the $CO_2$ diffusion resistance through the stomata is affected by the structural change in the leaf blade that is the increase in SLA

leads to an increase in stomatal resistance and a decrease in $CO_2$ conductance. Similar results on the relationship between changes in SLA and stomatal resistance were reported in wheat under NaCl treatment (Watanabe et al., 1992). Secondly, NAR is increased by the thickening of leaf blade through an increase in the nitrogen content per unit leaf area (NCLA). Based on these results in the present study, the difference in NAR under NaCl treatment would be attributed to that in SLA and NCLA as demonstrated by the nitrogen content of the leaf dry matter (Ehara et al., 1997).

In the present study, *Sorghum* plants under salt stress maintained a high $Na^+$ concentration in the roots and a lower $Na^+$ concentration in the stem and leaf blade (Table 2.3, Figs. 2.8 and 2.9). *Sorghum* plants under NaCl treatment might maintain a low $Na^+$ concentration in the leaf blade by retaining a higher concentration of $Na^+$ in the root and some in the stem. In the present experiment, the $Na^+$ concentration in the roots, was high suggesting no correlation with an increase in root dry weight, under NaCl treatment (Fig. 2.8C). The relative value of shoot (leaf + stem) dry weight also was not correlated with the $Na^+$ concentration in the shoot (Fig. 2.8A, B). These results are in agreement with those reported by Netondo et al. (2004a), which showed that *Sorghum* has the ability to maintain a high level of $Na^+$ in the roots and stem but allocates $Na^+$ to the leaf sheath for salt tolerance.

As shown in Fig. 2.2, root dry weight of *Sorghum* cultivars increased under NaCl treatment (relative value of dry weight was higher than 100%). Although the $Na^+$ concentration under NaCl treatment was higher in root (Table 2.3), the effect of NaCl stress on dry weight was most pronounced in leaf blade (Fig. 2.2). Cultivars that showed a larger increase in root dry weight under salinity had higher $Na^+$ concentration in root but lower $Na^+$ concentration in stem and leaf blade (Fig. 2.9). This result is in agreement with that of Rahnama et al. (2011) who concluded that the root of a bread wheat genotype showed a

positive response to moderate salinity (at 100 mM NaCl), but some genotypes decreased root biomass under higher level of NaCl treatment (200 mM NaCl); indicating that an increase in root biomass might be a main index of improvement of salt tolerance. Asfaw (2011) also reported that root dry weight of some *Sorghum* cultivars was increased under low levels of salinity. Studies on root branching under salt stress are limited but some authors indicated that the length and weight of primary root might be enhanced by moderate salinity (Kurth et al., 1986). In the present experiment, NaCl treatment reduced leaf dry weight in all the cultivars and increased root dry weight in 16 out of 22 *Sorghum* cultivars used, as well as in the other major crops of Poaceae. Nonetheless, in the present experiment, the physiological traits in relation to salt tolerance were not investigated. It is considered that salt tolerant cultivars could deposit excess $Na^+$ in the root. It is assumed that one of the mechanisms of salt tolerance of *Sorghum* plants is the tolerance of root to high internal $Na^+$ concentration.

In conclusion, *Sorghum* cultivars under NaCl treatment displayed reduced plant growth as demonstrated by decrease in dry weight, especially in leaf blade. Under salt stress, cultivars having high dry matter yield were recognized as tolerant to NaCl treatment although RGR was mostly decreased. RGR was correlated with NAR but not with LAR, which may be attributed to smaller SLA and thicker leaf blade under salt stress. *Sorghum* plants can retain $Na^+$ mainly in the roots, thereby, preventing the distribution of $Na^+$ to the leaf blade. A preferable leaf morphogenesis producing a thicker leaf blade and an apparent increase in root dry weight are main factors in the maintenance of dry matter yield and growth of *Sorghum* cultivars under NaCl treatment.

Table 2.1 Twenty-two cultivars of *Sorghum* plants used for the experiment.

| Number | Name of cultivar | *Sorghum* type[1)] | |
|---|---|---|---|
| 1 | Fain sorugo | Sorghum-sudangrass hybrid | (HB1) |
| 2 | Sudakkusu futushu | Sorghum-sudangrass hybrid | (HB2) |
| 3 | Sudakkusu 316 | Sorghum-sudangrass hybrid | (HB3) |
| 4 | Sudakkusu ryokuhiyou | Sorghum-sudangrass hybrid | (HB4) |
| 5 | Genki sorugo | Sorghum-sudangrass hybrid | (HB5) |
| 6 | Kumiai sorghum nyu 2 gou | Sorghum-sudangrass hybrid | (HB6) |
| 7 | Brown toumitsu | Sorghum-sudangrass hybrid | (HB7) |
| 8 | Lucky sorugo | Sorghum-sudangrass hybrid | (HB8) |
| 9 | Lucky sorugo 2 | Sorghum-sudangrass hybrid | (HB9) |
| 10 | King sorugo | Sorghum-sudangrass hybrid | (HB10) |
| 11 | Ryokuhiyou sorugo | Sorghum-sudangrass hybrid | (HB11) |
| 12 | Wind brake | Sorghum-sudangrass hybrid | (HB12) |
| 13 | BMR sweet (si-to) | Sorghum-sudangrass hybrid | (HB13) |
| 14 | Green sorugo | Sorghum-sudangrass hybrid | (HB14) |
| 15 | Tsuchi tarou | Sorghum-sudangrass hybrid | (HB15) |
| 16 | Kanmi sorghum | Sweet sorghum | (SS16) |
| 17 | Supersugar sorghum | Sweet sorghum | (SS17) |
| 18 | Koutoubun sorghum | Sweet sorghum | (SS18) |
| 19 | Haiguren sorghum | Grain sorghum | (GS19) |
| 20 | Mini sorghum | Grain sorghum | (GS20) |
| 21 | Summer baler hosokuki | Sudangrass | (SU21) |
| 22 | Oishii sudan | Sudangrass | (SU22) |

[1)] HB: sorghum-sudangrass hybrid, SS: sweet sorghum, GS: grain sorghum, SU: sudangrass.

Table 2.2 Partial regression coefficient, standard partial regression coefficient, and partial correlation coefficient of relationships between the relative values of increment from 13 to 29 DAG of plant dry weight (⊿W) and that of the leaf blade (⊿LW), stem (⊿SW), and root (⊿RW).

| Parameters | Partial regression coefficient [1)] | Standard partial regression coefficient | Partial correlation coefficient |
|---|---|---|---|
| ⊿LW | 0.5729 | 0.4388 | 0.9755** |
| ⊿SW | 0.3365 | 0.4910 | 0.9754** |
| ⊿RW | 0.0749 | 0.1432 | 0.9056** |

[1)] Y = (0.5729) (⊿LW) + (0.3365) (⊿SW) + (0.0749) (⊿RW) + 4.6194.

** indicates significant differences at the 0.01 probability level by multiple regression analysis.

Table 2.3 $Na^+$ concentration in different plant parts of *Sorghum* in the control and treated plants.

| No[1)] | $Na^+$ concentration (μmol $g^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | | | Treatment | | | |
| | Leaf | Stem | Root | Whole | Leaf | Stem | Root | Whole |
| HB1 | 10.3 | 20.6 | 26.6 | 16.1 | 190 | 537 | 798 | 467 |
| HB2 | 15.0 | 16.9 | 21.4 | 16.5 | 184 | 419 | 1003 | 462 |
| HB3 | 6.2 | 11.5 | 19.8 | 9.7 | 160 | 467 | 1165 | 500 |
| HB4 | 6.3 | 13.2 | 19.2 | 10.3 | 224 | 406 | 1166 | 517 |
| HB5 | 6.4 | 16.6 | 19.5 | 11.6 | 187 | 581 | 1031 | 561 |
| HB6 | 5.2 | 17.3 | 18.6 | 11.2 | 158 | 536 | 1021 | 537 |
| HB7 | 8.0 | 14.5 | 20.0 | 11.6 | 282 | 651 | 1047 | 615 |
| HB8 | 9.3 | 12.9 | 20.4 | 11.8 | 217 | 666 | 911 | 574 |
| HB9 | 8.8 | 13.9 | 19.1 | 11.8 | 286 | 611 | 863 | 568 |
| HB10 | 7.7 | 22.3 | 24.2 | 14.6 | 248 | 614 | 1017 | 569 |
| HB11 | 7.4 | 9.1 | 17.9 | 9.7 | 236 | 585 | 1092 | 571 |
| HB12 | 5.0 | 8.8 | 21.7 | 8.8 | 198 | 289 | 981 | 418 |
| HB13 | 9.0 | 9.5 | 22.9 | 10.9 | 294 | 677 | 789 | 578 |
| HB14 | 6.6 | 10.4 | 19.1 | 9.5 | 215 | 506 | 1108 | 544 |
| HB15 | 4.8 | 9.5 | 11.8 | 7.3 | 319 | 606 | 948 | 594 |
| SS16 | 11.1 | 14.0 | 15.7 | 12.6 | 234 | 493 | 1164 | 565 |
| SS17 | 5.8 | 9.0 | 17.8 | 8.3 | 207 | 489 | 1123 | 552 |
| SS18 | 6.2 | 10.9 | 21.3 | 9.7 | 232 | 484 | 1014 | 521 |
| GS19 | 6.3 | 12.5 | 17.0 | 9.5 | 248 | 455 | 1182 | 554 |
| GS20 | 5.6 | 9.7 | 15.0 | 7.9 | 211 | 482 | 808 | 446 |
| SU21 | 8.8 | 20.6 | 17.9 | 14.2 | 401 | 594 | 940 | 615 |
| SU22 | 5.7 | 17.5 | 22.5 | 12.2 | 296 | 656 | 945 | 579 |
| Average | 7.5 | 13.7 | 19.5 | 11.2 | 238 | 536 | 1005 | 541 |
| Maximum | 15.0 | 22.3 | 26.6 | 16.5 | 401 | 677 | 1182 | 615 |
| Minimum | 4.8 | 8.8 | 11.8 | 7.3 | 158 | 289 | 789 | 418 |
| Significance | | | | | ** | ** | ** | ** |

[1)] HB: sorghum-sudangrass hybrid, SS: sweet sorghum, GS: grain sorghum, SU: sudangrass.

** indicates significant differences in the values between the control and treated plants at 0.01 probability level by Student's *t*-test.

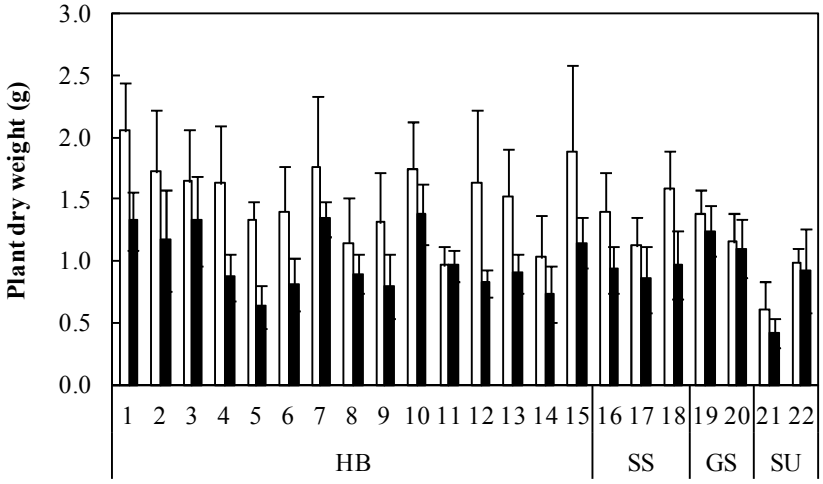


Fig. 2.1 Plant dry weight in all the cultivars in the control and the treated plants at the end of the experiment. HB: sorghum-sudangrass hybrid, SS: sweet sorghum, GS: grain sorghum, SU: sudangrass. □ , control group; ■ , treatment. The difference in the dry weight between the control and treated plants was significant at the 0.01 probability level by Student's *t*-test. Error bars in the figures indicate the mean standard deviation (STDEV).

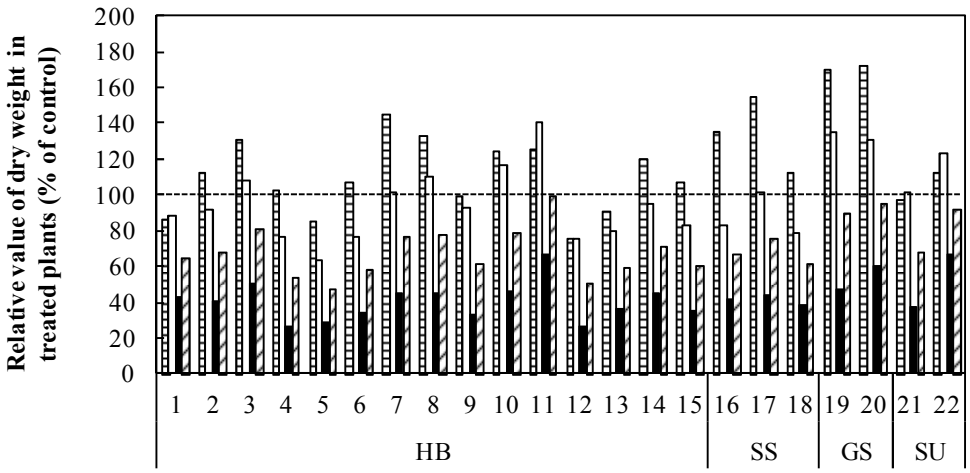


Fig. 2.2 Relative value of dry weight of different plant parts in the treated plants at the end of the experiment. ▤ , root; □ , stem; ■ , leaf blade; ▨ , whole plant. Relative value was expressed as % of dry weight under salt stress to that in the control: (treated/control) × 100.

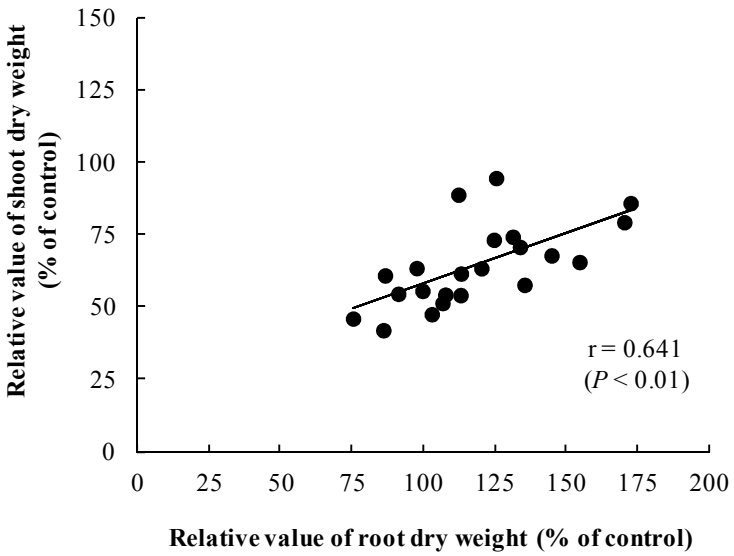


Fig. 2.3 Relationship between the relative value of root dry weight and that of shoot (stem and leaf) dry weight at 29 DAG.

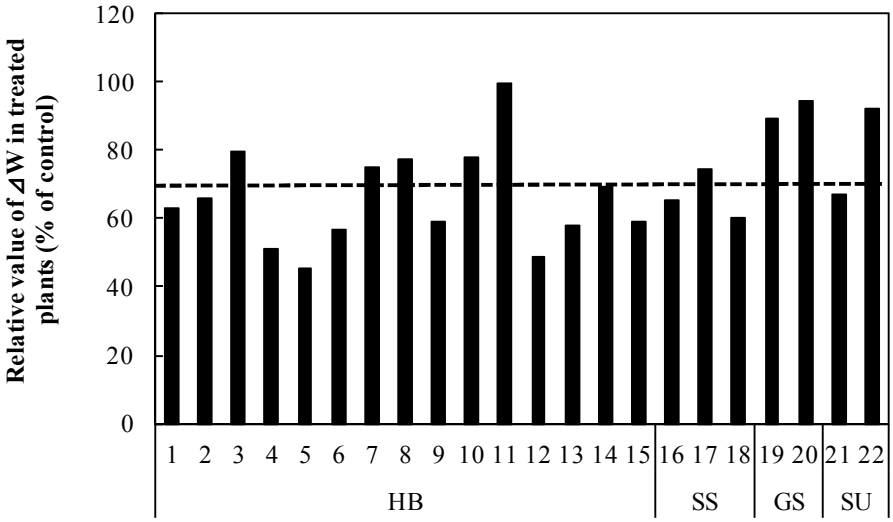


Fig. 2.4 Relative value of dry weight increment (⊿W) from 13 to 29 DAG in each cultivar. The horizontal broken line at 70% indicates the mean of the relative value of ⊿W. The difference in the ⊿W between the control and treated plants was significant at the 0.01 probability level by Student's *t*-test.

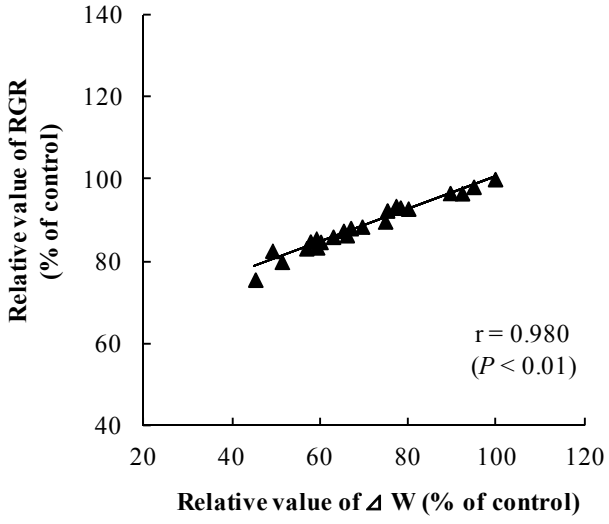


Fig. 2.5 Relationship between the relative value of dry weight increment (⊿W) and that of the relative growth rate (RGR) from 13 to 29 DAG.

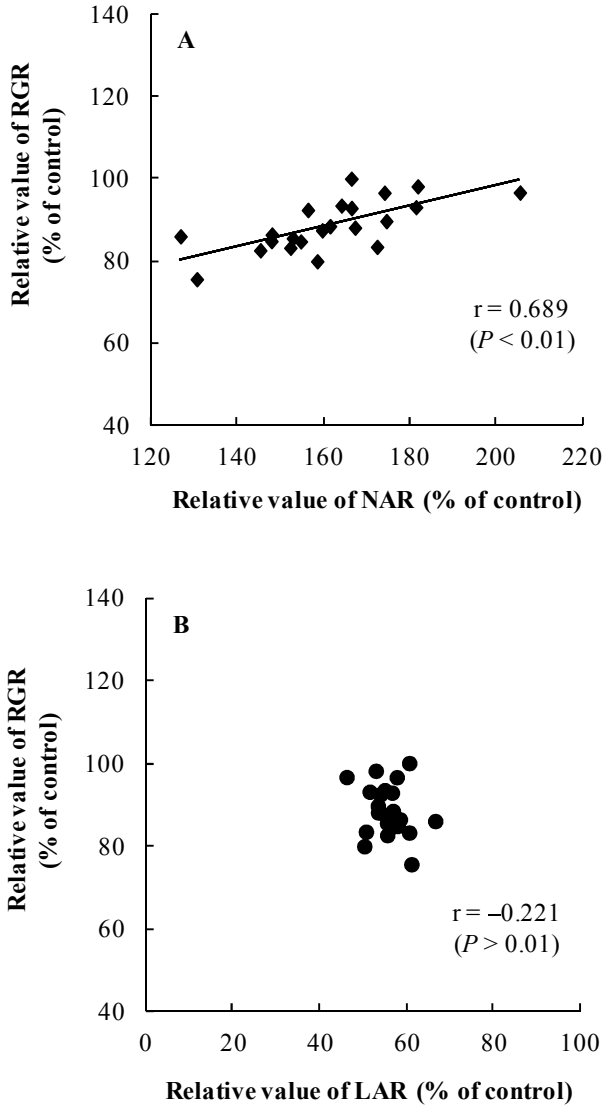


Fig. 2.6 Correlation of the relative value of relative growth rate (RGR) with that of [**A**], net assimilation rate (NAR), and [**B**], leaf area ratio (LAR) from 13 to 29 DAG.

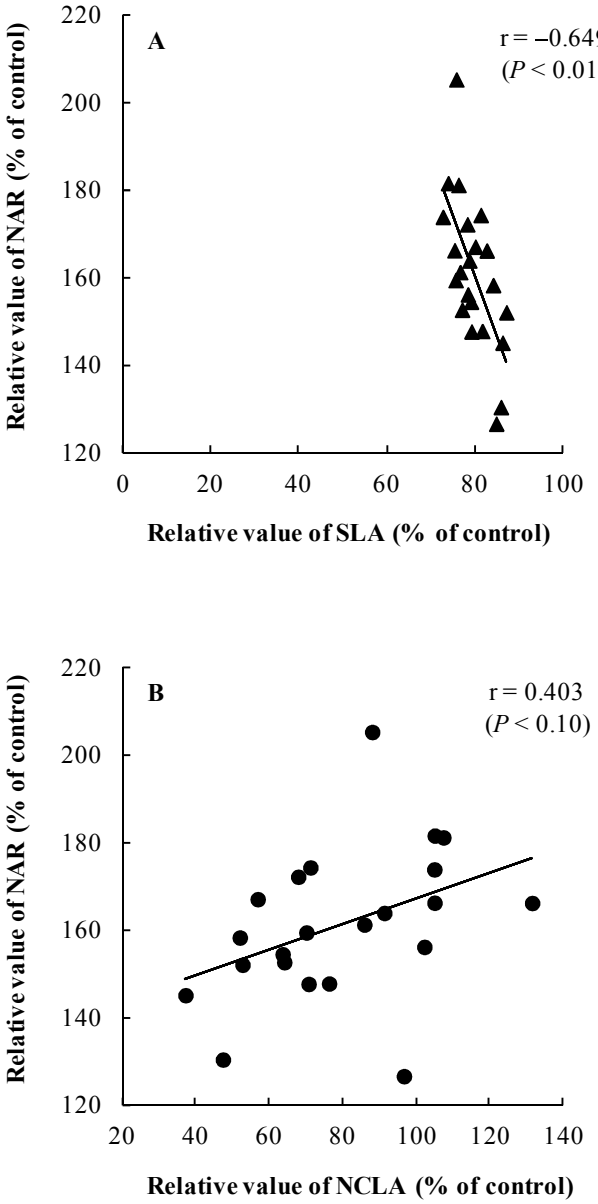


Fig. 2.7 Correlation of the relative value of net assimilation rate (NAR) with that of [**A**], specific leaf area (SLA), and [**B**], nitrogen content per unit leaf area (NCLA) from 13 to 29 DAG.

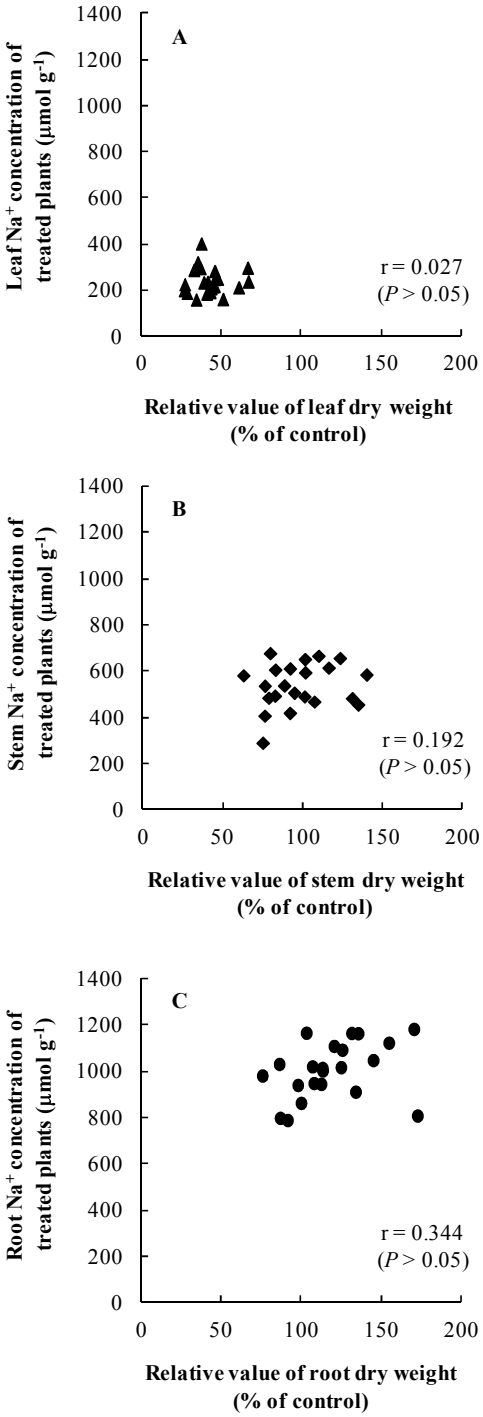


Fig. 2.8 Correlation of the relative value of dry weight of leaf, stem and root with the $Na^+$ concentration (μmol $g^{-1}$) in [**A**], leaf; [**B**], stem and [**C**], root, respectively, at 29 DAG.

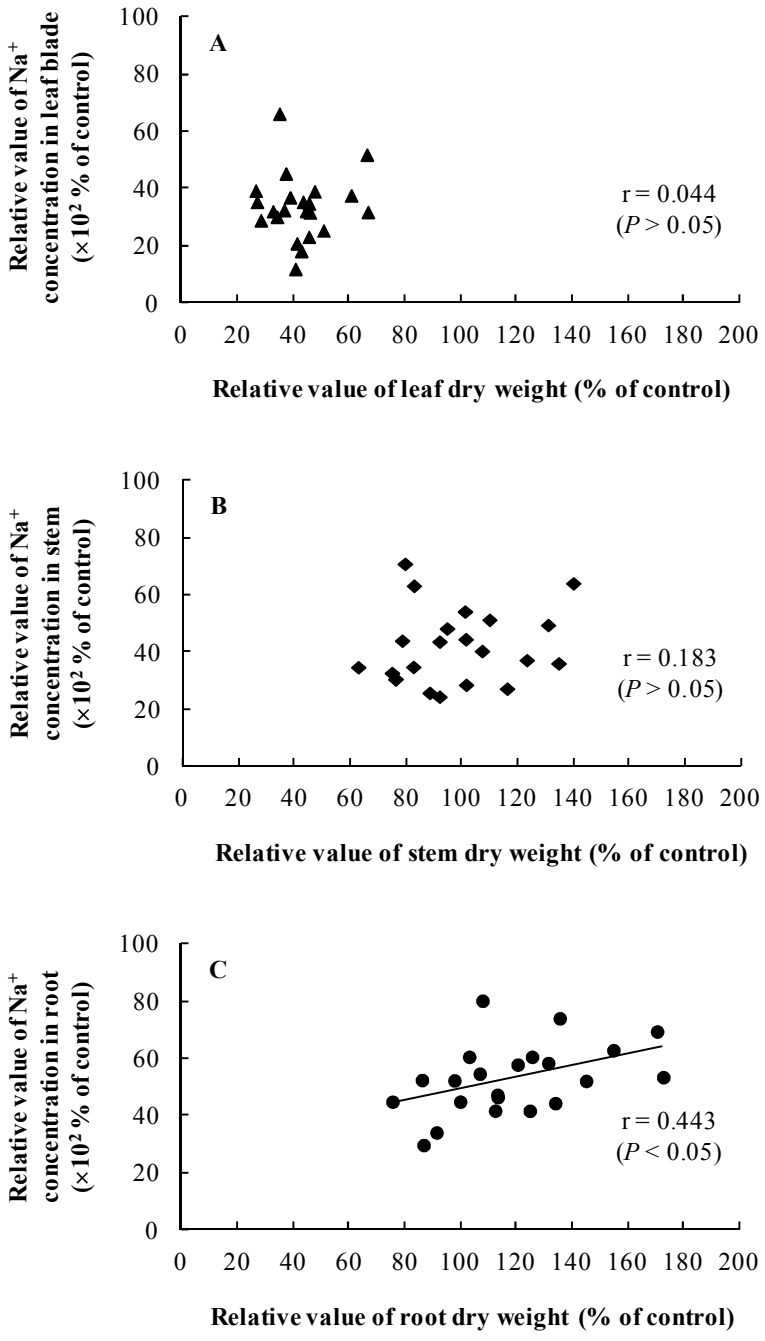


Fig. 2.9 Correlation of the relative values of dry weight of leaf, stem and root with those of $Na^+$ concentration in [**A**], leaf; [**B**], stem and [**C**], root, respectively, at 29 DAG.

# Chapter 3

# Physiological Response, Sodium Distribution, and Essential Nutrient Absorption of *Sorghum* Plants under Salinity Stress

## I. Introduction

Salinity is one of the severely abiotic stresses affecting a decrease in the growth rate of plants and can extremely limit the productivity of crop plants in large areas of the world's cultivated land (Plett and Møller, 2010; Tavakkoli et al., 2011). Salt's effect on plant response is associated with osmotic stress by reducing the ability of plants to take on water, ion toxicity and nutritional imbalance in cell, and this rapidly causes reduction in the rate of cell expansion in growing tissues and consequently, leads to decrease in photosynthetic efficiency and variously physiological disorders (Munns, 2002; Almodares et al., 2011). Salt resistance generally can be determined by assessment of the plant survival based on the rate of biomass production that is related with decrease in yield productivity (Munns, 2002). Furthermore, determination of physiological features to salinity could be contributed to a better understanding of complex phenomenon for salt stress resistance of plants (Naidoo et al., 2008; Munns, 2010). There have been several evidences reported that salt salinity reduced photosynthesis in salinized plants by the decrease of stomatal conductance or available $CO_2$ fixation and/or by the cumulative effects of non-stomatal factor (Sultana et al., 1999; Lu et al., 2009; Tavakkoli et al., 2011). Therefore, understanding of the mechanisms to salt salinity is needful or useful to improve crop for salt resistance and, importantly, could lead to increased productivity from plants growing in challenging environments (Tavakkoli et al., 2011).

Several studies have investigated the morphological and physiological characteristics to salinity stress under controlled conditions and also have focused on the accumulation of specific toxic ions, plant water relations, and photosynthesis in several monocotyledonous plants. Particularly, the major cereal crops of Poaceae for salt resistance, such as rice (Sultana et al., 1999), wheat (Fercha, 2011), barley (Khosravinejad et al., 2008), maize (Shahzad et al., 2012), and sorghum (Yan et al., 2012) have been extensively reported. Besides, the ability of resistance to salt salinity differs widely between species of plants even within a single species (Plett and Møller, 2010).

*Sorghum* is considered as moderately resistant to salinity (Maas et al., 1986; Almodares and Sharif, 2007). When *Sorghum* plants were grown under salinity conditions, Netondo et al. (2004b) studied that the remarkable reduction of total plant leaf area affected whole plant photosynthesis, contributing to the low biomass production. As an important component of major ions, salt sensitivity in *Sorghum* has been related to reduction in the accumulation of essential nutrients in leaf such as $K^+$ and $Ca^{2+}$, and/or concentration of specific toxic ions, particularly $Na^+$ (Lacerda et al., 2003; Netondo et al., 2004a). Most research also concluded that ion partitioning in different tissues contributed to the improved salt resistance of plant genotypes (Krishnamurthy et al., 2007; Rahnama et al., 2011). Nonetheless, the resistance to high saline soils in *Sorghum* plant seems to be related to the ability and variation of plant genotypes. The presence of large genotypic variations for salt resistance to salinity was found in *Sorghum* (Maiti et al., 1994; Krishnamurthy et al., 2007), therefore, the varietal differences in salt resistance of *Sorghum* plants used in this study would be affected on differential responses of physiological features.

As for the previous study, the results displayed that *Sorghum* cultivars could grow and survived under the 150 mM NaCl treatment in hydroponics for 29 days after germination

(DAG) (Chaugool et al., 2013). In the present study, few cultivars of *Sorghum* plants were selected to clarify difference in the physiological features whether the salt resistant cultivars or the salt sensitive ones. Young seedlings of three *Sorghum* cultivars selected were performed in hydroponics assembly under salinity conditions or without the NaCl treatment in order to clarify the effect of $Na^+$ distribution on absorption of element nutrients and some physiological traits which consisted of transpiration rate, stomatal conductance, and leaf water potential.

## II. Materials and Methods

### 1) Plant materials and growth conditions

The experiment was conducted in a greenhouse under natural light conditions at the Graduate School of Bioresources, Mie University, Japan, in August 2011. Three cultivars of *Sorghum* plants were selected, consisting HB2 as salt sensitive cultivar, and HB10 and GS20 as salt resistant ones (Table 3.1). Seeds were germinated on the surface of tap water in the plastic pots. The seedlings at 8 DAG or the second leaf stage were transplanted into a hole in a styrene board placed on an 80L plastic container filled with a 150% strength of Kimura A culture solution containing (μM) 182 $(NH_4)_2SO_4$, 283 $K_2SO_4$, 365 $MgSO_4$, 548 $KNO_3$, 182 $KH_2PO_4$, 182 $Ca(NO_3)_2$, and 14 $FeO_3$ (Baba and Takahashi, 1958). At 17 DAG or the fifth leaf stage, salt treatment was applied with 150 mM NaCl. The plants were grown until the end of the treatment (25 DAG or the seventh leaf stage). The nutrient solution without NaCl was used for the control. An air pump was supplied for 24hr to provide enough air into the nutrient solutions into both the control and treated plots throughout the experimental period. The culture solution was daily adjusted to pH6.5 by adding either 1N $H_2SO_4$ or 1N KOH, and renewed every two days.

**2) Measurements**

Measurements of plant growth, transpiration rate, stomatal conductance, and leaf water potential were determined at the end of the experiment (25 DAG or the seventh leaf stage).

**2.1 Plant growth**

Four replicated plants for each cultivar in both the control and treatment groups were corrected and carefully rinsed with distilled water. In each plant, the plant was separated into three parts: leaf blades, stem (including the leaf sheath), and roots. Dry weight was obtained after drying at $70^{o}$C for 72hr.

**2.2 Ion concentrations in different plant parts**

The dried samples ground into a powder were reduced to ash in a furnace (Yamato FO300, Japan), and then extracted with 1N $HNO_3$. In each plant part, concentrations of $Na^{+}$, $K^{+}$, $Ca^{2+}$, and $Mg^{2+}$ were measured using high performance liquid chromatography (HPLC) (Shimadzu CDD-10A, Japan).

**2.3 Transpiration rate**

The amount of transpiration of each plant per day was measured by weighing the whole pot including the *Sorghum* plant and the culture solution. For each cultivar, two seedlings were used and transferred into a 1/10,000a Wagner pot filled with a 1L of culture solution in both the control and treatment groups. Each pot was tightly sealed with a plastic sheet during the measurement for 6 hr between 10:00 and 16:00 h. Then, each container with grown plants was weighed once again to calculate the transpiration rate as the plant weight loss which was divided by leaf area.

### 2.4 Stomatal conductance

Three replicated plants from fully expanded leaves were used for analyzing the stomatal conductance and leaf water potential. Measurement of stomatal conductance was performed between 10:00 and 16:00 h. A portable leaf porometer (Decagon devices, Model SC-1, USA) was used to take measurement and made on upper (adaxial) surface of leaves.

### 2.5 Leaf water potential

Leaf water potential was measured with plant moisture stress by using the pressure chamber (PMS Instrument Company, Model 600, U.S.A). The leaf blade determined was cut closed to the leaf sheath and immediately put it in the chamber to measure the plant moisture stress. The measurement was carried out during midnight.

**3) Statistical analysis**

The data between the control and treatment groups were analyzed statistic using Student's *t*-test: paired samples as means. Analysis of variance (ANOVA) was performed on the parameters of the treated groups to determine the significant difference using SAS program and then tested the significance by using least significant difference (LSD).

## III. Results

**1) Plant growth**

The plant dry weight in different plant parts of the control and the treated plants at the end of experiment (25 DAG) is shown in Fig. 3.1. The plant (whole plant) dry weight of the treated plants was significantly decreased under the NaCl treatment ($P < 0.05$). Salt stress obviously reduced dry weights of the leaf blade and the stem ($P < 0.05$) but it was not affected on the roots. The salt resistant cultivars both HB10 and GS20 showed higher plant dry weight than salt sensitive one (HB2) (Fig. 3.1B). Fig. 3.2 shows the relative value of dry

weight in different plant parts of two sorghum-sudangrass hybrids (HB2 and HB10). The relative value was calculated from the percentage of dry weight of the treated plants versus that of the control, showing that the relative value of plant dry weight of HB10 was higher than that of HB2.

**2) Ion concentrations in different plant parts**

The relative value of $Na^+$ concentration in different plant parts of the treated plants under the NaCl treatment at 25 DAG is shown in Fig. 3.3. There was significant difference of the $Na^+$ concentration between the control and treated plants in the leaf blade, stem ($P < 0.01$), and roots ($P < 0.05$). The $Na^+$ concentration of the treated plants obviously increased in all plant tissues showing the highest $Na^+$ in the stem of all cultivars. The amount of $Na^+$ was the largest in all organs of salt sensitive (HB2). In contrast, HB10 tended to be less amount of $Na^+$ in the leaf blade.

Fig. 3.4 shows the relative value of $K^+$ concentration in different plant parts of the treated plants at 25 DAG. A significant decrease in $K^+$ concentration of the treated plants was observed in all tissues, compared to the control plants. The $K^+$ was high amount in the stem and leaf blade in all cultivars. Fig. 3.5 shows the relative value of $Ca^{2+}$ concentration in different plant parts of the treated plants at 25 DAG. Salt stress significantly reduced $Ca^{2+}$ concentration in the leaf blade and the stem ($P < 0.05$), but it was not significantly affected in the roots. The relative value of $Mg^{2+}$ concentration in different plant parts of the treated plants under salt treatment at 25 DAG is shown in Fig. 3.6. The concentration of $Mg^{2+}$ in the treated plants significantly decreased in the stem ($P < 0.05$) but it was not apparently significant in the leaf blade and the roots.

The $K^+$, $Ca^{2+}$, and $Mg^{2+}$to $Na^+$ ratios in different plant parts of the treated plants are shown in Fig. 3.7. The $K^+/Na^+$ ratio was the largest in the leaf blade, followed by the stem

and the roots (Fig. 3.7A). The highest $K^+/Na^+$ ratio was observed in all plant tissues of HB10, especially in the leaf blade and stem. In contrast, it was the lowest ratio in HB2, particularly in the leaf blade. The $Ca^{2+}/Na^+$ showed larger ratio in the roots and leaf blade, respectively (Fig. 3.7B). As for the $Mg^{2+}/Na^+$, the ratio was the largest in the leaf blade and was observed in HB10 (Fig. 3.7C).

**3) Transpiration rate**

The relative value of transpiration rate of the treated plants at the end of experiment is shown in Fig. 3.8. Salt stress significantly reduced transpiration rate in all cultivars ($P < 0.1$) compared with the control. Transpiration rate was highly decreased in both salt resistant cultivars and it was the lowest in HB10, whereas HB2 was not decreased as much as salt resistant.

**4) Stomatal conductance**

Fig. 3.9 shows stomatal conductance of the control and treated plants at 25 DAG. A significant reduction of stomatal conductance of the treated plants in all cultivars was due to the NaCl treatment ($P < 0.1$). In the treated plants, stomatal conductance was lower in both salt resistant cultivars.

**5) Leaf water potential**

The relative value of leaf water potential of the treated plants at 25 DAG is shown in Fig. 3.10 showing a significant reduction by NaCl treatment in all cultivars ($P < 0.05$), in comparison with the control. Leaf water potential was largely decreased in HB10.

## IV. Discussion

In the present study, young seedlings of three *Sorghum* cultivars were selected and evaluated under the NaCl treatment, which were considered as salt resistant cultivars: HB10

and GS20, and salt sensitive one: HB2 according to the previous study (Chaugool et al., 2013). The previous results showed that the plant dry weight of HB2 and HB10 was almost same under control conditions. But the plant dry weight of HB10 was apparently larger than that of HB2 under salt stress. While GS20 displayed that the dry weight of the control and treated plants was not largely different. The present study demonstrated that the NaCl treatment caused a significant difference in the plant growth of *Sorghum* plants (Fig. 3.1). Salt stress obviously affected by a large reduction in dry weight of leaf blade, but it was not apparent decrease in dry weight of root. Salt resistant cultivars performed higher plant production than salt sensitive one. The result was in agreement with several studies which reported that an increase in the root dry weight of plants could be occurred at moderate level of salinity (Hameed and Ashraf, 2008) and could be no significant effect in root fresh weight even under salt stress (Shahzad et al., 2012).

As for the distribution of ions, *Sorghum* plants maintained higher amount of $Na^+$ in the stem (including the leaf sheath) to prevent $Na^+$ influx more to the leaf blade (Fig. 3.3). In addition, salt resistant cultivars represented as HB10 and GS20 appeared storing lesser amount of $Na^+$ than salt sensitive one (HB2) in all plant tissues. It suggests that salt resistance of *Sorghum* cultivars in this study involved to the ability of assorting partition $Na^+$ into the stem and difference in $Na^+$ distribution of *Sorghum* can vary even though the plants that are same within plant species. The distribution of $Na^+$ can be accounted for that the main site of $Na^+$ toxicity for plants is the expanded leaves in which $Na^+$ accumulates after entering the root and transferring to the shoot in the transpiration steam (root xylem loading) and the preferential accumulation of $Na^+$ in the leaf sheath versus the leaf blade (Munns, 2002; Davenport et al., 2005). The result was consistent with Naidoo et al. (2008), who reported that $Na^+$ concentration of highly salt-tolerant grass increased with salinity increase

in both the roots and the shoots. In monocotyledonous species, the leaf is composed of the blade and the sheath which the bulk of the leaf's photosynthesis and transpiration occurs in the leaf blade (James et al., 2006). Also, elongation leaf tissue is a small region located near the leaf base, requires a continuous supply of nutrients to maintain cell expansion and is, therefore, highly susceptible to nutrient disturbances (Bernstein et al., 1995). When excessive amounts of salts enter the plant, salts will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and reduce the photosynthetic leaf area of the plant to a level that cannot sustain growth (Munns, 2002).

Salt resistance not only involves adaptation to $Na^+$ influx, but also acquisition of $K^+$, whose uptake is adversely affected by high external $Na^+$ concentration, due to the chemical similarity of these two ions (Naidoo et al., 2008). In addition to $K^+$ absorption, the uptake of essential nutrients such as $Ca^{2+}$ and $Mg^{2+}$can be also affected under saline soils due to the effect of ion selectivity (El-Hendawy et al., 2005). Under saline conditions, due to excessive amounts of exchangeable $Na^+$, high $Na^+$ to $K^+$ and $Ca^{2+}$ ratios can occur in the soils. Plants subjected to such environments, take up high amount of $Na^+$, whereas the uptake of $K^+$ and $Ca^{2+}$ is considerably reduced (Ashraf, 2004). Much study has been written about the importance of the ability of plants to discriminate between $Na^+$ and $K^+$, for which a simple index, thus, the $K^+/Na^+$ ratio can be determined in the whole plants and/or different plant parts for response to salt stress (Flowers, 2004).

As shown in Figs. 3.4-3.6, the accumulation of $K^+$ retained mainly in the stem and the leaf blade, whereas $Ca^{2+}$ and $Mg^{2+}$ maintained high amount in the leaf blade and the roots. The trend of ion partition among the cultivars, the $K^+/Na^+$ ratio was apparently large in the leaf blade and stem of salt resistant cultivars, especially in HB10 (Fig 3.7). The results in this study were considered that the restriction of $Na^+$ concentration into young leaf blades

and differences in ion partitioning by which *Sorghum* cultivars maintained higher $K^+/Na^+$ ratio in the shoot (leaf and stem) play an important role in physiological processes for resistance to salt stress (Wei et al., 2003; El-Hendawy, et al., 2005; Rahnama et al., 2011). The results were similar with of Rahnama et al. (2011), showing that $K^+$ concentration in wheat was decreased in roots and salt-tolerant genotypes maintained lower $Na^+$ concentration with higher $K^+/Na^+$ ratio in flag leaf blade. Wei et al. (2003) studied on the mechanism of ion partitioning in two near isogenic barley cultivars in response to increasing salinity, reporting that relatively salt tolerant cultivar maintained significantly lower $Na^+$ concentrations in young expanding tissues, but higher $K^+$ and $Ca^{2+}$ to $Na^+$ ratios in the young leaf blade and young sheath tissues than relatively salt sensitive one. The higher ratios of $Na^+$ to $K^+$, $Ca^{2+}$, and $Mg^{2+}$ under salt treatment reasonable that $K^+$, $Ca^{2+}$, and $Mg^{2+}$ transport is impaired by $Na^+$ and might disturb plant metabolism and reduce plant growth (Shahzad et al., 2012).

In addition to plant growth and ion distribution, measurements of major physiological traits can be used to observe non-instantaneous plant responses to salt stress (Belkhodja et al., 1994). As for the salt's effect on plant responses associated with alteration in water status, measurements of transpiration rate, stomatal conductance and leaf water potential were observed. In the present study, measurement of transpiration rate was carried out under natural sunlight, thus the amount of transpiration per plant could vary depending on changes of the temperature and humidity. Transpiration rate of the treated plants in all cultivars decreased under the NaCl treatment (Fig. 3.8) which supported by other studies (Netondo et al., 2004b). Comparing in sorghum-sudangrass hybrid types between HB2 (salt sensitive) and HB10 (salt resistant), HB10 have much reduction in transpiration rate, but it was lesser decreased in HB2 (Fig. 3.8). In addition, the reduction of stomatal conductance and leaf

water potential was apparently larger in HB10 than that of HB2 (Figs. 3.9 and 3.10). As the plant (whole plant) dry weight of HB10 was higher than that of HB2 in all plant tissues, especially in the leaf blade (Fig. 3.2), assuming that HB10 needed much water to maintain the plant water status. However, usually the water absorption under salt stress will be difficult or deficiency in all the cultivars. Therefore, HB10 had a high restriction of transpiration rate to prevent water loss as demonstrated by a decrease in stomatal conductance.

Under the NaCl treatment, salt resistant cultivars in this study displayed an increase in leaf thickness (data not shown). It assumes that a thicker leaf blade is associated with an increase in the ratio of mesophyll area available for the absorption of $CO_2$ to leaf area (Burslem et al., 1996). In addition, a high assimilation of $CO_2$ might be due to high $K^+$ concentration in leaf blade which was attributed to leaf stomatal conductance (Bayuelo-Jiménez et al., 2003). The $K^+$ is essential nutrient for cell enlargement, especially the young leaf blade, and relates to the maintenance of cell turgor, leaf stomatal regulation and water status in the plants (Bayuelo-Jiménez et al., 2003). Also, $K^+$ is specifically required for protein synthesis (Chow et al., 1990). The preference to accumulate $K^+$ might contribute to a better regulation of stomatal opening to achieve the normal regulation of turgor under salt stress and might maintain steady state photosynthetic rates (Bayuelo-Jiménez et al., 2003).

Such reduction of transpiration rate and stomatal conductance of salt resistant cultivars in this study might be attributed to smaller in stomatal aperture to restrict the water loss and maintain the water status in the plant body, under the NaCl treatment. Plants grown under salt stress, normally the closure of the stomata could be involved to the reduction of internal $CO_2$ concentration and $CO_2$ assimilation rate (Dionisio-Sese and Tobita, 2000). Several studies reported that the stomatal aperture might be small under salt stress to reduce

the transpiration rate and consequently resulted to the decrease in the photosynthetic rate (Ehara et al., 2008; Prathumyot et al., 2011). However, the results in this study were indicated that reduction of transpiration rate and stomatal conductance of salt resistant cultivars was attributed to an increase in leaf thickness. A thicker leaf blade could support high $CO_2$ assimilation rate to the leaf area and maintained plant growth under the NaCl treatment, even though stomatal conductance decreased.

A decrease in leaf water potential might be involved the reduction in stomatal conductance, resulting in turgor loss, and plants suffered from the restricted water availability of cells and leading to reduced photosynthetic rate (Sultana et al., 1999; Munns, 2002). Leaf water potential was adversely affected by salt stress which was supported by several studies in other plants of Poaceae (Netondo et al., 2004a; Hameed and Ashraf, 2008). In most response of plants to salinity, it is due to the disturbed water relations and the local synthesis of abscisic acid in the photosynthetic tissues (Munns and Tester, 2008). Netondo et al. (2004b) studied that salinity affected photosynthesis per unit leaf area of sorghum indirectly through stomatal closure and to a smaller extent through direct interference with the photosynthetic apparatus. A positive correlation between stomatal conductance and $CO_2$ assimilation rate was also reported, thus, it was suggested stomatal conductance as the primary factor limiting photosynthesis under salt stress. This was in similar with several studies; reporting that a decrease in photosynthetic rate of sorghum could be ascribed to stomatal limitation during salt treatment (Yan et al., 2012). In the present study, however, the photosynthetic rate was not estimated directly but it was assumed that, at least in part; the NaCl treatment might affect a decrease in photosynthetic efficiency of *Sorghum* plants by reduced the stomatal conductance and leaf water potential.

In conclusion, *Sorghum* plants used in this study were affected by the NaCl treatment, resulting in a decrease in plant growth by reducing dry weight of the leaf blade and stem, but it was not apparent in decrease of root dry weight. *Sorghum* plants stored $Na^+$ mainly in the stem to prevent the excess accumulation of $Na^+$ to the leaf blade, but maintained amount of $K^+$ higher in the shoot (leaf and stem). One of the mechanisms for adaptation to salt resistance of *Sorghum* plants was attributed to decreases in transpiration rate and leaf water potential through a reduction in stomatal conductance to avoid the water loss and keep the plant water status. Therefore, an accumulation of $Na^+$ in the stem, maintenance of higher $K^+/Na^+$ ratio in the leaf blades and keep the plant water status by decreased stomatal conductance were the key mechanisms for resistance to NaCl treatment and enhancement in the growth of *Sorghum* cultivars.

Table 3.1 Cultivar names of *Sorghum* plants used for the experiment.

| Number | Name of cultivar | *Sorghum* type[1)] | | Salt resistance classification |
|---|---|---|---|---|
| 2 | Sudakkusu futushu | Sorghum-sudangrass hybrid | (HB2) | sensitive |
| 10 | King sorugo | Sorghum-sudangrass hybrid | (HB10) | resistant |
| 20 | Mini sorghum | Grain sorghum | (GS20) | resistant |

[1)] HB: sorghum-sudangrass hybrid, GS: grain sorghum.

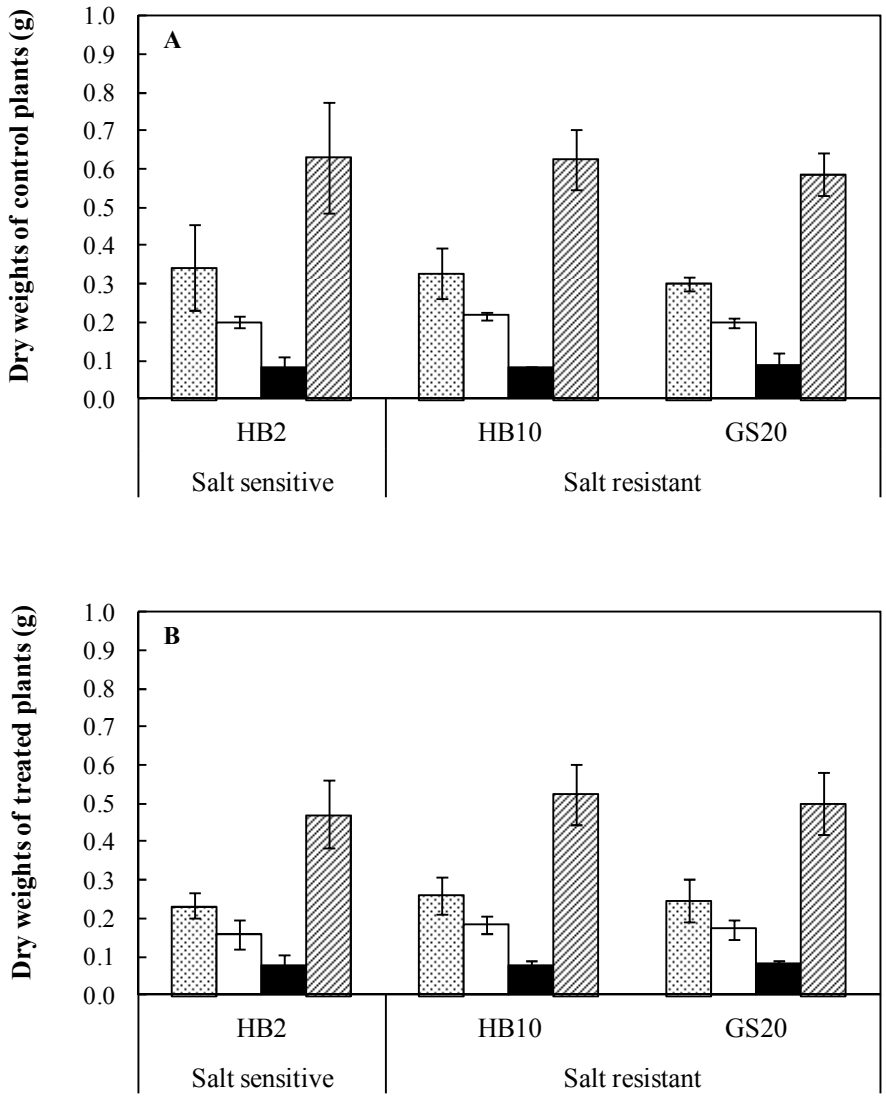


Fig. 3.1 The dry weight in different plant parts of the control [**A**] and the treated plants [**B**] at the end of the experiment. ▨ , leaf; □ , stem; ■ , root; ▨ , whole plant. HB: sorghum-sudangrass hybrid, GS: grain sorghum. The statistical difference between the control and treated plants in dry weights of leaf, stem, and root was analyzed by Student's *t*-test.

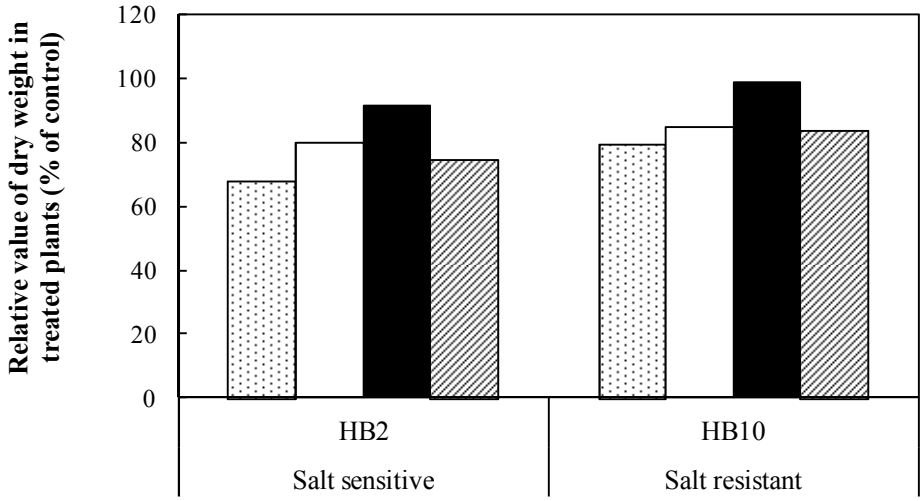


Fig. 3.2 The relative value of dry weight in different plant parts of sorghum-sudangrass hybrids between salt sensitive (HB2) and salt resistant (HB10) at the end of the experiment. ▨ , leaf; □ , stem; ■ , root; ▨ , whole plant.

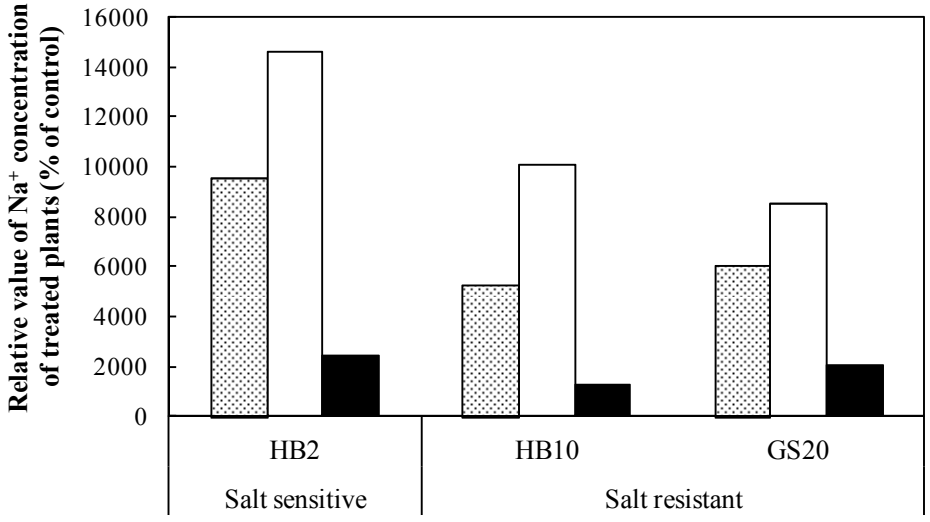


Fig. 3.3 Relative value of $Na^+$ concentration in different plant parts of treated plants at the end of the experiment. ▨ , leaf; □ , stem; ■ , root.

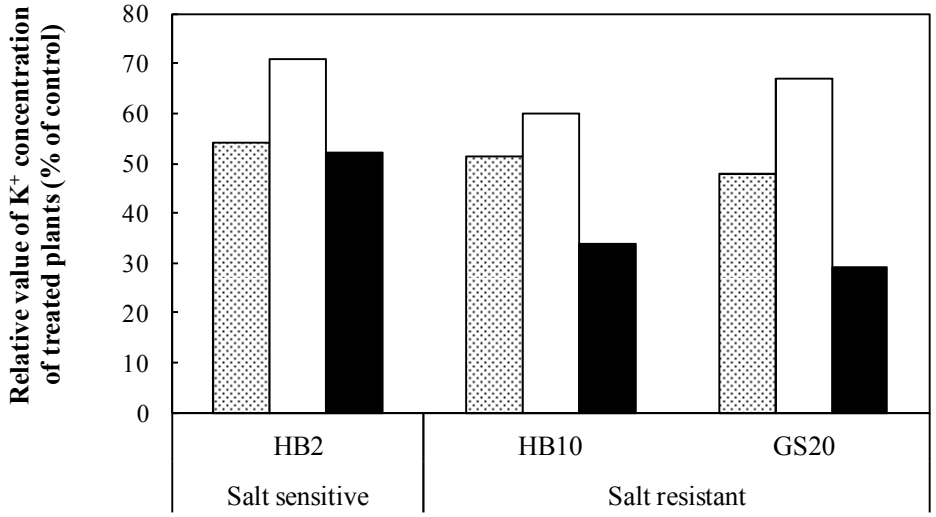


Fig. 3.4 Relative value of $K^+$ concentration in different plant parts of treated plants at the end of the experiment. ▨ , leaf; □ , stem; ■ , root.

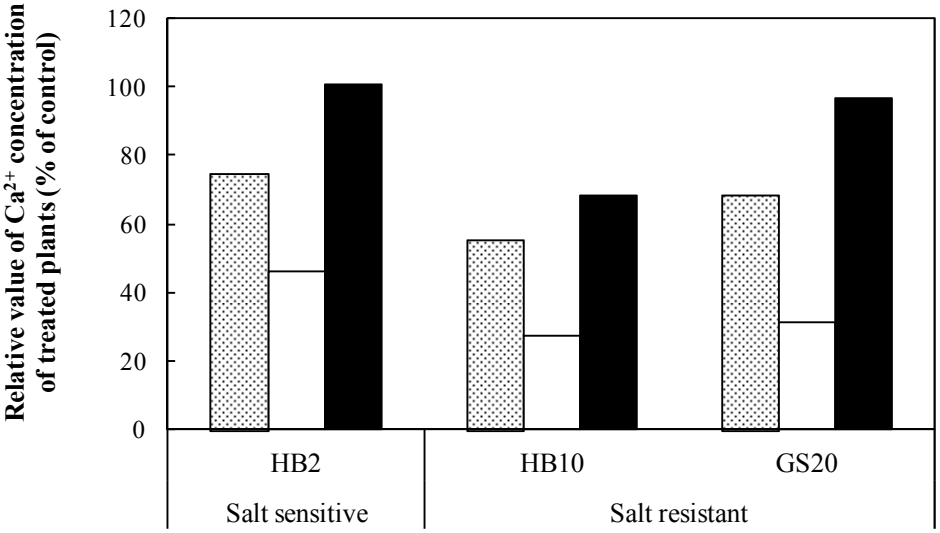


Fig. 3.5 Relative value of $Ca^{2+}$ concentration in different plant parts of treated plants at the end of the experiment. ▨ , leaf; □ , stem; ■ , root.

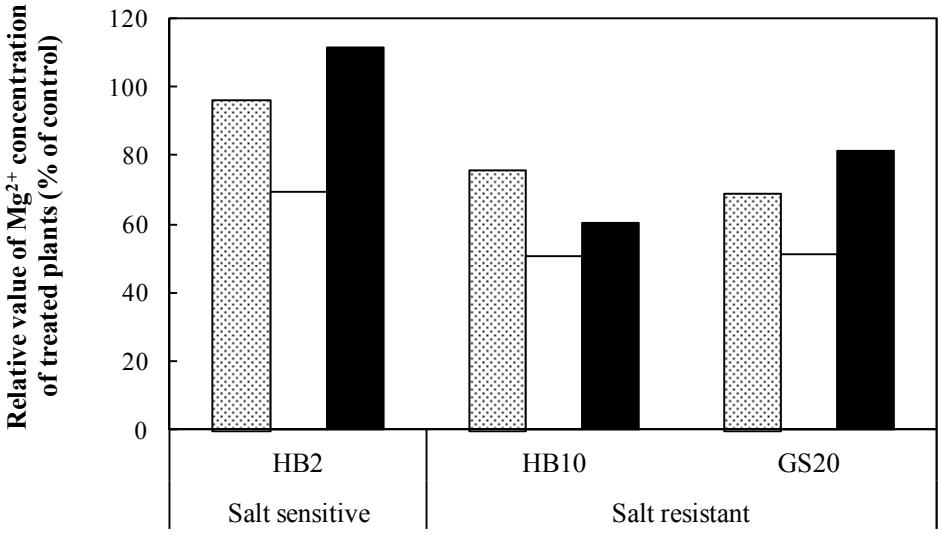


Fig. 3.6 Relative value of $Mg^{2+}$ concentration in different plant parts of treated plants at the end of the experiment. ▨ , leaf; □ , stem; ■ , root.

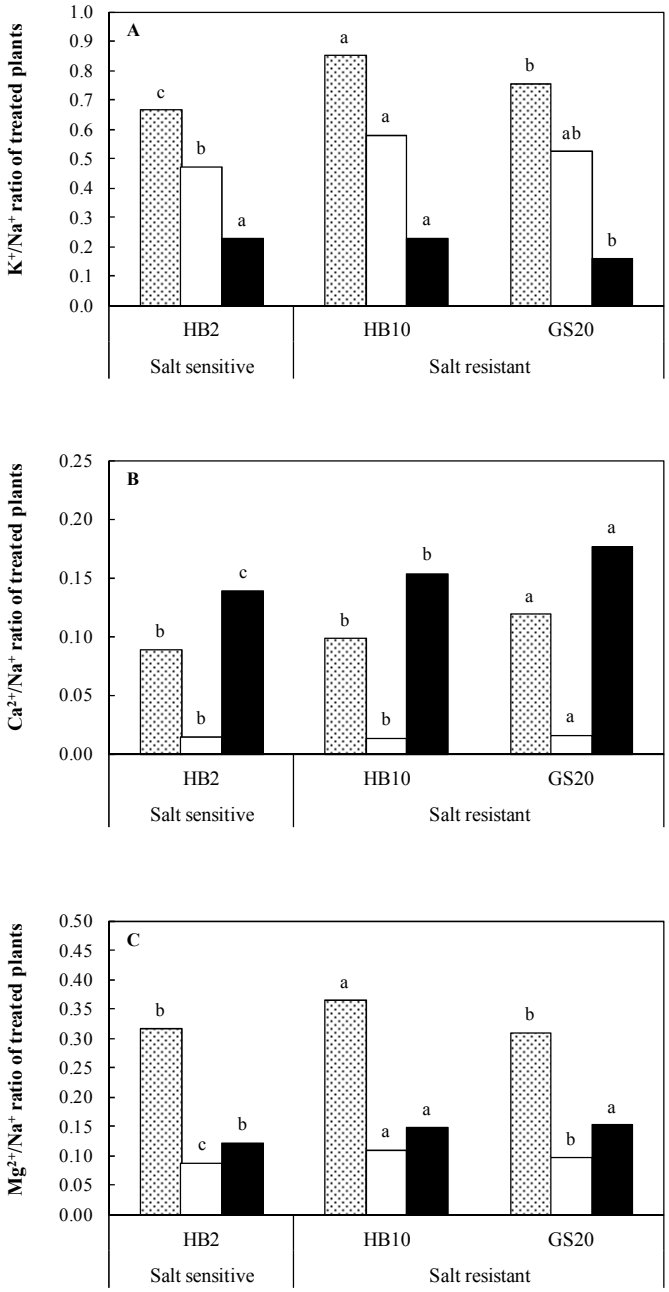


Fig. 3.7 The $K^+$, $Ca^{2+}$, and $Mg^{2+}$ to $Na^+$ ratios in different plant parts of treated plants at the end of the experiment. ▨ , leaf; □ , stem; ■ , root. Means followed by the same letter in each plant part are not significantly different at the 0.05 probability level by least significant difference (LSD).

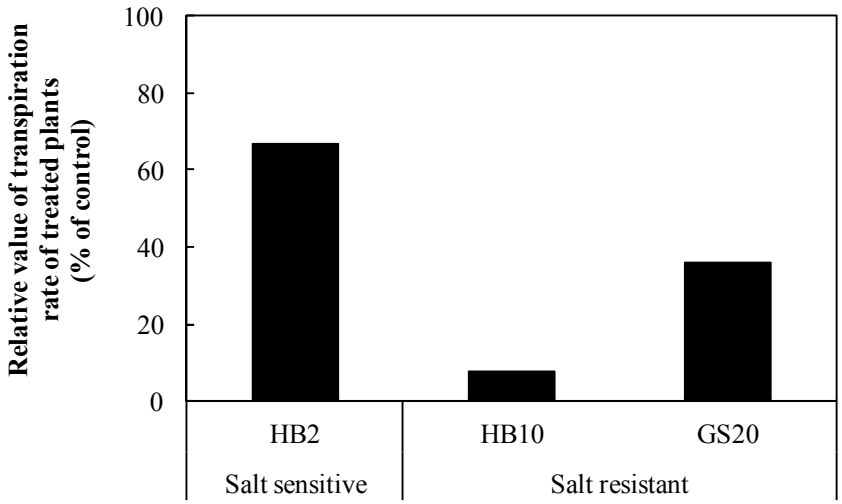


Fig. 3.8 Relative value of transpiration rate of the treated plants at the end of the experiment.

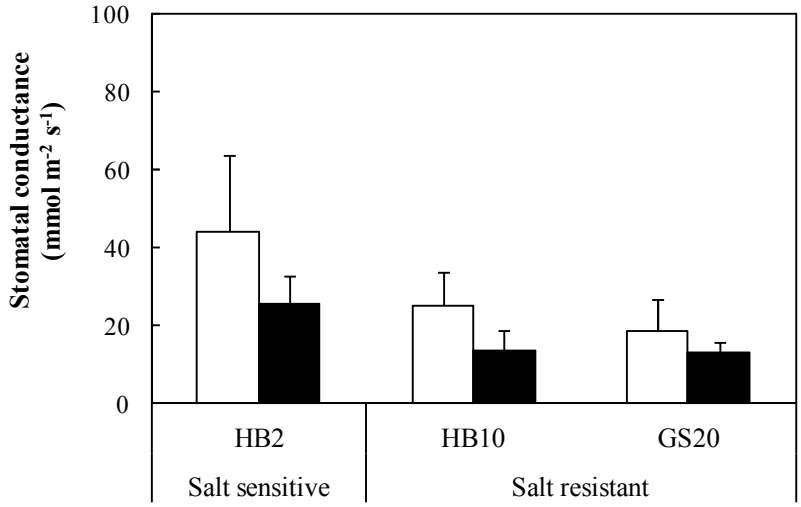


Fig. 3.9 Stomatal conductance of the control and treated plants at the end of the experiment. □ , control group; ■ , treatment.

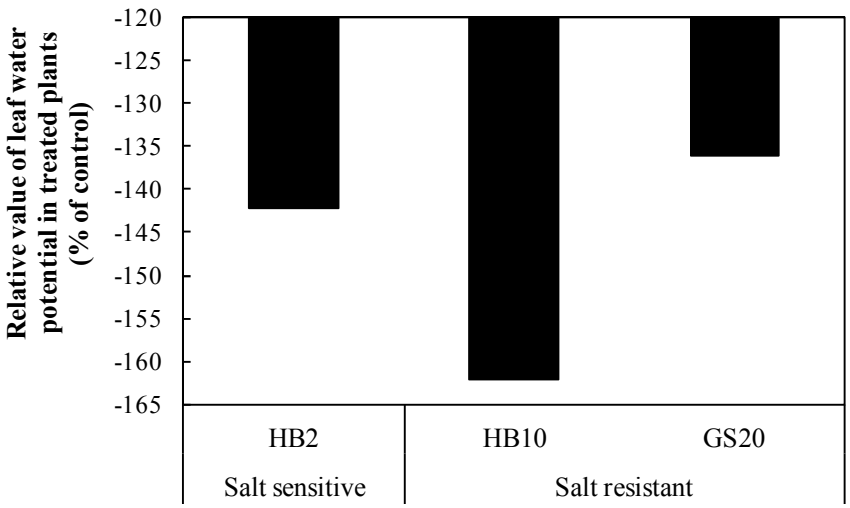


Fig. 3.10 Relative value of leaf water potential of the treated plants at the end of the experiment.

# Chapter 4

# Nutritional Evaluation and *In Vitro* Ruminal Fermentation of *Sorghum* Cultivars

## I. Introduction

*Sorghum* has become important increasingly in many countries with major advantages for growing under tropical and temperate climate conditions (Oliver et al., 2004). There are several types of *Sorghum* such as grain sorghum, sweet sorghum, sudangrass and particularly sorghum-sudangrass hybrids (Undersander, 2003), which crosses between the forage-type sorghum and sudangrass (Valenzuela and Smith, 2002; Clark, 2007). These hybrids have some variability in growth characteristics and can grow fast into 150-360 cm tall, with slender leaves (Valenzuela and Smith, 2002) and are medium in plant size in relation to sorghum and sudangrass (Undersander, 2003). Fiber fractions are primary component in *Sorghum* plants (NARO, 2009) and can vary among cultivars that are important with the nutritive constituents and influenced on the animal response. Due to these different features of *Sorghum* plants, it is necessary to investigate their nutritional characteristics as livestock forages in each plant.

*In vitro* rumen degradability and gas production techniques have been used for estimating the chemical composition of feed materials for the ruminants, particularly when many kinds or varieties of forage are compared (Getachew et al., 2004; Osuga et al., 2006; Huhtanen et al., 2008; Allam et al., 2012). The gas production provides an estimate of the extent and rate of degradation of forage, and can be used for predicting metabolizable energy content and voluntary feed intake in animals (Blümmel and Becker, 1997; Blümmel et al., 1997). This technique has been widely accepted to assess efficiency of rumen fermentation

of plants such as different varieties of straw cereals and chickpea (Kafilzadeh and Maleki, 2012), cultivars of bamboo leaves (Sahoo et al., 2010), wild sunflower (Osuga et al., 2012), and sorghum (Cabral Filho et al., 2005).

There have been different genotypes of *Sorghum* plants including sorghum-sudangrass hybrid types; therefore their nutritive values should be evaluated. This information can be provided for selecting cultivars and also the breeding program. The objectives of this study were 1) to determine the chemical composition, *in vitro* rumen degradability, gas and volatile fatty acid (VFA) production among *Sorghum* cultivars, 2) to investigate the characteristic difference in *Sorghum* plants by grouping types and 3) to elucidate the relationships between the chemical composition and fermentation characteristics of *in vitro* rumen degradability.

## II. Materials and Methods

### 1) Plant material and measurement of dry matter yield and morphological composition

Twenty-two cultivars of *Sorghum* were evaluated as follows; 15 from sorghum-sudangrass hybrids: HB, three from sweet sorghum: SS, two from grain sorghum: GS [*Sorghum bicolor* (L.) Moench], and two from sudangrass: SU [*Sorghum sudanense* Stapf] (Table 4.1). The study was conducted at the experimental field of 150 $m^2$ in Mie University, Japan, between June 5 and August 1 of 2010. For each cultivar, four seeds per hill were sown in a 0.8 m x 7 m plot following a planting distance of 0.2 m. Thinning was done three weeks after planting to maintain one plant per hill. A compound fertilizer ($N:P_2O_5:K_2O$ = 12:12:12) was applied at the rate of 50 kg $ha^{-1}$ during planting and repeated one month later.

Three replicate samples from each cultivar were harvested 88 days after planting and dried at 60$^oC$ a forced-air drying oven for 48 h to obtain dry matter (DM) yield. Different

plant parts such as leaf (consisted of blade and sheath), stem, inflorescence (grains) and tillers were separated to determine morphological composition. Dried different plant parts from each plant were pooled and allowed to pass through 1 mm screen with a Wiley mill and stored for further analysis.

**2) Determination of chemical composition**

The DM and organic matter (OM) contents of the different *Sorghum* cultivars were determined by oven-drying and ashing, respectively. The total nitrogen content of the samples was analyzed by the semi-micro Kjeldahl method and converted to crude protein (CP = N × 6.25). The amount of soluble sugars was determined by extraction using 80% ethanol and phenol-sulfuric assay. Ether extract (EE) was estimated by extraction with diethyl ether in a Soxhlet apparatus. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Van Soest et al. (1991). The ADF was treated with 72% $H_2SO_4$, dissolving the cellulose and leaving behind the acid detergent lignin (ADL). Hemicellulose was calculated from the difference between NDF and ADF. Non-fiber carbohydrate (NFC) was calculated by the equation:

NFC = OM−CP−NDF−EE.

**3) Determination of *in vitro* rumen degradability, gas production and volatile fatty acid concentration**

The rumen fluid for *in vitro* degradability was obtained from cattle before the morning feeding. The *in vitro* ruminal gas production and VFA concentration were measured according to the method of Uddin et al. (2010). Approximately 1.0 g of dried sample was placed in a 120-ml capacity serum bottle containing 50 ml incubation solution made up of rumen liquor and McDougal buffer at 1:2 ratio (v/v). Likewise, a blank sample

was included in the trial. Each *Sorghum* cultivar and the blank samples were replicated three times and incubated in a water bath maintained at 39$^{o}$C with $CO_2$ under anaerobic condition. Residual samples were collected at 96 h incubation and centrifuged two times at 2,320 × g for 10 min. The residues were dried and ashed to determine *in vitro* degradability of dry matter (IVDMD) and organic matter (IVOMD). The dry matter yield then was multiplied with IVDMD and IVOMD calculated as degradable DM yield and degradable OM yield, respectively.

The *in vitro* gas production at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation was subsequently recorded using a graduated syringe. The rate of gas production was calculated from the cumulative gas production expressed as ml $h^{-1}$ $g^{-1}$ DM at different incubation intervals such as, 0-9 h for early phase, 9-24 h for middle phase and 24-96 h for late phase. After 24 and 96 h of incubation, VFA production were determined from the 500 μL liquid cultures collected after centrifuging at 2,320 × g for 10 min. The supernatants at 24 and 96 h were deproteinized with percholic acid and subjected to VFA analysis using HPLC with a conductivity detector (Uddin et al., 2010).

**4) Statistical analysis**

Analysis of variance (ANOVA) was carried out on all parameters to determine the significant difference by using SAS. Significance between means was tested using the least significant difference (LSD) and difference between grouping types evaluated using the Student's *t*-test. Moreover, data were analyzed by simple linear regression and correlation at the 5% and 1% levels of significance (Snedecor and Cochran, 1967) using Microsoft Excel 2007 for Windows.

## III. Results and Discussion

### 1) Dry matter yield and morphological composition

The DM yield and morphological composition varied significantly among the 22 *Sorghum* cultivars (Table 4.1). Dry matter yield was average in 103 g plant$^{-1}$ DM and highest in SS16 at 146 g plant$^{-1}$ DM while the lowest was observed with SU21. In the morphological composition, the mean values of each plant part were 30.8 g plant$^{-1}$ for leaf, 57.9 g plant$^{-1}$ for stem, 7.3 g plant$^{-1}$ for inflorescence and 5.0 g plant$^{-1}$ for tiller. Cultivars used in this study showed higher DM yield such as HB3, HB4, SS16, SS18, and GS19 tended higher leaf and stem dry weights, whereas the highest grain and tiller dry weights were in GS20 and HB5, respectively. The determination in the yield variations across *Sorghum* types was investigated by grouping type comparison such as sorghum-sudangrass hybrid (HB) versus non-HB types and HB versus sorghum type (SS and GS). The results showed sorghum-sudangrass hybrid type was different in the plant production and some morphological composition such as leaf and tiller dry weights compared to sorghum type. In the current experiment, therefore, hybrid types were considered an intermediate plant between sorghum and sudangrass types which may account for the differently agronomic performances.

### 2) Chemical composition

The chemical composition of the different *Sorghum* plants expressed as the mean value is presented in Table 4.2. Among cultivars showed, the CP content averaged 57.9 g kg$^{-1}$ DM and the highest was apparent in SU21 while the lowest was in HB2. *Sorghum* cultivars used in this experiment show less CP content as compared to other reports (Ademosum et al., 1968; Wedig et al., 1988) and standard tables of feed composition in Japan (108 g kg$^{-1}$ DM at dough stage in grain type) as reported by NARO (2009). *Sorghum* cultivars in this study

were highly fibrous with average NDF and ADF contents of 690 and 362 g $kg^{-1}$ DM, respectively. The HB15 had the highest NDF and ADF contents while the lowest was in GS20. *Sorghum* cultivars contained 56.9 g ADL $kg^{-1}$ DM in average, with HB2 appearing to be the most lignified with 75.4 g $kg^{-1}$ DM while the lowest was in GS20 at 41.8 g $kg^{-1}$ DM. There was no significant difference in ADL content in comparison of HB versus non-HB and HB versus sorghum type (SS and GS), however, several cultivars such as HB7, HB13, and SS17 contained lower lignin even though their DM yield per plant were higher than the average of all tested cultivars.

The NFC (91 to 318 g $kg^{-1}$ DM) and sugars (17.1 to 94 g $kg^{-1}$ DM) contents of *Sorghum* cultivars were highly variable as compared to NDF and ADF considered from coefficient variance (data not shown). The highly significant difference in the fiber and NFC contents of across the different *Sorghum* cultivars could be due to the variously genotypic characteristics of individual plant which affected by the environment and expressed different responses on the agronomic performances and chemical component.

**3) *In vitro* rumen degradability, gas production and VFA concentration**

The IVDMD and IVOMD at 96 h, cumulative gas production, rate of gas production and VFA concentration at 24 and 96 h are summarized in Table 4.3. The results showed significant ($P < 0.01$) variations in the ruminal fermentation characteristics across *Sorghum* plant cultivars in all incubation periods. The mean IVDMD and IVOMD of SS17 and SU21 appeared to be the highest while the lowest was observed in HB2 and HB15. The cumulative gas production showed similar trend with IVOMD that were highest for SU21 at incubation periods 9, 24 and 96 h and lowest for HB15. Moreover, the average rate of gas production was 4.1 ml $h^{-1}$ $g^{-1}$ DM during the early phase (0-9 h) that gradually decreased to 2.6 ml $h^{-1}$ $g^{-1}$

DM in the middle phase (9-24 h) and 1.4 ml $h^{-1}$ $g^{-1}$ DM in the late phase (24-96 h). The highest rate of gas production was obtained by SU21 and lowest with HB15.

On the other hand, mean VFA production across cultivars was 54.8 mM after 24 h of incubation and eventually reached a remarkable level of 105 mM at 96 h. Variations in degradability of the various *Sorghum* plants can be attributed to their chemical composition, particularly its cell wall constituents. Results suggest that SU21 was by far the best cultivar in term of IVOMD, cumulative gas production and VFA concentration which are directly influenced by plant morphological characteristics. Apparently, the thin leaf and stem structures of SU21 provided more digestible cell wall compared with the other cultivars. In comparison across *Sorghum* plant types, HB type versus non-HB type did apparently differ in the rate of gas production during the middle phase (9-24 h) and VFA concentration at 24 h, whereas their almost measured rumen fermentation traits were not different. Moreover, HB type did not apparently differ to sorghum (SS and GS) in all the measured ruminal fermentation parameters.

The degradable DM yield and degradable OM yield among *Sorghum* plants expressed as g DM $plant^{-1}$ are shown in Table 4.4. The mean degradable DM and OM yield was 48.8 and 49.2 g DM $plant^{-1}$, respectively. From the results, few cultivars which were HB3, HB4, SS16, SS18, and GS19 appeared to be the highest in degradable DM and OM yield corresponding with dry matter yield. In grouping types across *Sorghum*, the degradation of rumen fermentation traits of HB type did not differ with non-HB type. Contrarily, there had an apparent difference in HB versus sorghum (SS and GS) by which non-HB type showed superior to that of hybrids.

The relationship among chemical composition, IVOMD at 96 h, cumulative gas production, rate of gas production and VFA concentration in *Sorghum* plants are presented as

correlation coefficients (r) in Table 4.5. The ruminal fermentation characteristics in *Sorghum* cultivars were not significantly correlated to fiber contents such as NDF or ADF. Results showed that the qualitative characteristics of the various fiber fractions are directly associated with rumen degradability and not the high amount of fiber in *Sorghum*. This is inconsistent with the previous reports which described that the amount of fibers was negatively associated with gas production found in other plants such as some sub-tropical browses (Allam et al., 2012) and forage maize (Boon et al., 2008; Cone et al. 2008). The fiber digestibility of stem parts along with internodes of forage maize were associated with differences in cell wall thickness, cell wall content, and cell wall digestibility because of the variation in anatomy and chemical composition in plants (Boon et al., 2005). In addition, cross-linking of fibers with lignin was also related to fiber degradability (Iiyama and Lam, 2001; Grabber et al., 2004). Huhtanen et al. (2008) reported that the volume of gas production had closely positive relation to the potential NDF digestibility. It means that the NDF content with high amount of digestible fiber fractions would greatly influence the higher degree of cumulative gas production or rate of gas production. Although the fiber digestibility had not been determined directly, it could be estimated that fibers among *Sorghum* cultivars with higher gas production or rate of gas production at the middle and late phase, such as HB8, HB13, SS17, SU21, and SU22 would relatively have high degradability (IVOMD) in the rumen fermentation.

The ADL contents among *Sorghum* plants were negatively related to IVOMD for 96 h, cumulative gas production and rate of gas production as well as VFA concentration. Results were in agreement with the findings of Boon et al. (2005) who studied in ADL around 40 to 90 g $kg^{-1}$. The relationships between lignin content and degradability that mainly influenced by the hydrophobicity of lignin, cross-linking to other cell wall

components, the polymerization conditions in the apoplast, and other chemical or structural factors (Grabber et al., 2004). By considering various groups of *Sorghum* plants, it was concluded that cultivars of HB7, HB13, SS16, and SS17 contained lower ADL content (less than 50 g $kg^{-1}$ DM) and similarly higher IVOMD, cumulative gas production and VFA concentration than the mean value.

In the current experiment, among 22 *Sorghum* cultivars, HB7 (Brown toumitsu), HB13 [BMR sweet (si-to)], and SU22 (Oishii sudan) were represented as brown midrib mutant (*bmr*) cultivar. The results showed that HB7 and HB13 contained rather less lignin than aother one. This suggests that *bmr* sorghum-sudangrass hybrid type performs lower lignin as compared to sudangrass type at the current experiment. Morphological differences in cell wall contents in normal and *bmr* sorghum-sudangrass hybrid were attributed to differences in the composition and concentration of lignin and carbohydrate fractions (Fritz, 1989).

Considering the correlation coefficients between chemical composition and ruminal fermentation characteristics of *Sorghum* plants, NFC contents was slightly correlated to gas production at early phase ($r = 0.53$, $P < 0.01$). Generally, NFC, which is easily fermentable carbohydratres such as starch is easily fermented after incubation and produced high amount of gas as well as soluble substances (Menke and Steingass, 1988). However, GS20 contained the highest NFC content but it was lower in IVOMD, gas production and VFA concentration which could be due to the antinutritional factors. It has been known that some cultivars in *Sorghum* plants contained high tannin and it can affect on the rumen fiber degradability (Cabral Filho et al., 2005; Oliveira et al., 2007).

The contents of soluble sugars were closely correlated with the cumulative gas production at 9 h or rate of gas production at the early phase of incubation (0-9 h) ($r = 0.87$, $P$

< 0.01). For instance, HB14 and SS16 contained high sugar contents (more than 90 g $kg^{-1}$ DM) and produced higher gas at early phase. This result was in agreement with reports of Boon et al. (2008) that sugar content was strongly correlated with gas production after 3 h of incubation. The VFA concentration at 24 and 96 h were also positively related to sugar contents (r = 0.67 and 0.70, $P < 0.01$, respectively). The soluble sugar contents were also correlated to not only the early phase but also the middle phase of incubation. Sugars are quickly fermentable and can supply ATP energy for microbial growth. Results assumed that the enhanced growth of microbes at early phase could promote the ruminal fermentation during middle phase of incubations. High sugar types such as HB14 and SS16 could be good resource to supply energy for rumen microbes.

In conclusion, cultivars that maintained high dry matter yield and degradable dry matter yield (or degradable organic matter yield) as well as lower ADL contents can be selected as potentially ruminant forage which were HB3 (Sudakkusu 316), HB4 (Sudakkusu ryokuhiyou), SS16 (Kanmi sorugo), SS18 (Koutoubun sorugo), and GS 19 (Haiguren sorugo). Among 22 cultivars of *Sorghum* plants, soluble sugars had positive responses on rate of gas production during early phase of incubation (0-9 h), whereas ADL content had negative influence on rumen degradability IVOMD; indicating that the qualitative characteristics of the various fiber fractions are directly associated with rumen degradability such as IVOMD and not the high amount of fiber in *Sorghum*. With respect of qualitative and quantitative fibrous traits, this result could be useful information for breeding program and to improve forage digestibility for livestock. Nonetheless, further deep studies are still required in the points of maturity and growing response to fertilizer application and environmental stress (such as drought, salt etc.) on nutrient production of *Sorghum* cultivars.

Table 4.1 Dry matter yield and morphological composition among 22 cultivars of *Sorghum* plants used in the experiment.

| No[1)] | Name of cultivar (Common name) | *Sorghum* type | Dry matter yield and morphological composition (g DM plant$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Total | Leaf | Stem | Inflorescence | Tillers |
| HB 1 | Fain sorugo | Sorghum-sudangrass hybrid | 78.6 | 18.1 | 45.7 | 13.6 | 0.6 |
| HB 2 | Sudakkusu futushu | Sorghum-sudangrass hybrid | 102.1 | 35.8 | 63.2 | 0.0 | 0.0 |
| HB 3 | Sudakkusu 316 | Sorghum-sudangrass hybrid | 139.4 | 40.6 | 84.9 | 0.0 | 11.1 |
| HB 4 | Sudakkusu ryokuhiyou | Sorghum-sudangrass hybrid | 139.7 | 41.5 | 96.2 | 0.0 | 0.0 |
| HB 5 | Genki sorugo | Sorghum-sudangrass hybrid | 109.6 | 14.8 | 49.7 | 9.9 | 33.4 |
| HB 6 | Kumiai sorghum nyu 2 gou | Sorghum-sudangrass hybrid | 94.2 | 24.4 | 51.1 | 9.2 | 8.6 |
| HB 7 | Brown toumitsu | Sorghum-sudangrass hybrid (bmr) | 111.4 | 27.2 | 76.1 | 7.1 | 0.0 |
| HB 8 | Lucky sorugo | Sorghum-sudangrass hybrid | 99.6 | 29.0 | 51.8 | 11.4 | 5.7 |
| HB 9 | Lucky sorugo 2 | Sorghum-sudangrass hybrid | 78.8 | 24.2 | 43.9 | 8.4 | 1.6 |
| HB 10 | King sorugo | Sorghum-sudangrass hybrid | 107.5 | 29.7 | 60.6 | 12.7 | 3.1 |
| HB 11 | Ryokuhiyou sorugo | Sorghum-sudangrass hybrid | 73.4 | 19.8 | 41.6 | 7.5 | 4.1 |
| HB 12 | Wind brake | Sorghum-sudangrass hybrid | 88.7 | 41.8 | 42.3 | 0.0 | 1.3 |
| HB 13 | BMR sweet (si-to) | Sorghum-sudangrass hybrid (bmr) | 100.7 | 29.9 | 51.1 | 6.5 | 11.6 |
| HB 14 | Green sorugo | Sorghum-sudangrass hybrid | 114.0 | 26.7 | 69.3 | 15.2 | 0.4 |
| HB 15 | Tsuchi tarou | Sorghum-sudangrass hybrid | 90.9 | 35.1 | 48.7 | 0.0 | 4.6 |
| SS 16 | Kanmi sorugo | Sweet sorghum | 146.2 | 43.4 | 95.5 | 3.8 | 0.0 |
| SS 17 | Supersugar sorugo | Sweet sorghum | 109.1 | 43.1 | 62.6 | 1.8 | 0.0 |
| SS 18 | Koutoubun sorugo | Sweet sorghum | 139.4 | 42.2 | 85.1 | 10.1 | 0.0 |
| GS 19 | Haiguren sorugo | Grain sorghum | 142.7 | 58.1 | 72.9 | 7.3 | 0.0 |
| GS 20 | Mini sorugo | Grain sorghum | 90.5 | 24.8 | 34.7 | 28.9 | 0.0 |
| SU 21 | Summer baler hosokuki | Sudangrass | 46.1 | 8.4 | 15.6 | 3.2 | 17.6 |
| SU 22 | Oishii sudan | Sudangrass (bmr) | 62.6 | 18.8 | 31.4 | 4.7 | 6.4 |
| | Mean | | 103.0 | 30.8 | 57.9 | 7.3 | 5.0 |
| | SEM | | 8.50 | 2.80 | 4.90 | 1.72 | 1.70 |
| | $LSD_{0.05}$ | | 25.97 | 9.20 | 16.77 | 6.54 | 8.32 |
| | Significance | | ** | ** | ** | ** | ** |
| | Mean of HB | | 101.9 | 29.2 | 58.4 | 6.8 | 5.7 |
| | Mean of non-HB | | 105.2 | 34.1 | 56.8 | 8.5 | 3.4 |
| | Mean of sorghum (SS and GS) | | 125.5 | 42.3 | 70.2 | 10.4 | 0.0 |
| | Significance of | | | | | | |
| | HB vs non-HB | | ns | ns | ns | ns | ns |
| | HB vs sorghum (SS and GS) | | * | * | ns | ns | * |

SEM: standard error of the means. ** indicates significant difference at the 0.01 probability level using least significant difference (LSD).

[1)] HB: sorghum-sudangrass hybrid; SS: sweet sorghum; GS: grain sorghum; SU: sudangrass.

Table 4.2 Chemical composition among 22 cultivars of *Sorghum* plants used in the experiment.

| No[1)] | OM[2)] | CP | EE | NDF | ADF | ADL | Cel | Hem | NFC | Sug |
|---|---|---|---|---|---|---|---|---|---|---|
| | (g kg$^{-1}$ DM) | | | | | | | | | |
| HB[2)] 1 | 938 | 57.4 | 14.6 | 696 | 364 | 57.7 | 307 | 332 | 170 | 59.0 |
| HB 2 | 942 | 41.5 | 15.0 | 741 | 404 | 75.4 | 328 | 337 | 144 | 58.3 |
| HB 3 | 943 | 43.0 | 12.8 | 725 | 390 | 66.2 | 324 | 334 | 162 | 71.1 |
| HB 4 | 941 | 53.7 | 12.8 | 744 | 403 | 68.6 | 335 | 341 | 130 | 63.3 |
| HB 5 | 939 | 69.8 | 15.0 | 682 | 365 | 61.0 | 304 | 317 | 172 | 76.1 |
| HB 6 | 939 | 57.7 | 14.8 | 695 | 370 | 61.9 | 308 | 325 | 171 | 69.9 |
| HB 7 | 945 | 55.2 | 15.3 | 682 | 356 | 46.4 | 310 | 326 | 192 | 84.2 |
| HB 8 | 945 | 59.9 | 14.7 | 675 | 354 | 52.8 | 301 | 321 | 195 | 86.5 |
| HB 9 | 945 | 54.8 | 16.2 | 687 | 350 | 52.8 | 298 | 336 | 187 | 69.9 |
| HB 10 | 944 | 52.6 | 16.2 | 687 | 353 | 55.6 | 297 | 334 | 188 | 71.9 |
| HB 11 | 939 | 64.9 | 16.6 | 682 | 347 | 52.8 | 294 | 335 | 175 | 64.1 |
| HB 12 | 899 | 67.0 | 12.6 | 708 | 383 | 62.9 | 320 | 325 | 111 | 17.1 |
| HB 13 | 940 | 61.2 | 15.4 | 695 | 378 | 44.1 | 334 | 317 | 168 | 56.1 |
| HB 14 | 943 | 50.5 | 16.6 | 650 | 347 | 59.8 | 287 | 303 | 227 | 94.0 |
| HB 15 | 909 | 57.2 | 11.2 | 750 | 411 | 73.6 | 337 | 339 | 91 | 24.8 |
| SS 16 | 933 | 57.1 | 13.9 | 665 | 354 | 47.4 | 306 | 311 | 197 | 92.1 |
| SS 17 | 933 | 52.7 | 13.9 | 686 | 349 | 44.8 | 305 | 337 | 180 | 86.6 |
| SS 18 | 929 | 52.9 | 14.6 | 681 | 353 | 53.9 | 299 | 328 | 181 | 66.8 |
| GS 19 | 934 | 61.3 | 14.3 | 726 | 370 | 62.0 | 308 | 357 | 132 | 50.9 |
| GS 20 | 937 | 65.0 | 23.3 | 531 | 250 | 41.8 | 208 | 281 | 318 | 30.6 |
| SU 21 | 936 | 75.0 | 15.6 | 675 | 344 | 52.6 | 291 | 332 | 170 | 87.4 |
| SU 22 | 940 | 62.5 | 13.4 | 713 | 369 | 57.0 | 312 | 344 | 151 | 74.3 |
| Mean | 936 | 57.9 | 15.0 | 690 | 362 | 56.9 | 305 | 328 | 173 | 66.1 |
| SEM | 1.83 | 2.57 | 0.62 | 8.83 | 6.32 | 1.98 | 5.61 | 3.89 | 9.82 | 7.23 |
| $LSD_{0.05}$ | 0.59 | 0.85 | 0.20 | 2.83 | 2.04 | 0.65 | 1.86 | 1.29 | 3.17 | 2.40 |
| Significance | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Mean of HB | 937 | 56.4 | 14.7 | 700 | 372 | 59.4 | 312 | 328 | 166 | 64.4 |
| Mean of non-HB | 935 | 60.9 | 15.6 | 668 | 341 | 51.4 | 290 | 327 | 190 | 69.8 |
| Mean of sorghum (SS and GS) | 933 | 57.8 | 16.0 | 658 | 335 | 50.0 | 285 | 323 | 202 | 65.4 |
| Significance of | | | | | | | | | | |
| HB vs non-HB | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| HB vs sorghum (SS and GS) | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

SEM: standard error of the means. ** indicates significant difference at the 0.01 probability level using least significant difference (LSD).

[1)] HB: sorghum-sudangrass hybrid; SS: sweet sorghum; GS: grain sorghum; SU: sudangrass.

[2)] OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; Cel: cellulose; Hem: hemicelluloses; NFC: non-fiber carbohydrate; Sug: soluble sugars.

Table 4.3 *In vitro* dry matter and organic matter degradability, cumulative gas production, rate of gas production, and volatile fatty acid concentration among *Sorghum* plants used in the experiment.

| No[1)] | IVDMD[2)] (%) | IVOMD (%) | Cumulative gas production (ml $g^{-1}$ DM) | | | Rate of gas production (ml $h^{-1}$ $g^{-1}$ DM) | | | VFA (mM) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 96 h | 96 h | 9 h | 24 h | 96 h | 0-9 h | 9-24 h | 24-96 h | 24 h | 96 h |
| HB 1 | 47.7 | 48.2 | 34.4 | 64.0 | 153 | 3.83 | 2.61 | 1.36 | 55.1 | 105 |
| HB 2 | 42.4 | 42.2 | 34.0 | 56.8 | 146 | 3.78 | 2.15 | 1.26 | 49.0 | 97 |
| HB 3 | 44.6 | 44.9 | 35.1 | 59.3 | 154 | 3.90 | 2.37 | 1.33 | 53.3 | 102 |
| HB 4 | 44.4 | 44.9 | 34.2 | 59.3 | 156 | 3.80 | 2.30 | 1.36 | 51.9 | 104 |
| HB 5 | 44.8 | 45.8 | 37.0 | 63.4 | 166 | 4.11 | 2.50 | 1.45 | 55.8 | 108 |
| HB 6 | 45.4 | 45.9 | 37.0 | 63.0 | 160 | 4.11 | 2.49 | 1.39 | 54.2 | 104 |
| HB 7 | 51.1 | 51.5 | 40.5 | 66.9 | 178 | 4.50 | 2.68 | 1.52 | 54.8 | 111 |
| HB 8 | 49.3 | 49.8 | 41.7 | 74.6 | 180 | 4.64 | 2.90 | 1.59 | 56.0 | 109 |
| HB 9 | 47.9 | 48.3 | 38.3 | 67.5 | 168 | 4.26 | 2.71 | 1.48 | 54.8 | 105 |
| HB 10 | 46.8 | 46.9 | 38.7 | 66.8 | 163 | 4.30 | 2.62 | 1.41 | 54.9 | 103 |
| HB 11 | 48.9 | 49.2 | 39.2 | 71.7 | 170 | 4.35 | 2.93 | 1.50 | 57.8 | 105 |
| HB 12 | 49.0 | 49.1 | 21.1 | 48.0 | 142 | 2.34 | 1.98 | 1.41 | 48.6 | 95 |
| HB 13 | 52.4 | 53.0 | 35.8 | 69.9 | 184 | 3.98 | 2.91 | 1.70 | 54.7 | 111 |
| HB 14 | 46.8 | 46.7 | 45.3 | 68.5 | 164 | 5.03 | 2.53 | 1.32 | 55.2 | 105 |
| HB 15 | 42.7 | 41.9 | 23.0 | 48.4 | 137 | 2.55 | 1.99 | 1.32 | 48.7 | 95 |
| SS 16 | 50.9 | 51.8 | 43.2 | 71.2 | 179 | 4.80 | 2.70 | 1.52 | 57.8 | 114 |
| SS 17 | 52.5 | 53.0 | 41.8 | 74.7 | 184 | 4.64 | 3.03 | 1.61 | 58.9 | 112 |
| SS 18 | 49.2 | 50.0 | 41.3 | 69.6 | 174 | 4.59 | 2.74 | 1.50 | 57.5 | 109 |
| GS 19 | 44.2 | 45.1 | 32.1 | 60.7 | 155 | 3.57 | 2.45 | 1.41 | 53.2 | 98 |
| GS 20 | 44.3 | 44.1 | 33.8 | 59.6 | 147 | 3.76 | 2.55 | 1.26 | 51.4 | 94 |
| SU 21 | 53.0 | 52.5 | 45.8 | 85.5 | 185 | 5.09 | 3.52 | 1.61 | 63.5 | 110 |
| SU 22 | 48.9 | 48.5 | 40.4 | 75.1 | 178 | 4.49 | 3.11 | 1.57 | 58.8 | 105 |
| Mean | 47.6 | 47.9 | 37.0 | 65.7 | 165 | 4.1 | 2.6 | 1.4 | 54.8 | 105 |
| SEM | 1.08 | 1.07 | 1.64 | 2.03 | 3.44 | 0.26 | 0.12 | 0.07 | 1.08 | 2.01 |
| $LSD_{0.05}$ | 3.72 | 3.59 | 5.57 | 7.24 | 11.6 | 0.62 | 0.32 | 0.13 | 3.76 | 6.67 |
| Significance | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Mean of HB | 46.9 | 47.2 | 35.7 | 63.2 | 161 | 3.97 | 2.51 | 1.43 | 53.6 | 104 |
| Mean of non-HB | 49.0 | 49.3 | 39.8 | 70.9 | 172 | 4.42 | 2.87 | 1.50 | 57.3 | 106 |
| Mean of sorghum (SS + GS) | 48.2 | 48.8 | 38.5 | 67.2 | 168 | 4.27 | 2.69 | 1.46 | 55.8 | 1.6 |
| Significance of | | | | | | | | | | |
| HB vs non-HB | ns | ns | ns | ns | ns | ns | * | ns | * | ns |
| HB vs sorghum (SS + GS) | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

SEM: Standard error of the means. ** indicates significant difference at the 0.01 probability level using least significant difference (LSD).

[1)] HB: sorghum-sudangrass hybrid; SS: sweet sorghum; GS: grain sorghum; SU: sudangrass.

[2)] IVDMD: *in vitro* dry matter degradability for 96 h; IVOMD: *in vitro* organic matter degradability for 96 h; VFA: volatile fatty acid concentration for 24 h and 96 h.

Table 4.4 The degradable dry matter yield and degradable organic matter yield in *Sorghum* plants used in the experiment.

| No[1)] | Degradable DM yield (g DM plant$^{-1}$) | Degradable OM yield (g OM plant$^{-1}$) |
|---|---|---|
| HB 1 | 37.5 | 37.9 |
| HB 2 | 43.2 | 43.0 |
| HB 3 | 62.1 | 62.6 |
| HB 4 | 62.0 | 62.8 |
| HB 5 | 48.8 | 49.9 |
| HB 6 | 42.8 | 43.3 |
| HB 7 | 57.0 | 57.5 |
| HB 8 | 49.1 | 49.5 |
| HB 9 | 37.8 | 38.1 |
| HB 10 | 50.2 | 50.4 |
| HB 11 | 36.2 | 36.3 |
| HB 12 | 43.5 | 43.6 |
| HB 13 | 52.8 | 53.4 |
| HB 14 | 53.3 | 53.1 |
| HB 15 | 38.8 | 38.0 |
| SS 16 | 74.4 | 75.8 |
| SS 17 | 57.5 | 58.1 |
| SS 18 | 68.4 | 69.6 |
| GS 19 | 63.3 | 64.6 |
| GS 20 | 40.4 | 40.3 |
| SU 21 | 24.3 | 24.1 |
| SU 22 | 31.0 | 30.7 |
| Mean | 48.8 | 49.2 |
| SEM | 4.40 | 4.40 |
| $LSD_{0.05}$ | 13.57 | 13.58 |
| Significance | ** | ** |
| Mean of HB | 47.7 | 48.0 |
| Mean of non-HB | 51.3 | 51.9 |
| Mean of sorghum (sweet and grain) | 60.8 | 61.7 |
| Significance of | | |
| HB vs non- HB | ns | ns |
| HB vs sorghum (sweet and grain) | * | * |

*, ** indicate significant difference at the 0.05 and 0.01 probability levels, respectively and ns indicates not significant difference using least significant difference (LSD).

[1)] HB: sorghum-sudangrass hybrid; SS: sweet sorghum; GS: grain sorghum; SU: sudangrass.

Table 4.5 The correlation coefficients of chemical composition, *in vitro* organic matter degradability, cumulative gas production, rate of gas production, and volatile fatty acid concentration in *Sorghum* plants used in the experiment.

| | Correlation coefficients | | | | | | |
|---|---|---|---|---|---|---|---|
| | NDF[1)] | ADF | ADL | Cel | Hem | NFC | Sug |
| IVOMD 96 h (%) | –0.21ns | –0.23ns | –0.70** | –0.03ns | –0.10ns | 0.16ns | 0.42ns |
| Cumulative gas production (ml $g^{-1}$ DM) | | | | | | | |
| 9 h | –0.38ns | –0.41ns | –0.50* | –0.32ns | –0.23ns | 0.53* | 0.87** |
| 24 h | –0.29ns | –0.37ns | –0.61** | –0.24ns | –0.07ns | 0.36ns | 0.72** |
| 96 h | –0.17ns | –0.21ns | –0.66** | –0.02ns | –0.04ns | 0.23ns | 0.67** |
| Rate of gas production (ml $h^{-1}$ $g^{-1}$ DM) | | | | | | | |
| 0-9 h (early phase) | –0.38ns | –0.41ns | –0.50* | –0.32ns | –0.23ns | 0.53* | 0.87** |
| 9-24 h (middle phase) | –0.30ns | –0.40ns | –0.66** | –0.26ns | –0.03ns | 0.33ns | 0.57** |
| 24-96 h (late phase) | 0.04ns | –0.01ns | –0.57** | 0.18ns | 0.13ns | –0.07ns | 0.33ns |
| VFA concentration (mM) | | | | | | | |
| 24 h | –0.22ns | –0.32ns | –0.55** | –0.19ns | 0.02ns | 0.25ns | 0.67** |
| 96 h | –0.11ns | –0.12ns | –0.57** | 0.07ns | –0.07ns | 0.20ns | 0.70** |

*, ** indicate significant difference at the 0.05 and 0.01 probability levels, respectively and ns indicates not significant difference.

[1)] NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; Cel: cellulose; Hem: hemicelluloses; NFC: non-fiber carbohydrate; Sug: soluble sugars; IVOMD: *in vitro* organic matter degradability for 96 h; VFA: volatile fatty acid concentration for 24 h and 96 h.

# Chapter 5
# General Discussion and Summary

Salinity is one of the major important environmental stresses that inhibits plant growth or crop productivity widely distributed throughout the world, importantly in the arid and semi-arid areas where soil salt content is naturally high and rainfall can be insufficient for leaching salt excess (Munns, 2002; Praxedes et al., 2010). There are a range of plants (legumes, grasses, and shrubs) that are capable of growing under conditions of saline soils and water, and many of these plants represent a feed resource for animal production (Masters et al., 2007). Some plants of Poaceae also have been utilized as forage or pasture for grazing livestock (Naidoo et al., 2008).

*Sorghum* is the world's fifth major cereal crop and also considered to be important forage for animal. *Sorghum* has wide adaptability and has a resistance to various environmental conditions such as high temperatures and drought. *Sorghum* also has been considered to be moderately resistant to salinity (Almodares and Sharif, 2007). There are several types of *Sorghum* plants and the presence of large genotypic variation for salinity resistance was found in *Sorghum*. Several studies have investigated the ability of resistance to salt stress of *Sorghum* under controlled conditions. Since the screening of large numbers of genotypes for salt resistance under field conditions is difficult, due to spatial heterogeneity of soil chemical and physical properties, and to seasonal rainfall distribution (Almodares et al., 2011).

In this study, the growth, ion distributions, and physiological traits as comparing criteria among different 22 *Sorghum* cultivars, namely sorghum-sudangrass hybrids, sweet sorghum, grain sorghum, and sudangrass, were investigated to clarify the response of

genotypic variation for resistance to salt stress as well as the importance of the assessment on feeding value of *Sorghum* cultivars using chemical composition and rumen fermentation. The determination of nutritive value of *Sorghum* has essential importance for improving cell wall digestibility and rumen degradation which is valuable information for breeding program of fodder production. To determine the feeding value in *Sorghum* cultivars, plants grown under field experiment was emphasized. But the evaluation of response for resistance to salinity of *Sorghum* plants was mainly conducted in hydroponics.

## Comparison of Young Seedling Growth and Sodium Distribution among *Sorghum* Plants under Salt Stress

Young seedlings of 22 *Sorghum* cultivars, namely, 15 of sorghum-sudangrass hybrids (HB), three of sweet sorghum (SS), two of grain sorghum (GS), and two of sudangrass (SU) were examined for their growth characteristics and $Na^+$ accumulation in different plant parts, under salt stress. The salt treatment was started with 100 mM NaCl and increased to 150 mM during the experiment in hydroponics. The experiment conducted in a greenhouse in June and July 2010.

The plant dry weight decreased under NaCl treatment in all cultivars, and especially the dry weight of leaf blade decreased markedly. At the mean of relative value of dry weight increment ($\Delta$W), nine cultivars, which were HB3 (Sudakkusu 316), HB7 (Brown toumitsu), HB8 (Lucky sorugo), HB10 (King sorugo), HB11 (Ryokuhiyou sorugo), SS17 (Supersugar sorghum), GS19 (Haiguren sorghum), GS20 (Mini sorghum), and SU22 (Oishii sudan), that maintained the higher relative value than the mean were considered to be resistant to salt stress. The cultivar difference in the plant dry weight under salt stress was affected by that in relative growth rate which was mainly changed by net assimilation rate (NAR). Cultivars that

maintained higher NAR under salt stress had a smaller specific leaf area and higher nitrogen content per unit leaf area. *Sorghum* plants under salt stress retained $Na^+$ mainly in roots preventing the distribution of excess amount of $Na^+$ to leaves, but the root dry weight was increased by salt stress. It was therefore considered that thicker leaf blades and apparent increases in root dry weight were the main contributors to the maintenance of dry matter yield and enhanced the growth of *Sorghum* cultivars under NaCl treatment.

**Physiological Response, Sodium Distribution, and Essential Nutrient Absorption of *Sorghum* Plants under Salinity Stress**

The objective of this experiment was to clarify the effect of $Na^+$ distribution on absorption of element nutrients, and some physiological characteristics of *Sorghum* plants differing in salt resistance. Young seedlings of three *Sorghum* cultivars were selected, which were HB2 as salt sensitive cultivar and HB10 and GS20 as salt resistant ones (sorghum-sudangrass hybrids: HB and grain sorghum: GS, respectively). *Sorghum* plants grown in hydroponics containing with Kimura A culture solution were submitted with 150 mM NaCl versus without salt for the control under the natural light condition in a greenhouse in 2011. During the experimental, the plant growth, accumulation of $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ were investigated in different plant parts. Physiological characteristics under NaCl treatment which consisted of transpiration rate, stomatal conductance, and leaf water potential were also determined.

Salt stress caused a decrease in plant growth by reduced the dry weight of the leaf blade and the stem, whereas in the root, it was not apparently significant. *Sorghum* plants stored $Na^+$ mainly in the stem preventing $Na^+$ influx more to the leaf blade but maintained higher $K^+$ in the shoot (leaf and stem). One of the mechanisms for adaptation to salt

resistance of *Sorghum* plants was attributed to a reduction in transpiration rate and leaf water potential by decreased stomatal conductance to keep the plant water status. It was therefore suggested that accumulation of $Na^+$ in the stem, maintenance of higher $K^+/Na^+$ ratio in the leaf blades and decrease in stomatal conductance were due to the important mechanisms for growth enhancement of *Sorghum* plants to resistance the salt stress.

**Nutritional Evaluation and *In Vitro* Ruminal Fermentation of *Sorghum* Cultivars**

There are several types of *Sorghum* such as grain sorghum, forage sorghum (including sweet sorghum), sudangrass, and sorghum-sudangrass hybrids. Feeding values including chemical composition and rumen degradability characteristics would be varied among these different types. The objectives of this study were to determine the chemical composition, *in vitro* rumen degradability, gas production and volatile fatty acid (VFA) concentration among various varieties of *Sorghum* plants.

Twenty-two cultivars of *Sorghum* consisted of 15 sorghum-sudangrass hybrids: HB, three sweet sorghum: SS, two grain sorghum: GS [*Sorghum bicolor* (L.) Moench], and two sudangrass: SU [*Sorghum sudanense* Stapf] were grown under natural field conditions and harvested at 88 days after planting. Significant difference in dry matter yield was observed and ranged from 46.1 to 146 g $plant^{-1}$ DM. The crude protein content averaged 57.9 g $kg^{-1}$ DM, contents of neutral detergent fiber (NDF) ranged from 531 to 750 g $kg^{-1}$ DM, acid detergent fiber (ADF) ranged from 250 to 411 g $kg^{-1}$ DM, acid detergent lignin (ADL) ranged from 41.8 to 75.4 g $kg^{-1}$ DM. Compared to fiber fractions, non-fiber carbohydrate and soluble sugar contents were highly variable (91 to 318 g $kg^{-1}$ DM and 17.1 to 94 g $kg^{-1}$ DM, respectively).

The *in vitro* organic matter degradability (IVOMD) at 96 h was different among cultivars ranged from 41.9 to 53.0%. Sorghum-sudangrass hybrid (HB) and non-hybrid types (non-HB) were not apparently different in the dry matter yield, morphological composition, and chemical composition. While the comparison between HB and sorghum types (sweet and grain) were different in the yield and some morphological composition but no difference in the chemical composition.

The content of soluble sugars in *Sorghum* plants was positively correlated to the *in vitro* ruminal gas production at early phase of incubation (0–9 h) ($r = 0.87$). Sugar contents were also highly correlated to cumulative gas production and VFA concentration ($r > 0.67$) compared to IVOMD ($r = 0.42$). Whereas ADL content had a negative relation to IVOMD ($r = -0.70$). The fiber contents (NDF or ADF) showed no relation between IVOMD and rate of gas production (24–96 h). Therefore, cultivars that maintained higher dry matter yield and degradable dry matter yield as well as higher soluble sugar and/or lower ADL contents should be selected as ruminant forages which were HB3 (Sudakkusu 316), HB4 (Sudakkusu ryokuhiyou), SS16 (Kanmi sorugo), SS18 (Koutoubun sorugo), and GS19 (Haiguren sorugo).

There are two contrasting physiological mechanisms to salt stress in plants (Touchette et al., 2009). Salt resistance is the responses to salt stress by which relates to either salt tolerance or salt avoidance mechanisms (Levitt, 1980). Salt tolerance mechanism is that the plants take up salt ions and can grow with high amount of $Na^+$ in the leaf tissues by which plants can be achieved through osmotic adjustment and changes in tissue elasticity (Touchette et al., 2009). Whereas salt avoidance mechanism is that the plants are able to exclude toxic ions from internal plant tissues by which plants restrict water loss through decreased stomatal conductance and changes in leaf morphology (Johnson, 1991; Touchette

et al., 2009). As for the general strategies, salt tolerance implicates with physiological and biochemical adaptations for maintaining protoplasmic viability as cells accumulate electrolytes, and salt avoidance relates with structural with physiological adaptations to minimize salt concentrations of the cells or physiological exclusion by root membrane (Ehara et al., 2007).

For most plants, salt exclusion is the key mechanism of salt resistance by which salt is kept away from meristem tissues, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing (Ashraf, 2004). The control of $Na^+$ transport and the potential exclusion from the mesophyll cells of leaves is an important requirement for salinity resistance (James et al., 2011). Sodium exclusion from the leaf blade is one of the major mechanisms for salt resistance in cereal crops (Gorham et al., 1990; Munns et al., 2006). In monocotyledon species without salt glands, response to salt tolerance depends on the control of $Na^+$ transport at four major points. First, selectivity of uptake by root cells in the cortex and stele; secondly, loading of the xylem by xylem parenchyma cells in roots; thirdly, removal of salt from the xylem in the upper part of the roots, the stem, or leaf sheaths by xylem parenchyma cells; and fourthly, loading of the phloem (Munns et al., 2006.) and the effect normally takes time to develop (Tester and Davenport, 2003; Munns and Tester, 2008).

The exclusion of salt from the phloem might be due to retranslocation but little of the import in the xylem. The presence of the rates of retranslocation of salt from the leaves that are low in relation to rates of import in the transpiration stream is as shown by the continued presence of salt in leaves long after the salt around the roots is removed (Munns et al., 2006). The strong evidence was supported with James et al. (2006) that retranslocation of $Na^+$ from the shoot to root was a relatively small component of shoot $Na^+$ uptake (around 2% to 6%).

There are studies which indicated that the major role of *Nax1* and *Nax2* genes have the potential to improve the salt tolerance in bread wheat (James et al., 2011) and durum wheat (James et al., 2006). They studied that there was the major gene, *Nax1*, in conferring salt tolerance which restricted the transport of $Na^+$ from the xylem in the roots to the shoots with a high selectivity for $K^+$ over $Na^+$, resulting in enhanced $K^+/Na^+$ discrimination in the leaf blade. The proportion of $Na^+$ stored in the leaf sheaths is increased because the physiological mechanism of the *Nax1* gene by which achieved low $Na^+$ concentration in the leaf blade and *Nax1* was also distinguished by a higher $Na^+$ concentration in the leaf sheath over the leaf blade than *Nax2*, thereby reducing $Na^+$ concentrations in the leaf blade.

Agronomic characteristics represent the combined genetic and environmental effects on plant growth, and include the integration of the physiological phenomena conferring salinity resistance, while physiological criteria are able to supply more reliable information than agronomic characteristics (Ashraf, 2004). In the results of the plant's response to salt stress, salt resistant cultivars of *Sorghum* displayed higher plant production than salt sensitive one, even though under salt stress, by which salt resistant cultivars have a mechanism for resistance to salinity by a reduction of transpiration rate and leaf water potential through decreased stomatal conductance. A reduction in transpiration rate was attributed to the smaller in stomatal aperture to control the plant water status. However, the other future studies should be evaluated deeply, such as the photosynthetic rate, $CO_2$ assimilation rate, and chlorophyll fluorescence etc., in order to make clear the knowledge of physiological mechanisms for resistance to salt stress in *Sorghum* plants.

As for the results in the feeding value of *Sorghum* cultivars, with respects of dry matter yield, nutritive value, and *in vitro* rumen degradability, several cultivars of the different *Sorghum* types at 88 days harvesting were considered as the potential forage crops

for ruminants, even though most of them were less in the content of crude protein. When the grouping types were compared, sorghum-sudangrass hybrid (HB) versus non-hybrid types (non-HB) were not apparent significance in dry matter yield and morphological composition, but HB type was almost different with sorghum type (sweet and grain). In other words, dry matter production of HB type had higher potential biomass than that of sudangrass type, but it was not as much as that of sorghum type. While the chemical composition and rumen degradability characteristics were not apparent difference across cultivars. It suggests that HB type had high performance in nutritional value and *in vitro* ruminal fermentation as well as sorghum type.

Under saline conditions, the high concentrations of NaCl in particular cause decreased feed intake, but under some conditions such as the moderate level of NaCl compromise animal health or have benefits to production (Masters et al., 2007). In a small ruminant, when NaCl and KCl were included as part of normal roughage based diet at 25% of the dry matter, the amount of wool grown per kg of organic matter intake increased by up 50% (Masters et al., 2005). Plants growing in salinity conditions also accumulate a range of secondary compounds such as vitamin E and betaine and these may have beneficial effects on grazing livestock (Masters et al., 2007). There was an evidence reported that crude protein of grain sorghum varieties at high saline soil under field experiment was not reduced in comparing with a low level of saline soil, whereas the *in vitro* feed was assessed as of medium quality (Begdullayeva et al., 2007). It suggests that contents of crude protein and/or digestible fiber of plants are probably not directly influenced by the level of salinity (Ashour et al., 1997; Masters et al., 2007).

In conclusion, salt resistance of *Sorghum* plants in this study could be due to salt avoidance mechanically by $Na^+$ exclusion from the leaf blade, that is, *Sorghum* retained

much $Na^+$ concentration mainly in the root and stem with higher $K^+/Na^+$ in the shoot, particularly in the leaf blade. A preferable leaf morphogenesis producing a thicker leaf blade, an apparent increase in root dry weight and a reduction of stomatal conductance were the main contributors to the maintenance of dry matter yield and enhanced the growth of *Sorghum* cultivars under salt stress. As for the nutritional value, several *Sorghum* cultivars from sorghum-sudangrass hybrid, sweet sorghum and grain sorghum types maintained high dry matter yield and degradable organic matter yield, but lower lignin content as grown under natural field conditions. Therefore, all of the results suggest that HB3 (Sudakkusu 316) and GS19 (Haiguren sorugo) can be selected as potential and valuable forages for ruminant production which can be grown under salt stress. The results can be useful in physiological mechanisms associated with resistance to salt stress, which is important for *Sorghum* plant breeding programmes and provide valuable information for improved digestibility for livestock. However, the further study under saline soils should be conducted to make a better understanding and could obtain some advantages for growing fodder crops in salt-affected areas.

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