

**Studies on an improvement of sugarcane quality  
through fertilizer management and cultivar selection**

**施肥管理および品種選択を通じたサトウキビの糖度向上に関する研究**

**Doctoral Thesis**

**学位論文**

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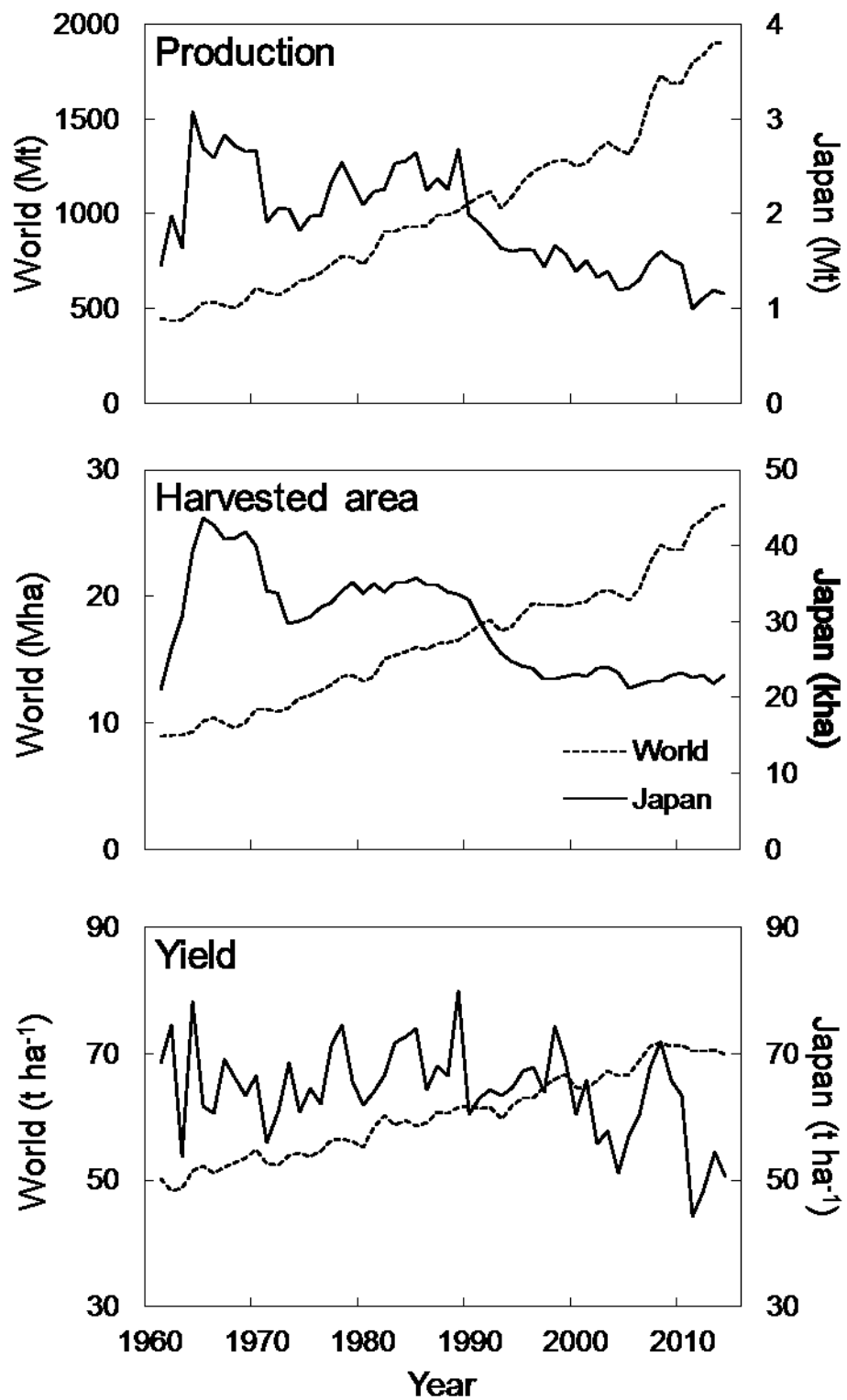
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# Chapter 1

## General introduction

Sugarcane (*Saccharum officinarum* L.) is an important sugar accumulating plant which belongs to grass family Poaceae, as it accounts for about 80% of sugar produced in the world (European Commission's Directorate General for Agriculture and Rural Development, 2016). Sugarcane is the world's largest crop by production quantity. Currently, it is cultivated in nearly all tropical and sub-tropical regions, with a total production of 1.9 billion tons and harvested area of 27 million ha (Food and Agricultural Organization, 2016) and it is likely the production will still keep increasing (Fig. 1-1).

In Japan, sugarcane is mainly grown in Nansei Islands, which is located in southwest Japan and composed of many small islands in Kagoshima and Okinawa prefectures. Sugarcane is one of the most important agricultural products in this region, with an economic impact greater than that of any other crop (Iesaka, 2001), because of its relatively strong resistance to natural disasters such as typhoons and drought (Oshiro, 2001; Ueno et al., 2005). Sugar mills to produce raw sugar or brawn sugar are located in most of the inhabited islands and it is of particular importance in disadvantaged small islands (Inoue, 2006). Despite its essential role in the economy, both the production and the harvested area are decreasing due to inappropriate and extensive managements by the increasing ratio of part-time farmers and labor shortage by the decreasing number of younger farmers as well as the aging of sugarcane producers and change in land use toward more marketable plants (Inoue, 2006; Sugimoto and Terajima, 2006; Kikuchi, 2009; Matsuoka, 2006). Besides that, sugarcane yield per ha widely fluctuates year by year and has not been increased long or even slightly decreased though many attempts such as breeding, disease and pest control, soil and water management, have been done. The production of raw sugar is roughly calculated by a product of sugarcane production, consisting of harvested area and cane yield, and Pol in cane (PIC). Considering about the current situation of sugarcane



**Fig. 1-1.** Changes in sugarcane production, harvested area, and yield in the world and Japan over recent 50 years.

industry, it is quite difficult to increase the production by extending the harvested area; therefore, increasing raw sugar production requires technical improvements of sugarcane yield or quality. The sugarcane price in Japan is determined by not only sugarcane weight but also PIC based on the quality payment system. Currently, the standard PIC value is set from 13.1 to 14.3% and, above the standard value, each increment of 0.1% PIC increases the price by 130 yen (Matsuoka, 2006), so the improvement of sugarcane quality can contribute to increasing producer profit as well.

Plants need a number of essential element nutrients for their growth and development. An element considered essential if (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium (Arnon and Stout, 1939). There are 16 essential nutrients for higher plants and those required by plants in large amounts is called macronutrients, while those in minute amount are termed micronutrients. Nutrient diagnosis is used to identify deficiency, sufficiency or excess of nutrients, optimize crop production and evaluate fertilizer requirements.

Fertilizer management practice is one of the factors that influence sugarcane quality (Meyer and Wood, 2001), but the application of suitable fertilizer management practices requires the knowledge of the nutritional status of each sugarcane field. Foliar diagnosis is a common practice in agriculture to manage the mineral nutrition of plants (Oliveira et al., 2010) and it allows for early detection of nutritional problems and enables the grower to add supplemental fertilizer to the current year's crop or to adjust next year's fertilizer application (McGray et al., 2010a) so that nutrient limitations can be corrected. The method is well established and is widely used as a diagnostic tool in sugarcane production both within and

outside Japan (Oshiro et al, 1994; Anderson et al., 1995; Kumar and Verma, 1997; Ota et al., 2000; Bokhitar, 2004; McGray and Mylavarapu, 2010b). However, foliar diagnosis can be a complex exercise because of the dynamic nature of foliar composition, which is strongly influenced by aging processes as well as interactions affecting nutrient uptake and distribution (Walworth and Sumner, 1987; McGray et al., 2010a) and the recommended sampling time and leaf position for diagnosis differed : according to Samuels et al. (1955), for example, fourth, fifth, and sixth leaves are used for diagnosis and leaf samples taken at a cane age of 3 months were best for indicating the fertilizer needs of the cane, while McGray et al. (2011) stated that the blade of the top visible dewlap (TVD) leaf—the uppermost fully expanded leaf that has a visible dewlap or distinct collar—is often sampled during the ground growth period of June to September. The method also requires technical knowledge, skill, and machines. Nutrient diagnosis should be as easy and precise as possible for anyone needing to know the nutritional status of sugarcane. Accordingly a new method for sugarcane nutrient diagnosis on the basis of the sugarcane juice obtained at harvest was investigated. The advantages of using this method are as follows:

- Nutrients in the juice more precisely represent the nutritional status of the whole plant, given that it reveals the total amount of nutrients absorbed and stored into stem parts during cultivation, while leaf analysis evaluates temporary leaf nutritional status
- Juice quality depends on the amount and type of nutrients present in the juice (Gomathi and Thandapani, 2005) as sucrose is also accumulated into the stems
- It is not necessary to take samples for diagnosis, given that the stalk is an economic part of the sugarcane and stalks are sent to sugar mills
- Sample preparation is easier because juice samples are liquid and directly used for analyses without extraction

The principle objective of this study is to improve sugarcane quality based on the results obtained by this new method using sugarcane juice. In Chapter 2, major nutrients present

in juice of field sugarcane and the relationships between the nutrients and sugar content were first revealed to identify the key factors in sugarcane quality. Chapters 3 to 5 contain pot and field experiments to test the hypothesis that the factors suggested from the results of Chapter 2 certainly affected sugarcane quality and to see the effects of treatments on the quantitative parameters. These include pot experiments by applying different fertilizers, field experiments with different fertilizers, and pot experiments with different water qualities based on the survey of rain and irrigation water collected from sugarcane producing areas in Japan, shown in Chapters 3, 4, and 5, respectively. I also attempted to reveal the mechanism how the factors affected sugarcane quality in these chapters. While Chapters 3 to 5 propose the ways for the quality improvement in terms of cultivation management, Chapters 6 and 7 highlight cultivar selection which could be another way toward a solution of the problem. In Chapter 6, nutrient balances and sugar accumulating ability of each sugarcane cultivar grown in Japan are revealed. Then, I conducted a pot trial using some of the cultivars which were supposed to have specific characteristics from the results of Chapter 6 or important backgrounds of sugarcane production in Japan. Chapter 7 only discusses a possibility or an expectation for the effectiveness of cultivar selection against the problem as this trial is currently under way and the results are still unknown. All these surveys and experiments above taken together, Chapter 8, general discussion, gives suggestions to achieve the objectives through sugarcane cultivation management and cultivar selection. Future perspectives will be also presented in this chapter.

## **Chapter 2**

### **Relationships between nutrients and sugar content in sugarcane juice**

#### **2.1. Analysis of sugarcane juice from sugarcane producing areas in Japan**

##### **Introduction**

Leaf nutrient analysis has been widely used as a diagnostic tool to complement soil testing in sugarcane production. However, the method can be a complex exercise since it requires technical knowledge, skill, and machines. It is thus needed to establish a new method for ordinary sugarcane growers. Juice analysis can be substituted for foliar diagnosis because it has some advantages as described in Chapter 1, however, we have not yet understood the nutrient composition in sugarcane juice. To utilize the data based on juice analysis for the quality improvement of sugarcane, we first need to understand what nutrients are present in sugarcane juice and how they are related to sugar content, so that we can apply appropriate amounts of fertilizer for the next planting season to maximize the quality efficiently. In this study, I collected stalk samples from the sugarcane planted areas producing raw sugar and investigated the nutrient composition and the relationships between nutrients and sugar content to identify the key factors affecting sugarcane quality.

##### **Materials and methods**

This study was performed over three harvest seasons: from February to March in 2013, 2014, and 2015. Each sample was composed of a 3- to 4-kg bundle of clean cane stalks, and 10 samples differing in sampling sites, cropping types, and cultivars were randomly chosen and sent from the 17 sugar mills (Table 2.1-1) to the University of the Ryukyus (26°25'N, 127°77'E; 125 m a.s.l.) in each of the harvest seasons.

The samples were shredded with a cutter grinder (CG03, Jeffco) and 100 g of



**Table 2.1-1.** List of 17 raw sugar mills under operation in Japan.

Mill No.	Name	Location
1	Shinko Togyo Co., Ltd.	30°51'N, 130°96'E
2	Fukoku Seito Co., Ltd.	28°45'N, 129°68'E
3	Showa Togyo Co., Ltd.	28°33'N, 129°95'E
4	Nansei Togyo Co., Ltd., Tokuwase Factory	27°76'N, 129°02'E
5	Nansei Togyo Co., Ltd., Isen Factory	27°68'N, 128°92'E
6	Nanei Togyo Co., Ltd.	27°37'N, 128°64'E
7	Yoronjima Seito Co., Ltd.	27°04'N, 128°41'E
8	Okinawa Agricultural Coop. Assoc., Izena Branch	26°93'N, 127°94'E
9	Kyuyo Sugar Mfg. Co., Ltd.	26°34'N, 127°86'E
10	Kumejima Sugar Mfg. Co., Ltd.	26°33'N, 126°77'E
11	Shonan Sugar Mfg. Co., Ltd.	26°19'N, 127°70'E
12	Kita-Daito Sugar Mfg. Co., Ltd.	25°95'N, 131°30'E
13	Daito Sugar Mfg. Co., Ltd.	25°83'N, 131°20'E
14	Miyako Sugar Mfg. Co., Ltd., Irabu Factory	24°81'N, 125°27'E
15	Okinawa Sugar Mfg. Co., Ltd.	24°75'N, 125°29'E
16	Miyako Sugar Mfg. Co., Ltd., Gusukube Factory	24°73'N, 125°35'E
17	Ishigakijima Sugar Mfg. Co., Ltd.	24°39'N, 124°16'E

Note that Shonan Sugar Mfg. Co., Ltd. (No. 11) was closed in 2015 is currently combined with Kyuyo Sugar Mfg. Co., Ltd. (No. 9) with a new name, Yugafu Sugar Mfg. Co., Ltd.

carefully mixed subsample was filled a cup with to measure PIC by near infrared spectroscopy (InfraXact, Foss). The measurement was performed three times for each sample and PIC was expressed as the averaged value. Then, 550 g of subsample including those used for the measurement of PIC was pressed to obtain juice and 15 ml of the press juice was stored at  $-20^{\circ}\text{C}$  for the measurement of electrical conductivity (EC) and ion analysis. After complete thawing, EC of juice samples was measured with an EC meter (WM-32EP; Toa). In 2013, 40 of 170 data on EC were missing, but this loss had little effect on the results because the sample number was sufficient enough to understand the general tendency. Juice samples for ion analysis were diluted 100 times with extra-pure water and filtered with a membrane filter (diameter, 13 mm; pore size,  $0.45\text{ }\mu\text{m}$ ; Advantec). Ion chromatographs (ICS-1600; Thermo Fisher Scientific) were used to determine the concentrations of major ions present in sugarcane juice (sodium ( $\text{Na}^{+}$ ), ammonium ( $\text{NH}_4^{+}$ ), potassium ( $\text{K}^{+}$ ), magnesium ( $\text{Mg}^{2+}$ ), calcium ( $\text{Ca}^{2+}$ ), chloride ( $\text{Cl}^{-}$ ), nitrite ( $\text{NO}_2^{-}$ ), nitrate ( $\text{NO}_3^{-}$ ), phosphate ( $\text{PO}_4^{3-}$ ), and sulfate ( $\text{SO}_4^{2-}$ ) ions). The columns and eluents used were Ion Pac CS12 and 20 mM methane sulfonic acid solution for cation analysis and Ion Pac AS22 and a solution of 4.5 mM sodium carbonate and 1 mM sodium hydrogen carbonate for anion analysis. The samples were also categorized by harvest year, production area, cropping type, and cultivar for detailed analyses.

Mean and standard deviations (SD) of the samples were calculated and statistical analysis was performed with R (R Core Team, 2015). Data were subjected to one-way analysis of variance (ANOVA) to test differences among sugar mills. Significance was accepted based on a P value  $<0.05$ .

## Results

In all the 3 years,  $\text{K}^{+}$  was the most abundant ion present in sugarcane juice, with the mean concentration ranging from 58.1 to 65.7 mM (Table 2.1-2). The second most abundant was  $\text{Cl}^{-}$  (35.2 to 39.7 mM), whose mean was highest among the three anions. These two ions were

**Table 2.1-2. Means of ion concentrations (mmol L<sup>-1</sup>) in sugarcane juice.**

	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>
2013	3.5 ± 2.6	1.3 ± 1.7	65.7 ± 18.6	8.4 ± 2.5	4.7 ± 1.9	39.7 ± 11.7	0.0 ± 0.0	0.1 ± 0.2	5.5 ± 3.6	16.1 ± 4.7
2014	2.1 ± 1.2	1.3 ± 1.7	58.1 ± 18.8	9.4 ± 2.9	5.9 ± 2.1	35.2 ± 10.8	0.1 ± 0.5	0.1 ± 0.1	4.1 ± 2.5	15.5 ± 4.2
2015	3.3 ± 2.2	5.5 ± 10.6	63.9 ± 19.0	7.7 ± 2.6	4.5 ± 1.6	38.0 ± 11.5	0.0 ± 0.1	0.1 ± 0.2	4.7 ± 2.5	13.8 ± 3.5
<b>Average</b>	<b>3.0 ± 2.2</b>	<b>2.7 ± 6.5</b>	<b>62.1 ± 19.3</b>	<b>8.5 ± 2.8</b>	<b>5.0 ± 1.9</b>	<b>37.5 ± 11.7</b>	<b>0.0 ± 0.3</b>	<b>0.1 ± 0.2</b>	<b>4.8 ± 3.0</b>	<b>15.1 ± 4.3</b>

Means ± SD.

thought to be dominant, as their sum accounted for more than 70% of total ion concentrations.  $\text{SO}_4^{2-}$  was the third most abundant with a mean from 13.8 to 16.1 mM. The mean concentrations of the other ions were lower than 10 mM. The correlation coefficients between the ion concentrations and PIC for the three years are given in Table 2.1-3. Through the three years, the concentrations of  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{NO}_3^-$  negatively correlated with PIC with significance at the 1% level, while  $\text{PO}_4^{3-}$  significantly positively correlated. These ions were thought to be affecting PIC; however, the concentrations of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  were much lower than those of  $\text{K}^+$  and  $\text{Cl}^-$ , implying that changing nitrogen and phosphorus management would make small effect. The other ions showed significant correlations with PIC in some years, but the results were not constant enough. The concentrations of  $\text{K}^+$  and  $\text{Cl}^-$  were the most abundant among the cations and the anions respectively and the correlations of these ions with PIC were significantly negative at the 1% level in all the three harvest seasons; therefore, I concluded that they are the factors to be most concerned for improving sugarcane quality and accordingly focused on these two ions for further analyses.

The correlations between these factors and PIC are illustrated in Fig. 2.1-1. PIC ranged widely from 7.6 to 17.7%.  $\text{K}^+$  and  $\text{Cl}^-$  scattered in a wide range as well, namely  $\text{K}^+$  from 805 to 4749  $\text{mg L}^{-1}$  and  $\text{Cl}^-$  from 380 to 3121  $\text{mg L}^{-1}$ . As previously stated,  $\text{K}^+$  and  $\text{Cl}^-$  negatively correlated with PIC in all the three years. The slopes of the regression lines varied depending on the years and those in 2013 were slightly steeper.

I also plotted the mean values of the two factors and PIC when samples were categorized by sugar mill, resulting in 17 circles in a plot (Fig. 2.1-2). The means varied greatly and, similarly to the tendency in Fig. 2.1-1, those two factors were negatively associated with PIC, so that cane from areas with high  $\text{K}^+$  or  $\text{Cl}^-$  generally had a low PIC.

From Fig. 2.1-2, it was suggested that there were differences in  $\text{K}^+$  and  $\text{Cl}^-$  concentrations between sugar mills. Then, the means of each mill over three harvest seasons were given in Table 2.1-4. As a result of ANOVA, the means significantly differed depending

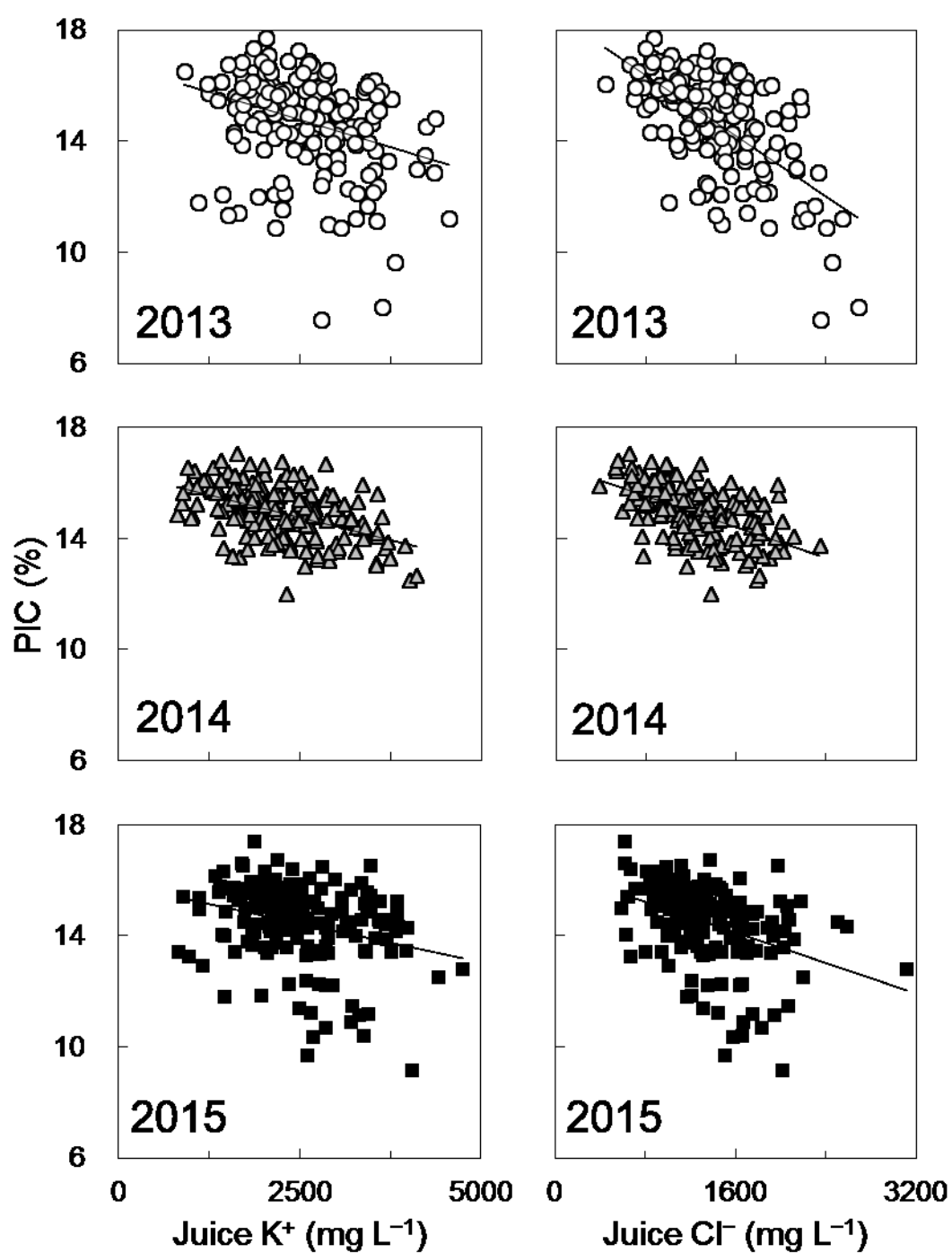
**Table 2.1-3. Correlation coefficients of ion concentrations in sugarcane juice with PIC.**

	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>3-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	EC
2013	-0.57 **	0.01	-0.32 **	0.04	-0.01	-0.63 **	0.04	-0.36 **	0.18 *	0.06	-0.59 **
2014	-0.13	-0.11	-0.48 **	0.18 *	0.20 **	-0.54 **	-0.01	-0.34 **	0.19 *	0.05	-0.60 **
2015	-0.03	-0.09	-0.29 **	0.16 *	0.27 **	-0.39 **	-0.06	-0.44 **	0.38 **	0.22 **	-0.46 **

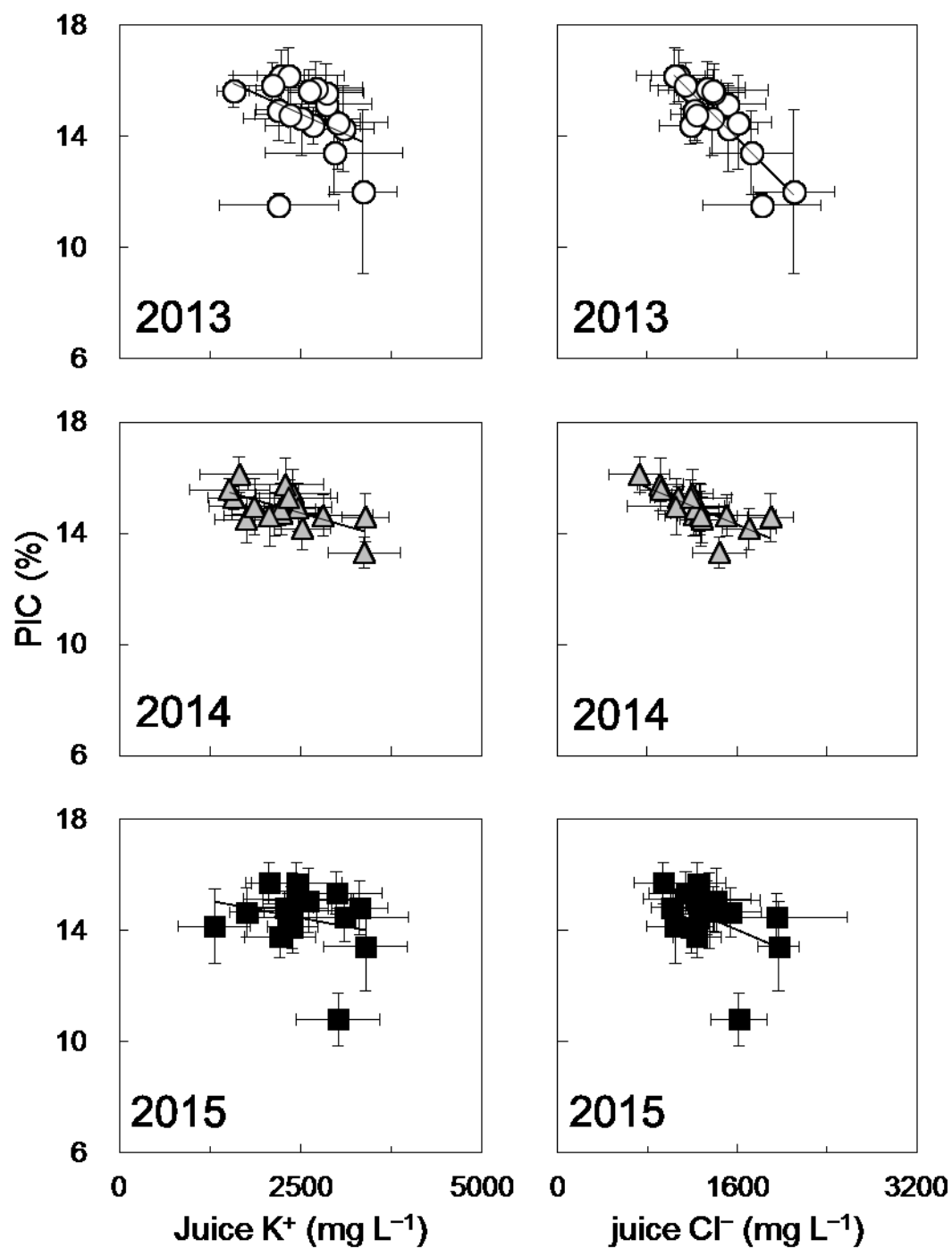
\* and \*\* mean significances at the 5% and the 1% levels, respectively.

**Table 2.1-4.** Means of K<sup>+</sup> and Cl<sup>-</sup> concentrations  
in sugarcane juice of each sugar mill.

Mill No.	Juice K <sup>+</sup>			Juice Cl <sup>-</sup>		
	2013	2014	2015	2013	2014	2015
1	2672	3378	3016	1188	1444	1614
2	2231	2392	2373	1081	1206	1384
3	1573	1574	1754	1203	1083	1541
4	2851	2143	2212	1519	1217	1240
5	2849	2464	2380	1396	1268	1349
6	3361	2238	3394	2107	1269	1968
7	2201	1751	1314	1819	1289	1046
8	3087	2228	2444	1525	1234	1241
9	2962	1866	2456	1731	1059	1238
10	2513	1648	2318	1380	737	1259
11	3015	2291	3312	1612	919	1329
12	2710	2341	3109	1337	1189	1948
13	2332	3394	2605	1048	1900	1413
14	2195	2522	2282	1225	1710	1010
15	2355	2075	2389	1246	1282	1186
16	2111	1519	2063	1145	925	938
17	2618	2814	2992	1388	1510	1141
Average	2567	2273	2496	1409	1250	1346



**Fig. 2.1-1.** Relationships of K<sup>+</sup> and Cl<sup>-</sup> concentrations in sugarcane juice with PIC.



**Fig. 2.1-2.** Relationships of the mean  $K^+$  and  $Cl^-$  concentrations in sugarcane juice with the mean PIC. Each symbol represents the mean of each sugar mill. Horizontal bars indicate SD of juice  $K^+$  and  $Cl^-$  concentrations and vertical bars indicate SD of PIC.



**Table 2.1-4.** Means of K<sup>+</sup> and Cl<sup>-</sup> concentrations in sugarcane juice of each sugar mill.

Mill No.	Juice K <sup>+</sup>			Juice Cl <sup>-</sup>		
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7	2201	1751	1314	1819	1289	1046
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9	2962	1866	2456	1731	1059	1238
10	2513	1648	2318	1380	737	1259
11	3015	2291	3312	1612	919	1329
12	2710	2341	3109	1337	1189	1948
13	2332	3394	2605	1048	1900	1413
14	2195	2522	2282	1225	1710	1010
15	2355	2075	2389	1246	1282	1186
16	2111	1519	2063	1145	925	938
17	2618	2814	2992	1388	1510	1141
Average	2567	2273	2496	1409	1250	1346

on sugar mills in all the three years. Although each of the means greatly varied depending on the years, some sugar mills constantly had higher values than the means, namely higher  $K^+$  concentrations were observed in Shinko Togyo Co., Ltd., Shonan Sugar Mfg. Co., Ltd., Kita-Daito Sugar Mfg. Co., Ltd., and Ishigakijima Sugar Mfg. Co., Ltd. and  $Cl^-$  in Nanei Togyo Co., Ltd.

To obtain more information, I categorized the juice samples by cropping type and cultivar. More than 30 cultivars were analyzed and NiF8, Ni21, Ni22, Ni23, and Ni27 were the five most dominant cultivars. The correlation coefficients of juice  $K^+$  and  $Cl^-$  with PIC, when the samples were categorized by cropping type or cultivar are shown in Table 2.1-5. Most of the correlations were negative and, in particular, the correlations between  $Cl^-$  and PIC were all significant at the 1% level except for summer planting in 2015 when the samples were categorized by cropping type. The correlation coefficients were high in spring planting and ratooning among cropping type and Ni22 and Ni23 among cultivars.

For all the samples in the three years, linear regression was performed by using EC as an independent variable and  $K^+$  and  $Cl^-$  concentrations as a dependent variable (Fig. 2.1-3). The coefficients of determination were high enough to predict  $K^+$  and  $Cl^-$  concentrations based on EC values. The equations were  $y = 3.9922x - 424.65$  and  $y = 2.0785x - 162.33$ , respectively.

## Discussion

This study focused on identifying factors affecting sugarcane quality and, following juice analysis for three years, found that  $K^+$  and  $Cl^-$  were the most abundant cation and anion in sugarcane juice and that the concentrations of these two ions negatively correlated with PIC. It is noteworthy that similar results were observed for all the three years, although the sugarcane samples differed in cropping type and cultivar and were derived from different fields every year. From these, it was concluded that  $K^+$  and  $Cl^-$  are influential ions and exert

**Table 2.1-5.** Correlation coefficients of K<sup>+</sup> and Cl<sup>-</sup> concentrations in sugarcane juice with PIC when sorted by cropping type and cultivar.

		Juice K <sup>+</sup>			Juice Cl <sup>-</sup>		
		2013	2014	2015	2013	2014	2015
Cropping type	Spring planting	-0.24 **	-0.60 **	-0.55 **	-0.63 **	-0.52 **	-0.67 **
	Summar planting	-0.06	-0.43 **	-0.11	-0.34 *	-0.55 **	-0.23
	Ratooning	-0.45 **	-0.46 **	-0.22	-0.73 **	-0.60 **	-0.28 *
Cultivar	NiF8	0.07	-0.49 **	-0.18	-0.36 *	-0.42 **	-0.35 *
	Ni21	-0.12	-0.61 *	0.02	-0.11	-0.85 **	-0.79 **
	Ni22	-0.48 *	-0.61 **	-0.50 *	-0.80 **	-0.54 **	-0.32
	Ni23	-0.08	-0.36	-0.54 *	-0.77 **	-0.68 **	-0.43
	Ni27	-0.50 *	-0.22	-0.39 *	-0.37	-0.16	-0.64 **

\* and \*\* mean significances at the 5% and the 1% levels, respectively.

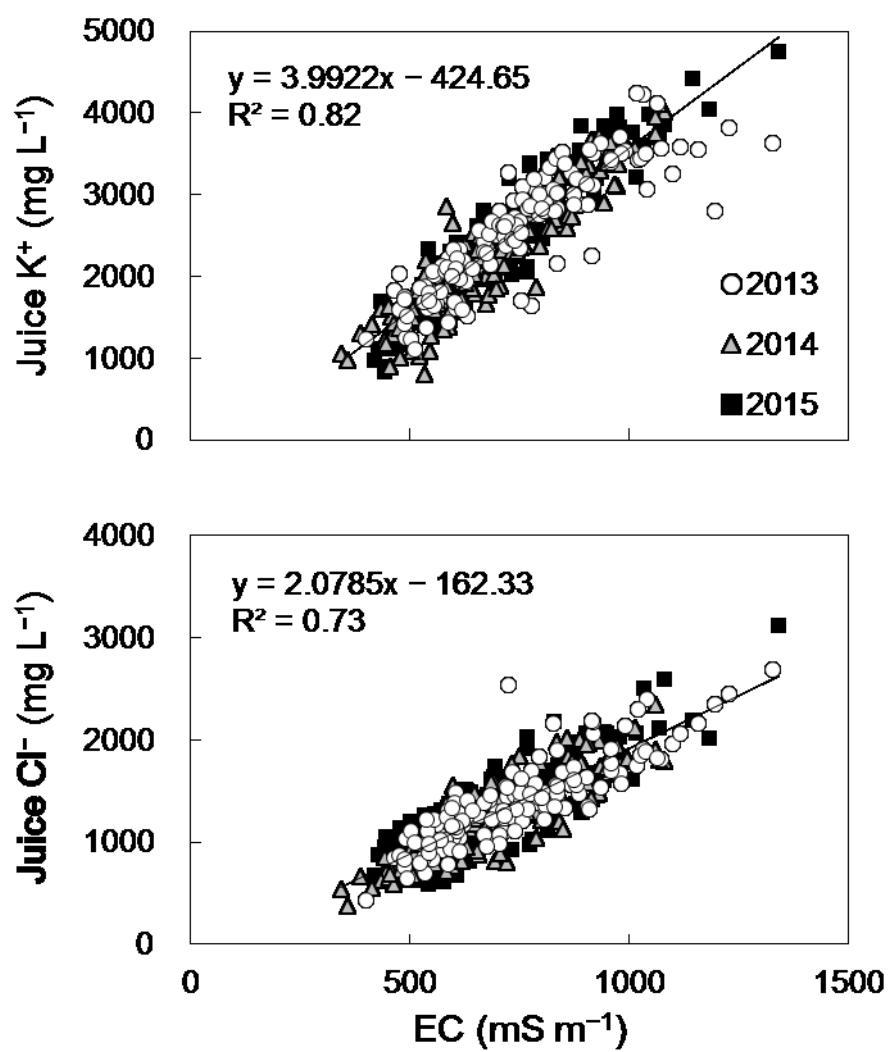


Fig. 2.1-3. Relationships of EC with K<sup>+</sup> and Cl<sup>-</sup> concentrations in sugarcane juice.

adverse effects on sugarcane quality.

Soluble inorganic salts such as K and chloride (Cl) in sugarcane juice are defined as ash (Jackson et al., 2008). Especially K is of great concern because it is the main contributor to the high ash problem by comprising about 40% of the total ash (Stevenson et al., 1970). Higher levels of ash are undesirable because of deleterious effects on processing in raw sugar mills and sugar refineries (Hogarth and Kingston, 1983). In the raw sugar mill, exhaustion of molasses is impaired by increasing ash levels due to enhanced solubility of sucrose and increased viscosity of massecuites. In sugar refineries, ash can also affect color of refined sugar through impairment of the decolorizing process due to incorporation of ash on the char matrix. Additional process energy is required to evaporate the required extra washings and to recrystallize sugar made from high ash raw sugar. These two factors therefore could have a negative impact on the production of sugar, one of the final product of sugarcane, through both sugarcane cultivation and sugar producing process.

Depending on production area, the concentrations of  $K^+$  and  $Cl^-$  varied greatly and areas with higher juice  $K^+$  and  $Cl^-$  concentrations showed lower PIC. The concentrations of  $K^+$  and  $Cl^-$  in soil or irrigation water highly correlated with concentrations of those in the juice (Stevenson et al., 1970; Kingston, 1982a; Lingle and Wiegand, 1997), suggesting that  $K^+$  and  $Cl^-$  had been supplied in excess in those areas, eventually leading to reduction in PIC. Ota et al. (2000) performed foliar and soil diagnoses in some sugarcane-producing regions in Japan and found that levels of potassium (K) in sugarcane leaves and exchangeable K in soils were mostly sufficient or even overabundant. Kafkafi (2001) reported that negative effects of  $Cl^-$  are observed in coastal regions where airborne  $Cl^-$  is transported from the ocean. Sugarcane cultivation in Japan, as already mentioned, is limited to island areas. These partly support our results and indicate that some sugarcane production areas in Japan may contain large amounts of soil  $K^+$  and  $Cl^-$ . One of the sources of  $K^+$  and  $Cl^-$  is a common K fertilizer, potassium chloride (KCl), which is widely used for sugarcane production in Japan.

Recommended K dose is precisely decided depending upon soil and cropping type, ranging from 50 to 250 kg ha<sup>-1</sup> (Ministry of Agriculture, Forestry and Fisheries, 2003; Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries, 2015), and sugarcane is generally fertilized with compound fertilizers including 6 to 15 % of K by KCl (Japan Agriculture, Kagoshima Prefectural Economic Federation of Agricultural Co-operatives, 2015a; Japan Agriculture, Kagoshima Prefectural Economic Federation of Agricultural Co-operatives, 2015b; Ryukyu Fertilizer Co., Ltd., 2015) in this region, so that use of fertilizers with lower K percentage could contribute to an increase in PIC in such areas. This measure would benefit not only the sugar industry but also cane growers and the environment because it is more cost-effective and environment-friendly. Negative correlations of K<sup>+</sup> and Cl<sup>-</sup> concentrations with PIC were also confirmed when samples were categorized by cropping type or cultivar. The proposed practice would thus be effective irrespective of cropping type or sugarcane cultivar.

## **2.2. Field surveys in Miyako Island for sugarcane juice and soil analysis**

### **Introduction**

From Chapter 2.1, it was revealed that  $K^+$  and  $Cl^-$  were the most abundant cation and anion and they negatively correlated with PIC. These results suggest that the two ions are the factors most concerned for sugarcane quality. In the study, however, these samples were collected from different areas as well as their cropping types and cultivars were different.  $K^+$  and  $Cl^-$  concentrations obviously had regional differences in the study of Chapter 2.1. It indicates that, in some production areas with high  $K^+$  and  $Cl^-$ , KCl –the major source of  $K^+$  and  $Cl^-$  from fertilizer– has been overdosed or  $K^+$  and  $Cl^-$  derived from other sources such as rain and irrigation water and saline soil tend to be excessively supplied in the environments, which can contribute to the accumulation of  $K^+$  and  $Cl^-$  in the sugarcane fields. Cropping type and cultivar may also affect ion composition and its relation with PIC. Besides, it is necessary to reveal the relationships of  $K^+$  and  $Cl^-$  with quantitative parameters since both yield and quality are concerned with sugar production.

Miyako Island lies approximately 300 km south west from Okinawa Island. This island is the largest sugarcane producing area in Japan, accounting for about 20% of its total sugarcane production (Agriculture and Livestock Industries Cooperation, 2016a), and has two raw sugar mills currently under operation. I conducted field surveys for a number of sugarcane fields in Miyako Island, collecting soil samples as well as plants to examine the yields, sugar contents and ion compositions in sugarcane juice, and soil inorganic nutrients and to see the relationships of juice nutrients with sugarcane yield and quality and soil nutrients.

### **Materials and methods**

This surveys were conducted three times: October, 2014 ( $T_1$ ); January, 2015 ( $T_2$ ); and March, 2016 ( $T_3$ ) and the numbers of sample fields were 37, 43, and 14, respectively. The information

of cropping type and cultivar of the survey was shown in table 2.2-1. The plot size was 4.2 m<sup>2</sup>, 3 m row length with 1.4 m of inter-row space, assuming that the row width is all 1.4 m in Miyako Island's sugarcane fields and, from each field, three plots in T<sub>1</sub> and T<sub>2</sub> and four plots in T<sub>3</sub> were established. The number of millable stalks per plot was counted and three average stalks were chosen by visual observation to measure stalk weight and length and to obtain juice. Stalk number and weight were used for the rough estimation of yield per area. The three cane stalks from each plot were then pressed together. Bagasse weight after squeezing was measured to calculate extraction efficiency and sugar yield by the equations below.

$$\text{Extraction efficiency} = 1 - \text{bagasse weight} / \text{millable stalk weight}$$

$$\text{Sugar yield} = \text{yield} * \text{extraction efficiency} * \text{sucrose concentration}$$

Approximately 100 g of soil samples were taken from three points at 5–10 cm depth and air-dried for soil analysis.

Juice samples were diluted 50-fold with extra-pure water and filtered with a membrane filter (diameter, 13 mm; pore size, 0.45 µm; Advantec) for sugar analysis. Sucrose content was determined by high-pressure liquid chromatography (HPLC, Shimadzu) with a liquid chromatograph (LC-20AD), a column oven (CTO-20A), a refractive index detector (RID-10A), and an autosampler (SIL-20A). The column and eluent used were SCR-101H and degassed extra-pure water. The remaining filtrates were further diluted four times and used for ion analysis. The concentrations of K<sup>+</sup> and Cl<sup>-</sup> were measured by ion chromatography method as described in Chapter 2.1.

Soil samples were ground and passed through a 2 mm sieve. Then, 5 g of each sample was shaken with 25 mL of extra-pure water for one hour (1:5, soil:water; U.S. Salinity Laboratory Staff, 1954). pH and EC of saturated water extracts were measured with a pH meter (HM-20S, Toa) and an EC meter (CM-14P, Toa).

Mean and standard deviations of the samples were calculated and statistical analysis was performed with R (R Core Team, 2015). Data were subjected to one-way ANOVA to test



differences among sugar mills. Significance was accepted based on a P value <0.05.

## Results

The major sugarcane cultivars in the surveys were Ni21, NiH25, and Ni27 (Table 2.2-1). Especially Ni27 accounted for more than half of the samples. Except for T<sub>3</sub>, most were ratooning and summer planting fields and few fields were categorized in spring planting. Taken together, the major combinations of cropping type and cultivar were considered to be summer planting and ratooning of Ni 21 and Ni27 in T<sub>1</sub> and T<sub>2</sub> and ratooning of Ni27 in T<sub>3</sub>, given that three or more than three fields were examined for the surveys.

The means of cane yield and its components are shown in Table 2.2-2. Yields were relatively high as compared to the average cane yield in Japan (2014/15: 5.07 t 10a<sup>-1</sup>, 2015/16: 5.39 t 10a<sup>-1</sup>; Agriculture and Livestock Industries Cooperation, 2016b). Stalk length and weight were the highest in T<sub>2</sub>, while the highest number of stalks and the highest yield were observed in T<sub>3</sub> and T<sub>1</sub>, respectively. Sucrose contents of T<sub>2</sub> and T<sub>3</sub> were higher than that of T<sub>1</sub> probably because canes were matured when harvested. The highest sugar yield was obtained in T<sub>2</sub>.

Then, I examined the relationships of the two ions in juice, K<sup>+</sup> and Cl<sup>-</sup>, with these parameters (Table 2.2-3). There were some significant relationships between juice K<sup>+</sup> and quantitative parameters, but the correlation coefficients were relatively low. In comparison with K<sup>+</sup>, Cl<sup>-</sup> was thought to be more influential in quantitative parameters, especially stalk length and stalk weight, resulting in significances at the 1% level in all the sampling times. Yield was also positively associated with Cl<sup>-</sup>. When it comes to the quality, though juice sucrose content of T<sub>1</sub> had a significantly positive correlation with K<sup>+</sup> and no significant correlation with Cl<sup>-</sup>, the relationships completely changed in harvest seasons of T<sub>2</sub> and T<sub>3</sub>: significant negative relations at the 1% level were observed both with K<sup>+</sup> and Cl<sup>-</sup> and the correlation coefficients were relatively high. Sugar yield was significantly positively related

**Table 2.2-1.** Compositions of cropping types and cultivars in the surveys.

		Ni21	NiH25	Ni27	Others	Total
	Spring planting	0	0	1	0	
T <sub>1</sub>	Summer planting	4	1	8	1	37
	Ratooning	5	2	13	2	
	Spring planting	1	0	1	0	
T <sub>2</sub>	Summer planting	5	1	12	2	43
	Ratooning	3	2	14	2	
	Spring planting	0	2	0	0	
T <sub>3</sub>	Summer planting	1	0	1	0	14
	Ratooning	1	2	5	2	

**Table 2.2-2. Sugar yield and its components of the surveys.**

	Stalk length cm	Stalk weight g stalk <sup>-1</sup>	No. of stalks stalks 10a <sup>-1</sup>	Yield t 10a <sup>-1</sup>	Sucrose %	Sugar yield t 10a <sup>-1</sup>
T <sub>1</sub>	226 ± 58	1218 ± 390	7473 ± 1707	9.19 ± 3.86	13.5 ± 2.9	0.64 ± 0.32
T <sub>2</sub>	279 ± 59	1552 ± 488	6819 ± 1959	8.91 ± 3.73	18.3 ± 1.9	0.82 ± 0.32
T <sub>3</sub>	269 ± 61	1294 ± 461	7543 ± 1809	8.00 ± 3.57	16.9 ± 1.8	0.65 ± 0.31

Values are means ± SD.

**Table 2.2-3.** Correlation coefficients of juice  $K^+$  and  $Cl^-$  with sugar yield and its components.

		Stalk length	Stalk weight	No. of stalks	Yield	Sucrose	Sugar yield
Juice $K^+$	T <sub>1</sub>	0.19 *	0.07	0.15	0.16	0.33 **	0.27 **
	T <sub>2</sub>	0.24 **	0.19 *	0.11	0.22 *	-0.52 **	0.09
	T <sub>3</sub>	0.06	0.01	-0.20	-0.04	-0.54 **	-0.19
Juice $Cl^-$	T <sub>1</sub>	0.37 **	0.25 **	0.25 **	0.36 **	0.03	0.31 **
	T <sub>2</sub>	0.45 **	0.38 **	0.03	0.33 **	-0.62 **	0.15
	T <sub>3</sub>	0.59 **	0.45 **	0.12	0.43 **	-0.51 **	0.31 *

\* and \*\* mean significances at the 5% and the 1% levels, respectively.

to  $K^+$  and  $Cl^-$  in  $T_1$  and  $Cl^-$  in  $T_3$ .

Further correlation analysis between the ions and yield components was performed for the major combinations of cropping type and cultivar (Table 2.2-4). Few significant correlations were found between the juice ions and quantitative parameters including sugar yield and it was hard to tell the general tendencies. As compared to quantitative parameters, the qualitative parameter –sucrose content– was likely to be influenced by  $K^+$  and  $Cl^-$  when samples were categorized by cropping type and cultivar as well as when all samples were used for analysis, giving consistency with the previous results shown in Table 2.2-3. In  $T_1$ , the relations were not significant or significantly positive, but most of the relations of  $K^+$  and  $Cl^-$  with sucrose content in  $T_2$  and  $T_3$  were significantly negative.  $Cl^-$  could have stronger effects on quality than  $K^+$ , given that the correlation coefficients were all higher in  $Cl^-$ .

Fig. 2.2-1 to 3 show the relationships between the two ions and yield, sucrose content, and sugar yield, respectively.  $K^+$  and  $Cl^-$  concentrations in sugarcane juice varied widely, ranging from 394 to 4153  $mg\ L^{-1}$  and 395 to 2158  $mg\ L^{-1}$  at  $T_1$ , from 657 to 4037  $mg\ L^{-1}$  and 470 to 2352  $mg\ L^{-1}$  at  $T_2$ , and 619 to 3301  $mg\ L^{-1}$  and 386 to 1657  $mg\ L^{-1}$  at  $T_3$ , respectively. As stated before, it is difficult to tell the tendencies of the correlations with yield and sugar yield when samples are sorted by cropping type and cultivar. For instance, comparatively strong correlations were observed between juice  $K^+$  or  $Cl^-$  and yield in summer planting of Ni21, while other combinations had lower values. Compared to this, juice sucrose content, irrespective of cropping type and cultivar, adversely correlated with  $K^+$  or  $Cl^-$  at harvest of  $T_2$  and  $T_3$ . Each of the major combinations had a wide range of  $K^+$  and  $Cl^-$  and sucrose content decreased with increases of  $K^+$  and  $Cl^-$  concentrations.

The relationships between  $K^+$  and  $Cl^-$  of soil and those of sugarcane juice were also examined (Fig. 2.2-4). Between soil  $K^+$  and juice  $K^+$ , positive correlations were consistently confirmed from  $T_1$  to  $T_3$ , while those between  $Cl^-$  of soil and juice were positive to negative and significantly positive only in  $T_2$ . It is likely that in  $T_1$  and  $T_2$  the relations between soil  $K^+$

**Table 2.2-4.** Correlation coefficients of juice  $K^+$  and  $Cl^-$  with sugar yield and its components when samples are sorted by cropping type and cultivar.

		Stalk length	Stalk weight	No. of stalks	Yield	Sucrose	Sugar yield	
Juice K <sup>+</sup>	T <sub>1</sub>	Ni21, Summer	0.33	0.07	0.23	0.27	0.00	0.32
		Ni21, Ratoon	−0.20	−0.08	0.07	−0.01	−0.05	0.00
		Ni27, Summer	0.21	0.13	0.28	0.23	0.58 **	0.33
		Ni27, Ratoon	−0.11	−0.26	0.20	−0.08	0.28	0.20
	T <sub>2</sub>	Ni21, Summer	0.59 *	0.38	0.05	0.34	−0.69 **	0.16
		Ni21, Ratoon	0.83 **	0.32	0.02	0.16	−0.71 *	−0.01
		Ni27, Summer	−0.17	−0.15	0.42 *	0.18	−0.52 **	0.06
		Ni27, Ratoon	−0.15	−0.14	−0.14	−0.19	−0.38 *	−0.32 *
	T <sub>3</sub>	Ni21, Summer	0.14	0.23	−0.41	−0.09	−0.46	−0.27
	Juice Cl <sup>−</sup>	T <sub>1</sub>	Ni21, Summer	0.53	0.21	0.18	0.34	−0.16
Ni21, Ratoon			−0.34	−0.02	−0.12	−0.10	−0.02	−0.05
Ni27, Summer			0.20	0.02	0.51	0.30	0.35	0.30
Ni27, Ratoon			−0.02	−0.24	0.11	−0.11	0.05	0.05
T <sub>2</sub>		Ni21, Summer	0.65 **	0.49	0.11	0.47	−0.70 **	0.27
		Ni21, Ratoon	0.63	0.13	−0.25	−0.15	−0.75 *	−0.34
		Ni27, Summer	−0.03	−0.01	0.31	0.19	−0.71 **	−0.26
		Ni27, Ratoon	0.18	0.01	−0.21	−0.13	−0.57 **	0.06
T <sub>3</sub>		Ni21, Summer	0.39	0.43	−0.17	−0.09	−0.62 **	−0.06

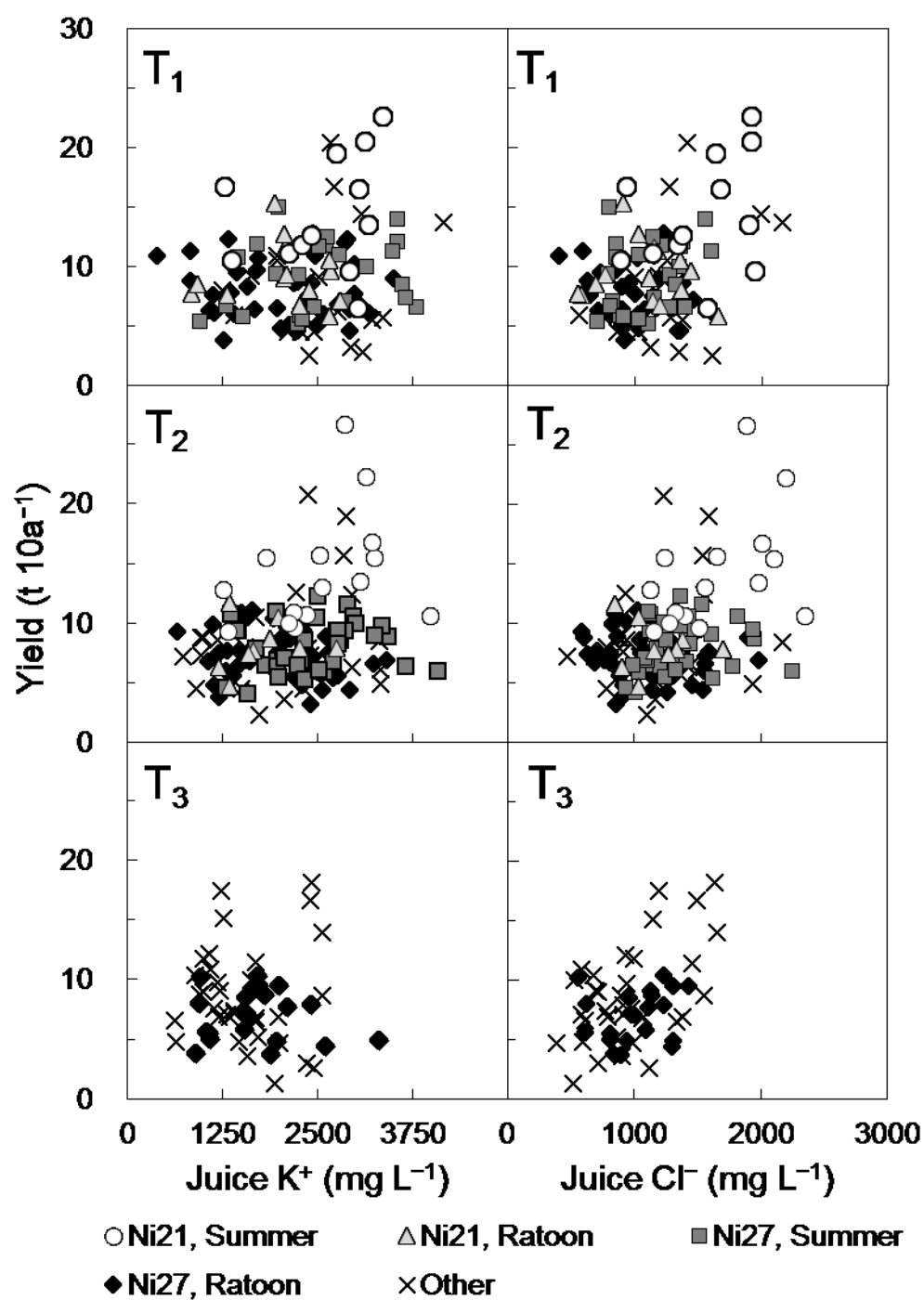


Fig. 2.2-1. Relationships of juice  $K^+$  and  $Cl^-$  with yield.

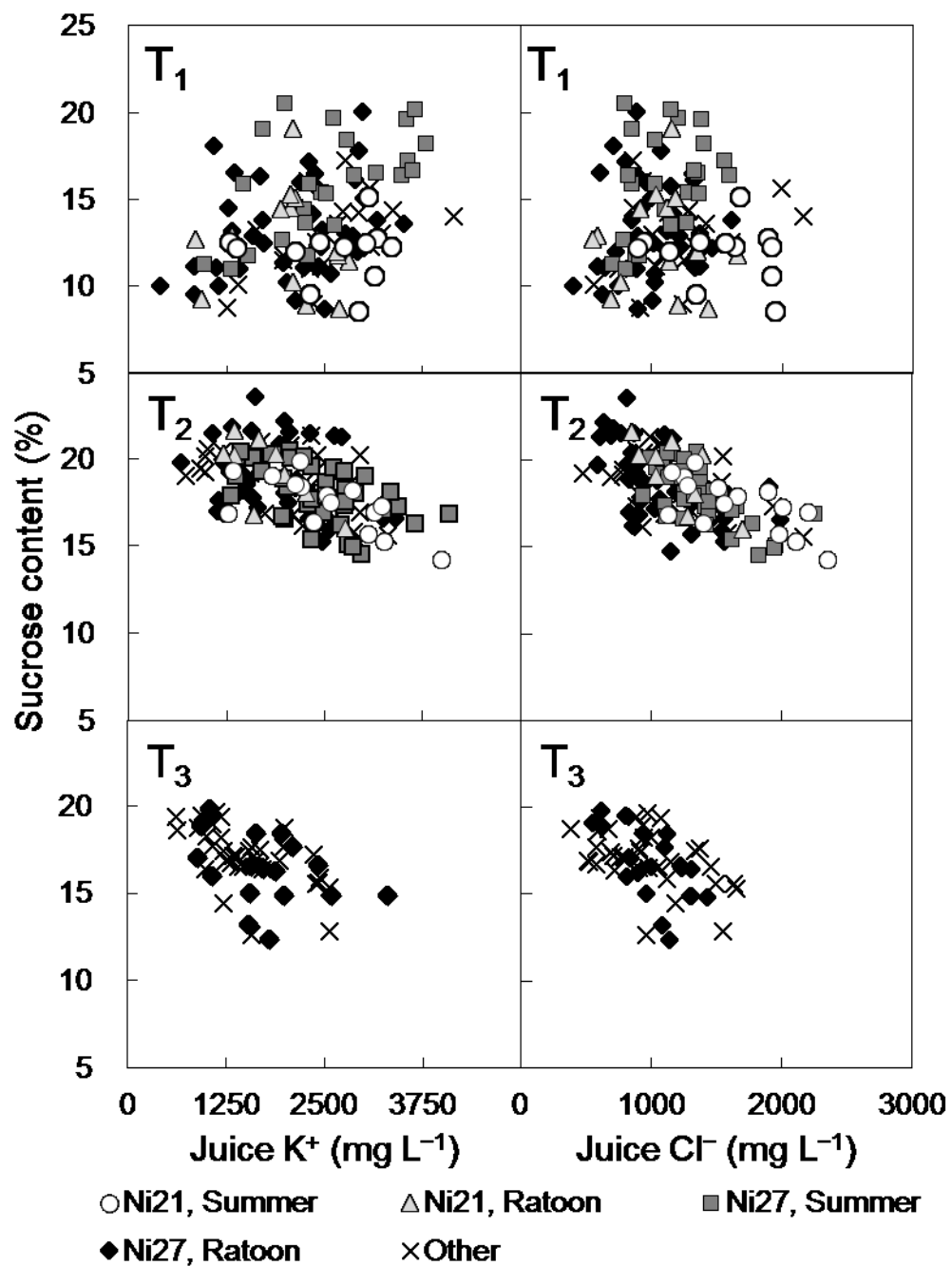


Fig. 2.2-2. Relationships of juice K<sup>+</sup> and Cl<sup>-</sup> with sucrose content.



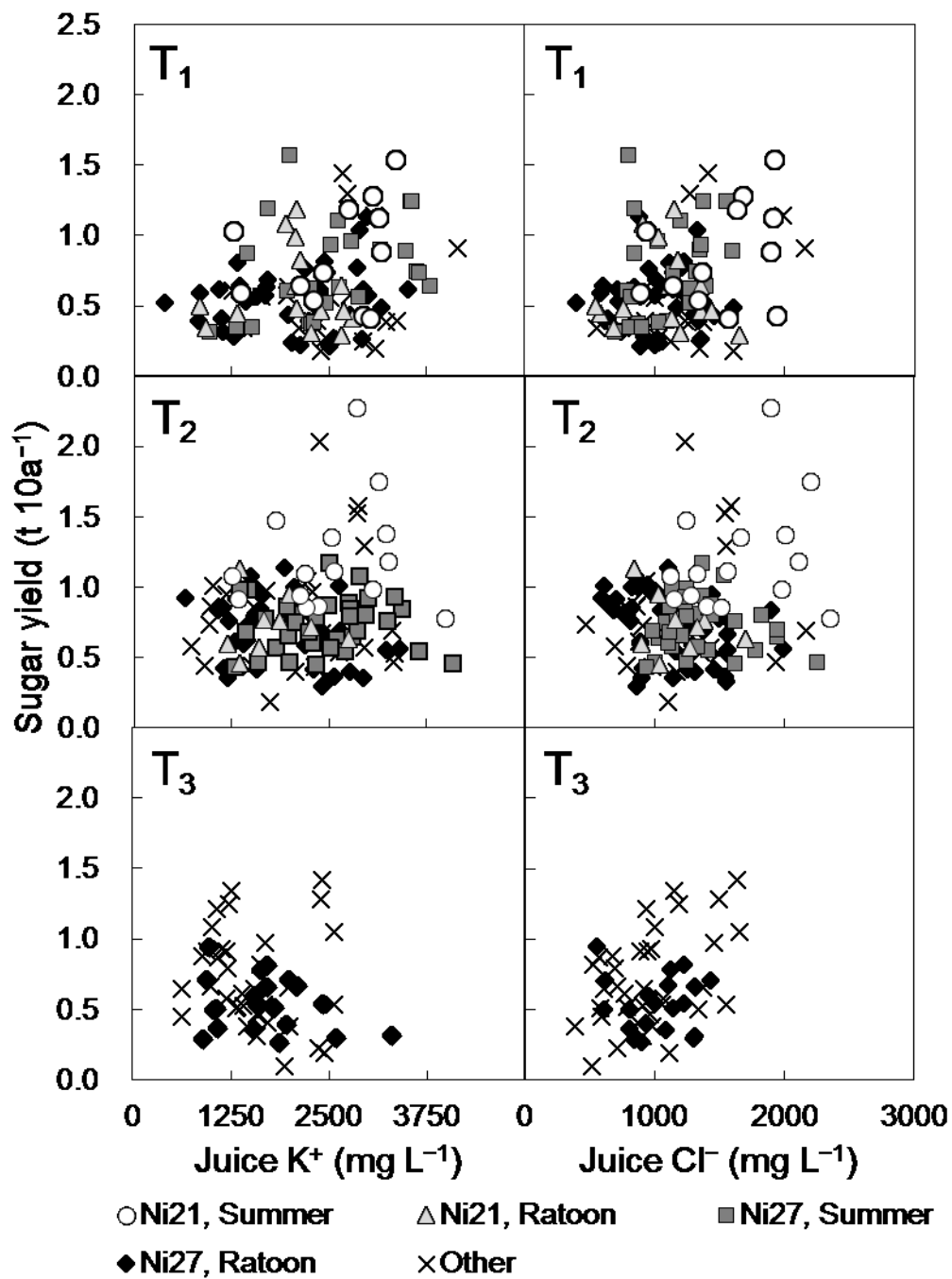


Fig. 2.2-3. Relationships of juice  $K^+$  and  $Cl^-$  with sugar yield.

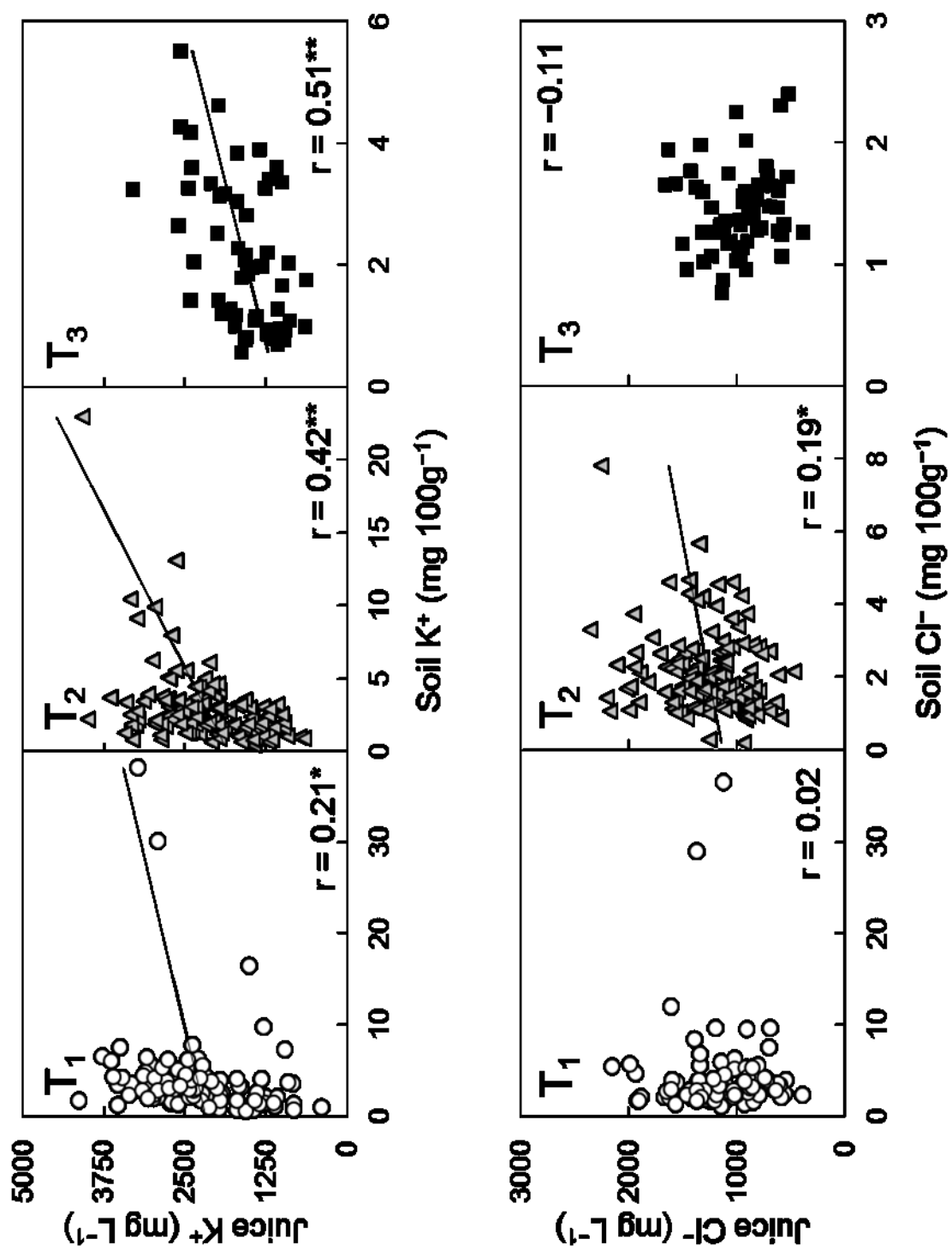


Fig. 2.2-4. Relationships of K<sup>+</sup> and Cl<sup>-</sup> in soil with those in juice.

and juice  $K^+$  were significant due to a few samples with extremely high soil  $K^+$  plotted away from the average. As compared to  $T_1$  and  $T_2$ , soil  $K^+$  of  $T_3$  scattered in a smaller range probably because of an improvement of soil sampling and had a stronger relationship with juice  $K^+$ . Besides that, in  $T_3$ , juice  $Cl^-$  was significantly positively associated with soil  $K^+$  at the 1% level ( $r = 0.49$ ; data not shown).

## Discussion

Through the field surveys in Miyako Island three times, it was confirmed that both  $K^+$  and  $Cl^-$  in sugarcane juice at harvest seasons were negatively associated with sugarcane quality, which is consistent with the results of Chapter 2.1 in which samples were collected from a number of sugarcane producing areas. It is notable that similar results were obtained in two years, namely  $T_2$  and  $T_3$ , even when focusing on one producing area. Moreover, in the major combinations of cropping type and cultivar, summer planting of Ni21 and Ni27 and ratooning of Ni21 and Ni27 in  $T_2$  and ratooning of Ni27 in  $T_3$ , all the relations became obviously significant except for the one between  $K^+$  and sucrose for ratooning of Ni27 in  $T_3$ , implying that this tendency can be observed irrespective of cropping type and cultivar when sample number is many enough.

These surveys also revealed the relationships between the ions and quantitative parameters such as stalk length, stalk weight, number of stalks, cane yield, and sugar yields. Somehow, juice  $Cl^-$  concentration was associated with increases of cane yield when all samples were used for correlation analysis; however, the results were unclear when samples were sorted by cropping type and cultivar. This indicates that  $Cl^-$  itself may not strongly influence sugarcane quantitative parameters. From Fig. 2.2-1,  $K^+$  and  $Cl^-$  concentrations were apparently high in Ni21's summer planting, which makes the correlations positive, sometimes even with significances. However, I can neither prove that  $Cl^-$  did not affect sugarcane growth as Cl is known as one of the essential nutrients for higher plants, by functioning stomatal

regulation, photosynthetic O<sub>2</sub> evolution, osmoregulation, and interaction with other nutrient ions and by enhancing disease resistance and crop yield (Chen et al., 2010), so it is remarkably important to conduct experiments changing Cl<sup>-</sup> levels to see its effects. In Chapter 3 and 4

I tried to identify the sources of juice ions by soil analysis as well. Human practices, such as fertilization and irrigation, significantly contribute to Cl<sup>-</sup> deposition. The vast majority of crops are fertilized with KCl (Kafkafi, 2001), which is composed of K<sup>+</sup> and Cl<sup>-</sup>. Furthermore, natural inputs of ions are not ignorable. For example, natural Cl<sup>-</sup> mainly comes from rainwater, sea spray, dust, and air pollution (White and Broadley, 2001). Saline soil, containing a number of salts, also have effects on ion composition in sugarcane juice. These all account for increments of both K<sup>+</sup> and Cl<sup>-</sup>. Apart from growth environmental factors, selections of cropping type and cultivar can be another factor affecting ion composition. Cropping type is directly related to the length of plant growth period, so probably it affects ion concentrations in juice as well because, as mentioned earlier, nutrient contents change during the crop growth cycle (Walworth and Sumner, 1987; McGray et al., 2010a). Each cultivar has a different characteristic to accumulate nutrients; this part will be documented in detail in Chapter 6 and 7.

Significant positive correlations were found between soil K<sup>+</sup> and juice K<sup>+</sup> but hardly seen between soil Cl<sup>-</sup> and juice Cl<sup>-</sup>. Considering that at T<sub>3</sub> juice Cl<sup>-</sup> was closely associated with soil K<sup>+</sup>, there is a possibility that juice Cl<sup>-</sup> is rather determined by K<sup>+</sup> ions present in fields since Cl<sup>-</sup> is known as the counter ion of K<sup>+</sup> (Laties et al., 1964; Stuart and Jones, 1978; Schnabl and Raschke, 1980). The latter chapters will be focusing on these points.

## **Chapter 3**

### **Effects of different kinds of potassium fertilizers on the growth and quality of sugarcane under pot conditions**

#### **3.1. Pot experiments by changing types and levels of potassium fertilizers**

##### **Introduction**

K and chlorine (Cl) are essential nutrients for plant growth and play numerous roles in plants, for instance, photosynthesis, stomatal activity, transport of sugars, protein, and starch synthesis, and activation of more than 60 enzymes by K (Prajapati and Modi, 2012) and stomatal regulation, photosynthetic O<sub>2</sub> evolution, osmoregulation, interaction with other nutrient ions, disease resistance and improvement of crop yield (Chen et al., 2010). These two nutrients are also known as the components of the most important K fertilizer used for sugarcane production in Japan, KCl. From the sugarcane juice analysis in Chapter 2, however, showing consistently negative relationships with sugar content in juice at harvest, K<sup>+</sup> and Cl<sup>-</sup> were considered to be unfavorable ions for sugarcane quality when their levels in the soils increased. On the contrary, some data suggested Cl<sup>-</sup> may enhance sugarcane growth, though the results were not persuasive enough. It is thus necessary to understand the effects of K<sup>+</sup> and Cl<sup>-</sup> on both cane yield and quality to utilize the data for determining appropriate fertilizer amounts in order for better sugarcane production.

Since KCl is the main source of K as already mentioned, it is hypothesized that regional differences of juice K<sup>+</sup> and Cl<sup>-</sup> concentrations observed in Chapter 2.1 were derived from different doses of KCl. In addition to KCl, potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) is another form of K fertilizer, known as more valuable because it does not contain Cl that gives adverse effects like quality deterioration to some crops such as tobacco (Peele et al., 1960). K from these two fertilizers are equally effective, but they may give plants different effects due to the different

sub-components  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ . Using  $\text{K}_2\text{SO}_4$  enables to see the effects of only  $\text{K}^+$  apart from  $\text{Cl}^-$ , while  $\text{KCl}$  releases both  $\text{K}^+$  and  $\text{Cl}^-$  to the soil.

In the present study, to evaluate the effects of increasing levels of  $\text{K}^+$  and  $\text{Cl}^-$  on sugarcane yield and quality, I conducted several pot experiments applying different amounts of K by two kinds of fertilizers:  $\text{KCl}$  and  $\text{K}_2\text{SO}_4$ .

### **Materials and methods**

I performed two pot experiments: one from April 2010 to February 2011 (experiment 1) and the other from January 2013 to January 2014 (experiment 2) under greenhouse conditions at the University of the Ryukyus, Okinawa, Japan (26°25'N, 127°77'E; 125 m a.s.l.).

Seedlings of a commercial sugarcane cultivar (*Saccharum* spp. cv. NiF8) were collected from fields at the Subtropical Field Science Center of the University of the Ryukyus. One-bud seedlings were immersed in a solution of Benlate-R (5 g L<sup>-1</sup>, Sumitomo Chemical) and in tap water for 24 hours each for sterilization and to improve germination rate. These seedlings were planted and grown in containers from April 15 to May 12, 2010 in experiment 1 and from January 27 to March 5, 2013 in experiment 2. After the first fully expanded leaves were confirmed, seedlings were transplanted into 1/2000a Wagner pots filled with mixed soil of three materials: dark red soil (Shimajiri mahji), sea sand, and peat moss (1:1:1, v v<sup>-1</sup>). Tillers were immediately removed after emergence. Irrigation was carefully performed with tap water ( $\text{Na}^+$ , 19;  $\text{K}^+$ , 1;  $\text{Mg}^{2+}$ , 3;  $\text{Ca}^{2+}$ , 9;  $\text{Cl}^-$ , 29;  $\text{SO}_4^{2-}$ , 8 mg L<sup>-1</sup>) through daily soil moisture evaluation to prevent water stress.

Fertilization was performed approximately once a month after transplanting. The same amounts of nitrogen (N) and phosphorus (P) were supplied for all pots, and treatments consisted of changing types and levels of K fertilizer. In experiment 1, 2.5 g pot<sup>-1</sup> of N and 0.75 g pot<sup>-1</sup> of P were applied with ammonium sulfate and magnesium multi-phosphate,

respectively. Eight plots were established using two types of K fertilizer: KCl and K<sub>2</sub>SO<sub>4</sub>, and four levels of K: 0.19, 0.75, 1.5, and 7.5 g pot<sup>-1</sup> (expressed as i, ii, iii, and iv, respectively). Fifteen pots for each of the plots were prepared. In experiment 2, 2.9 g pot<sup>-1</sup> of N and 1.16 g pot<sup>-1</sup> of P were applied as ammonium nitrate and ammonium phosphate to exclude the effects of SO<sub>4</sub><sup>2-</sup> and other nutrients from N and P fertilizers. Thirteen plots, including one without K fertilizer (expressed as K-0), were established with three types of K fertilizer: KCl, K<sub>2</sub>SO<sub>4</sub>, and a mixture of KCl and K<sub>2</sub>SO<sub>4</sub> (1:1, based on K amount; expressed as Mix) and four levels of K: 0.87, 2.61, 8.7, and 26.1 g pot<sup>-1</sup> (expressed as I, II, III, and IV, respectively). Six pots for each of the plots were prepared. The names of the plots are described as combinations of the supplied K type and level; for example, KCl-i.

After transplanting, stem height from the ground to the base of the top visible dewlap (TVD) leaf, number of green leaves, and soil-plant analysis development (SPAD) value of the TVD leaf using a SPAD meter (SPAD-502, Minolta Camera Co., Ltd.) were measured every 4 weeks to evaluate the effects of the treatments on plant growth.

To investigate how the treatments affected quality as plants matured, I performed sampling four times in experiment 1: three plants from each of the plots on August 20, October 31, and December 20, 2010 and the remaining four to six plants on February 22, 2011. In experiment 2, four to six healthy plants from each of the plots were sampled only once on January 9, 2014. Samples were cut at the ground level. After removal of parts unnecessary for sugar refining, stalk length, diameter, and weight were measured and squeezed. Juice samples were stored at -80°C until used for juice analysis to prevent deterioration in quality. In experiment 2, approximately 20 g of soil was collected from each pot after harvesting plants and was air-dried for soil analysis.

After juice samples were completely melted, EC of juice was measured with an EC meter (CM-14P, Toa) only in experiment 2. Juice samples were prepared for ion and sugar analyses as stated in Chapter 2.2. In experiment 1, K<sup>+</sup> concentration was measured by

inductively coupled plasma emission spectrometry (ICPS-8100, Shimadzu), and  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  concentrations were measured with an ion analyzer (IA-300, Toa). In experiment 2, ion concentrations were determined by ion chromatography as described in Chapter 2.1. Sucrose concentration was determined by HPLC in Chapter 2.2. Soil analysis was performed, following Chapter 2.2.

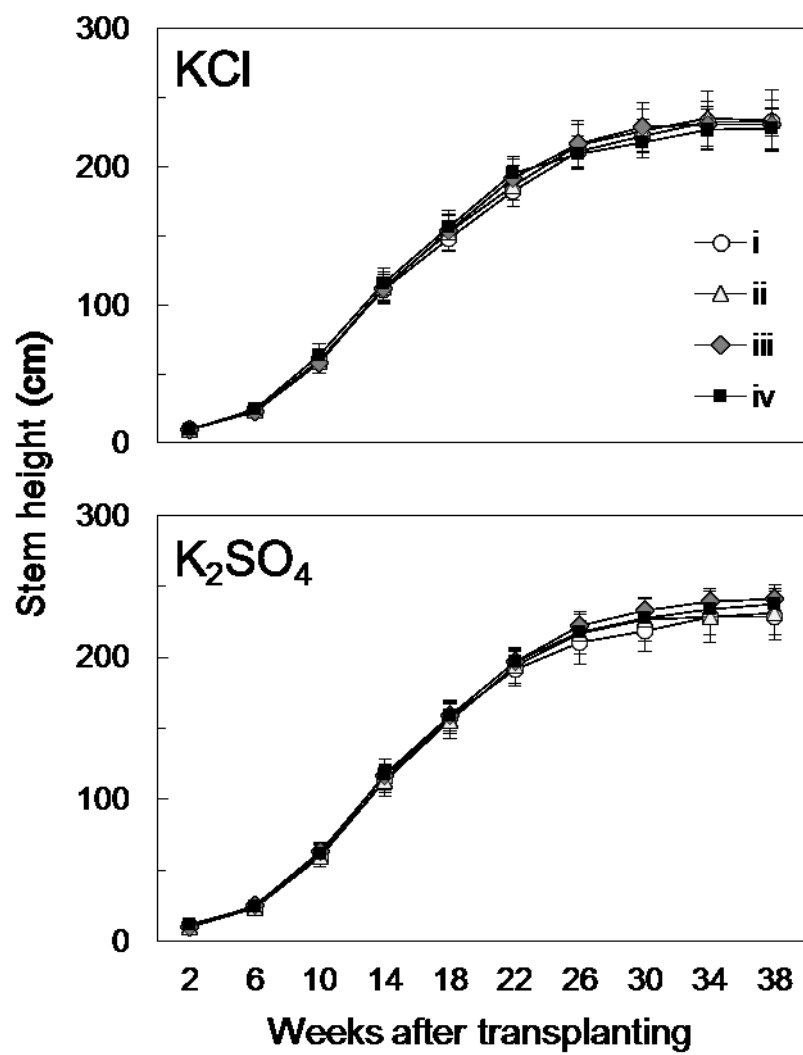
Means and SD of the replications were calculated, and statistical analysis was performed using the software R (R Core Team, 2015). Data were subjected to a *t*-test between two types of K fertilizer or one-way ANOVA between three types of K fertilizer and between K levels. When significances were found, the Tukey test or Tukey–Kramer test for groups with different sample sizes were conducted and significant differences were accepted based on a P value  $<0.05$ .

## Results

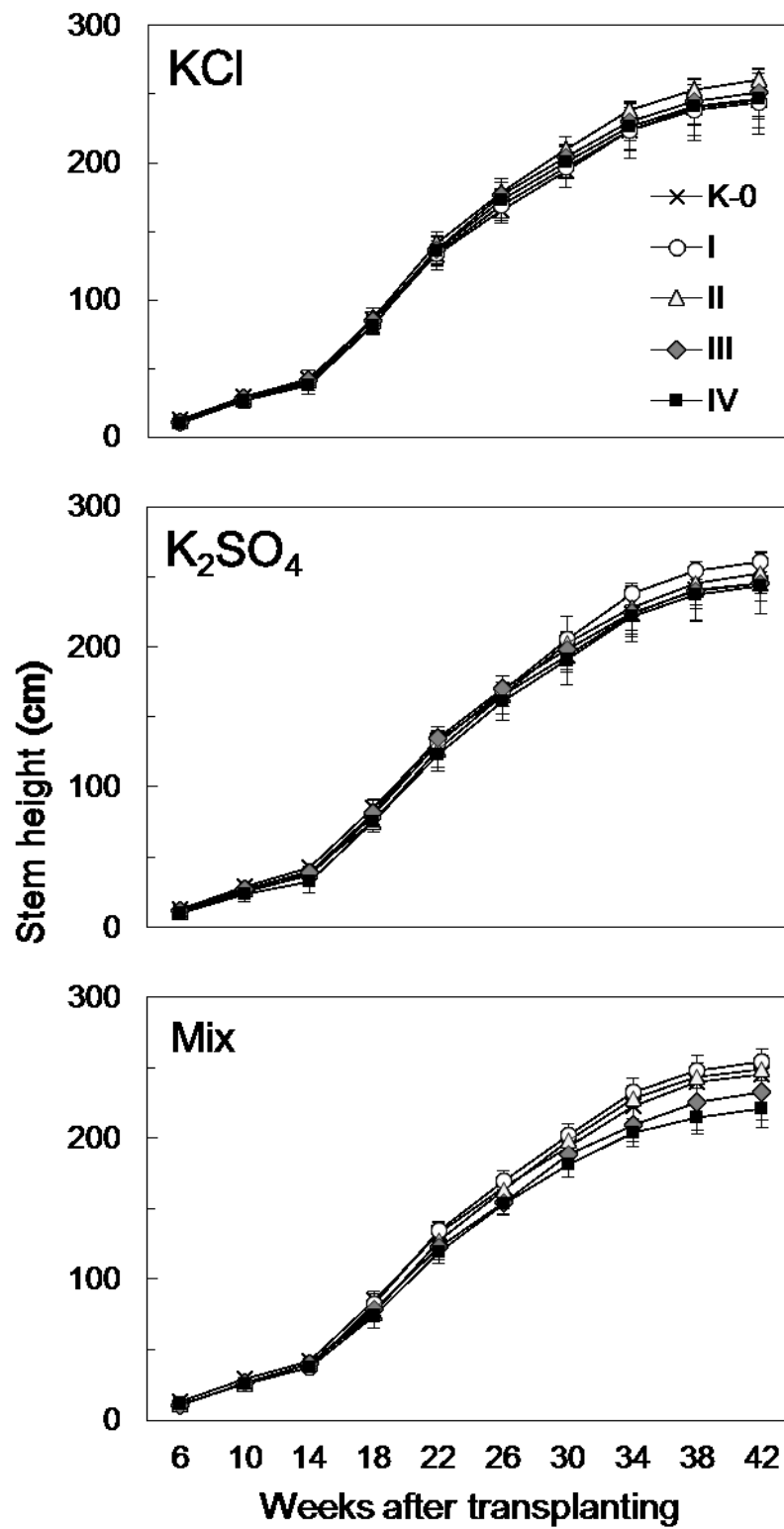
Visual observation revealed that all the plants appeared to be growing well. In experiment 1, stem height increased slowly in the early growth period; however, the speed of growth increased 10 weeks after transplanting and gradually decreased again (Fig. 3.1-1). All the plots showed similar changes, and stem heights finally reached approximately 230 cm. In experiment 2, the change of stem height was similar to that in experiment 1; growth was vigorous during 14–34 weeks after transplanting but sluggish at earlier and later periods of the growing season (Fig. 3.1-2). The final values ranged from 220 cm to 260 cm in experiment 2, which were wider than in experiment 1. In particular, Mix-III and Mix-IV had lower stem height, although there was no clear difference either in their SPAD values of the TVD leaf or in the numbers of green leaves (data not shown).

Table 3.1-1 shows yield and its components at harvest, namely February in 2011. The highest stalk weight was obtained in  $\text{K}_2\text{SO}_4\text{-iv}$  (789 g) and the lowest in  $\text{K}_2\text{SO}_4\text{-i}$  (598 g). Though the difference among the plots was not that small, it was not significant perhaps





**Fig. 3.1-1.** Change of stem height during the experimental period (Experiment 1).



**Fig. 3.1-2.** Change of stem height during the experimental period (Experiment 2).

**Table 3.1-1.** Sugar yield and its components.

Sampling time	K type	K level	Length cm	Diameter mm	Weight g	Sucrose %	Sugar yield g
Experiment 1 Feb., 2011	KCl	i	200 a	19.90 a	730 a	22.9 a	108 a
		ii	196 a	20.29 a	733 a	21.8 ab	103 a
		iii	194 a	19.17 a	664 a	22.0 ab	94 a
		iv	186 a	19.60 a	641 a	21.7 ab	90 a
	K <sub>2</sub> SO <sub>4</sub>	i	189 a	18.98 a	598 a	21.2 b	82 a
		ii	184 a	19.36 a	646 a	21.9 ab	91 a
		iii	190 a	20.26 a	765 a	22.1 ab	109 a
		iv	206 a	20.10 a	789 a	22.5 ab	114 a
Experiment 2	K-0		211 ab	23.59 a	973 a	20.3 c	118 a
	KCl	i	205 ab	22.94 a	939 a	20.5 bc	115 a
		ii	220 a	22.52 a	967 a	22.0 a	127 a
		iii	213 ab	23.58 a	1040 a	21.1 abc	132 a
		iv	204 ab	23.29 a	998 a	20.4 bc	123 a
	K <sub>2</sub> SO <sub>4</sub>	i	223 a	23.21 a	1014 a	21.2 abc	103 a
		ii	215 ab	23.84 a	1032 a	21.6 a	134 a
		iii	210 ab	23.29 a	1001 a	21.6 ab	130 a
		iv	202 ab	22.90 a	940 a	21.7 a	122 a
	Mix	i	217 a	22.45 a	954 a	21.5 ab	123 a
		ii	214 ab	23.27 a	998 a	21.8 a	131 a
		iii	198 ab	24.06 a	989 a	21.2 abc	126 a
		iv	186 b	23.48 a	930 a	21.1 abc	118 a

Means with different alphabets are significantly different at the 5% level (Tukey test or Tukey-Kramer method).

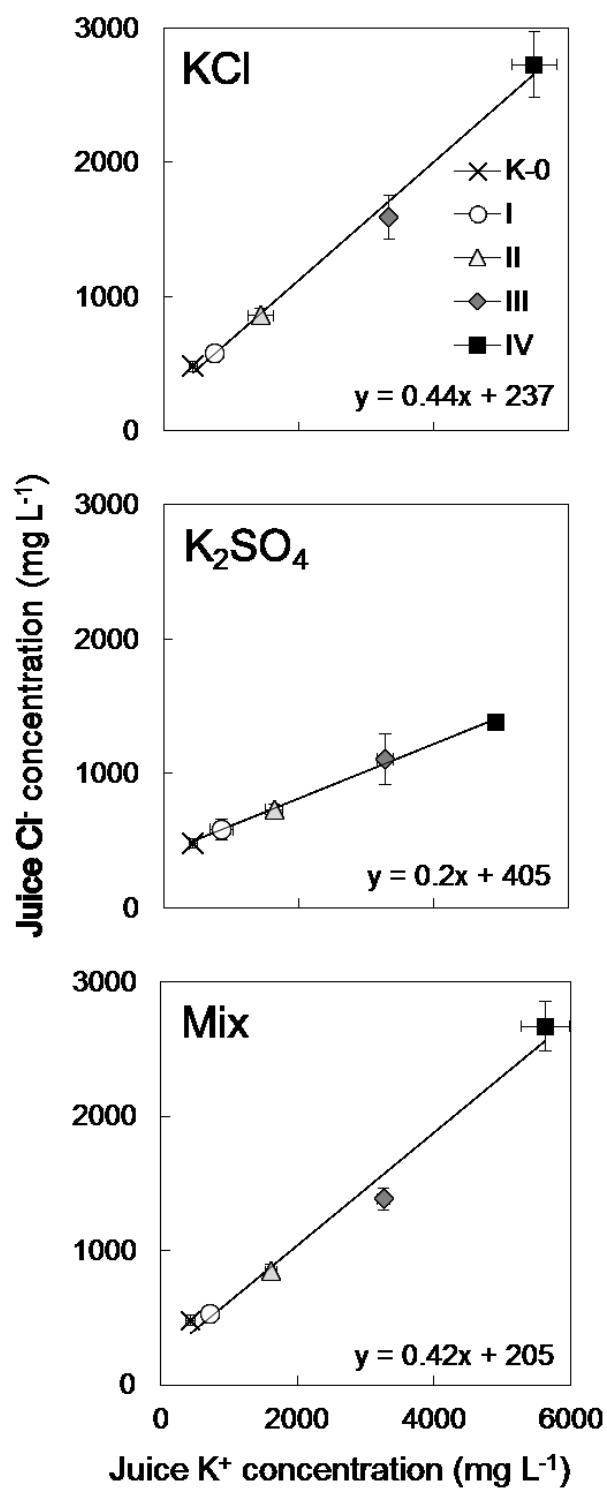
because of large variations within the plots. As a result, no significance was found in quantitative parameters including sugar yield. Qualitative parameter, sucrose concentration in juice scattered from 21.2 to 22.9, with a significance between KCl-i and K<sub>2</sub>SO<sub>4</sub>-i.

Because juice ion compositions were similar among the sampling times in experiment 1, only that of the final sampling on February, 2011 is shown in Table 3.1-2. In experiment 1, irrespective of sampling time, juice K<sup>+</sup> concentration increased with K level both in the KCl and K<sub>2</sub>SO<sub>4</sub> plots and this tendency lasted until February. K<sup>+</sup> concentrations varied widely from 200 to 3100 mg L<sup>-1</sup> throughout the experiment. Juice Cl<sup>-</sup> concentration also increased with K levels, ranging from 400 to 2000 mg L<sup>-1</sup>. This tendency was observed even in the K<sub>2</sub>SO<sub>4</sub> plots where no Cl<sup>-</sup> was administered. The Cl<sup>-</sup> concentration of the KCl-iv plot was significantly higher than that of the K<sub>2</sub>SO<sub>4</sub>-iv plot, although there were little effects of K type on Cl<sup>-</sup> concentration in the other K level plots. In contrast to Cl<sup>-</sup>, juice SO<sub>4</sub><sup>2-</sup> concentration seemed to show smaller effects of increasing K levels; K<sub>2</sub>SO<sub>4</sub> application contributed little to increasing SO<sub>4</sub><sup>2-</sup> concentrations. However, SO<sub>4</sub><sup>2-</sup> concentration tended to decrease in both the KCl and K<sub>2</sub>SO<sub>4</sub> plots as K levels increased. Similar results were confirmed in experiment 2: juice K<sup>+</sup> and Cl<sup>-</sup> concentrations responded positively to increasing K levels, irrespective of K type. Compared to experiment 1, wider ranges of K<sup>+</sup> from 400 to 5700 mg L<sup>-1</sup> and of Cl<sup>-</sup> from 400 to 3000 mg L<sup>-1</sup> were obtained in experiment 2 because of the higher amounts of K fertilizer. There was no obvious difference in K<sup>+</sup> concentration between K types; however, Cl<sup>-</sup> concentrations varied greatly depending on K type. K<sub>2</sub>SO<sub>4</sub>- III and IV had a significantly lower Cl<sup>-</sup> concentration than the plots of KCl and Mix at the same K level. In the KCl plots, juice SO<sub>4</sub><sup>2-</sup> concentration decreased with an increase of K level, whereas there was no significant difference in SO<sub>4</sub><sup>2-</sup> in the other plots. EC of juice also increased significantly with K levels, and EC of juice of the K<sub>2</sub>SO<sub>4</sub>-IV plot was significantly lower than those of the KCl and Mix-IV plots. In experiment 2, juice K<sup>+</sup> and Cl<sup>-</sup> were highly correlated in all the K types and all the correlations were significant at the 1% level (Fig. 3.1-3). However, the slopes of

**Table 3.1-2.** Ion compositions and EC in sugarcane juice.

Sampling time	K type	K level	Juice K <sup>+</sup>	Juice Cl <sup>-</sup>	Juice SO <sub>4</sub> <sup>2-</sup>	Juice EC
			mg L <sup>-1</sup>			mS m <sup>-1</sup>
Experiment 1 Feb., 2011	KCl	i	440 de	501 d	2294 ab	—
		ii	580 cd	749 cd	2388 ab	—
		iii	890 b	898 c	2130 ab	—
		iv	2791 a	1837 a	1835 b	—
	K <sub>2</sub> SO <sub>4</sub>	i	222 e	649 cd	2528 a	—
		ii	398 de	729 cd	2351 ab	—
		iii	828 bc	758 cd	2124 ab	—
		iv	2558 a	1276 b	2049 ab	—
Experiment 2 Jan., 2014	K-0		435 f	481 f	1736 a	251 g
	KCl	i	758 ef	579 ef	1695 a	297 efg
		ii	1435 d	856 d	1334 ab	347 def
		iii	3334 c	1592 b	1272 ab	607 c
		iv	5488 a	2725 a	1069 b	935 a
	K <sub>2</sub> SO <sub>4</sub>	i	854 e	582 ef	1545 ab	277 fg
		ii	1633 d	733 de	1505 ab	367 de
		iii	3277 c	1108 c	1767 a	626 c
		iv	4909 b	1381 b	1547 ab	772 b
	Mix	i	722 ef	526 ef	1624 a	270 g
		ii	1611 d	844 d	1616 a	376 d
		iii	3262 c	1385 b	1664 a	597 c
		iv	5635 a	2670 a	1575 ab	972 a

Means with different alphabets are significantly different at the 5% level (Tukey test or Tukey-Kramer method).



**Fig. 3.1-3.** Relationship between K<sup>+</sup> and Cl<sup>-</sup> in sugarcane juice.

the regression lines were markedly different; the regression coefficient was 0.44 in the KCl plots and 0.42 in the Mix plots, whereas the value in the K<sub>2</sub>SO<sub>4</sub> plots was 0.2. The similar tendency was observed in experiment 1 also (data not shown).

With any type of K fertilizer, similarly to those in the juice samples, soil K<sup>+</sup> concentration and EC increased with K levels (Table 3.1-3). Soil K<sup>+</sup> was less than 2 mg 100 g<sup>-1</sup> in the K-0, I, and II plots but rose to approximately 10 mg 100 g<sup>-1</sup> in the III plots, although the values were not significantly different from those of the lower K plots. K<sup>+</sup> and EC of the K<sub>2</sub>SO<sub>4</sub>-IV plot were significantly higher than those of the KCl-IV plot. Soil Cl<sup>-</sup> concentrations increased with K levels in the KCl and Mix plots and reached 48.3 and 30.4 mg 100 g<sup>-1</sup> in the KCl-IV and Mix-IV plots, respectively, resulting in a significant difference from that of the K<sub>2</sub>SO<sub>4</sub>-IV plot. Similarly, increasing K levels increased SO<sub>4</sub><sup>2-</sup> concentrations in the K<sub>2</sub>SO<sub>4</sub> and Mix plots, which was not seen in juice samples.

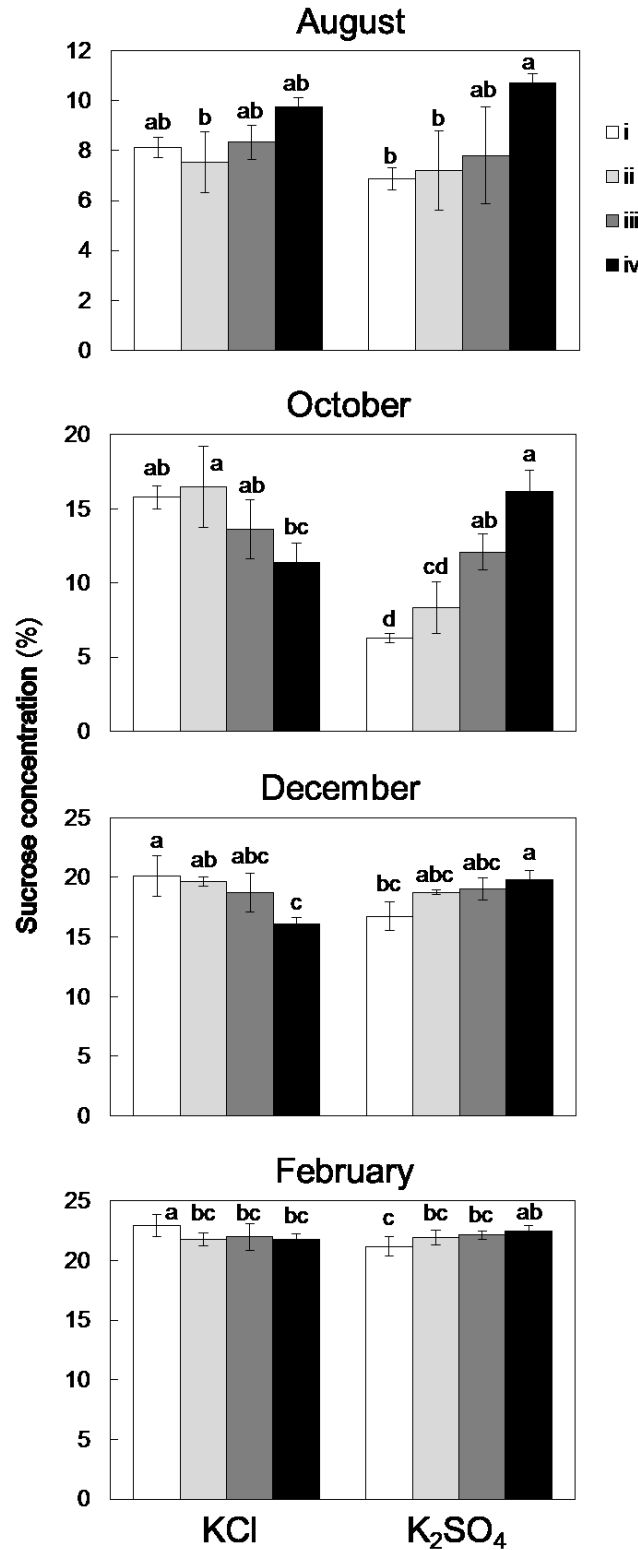
In experiment 1, sucrose concentration in the August sampling tended to increase with K levels and K<sub>2</sub>SO<sub>4</sub>-iv plots showed a significantly higher sucrose concentration than some of the lower K level plots (Fig. 3.1-4). At the October sampling, however, the relationships were markedly different depending on K type. As K levels increased, the sucrose concentration in the KCl plots tended to decrease from 16.5 to 11.4%, whereas that in the K<sub>2</sub>SO<sub>4</sub> plots increased from 6.3 to 16.2%. In December, sucrose concentration spanned a smaller range; however, the difference in sucrose concentration between the K types still remained. As a result, the KCl-iv and K<sub>2</sub>SO<sub>4</sub>-i plots showed significantly lower sucrose concentrations than the KCl-i and K<sub>2</sub>SO<sub>4</sub>-iv plots. At the last sampling in February, sucrose concentration rose above 21% and the variation among plots diminished greatly compared to the previous samplings. The KCl-iv plot seemed to have caught up with the other plots, whereas the concentration of the K<sub>2</sub>SO<sub>4</sub>-i plot still stayed relatively low. At the end of the experiment, sucrose concentration was highest in the KCl-i plot and lowest in the K<sub>2</sub>SO<sub>4</sub>-i plot. In experiment 2, the range of sucrose concentration was narrower than that in experiment 1 (Fig.

**Table 3.1-3.** Ion compositions and EC in pot soil.

K type	K level	Soil K <sup>+</sup>	Soil Cl <sup>-</sup>	Juice SO <sub>4</sub> <sup>2-</sup>	Juice EC
		mg 100g <sup>-1</sup>			mS m <sup>-1</sup>
	K-0	0.6 c	4.9 b	8.0 d	29.4 c
KCl	i	0.6 c	5.0 b	9.3 d	30.4 c
	ii	1.6 c	9.7 b	9.1 d	32.6 c
	iii	9.2 c	25.6 ab	10.1 d	41.9 c
	iv	46.2 b	48.3 a	5.4 d	58.4 bc
K <sub>2</sub> SO <sub>4</sub>	i	0.5 c	6.5 b	11.3 d	32.4 c
	ii	0.7 c	8.6 b	22.0 cd	37.4 c
	iii	10.3 c	10.4 b	84.5 bc	60.1 bc
	iv	68.0 a	10.6 b	166.4 a	97.8 a
Mix	i	1.0 c	12.3 b	11.2 d	36.5 c
	ii	1.1 c	14.9 b	17.1 d	39.3 c
	iii	11.6 c	15.8 ab	46.6 bc	53.8 bc
	iv	55.2 ab	30.4 ab	92.1 b	85.1 ab

Means with different alphabets are significantly different at the 5% level (Tukey-Kramer method).





**Fig. 3.1-4.** Effects of different types and levels of K fertilizer (Experiment 1). Vertical bars indicate SD. Means with different alphabets are significantly different at the 5% level (Tukey test).

3.1-5). Sucrose concentration was lowest in the K-0 plot and, irrespective of K type, tended to increase with K levels up to the II plots. However, when more K was supplied, different effects of K fertilizer appeared, which were similar to experiment 1, thereby indicating that sucrose concentration decreased significantly in the KCl plots but remained stable or even slightly increased in the K<sub>2</sub>SO<sub>4</sub> plots, whereas that in the Mix plots tended to decrease similarly to that in the KCl plots. As a result of increasing K levels from II to IV, 1.6 and 0.6% of sucrose reduction was observed in the KCl and Mix plots, respectively. The highest sucrose concentration was observed in the KCl-II plot.

### **Discussion**

In the present study, a reduction of sucrose concentration in sugarcane juice with increasing KCl levels was observed; however, there was no negative effect of increasing K<sub>2</sub>SO<sub>4</sub> levels. Similar results were also consistently observed in previous studies: pot experiments have shown that increasing K levels with potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) did not adversely affect sucrose concentration (Nagae et al., 1997; Uehara et al., 2004; Kawamitsu et al., 2006; Azama et al., 2007). The Mix plots in experiment 2 also showed a tendency of sucrose reduction as K levels rose, although the supplied Cl<sup>-</sup> levels were half of those in the KCl plots. These findings suggest that Cl<sup>-</sup> and not K<sup>+</sup> is the factor most responsible for sucrose reduction. However, it still remains unclear whether the combination of K<sup>+</sup> and Cl<sup>-</sup> or Cl<sup>-</sup> alone reduced sucrose content. This point was further examined with a pot experiment using different K and Cl fertilizers and it will be discussed in Chapter 3.2.

In addition to the sucrose reduction with KCl application, I also confirmed the positive effects of K<sub>2</sub>SO<sub>4</sub> on sucrose concentration. Although there is no clear explanation for this finding, it may have resulted from the positive effects of SO<sub>4</sub><sup>2-</sup> because sulfur plays an important role in plant metabolism (for example, in photosynthesis and synthesis of amino acids and proteins) as an essential macronutrient (Hamid and Dagash, 2014), though SO<sub>4</sub><sup>2-</sup>

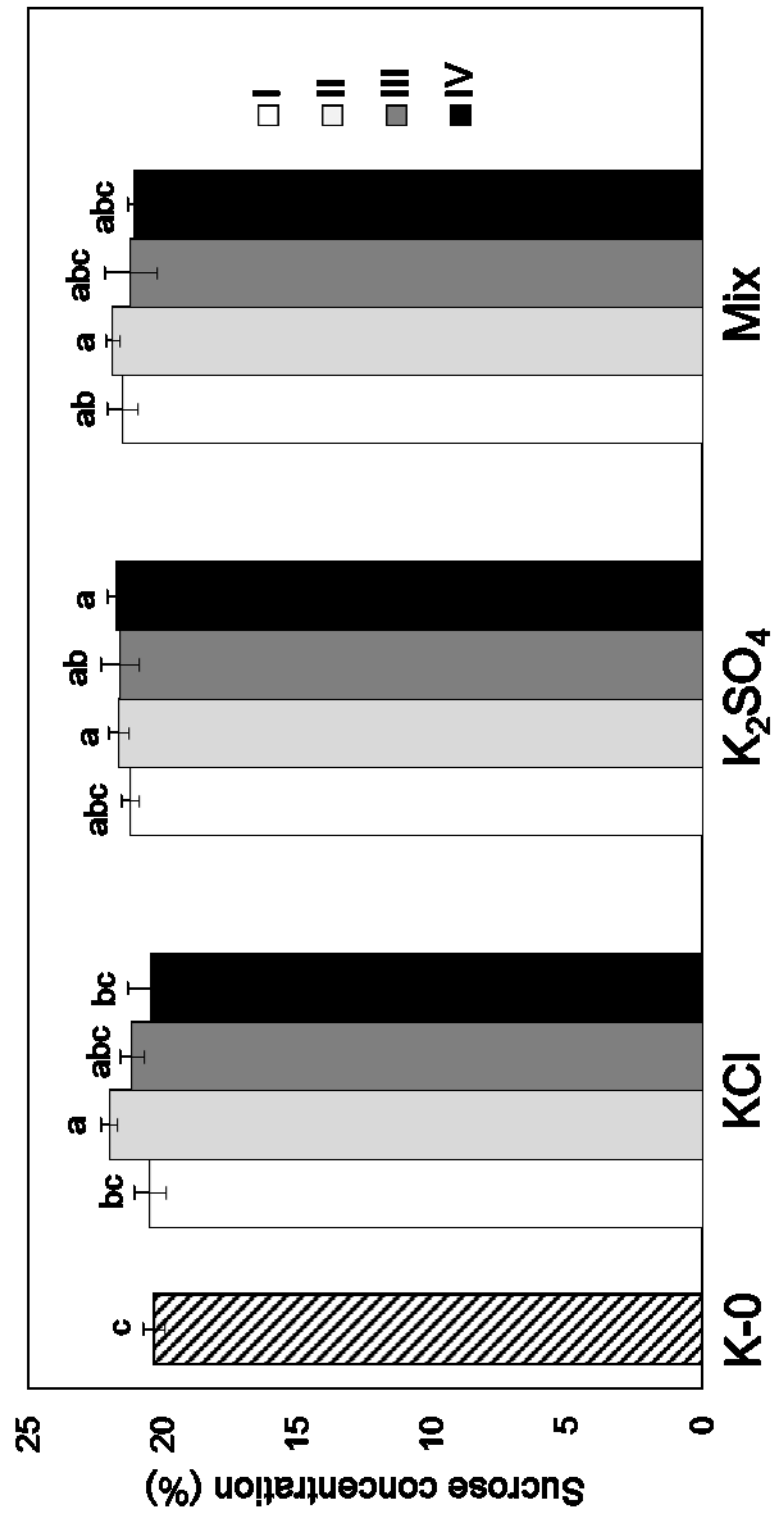


Fig. 3.1-5. Effects of different types and levels of K fertilizer (Experiment 2). Means with different alphabets are significantly different at the 5% level (Tukey-Kramer method).

concentration in juice was not increased by  $K_2SO_4$  application.

Surprisingly, the close relationship between  $K^+$  and  $Cl^-$  was also confirmed in the  $K_2SO_4$  plots, although the plants in the  $K_2SO_4$  plots were not given  $Cl^-$  by fertilization. This result indicates that they positively absorbed  $Cl^-$  from irrigation water with the uptake of K, which is luxuriously consumed by sugarcane (Hunsigi, 2011). This absorption most likely follows the principle of electrical neutrality by which a bulk solution always contains equal numbers of anions and cations (Poole, 2010). However, the increase in  $Cl^-$  concentration was smaller with the  $K_2SO_4$  than with the KCl treatments. These results suggest that it is possible to make sugarcane absorb less  $Cl^-$  and accumulate more sucrose by  $K_2SO_4$  application instead of KCl, although currently a great majority of the crops are fertilized with KCl (Kafkafi, 2001). White and Broadly (2001) defined  $Cl^-$  transport as “passive” when  $Cl^-$  moves in the direction of its electrochemical gradient and as “active” when  $Cl^-$  is accumulated against its electrochemical gradient. They also stated that active  $Cl^-$  transport dominates  $Cl^-$  influx to root cells at low  $Cl^-$  concentrations in the external medium and that passive  $Cl^-$  influx to root cells occurs under more saline conditions.  $Cl^-$  influx to root cells was passive at  $Cl^-$  concentrations in the external medium between 1 and 40 mM when  $Cl^-$  was supplied as KCl (Laties et al. 1964; Macklon and MacDonald, 1966). Taken together with our results, these reports suggest that sucrose reduction occurs via passive  $Cl^-$  influx, for example when KCl is applied, and that actively absorbed  $Cl^-$  does not lead to negative effects.

Lingle and Wiegand (1997) investigated the effects of soil salinity on juice quality of sugarcane from a salt-affected field and reported that EC of soil increased EC of juice and markedly lowered sugarcane quality such as Pol, Brix, and apparent purity, and that most of the increase of EC in juice was explained by  $Cl^-$ . These findings partly support ours; however, the authors also speculated that the effect of EC of soil on sugarcane quality is an osmotic rather than a specific ion effect. In our study, sucrose reduction was observed only when KCl was supplied, whereas increases of EC of juice and soil were observed for all the K types,

indicating a possibility of  $\text{Cl}^-$  ionic stress. Because the ion concentrations varied widely among the treatments, the change of ion composition may have affected physiological functions associated with sucrose accumulation. Considering that sucrose is both a product of photosynthesis and a reserve substance after translocation of photosynthate (Stewart et al. 1973), the reduction of sucrose concentration may have resulted from photosynthesis inhibition or sucrose allocation. In addition, sucrose reduction by  $\text{Cl}^-$  is likely to occur during the progress of sucrose accumulation as the weather becomes colder because the negative effects of higher KCl applications appeared at the October sampling in experiment 1. Elucidation of the mechanism, however, awaits further study.

In contrast to qualitative parameters like ion and sucrose concentrations in juice, the results of quantitative parameters seemed to be unclear and inconsistent: stalk weights tended to decrease in experiment 1, but the highest was obtained in KCl-iii, though juice  $\text{Cl}^-$  increased significantly by KCl application. Based on the results of this study, therefore, it is difficult to prove a hypothesis that  $\text{Cl}^-$  is involved in the enhancement of sugarcane yield, which came up from Chapter 2.2. A possible reason associated with the different results between the surveys in Chapter 2.2 and the current experiments may have resulted from the difference between pot and field conditions. However, it is definitely worth revealing the effects of the treatments on qualitative parameters for a better K management aimed at improving sugar production. It is also unknown whether or not I can observe the same results, including tillers as well as mother stems. To answer this question, field studies assessing the effects of types and levels of K fertilizer on sugarcane yield and quality were conducted and these will be examined in Chapter 4.

### 3.2. Pot experiment by changing types of potassium and chloride salts

#### Introduction

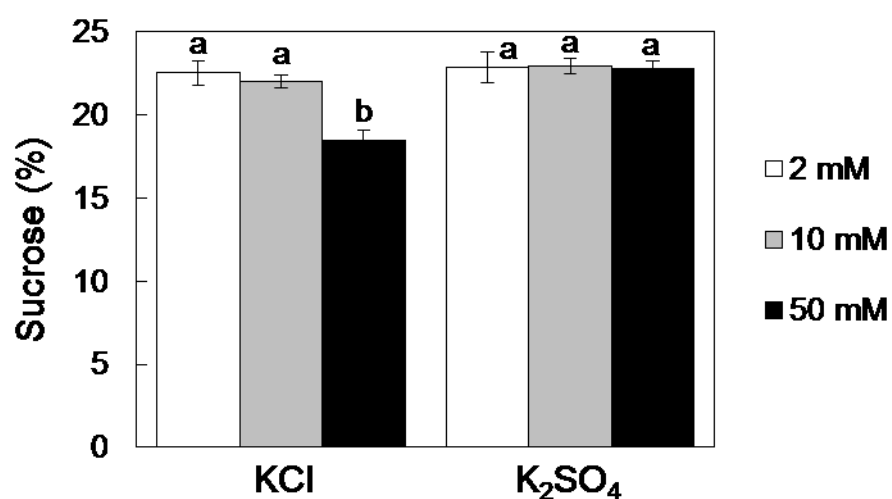
To examine the effects of  $K^+$  independent of  $Cl^-$ , pot experiments were conducted with variable potassium levels using KCl and  $K_2SO_4$ , which resulted in a significant reduction in juice sucrose content only when plants were treated with higher doses of KCl (Chapter 3.1), suggesting that  $Cl^-$ , not  $K^+$ , is more responsible for sucrose reduction. However, it is difficult to conclude whether the combination of  $K^+$  and  $Cl^-$  or only  $Cl^-$  is involved in this process since both the ions are closely associated, with  $Cl^-$  considered to be the counterion of  $K^+$  (Laties et al., 1964; Stuart and Jones, 1978; Schnabl and Raschke, 1980). In order to improve the quality through fertilizer management practices, it is important to determine whether we need to lower both  $K^+$  and  $Cl^-$  or  $Cl^-$  alone. Some studies have revealed specific ion toxicities by separately using different types of salt, e.g., individual effects of  $Na^+$  and  $Cl^-$  (Joshi and Nail, 1980; Sümer et al., 2004; Tavakkoli et al., 2011; García and Medina, 2013). Therefore, the use of different types of  $Cl^-$  salts could help in examining the effects of only  $Cl^-$ . Moreover, the process of sucrose reduction has not been fully characterized. Sucrose is one of the major products of photosynthesis (Fewkes et al. 1971), so sucrose reduction by KCl overdose may occur through changes in the physiological functions associated with photosynthesis, for example, the inhibition of  $CO_2$  assimilation by stomatal closure (Meinzer et al. 1994; Du et al. 1996; Gomathi et al. 2010; Vasantha et al. 2010; Ribeiro et al. 2013), metabolism impairment (Meinzer et al. 1994; Du et al. 1996; Gomathi et al. 2010; Markelz et al. 2011; Ribeiro et al. 2013), and accumulation of carbohydrates in leaves (Hartt and Burr 1967; Goldschmidt and Huber 1992; Paul and Pellny 2003). The objectives of this study were to determine the individual effects of  $Cl^-$  on sugarcane quality and investigate a possible mechanism for sucrose reduction in terms of photosynthesis, using different kinds of  $K^+$  and  $Cl^-$  salts.

## Materials and methods

A pot experiment was conducted under a greenhouse condition at the University of the Ryukyus, Okinawa, Japan from April to November, 2015. Seedlings of *S. spp.* cv. NiF8 were collected from the fields at the Subtropical Field Science Center of the University of the Ryukyus. The latter procedure was performed following Chapter 3.1.

Fertilization was performed once a week after transplantation using a modified Hoagland solution containing 6 mM  $\text{Ca}(\text{NO}_3)_2$ , 4 mM  $\text{KNO}_3$ , 2 mM  $\text{KH}_2\text{PO}_4$ , 2 mM  $\text{MgSO}_4$ , 100  $\mu\text{M}$   $\text{C}_{10}\text{H}_{12}\text{FeN}_2\text{NaO}_8$ , 25  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 10  $\mu\text{M}$   $\text{MnSO}_4$ , 2  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.5  $\mu\text{M}$   $\text{CuSO}_4$ , and 0.5  $\mu\text{M}$   $\text{H}_2\text{MoO}_4$ . The treatments were started seven weeks after transplantation, and five treatments were established by dissolving different types of  $\text{K}^+$  and  $\text{Cl}^-$  salts: 50 mM KCl, 25 mM  $\text{K}_2\text{SO}_4$ , a mixture of 12.5 mM magnesium chloride ( $\text{MgCl}_2$ ) and 12.5 mM calcium chloride ( $\text{CaCl}_2$ ), and 50 mM sodium chloride (NaCl) (the treatments as well as the plants treated with these solutions are referred to as *KCl*, *K<sub>2</sub>SO<sub>4</sub>*, *MgCl<sub>2</sub>+CaCl<sub>2</sub>*, and *NaCl*, respectively) into the Hoagland solution. The *Control* plants were given only the Hoagland solution. The concentrations of these salts in the Hoagland solution were determined based on the results of a pretest from July, 2014 to March, 2015, in which we had applied 2, 10, and 50 mM of  $\text{K}^+$  either by KCl or  $\text{K}_2\text{SO}_4$ . We observed a significant reduction of sucrose following 50 mM KCl application while  $\text{K}_2\text{SO}_4$  application barely affected the sucrose concentration (Fig. 3.2-1). Hence, the salt treatments contained 50 mM  $\text{K}^+$  and/or 50 mM  $\text{Cl}^-$  given in different forms. Eight pots for each of the treatments were prepared.

Stem height from the ground to the base of the top visible dewlap leaf, number of green leaves, and SPAD value of the top visible dewlap leaf using a SPAD meter (SPAD-502, Minolta Camera) were measured monthly to evaluate the effects of the treatments on plant growth. Four plants were harvested on September 19 ( $T_1$ ) and then on November 19 ( $T_2$ ), and the weight of millable stalks and leaf area were measured. Based on their length, the stems were divided into three parts: upper, middle, and lower, and the internodes located at the center of each part were



**Fig. 3.2-1.** Effects of different levels of K<sup>+</sup> (2, 10, and 50 mM) either by KCl or K<sub>2</sub>SO<sub>4</sub> on sucrose concentration in juice of the pretest from July 2014 to March 2015. Vertical bars indicate SD. Different letters mean significant differences at the 5% level (Tukey test).



squeezed separately. Juice samples were diluted 50 fold with extra-pure water and passed through a membrane filter (diameter, 13 mm; pore size, 0.45  $\mu\text{m}$ ; Advantec) for ion and sugar analyses.

Photosynthesis and chlorophyll fluorescence measurements were performed on the second to the fourth visible dewlap leaves before and one month after the treatments started (July and August, respectively) and before T<sub>1</sub> (September) and T<sub>2</sub> (November), using an infrared open gas exchange system (Li-6400, LI-COR). Minimum fluorescence ( $F_o$ ) and maximum fluorescence ( $F_m$ ) were measured before dawn, and variable fluorescence ( $F_v$ ,  $F_m - F_o$ ) and the maximum photochemical quantum yield of photosystem II ( $F_v/F_m$ ) were calculated. The CO<sub>2</sub> assimilation rate ( $A$ ), stomatal conductance ( $g_s$ ), and intercellular CO<sub>2</sub> concentration ( $C_i$ ) were measured at a photosynthetic photon flux density of 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , using a constant flow rate of 400  $\mu\text{mol s}^{-1}$ . The means  $\pm$  SD of leaf temperature and vapor pressure deficit based on leaf temperature were  $35.2 \pm 1.1$  °C and  $2.2 \pm 0.5$  kPa, respectively. The measurements were performed at 400 ( $A_{400}$ ) and 2000 ( $A_{2000}$ )  $\mu\text{mol mol}^{-1} \text{CO}_2$  to estimate stomatal limitation (SL), calculated using the following formula by referring to Long and Bernacchi (2003) with a slight modification.

$$\text{SL} = (A_{2000} - A_{400})/A_{2000}$$

One day after the photosynthesis measurements in September and November, approximately 10 cm<sup>2</sup> of green leaf pieces were taken from the measured leaves at 08:00, 14:00, and 20:00 h. This temporal sampling was necessary because sugar compositions in sugarcane leaves undergo diurnal changes (Du et al., 2000). Freeze-dried leaf samples were cut into small pieces, and 50 mg of a sample was extracted with 3 ml of boiled water for 10 min. After centrifugation at 3000 rpm for 10 min, the supernatant was collected. This cycle was repeated twice with 1 ml of boiled water, and then 5 ml of the combined supernatant was used for sucrose, glucose, and fructose analyses. The pellet was retained for starch analysis. Starch was extracted

following Du et al. (1998), and the measurement was performed photometrically (UV-1800, Shimadzu) using a modified enzymatic method (Jones et al., 1977). Starch concentration was expressed as glucose weight. The extracts of leaf samples at 14:00 h were used for ion analysis.

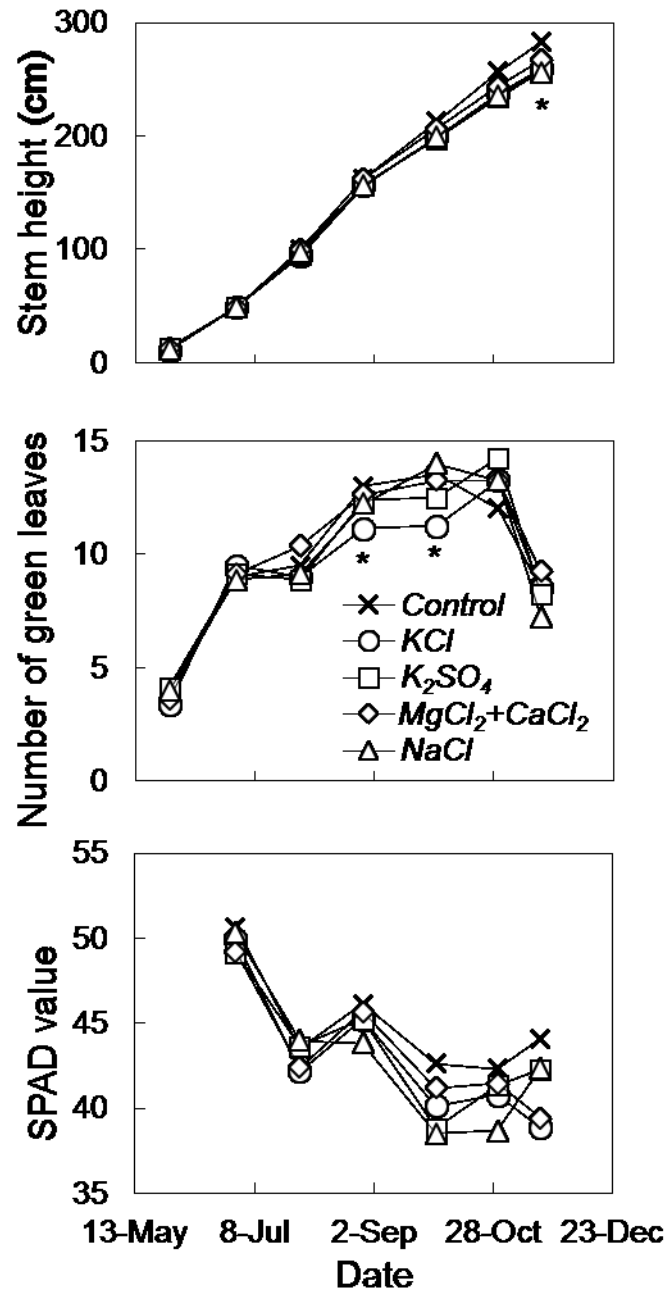
The concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$  were determined by ion chromatography, following Chapter 2.1. The concentrations of sucrose, glucose, and fructose were determined by HPLC as described in Chapter 2.2. Ion and sugar concentrations in juice were expressed as the averaged values of the three stem parts.

Statistical analysis was performed using the software R (R Core Team, 2015). Data were subjected to one-way ANOVA between the treatments. When significances were found, the Tukey test was conducted, and the significant differences were accepted based on a P value  $< 0.05$ .

## Results

Visual observation suggested that all the plants were growing well. Stem height gradually increased and finally reached 250–280 cm (Fig. 3.2-2). At  $T_2$ , *Control* had a significantly higher stem height value than  $K_2\text{SO}_4$  and  $\text{NaCl}$ . Stem weight at  $T_2$  ranged from 1007 to 1152 g and was the highest in *Control* and lowest in  $\text{NaCl}$ , but there was no significant treatment effect (data not shown). The number of green leaves and SPAD value showed similar changes in all the treatments, except that the numbers of green leaves in  $\text{KCl}$  were significantly lower than those in *Control* and  $\text{MgCl}_2 + \text{CaCl}_2$  at 14 weeks after transplantation and in  $\text{NaCl}$  at 18 weeks after transplantation. The leaf area was approximately 5000  $\text{cm}^2$  and did not differ significantly between the treatments (data not shown).

Juice ion compositions were similar at  $T_1$  and  $T_2$ , except that  $\text{K}^+$  and  $\text{Cl}^-$  concentrations increased in all the treatments from  $T_1$  to  $T_2$  (Table 3.2-1).  $\text{KCl}$  and  $\text{K}_2\text{SO}_4$  accumulated a great deal of  $\text{K}^+$ . Juice  $\text{Cl}^-$  concentration was remarkably high only in  $\text{KCl}$ , and the other  $\text{Cl}^-$  salt treatments, namely  $\text{MgCl}_2 + \text{CaCl}_2$  and  $\text{NaCl}$ , had lower  $\text{Cl}^-$  concentrations than  $\text{K}_2\text{SO}_4$ ,



**Fig. 3.2-2.** Changes of stem height, the number of green leaves, and SPAD value during the experimental period. \* means a significant difference at the 5% level.

**Table 3.2-1.** Effects of different salts on juice ion composition.

Sampling time	Treatment	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
T <sub>1</sub>	Control	27 ab	1511 c	225 a	228 a	903 d	848 ab
	KCl	27 ab	5363 a	219 a	231 a	4006 a	664 b
	K <sub>2</sub> SO <sub>4</sub>	29 ab	4195 b	153 a	155 a	2034 b	1000 a
	MgCl <sub>2</sub> + CaCl <sub>2</sub>	19 b	1988 c	214 a	197 a	1548 c	718 b
	NaCl	34 a	1832 c	223 a	192 a	1409 c	793 b
T <sub>2</sub>	Control	116 a	2033 c	208 ab	197 ab	1079 c	868 b
	KCl	37 a	7914 a	211 ab	196 ab	5032 a	663 b
	K <sub>2</sub> SO <sub>4</sub>	51 a	5584 b	118 b	105 b	1900 b	1165 a
	MgCl <sub>2</sub> + CaCl <sub>2</sub>	57 a	2753 c	229 a	210 a	1735 b	848 b
	NaCl	57 a	2610 c	240 a	210 a	1614 b	876 b

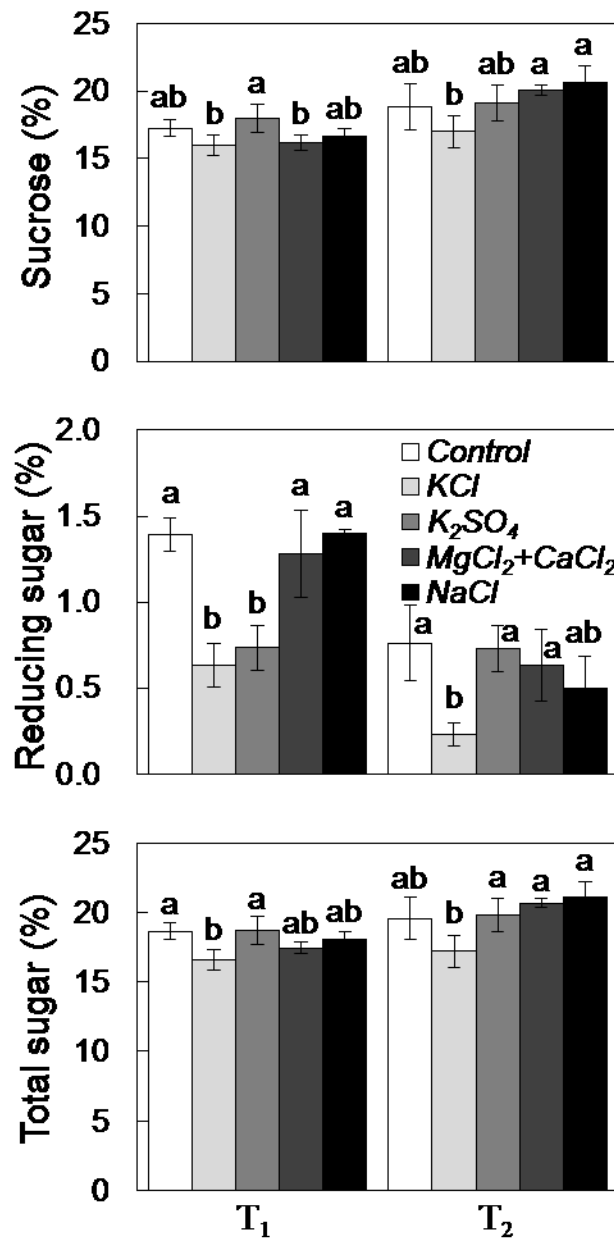
Means with different alphabets are significantly different at the 5% level (Tukey test).

although those were all significantly higher than that of *Control*.  $\text{SO}_4^{2-}$  concentration was low in *KCl* and high in  $\text{K}_2\text{SO}_4$ , and the other treatments had similar values. We did not see the effects of  $\text{MgCl}_2+\text{CaCl}_2$  and *NaCl* as these salts barely affected  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Na}^+$  concentrations.

Sucrose concentration ranged from 16.0% to 18.0% at  $T_1$  and from 17.0% to 20.6% at  $T_2$  (Fig. 3.2-3). At  $T_1$ , sucrose concentration in  $\text{K}_2\text{SO}_4$  was the highest and was significantly higher than those in *KCl* and *NaCl*. Compared to the values at  $T_1$ , sucrose concentration increased in all the treatments at  $T_2$ ; however, the increment was smallest in *KCl*. Sucrose concentrations in  $\text{MgCl}_2+\text{CaCl}_2$  and *NaCl* rose over 20% and were significantly higher than in *KCl*. At both  $T_1$  and  $T_2$ , sucrose concentration in *KCl* was the lowest. The concentration of reducing sugar, which was composed of glucose and fructose, decreased from 0.6%–1.4% at  $T_1$  to 0.2%–0.4% at  $T_2$ . Reducing sugar concentration was significantly lower in *KCl* and  $\text{K}_2\text{SO}_4$  at  $T_1$ , with *KCl* decreasing to 0.2% while  $\text{K}_2\text{SO}_4$  stayed at the same level, resulting in significant differences between *KCl* and other treatments at  $T_2$ . Similar results to sucrose concentration were obtained for total sugar concentration: *KCl* had a significantly lower total sugar concentration than plants treated by other treatments except for *Control* at  $T_2$ .

The  $\text{K}^+$  and  $\text{Cl}^-$  salt treatments tended to increase leaf  $\text{K}^+$  and  $\text{Cl}^-$  concentrations, respectively; yet, the differences were less significant than juice ion composition (Table 3.2-2).  $\text{MgCl}_2+\text{CaCl}_2$  and *NaCl* also had some effects on leaf ion compositions, and the results were clearer at  $T_2$  than at  $T_1$ : high leaf  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  concentrations in  $\text{MgCl}_2+\text{CaCl}_2$  and high  $\text{Na}^+$  concentration in *NaCl*, respectively.

In the leaf, sucrose concentration was low at 08:00 h, increased at 14:00 h, and decreased again at 20:00 h, whereas starch concentration constantly increased from morning to night (Fig. 3.2-4), which is consistent with Du et al. (2000). Reducing sugar remained relatively stable irrespective of the sampling time. All of the sugar concentrations, especially reducing sugar, tended to decrease from  $T_1$  to  $T_2$ . *KCl* had the highest starch concentration at  $T_2$ , and only small differences between treatments were observed.

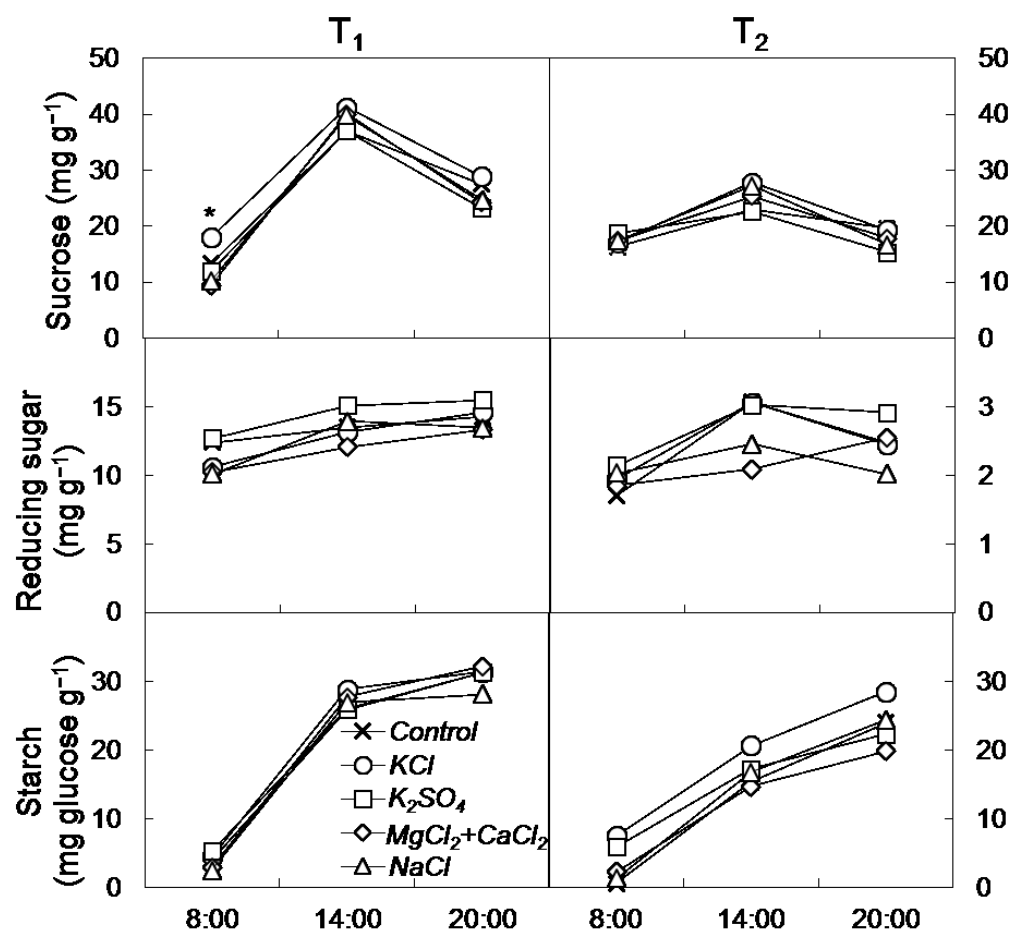


**Fig. 3.2-3.** Effects of different salts on juice ion composition. Vertical bars indicate SD. Means with different alphabets are significantly different at the 5% level (Tukey test).

**Table 3.2-2.** Effects of different salts on leaf ion composition.

Sampling time	Treatment	Na <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
T <sub>1</sub>	Control	0.22 a	1.41 a	2.20 ab	7.37 b	5.67 bc	0.31 a
	KCl	0.21 a	1.18 ab	1.98 ab	9.59 a	7.52 a	0.16 a
	K <sub>2</sub> SO <sub>4</sub>	0.18 a	1.00 b	1.63 b	9.32 a	5.36 c	0.35 a
	MgCl <sub>2</sub> + CaCl <sub>2</sub>	0.17 a	1.45 a	2.78 a	7.29 b	6.57 ab	0.25 a
	NaCl	0.19 a	1.47 a	2.71 a	7.31 b	6.77 ab	0.28 a
T <sub>2</sub>	Control	0.09 a	1.50 ab	1.88 ab	8.12 bc	4.55 b	0.46 a
	KCl	0.37 a	0.79 c	0.91 c	9.89 ab	5.05 ab	0.12 bc
	K <sub>2</sub> SO <sub>4</sub>	0.45 a	0.82 c	0.89 c	10.17 a	4.40 b	0.27 ab
	MgCl <sub>2</sub> + CaCl <sub>2</sub>	0.14 a	1.58 a	2.29 a	7.25 c	5.78 a	0.33 ab
	NaCl	0.41 a	1.04 b	1.39 b	9.16 ab	4.96 ab	0.04 c

Means with different alphabets are significantly different at the 5% level (Tukey test).



**Fig. 3.2-4.** Effects of different salts on sugar and starch content in leaf.

\* means a significant difference at the 5% level.



$A$  linearly decreased as plants matured (Fig. 3.2-5). The differences between the treatments became greater, and  $A_{400}$  ranged from 25.2 to 30.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $A_{2000}$  from 29.4 to 35.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in November. All the treatments had similar changes in  $g_s$  and  $C_i$  at 400  $\mu\text{mol mol}^{-1}$  of atmospheric  $\text{CO}_2$  concentration.  $C_i$  varied depending on the treatments but with no clear pattern. The increasing  $\text{CO}_2$  level caused an increase in  $C_i$  and reduction in  $g_s$ , which resulted in higher  $A$ . Both  $A_{400}$  and  $A_{2000}$  were the lowest in *KCl* in September and November although the differences were not significant. SL increased from 0.06 in July to 0.08–0.16 in the later periods, and we observed a significant difference between *KCl* and *NaCl* only in August (Fig. 3.2-6).  $F_v/F_m$  was barely changed by the treatments and was always 0.78–0.80 irrespective of the date and treatment, and no significances were found.

## Discussion

This study was conducted using different types of  $\text{K}^+$  and  $\text{Cl}^-$  salts to examine the individual effects of  $\text{Cl}^-$  on sugarcane. Many salinity stress studies, mainly using *NaCl*, have been conducted in sugarcane, and it is generally reported that *NaCl* stress induces inhibition of growth (e.g., Wahid, 2004), yield (e.g., Akhtar et al., 2001), and photosynthesis (e.g., Medeiros et al., 2014), as well as quality deterioration (e.g., Gomathi and Thandapani 2005) with accumulation of  $\text{Cl}^-$ . In the present study, however, visual observation and quantitative parameters revealed no obvious treatment effects on sugarcane growth. Nevertheless, ion and sugar compositions in the juice were greatly affected, indicating that the treatment effects observed in this study differed from those induced by common salt stress.

*KCl* application significantly increased both  $\text{K}^+$  and  $\text{Cl}^-$  concentrations in juice as previously reported in Chapter 3.1; yet the other  $\text{Cl}^-$  salt treatments, *MgCl*<sub>2</sub>+*CaCl*<sub>2</sub> and *NaCl*, did not induce sugarcane to absorb as much  $\text{Cl}^-$  as *KCl*. Notably, the counterions of  $\text{Cl}^-$ — $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Na}^+$ —were barely absorbed, but  $\text{K}^+$  concentrations in *MgCl*<sub>2</sub>+*CaCl*<sub>2</sub> and *NaCl* were higher than that in *Control*. On the other hand,  $\text{K}^+$  accumulation was markedly enhanced by

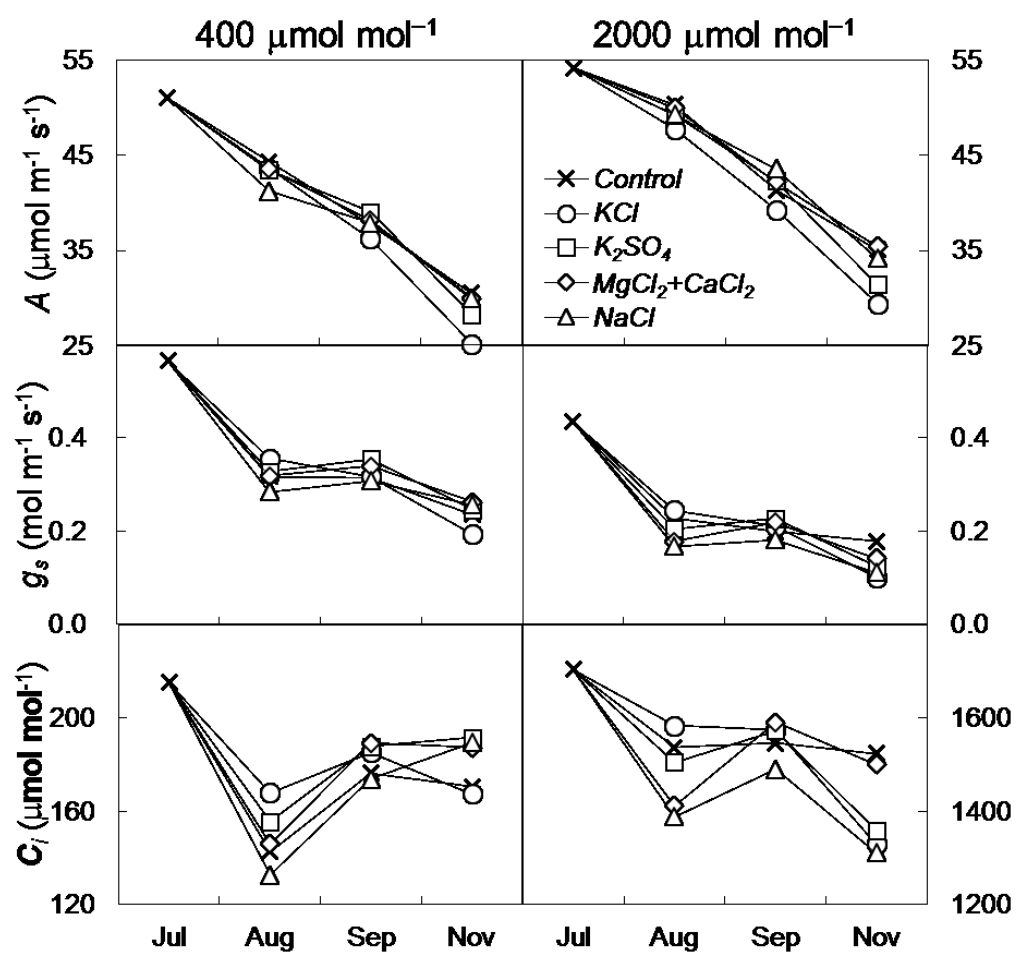
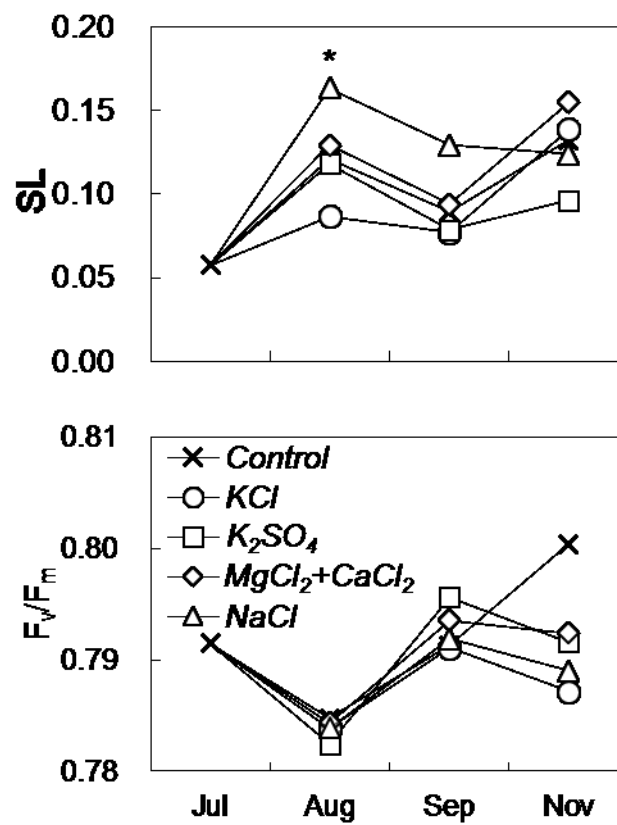


Fig. 3.2-5. Effects of different salts on photosynthetic parameters.



**Fig. 5.2-0.** Effects of different salts on SL and  $F_v/F_m$ . \* means a significant difference at the 5% level.

K<sub>2</sub>SO<sub>4</sub> application, which led to increasing Cl<sup>-</sup> concentration in accordance with Chapter 3.1. These results suggest that K<sup>+</sup> can primarily move into sugarcane alone, but Cl<sup>-</sup> needs K<sup>+</sup> to be efficiently absorbed and other cations, such as Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup>, hardly accompany Cl<sup>-</sup>. As a result, only the *KCl* treatment lowered sucrose concentration with a great increase in Cl<sup>-</sup> in juice. Therefore, from the present study, we could not evaluate the effects of Cl<sup>-</sup> independent of K<sup>+</sup> accumulation and conclude whether Cl<sup>-</sup> alone or both K<sup>+</sup> and Cl<sup>-</sup> have adverse effects on sugarcane quality.

Although the differences were not significant, both *A*<sub>400</sub> and *A*<sub>2000</sub> were lower in *KCl*. We observed the decline of neither stomatal conductance nor *F<sub>v</sub>/F<sub>m</sub>*, but *KCl* showed a slightly higher leaf starch concentration in the later growth period, which may have resulted in the lower *A*<sub>400</sub> and *A*<sub>2000</sub> readings. This indicates the possibility that the sucrose reduction was induced through feedback regulation of photosynthesis because starch accumulation in leaves is correlated with a decline in CO<sub>2</sub> fixation (Hartt and Burr 1967; Goldschmidt and Huber 1992; Paul and Pellny 2003). However, the results are not strong enough to prove this hypothesis, and Lunn and Hatch (1995) found no evidence for feedback inhibition of photosynthesis as carbohydrate accumulated in leaves of various C<sub>4</sub> plants; thus, a more detailed study is needed to confirm this hypothesis.

Furthermore, given that sucrose is not only one of the major products of photosynthesis (Fewkes et al., 1971) but also a translocated photosynthate and a soluble reserve substance (Stewart et al., 1973), the physiological functions related to translocation and distribution of sugar may have also been affected. The sucrose reduction, however, was not simply induced by the decomposition of sucrose to its components, glucose and fructose, because the reducing sugar concentration in juice also markedly declined in *KCl*. To further characterize this, studies assessing the effects of *KCl* application on sucrose accumulation are also needed.

The Cl<sup>-</sup> salt treatments other than *KCl*, namely MgCl<sub>2</sub>, CaCl<sub>2</sub>, and NaCl, showed lower juice Cl<sup>-</sup> concentrations than plants in K<sub>2</sub>SO<sub>4</sub> that were given no Cl<sup>-</sup> through fertilization. Also,

in these two treatments, concentrations of up to 50 mM  $\text{Cl}^-$  did not adversely affect sugarcane quality, but sucrose concentration in juice declined with 50 mM KCl treatment. Taken together, it appears that juice  $\text{Cl}^-$  concentration is determined by available  $\text{K}^+$  rather than by available  $\text{Cl}^-$  in soil, which also supports the results of soil analysis in Chapter 2.2, and that  $\text{Cl}^-$  could have a negative impact on sugarcane quality only when  $\text{K}^+$  is supplied above sufficiency levels. Therefore, we need to reduce  $\text{K}^+$  as well as  $\text{Cl}^-$  concentrations to improve sugarcane quality through fertilizer management practices when both  $\text{K}^+$  and  $\text{Cl}^-$  are excessively accumulated in the environment.

## **Chapter 4**

### **Effects of different kinds of potassium fertilizers on the yield and quality of sugarcane under field conditions**

#### **4.1. Field experiment by changing types of potassium fertilizers**

##### **Introduction**

From the previous chapters, it is suggested that  $K^+$  and  $Cl^-$  are highly influential factors in sugarcane quality. Chapter 2 revealed that negative relationships were found to exist between these ions and sugar content in juice of sugarcane stalk samples collected from raw sugar mills in Japan and the ions concentrations in juice varied greatly depending on producing area. In Chapter 3, I hypothesized the differences of ion concentrations among areas were derived from a potassium fertilizer, KCl, and thus conducted some pot experiments with different levels of K. The pot experiments successfully showed the reduction of sucrose with higher KCl doses. On the other hands, pot conditions may differ from field ones due to unlimited rhizosphere and direct impact of climatic conditions such as rain and typhoon. Besides that, tillering ability is of remarkable value in the profitability of sugarcane (Matsuoka and Stolf, 2012). In the previous pot experiments in Chapter 3, however I only focused on mother stems, implying that results may change when tillers as well as mother stems are used for analysis. It is also difficult to discuss whole cane yield excluding the effects of tillers. These all suggest the necessity of field experiments accessing the effects of types and levels of K fertilizers on yield and quality of whole sugarcane plants including mother stems and tillers. In the present chapter, first a field experiment was conducted using KCl and  $K_2SO_4$  to briefly see the effects of different K fertilizes on sugarcane quality.

## Materials and methods

On April 29 in 2011, one-bud seedlings of sugarcane (*Saccharum* spp. cv. NiF8) were planted 30 cm apart on 1.2 m wide rows in an experimental field (No.13; 26°25'N, 127°76'E; 123 m a.s.l.; Shimajiri mahji) of the University of the Ryukyus. Fertilization was performed with a common compound fertilizer for sugarcane production in Japan called BB666 (Ryukyu Fertilizer; expressed as *Control*) and mixtures of single fertilizers of N by ammonium nitrite; of P<sub>2</sub>O<sub>5</sub> by multiple phosphate; and of K<sub>2</sub>O by KCl (expressed as *KCl*) or K<sub>2</sub>SO<sub>4</sub> (expressed as *K<sub>2</sub>SO<sub>4</sub>*). The total amounts of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O were 20.8, 7.8, and 7.8 kg 10a<sup>-1</sup>, respectively. Approximately 70 m<sup>2</sup> of a plot was prepared for each treatment with one replication. Given that it was found from Chapter 3.1 that the effects of the K treatments on sugar content differed depending on sampling times, samplings were performed two times: December 7 in 2011 (T<sub>1</sub>) and March 5 in 2012 (T<sub>2</sub>), and 15 plants were taken from each treatment. After measurements of stalk length and weight, PIC was measured by near infra-red spectroscopy (NIR; Foss, InfraXact) for all stalks at T<sub>1</sub> and for 15 stalks at T<sub>2</sub>. Pressed juice was used for Brix measurement and diluted and filtered for determining K<sup>+</sup> by ICP method at T<sub>1</sub> and K<sup>+</sup>, Cl<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> by ion chromatography method at T<sub>2</sub>, following the methods shown in the previous chapters.

Means and SD of the replications were calculated, and statistical analysis was performed using the software R (R Core Team, 2015).

## Results

The numbers of stalks were 28 in *Control*, 24 in *KCl*, and 38 in *K<sub>2</sub>SO<sub>4</sub>* at T<sub>1</sub> and 32 in *Control*, 39 in *KCl*, and 43 in *K<sub>2</sub>SO<sub>4</sub>* at T<sub>2</sub>. Stalk length and weight and the number of stalks were consistently higher in *K<sub>2</sub>SO<sub>4</sub>*, which resulted in the highest yield (Table 4.1-1). By contrast to quantitative parameters, PIC of *K<sub>2</sub>SO<sub>4</sub>* was significantly lower than those of the other treatments in T<sub>1</sub> but no significant difference in T<sub>2</sub>. Because PICs were not remarkably

**Table 4.1-1.** Sugar yield and its components.

		Stalk length	Stalk weight	No. of stalks	Yield	PIC	Sugar yield
		cm	g stalk <sup>-1</sup>	stalks plant <sup>-1</sup>	g plant <sup>-1</sup>	%	g plant <sup>-1</sup>
T <sub>1</sub>	<i>Control</i>	147 b	615 b	1.87	1148	13.8 a	141
	<i>KCl</i>	145 b	556 b	1.60	889	13.4 a	107
	<i>K<sub>2</sub>SO<sub>4</sub></i>	171 a	735 a	2.53	1862	12.9 b	201
T <sub>2</sub>	<i>Control</i>	139 b	575 b	2.13	1228	16.7 a	192
	<i>KCl</i>	146 b	558 b	2.60	1452	16.4 a	222
	<i>K<sub>2</sub>SO<sub>4</sub></i>	173 a	723 a	2.87	2073	16.4 a	316

Means with different alphabets are significantly different at the 5% level (Tukey-Kramer method).



different among the treatments, sugar yield seemed to be rather affected by cane yield and that of  $K_2SO_4$  was highest.

At  $T_1$ , a significant lower  $K^+$  concentration in juice was obtained in  $K_2SO_4$  despite giving the equal amount of  $K_2O$  to all the treatments (Table 4.1-2). In contrast to  $T_1$ ,  $K^+$  concentration at  $T_2$  was lowered; however, significantly higher  $K^+$  of  $K_2SO_4$  was consistently observed.  $K_2SO_4$ , furthermore, had a higher  $Cl^-$  concentration with significance even though the fertilizer applied to  $K_2SO_4$  did contain no  $Cl^-$ .  $SO_4^{2-}$  was significantly lower in *Control*.

Both at  $T_1$  and  $T_2$ , juice  $K^+$  and  $Cl^-$  concentrations and PIC scattered in wide ranges (Fig. 4.1-1). While clear relationships between  $K^+$  and PIC were not found in *Control* and *KCl*, that of  $K_2SO_4$  was significantly negative at the 1% level. On the other hands, at  $T_2$  PIC tended to be decreased with an increase of juice  $K^+$ , particularly with significance in *Control* and *KCl* (Fig. 4.1-2). Moreover, I checked the correlations between  $K^+$  and Brix for all the samples of  $T_2$  and found that all the treatments had significantly negative relations. Similar results were obtained between  $Cl^-$  and Brix as well.  $K^+$  and  $Cl^-$  in juice positively correlated in all the treatments, but the regression coefficients were not statistically different (Fig. 4.1-3).

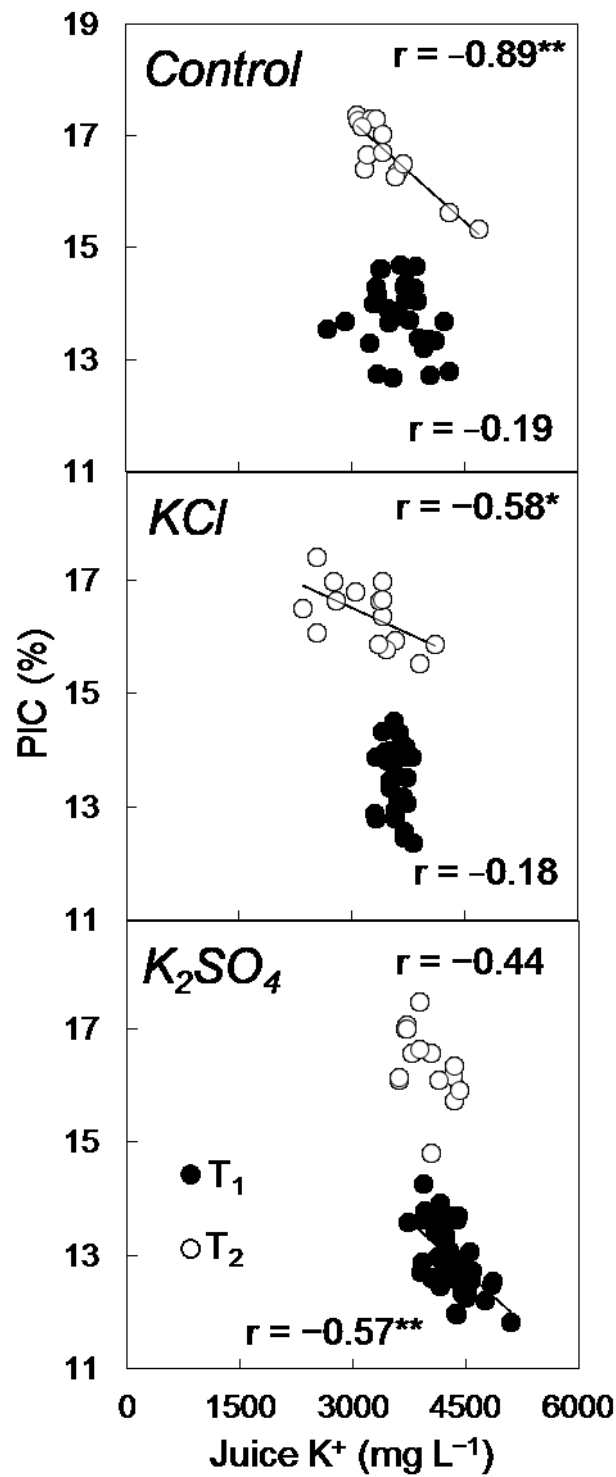
## Discussion

In this study, I established treatments by using different kinds of K fertilizer but applying the equal amounts of nutrients, so not juice  $K^+$  but  $Cl^-$  were expected to differ between the treatments. At both of the samplings, however, it resulted in significantly higher  $K^+$  and  $Cl^-$  concentrations in  $K_2SO_4$  where no  $Cl^-$  was given. It was also revealed that the ion composition in sugarcane juice greatly varied within the treatments. These results suggest a possibility of the inequality of soil fertility that may have affected by the preceding cropping, though no data to support this elucidation can be shown here because soil sampling was not performed in this study. Referring to Lingle and Wiegand (1997), soil ion composition varied within a field and positive correlations were found to exist between ions in soil and those in

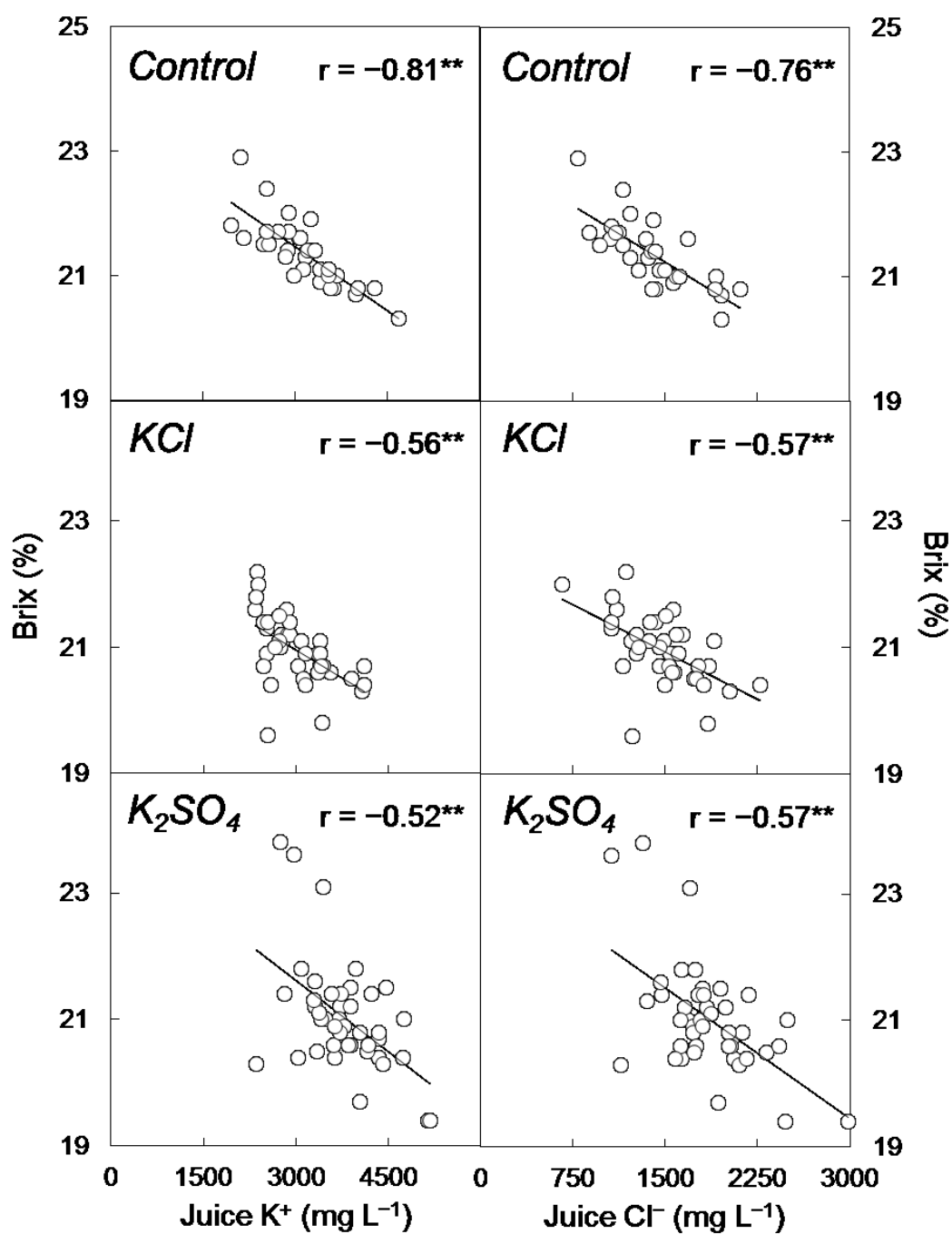
**Table 4.1-2.** Effects of different types of K fertilizer on juice ion concentrations.

	T <sub>1</sub>	T <sub>2</sub>		
	K <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
<i>Control</i>	3635 b	3152 b	1403 b	1547 b
<i>KCl</i>	3577 b	3019 b	1482 b	1830 a
<i>K<sub>2</sub>SO<sub>4</sub></i>	4314 a	3784 a	1852 a	1807 a

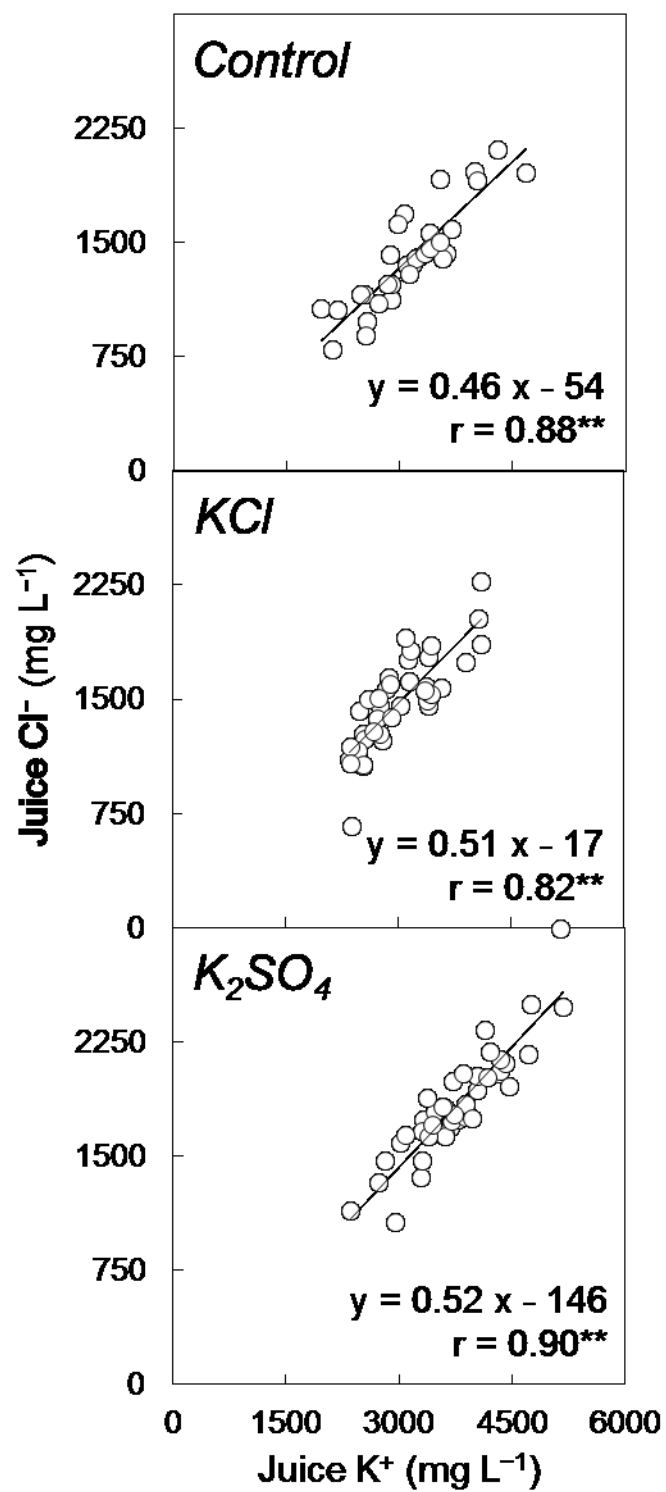
Means with different alphabets are significantly different at the 5% level (Tukey-Kramer method).



**Fig. 4.1-1.** Relationships between juice K<sup>+</sup> and PIC. \* and \*\* mean significance at the 5% and 1% level, respectively.



**Fig. 4.1-2.** Relationships of juice K<sup>+</sup> and Cl<sup>-</sup> with Brix. \*\* means significance at the 1% level.



**Fig. 4.1-3.** Relationships between  $K^+$  and  $Cl^-$  in sugarcane juice. **\*\*** means significance at the 1% level.

the juice, indicating that sugarcane juice ion composition were determined by soil ion concentrations of small-scale plots within a sugarcane field.

Highly positive correlations between juice  $K^+$  and  $Cl^-$  were also confirmed in all the treatments, but differing from Chapter 3.1, the regression coefficients in  $KCl$  and  $K_2SO_4$  were not statistically different, meaning the treatments eventually did not make any difference. Considering that  $Cl^-$  is functioning as the counterion of  $K^+$  (Laties et al., 1964; Stuart and Jones, 1978; Schnabl and Raschke, 1980) and soil solution (Goos, 1987; Wiegand et al., 1996; Lingle and Wiegand, 1997), atmosphere (Kafkafi, 2001), and irrigation water (Thomas et al., 1981; Lingle et al., 2000; Golabi et al., 2009) can be its sources other than fertilizers,  $Cl^-$  is thought to be passively absorbed with  $K^+$  when  $Cl^-$  is present enough in the environment, even without supplying  $Cl^-$  by fertilization; as a result, sugarcane quality was lowered with increases of juice  $K^+$  and  $Cl^-$  in the  $K_2SO_4$  plot as well as the other plots.

From the results of the current study, it is difficult to explain why the concentrations of  $K^+$  and  $Cl^-$  in sugarcane juice widely differed within the treatments due to the lack of soil testing. Hence, it will be of particular importance to precisely monitor the changes of soil ion compositions throughout experiments. Another possible determinant of juice ion concentrations is cane maturity. It is well known that K and Cl are kept ionic steady-state or to be ionized in plants and easily retranslocated from older to younger organs (Miyazato, 1986; Bloom, 2010), so it was hypothesized that accumulations of  $K^+$  and  $Cl^-$  differ among old and young parts, in other words, mother stems and tillers. The next chapter will demonstrate these points by conducting a field experiment with different kinds and levels of K fertilizer.

## 4.2. Field experiment by changing types and levels of potassium fertilizers

### Introduction

To examine the reproducibility of the pot experiments, a field trial by using KCl and K<sub>2</sub>SO<sub>4</sub> was conducted (Chapter 4.1). The results were that PIC had a decreasing trend with increases of K<sup>+</sup> and Cl<sup>-</sup> concentrations in juice, meaning these two ions can be factors adversely affecting sugarcane quality under a field condition as well. However, since no treatment effect was observed, e.g. the KCl-treated sugarcanes had higher Cl<sup>-</sup> concentration in juice, in order to investigate whether K<sup>+</sup> and Cl<sup>-</sup> derived from fertilizers increase juice K<sup>+</sup> and Cl<sup>-</sup> and reduce sugar content, it is necessary to change levels of K fertilizer as well as types, assuming fields containing different levels of K<sup>+</sup> and Cl<sup>-</sup>. In Chapter 4.1, I also failed to perform soil sampling and thus could not see treatment effects on the changes of soil ion composition. In this study, therefore soil testing was constantly performed through the experimental period. Moreover, juice K<sup>+</sup> and Cl<sup>-</sup> concentrations varied widely within the treatments. The difference in juice ion composition between mother stems and tillers has been barely understood, which may have accounted for it. In this chapter, I will talk about a field experiment with different levels of K<sub>2</sub>O by KCl and K<sub>2</sub>SO<sub>4</sub> to prove that sugarcane quality is adversely affected by K<sup>+</sup> and Cl<sup>-</sup> derived from KCl application.

### Materials and methods

On April 12 in 2014, one-bud seedlings of sugarcane (*Saccharum* spp. cv. NiF8) were planted 30 cm apart on 1.2 m wide rows in an experimental field (No.15; 26°25'N, 127°76'E; 127 m a.s.l.) of the University of the Ryukyus. Fertilization was performed with ammonium sulfate and ammonium phosphate and 20 kg 10a<sup>-1</sup> of N and 6 kg 10a<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> were given to all the treatments. Then, seven treatments, including one without K fertilizer (expressed as 0K), were established with two types (KCl and K<sub>2</sub>SO<sub>4</sub>) and three levels (6, 18 and 60 kg 10a<sup>-1</sup>;

expressed as 1K, 3K, and 10K, respectively) of  $K_2O$ . The plot area was  $72\text{ m}^2$  for 0K and  $43\text{ m}^2$  for the others and only one replication was prepared for each treatment. The height of mother stems and the number of tillers were measured every month. Four plants on October 9 ( $T_1$ ) and six plants on March 13 ( $T_2$ ) were taken and classified into mother stems and tillers. All stalk samples were weighed and squeezed separately. Ion and sucrose concentrations in juice were determined by ion chromatography and HPLC as described in Chapter 2.1 and 2.2, respectively, and sucrose content was used for the calculation of PIC. Soil samples were taken before planting (April 29, 2014), about one month after the treatments started (July 3), after big typhoons in July (July 9), August (August 9), and October (after  $T_1$  sampling; October 15), and after  $T_2$  sampling (March 14, 2015). Soil analysis was performed following Chapter 2.2.

## Results

The plant height increased gradually, but the growth speed became higher from July to September (Fig. 4.2-1). Meanwhile, the difference between the treatments increased and the KCl 10K plot had a lower height than the others after August. Then, the elongation remained sluggish and the height hardly changed at the latter period, resulting in the final values from 220 to 250 cm. Through the period, the number of tillers was high in the 0K plot. The KCl 10K plot had a lower number from November,  $1.5\text{ stalks plant}^{-1}$ , as compared to more than 2 stalks  $\text{plant}^{-1}$  in the other plots.

At  $T_1$ , the lowest PIC was seen in the 0K plot, while those of KCl 1K and KCl 10K were high; at  $T_2$ , on the other hands, KCl 10K had the lowest PIC (Table 4.2-1). The stalk weight of 0K was significantly lower than that of  $K_2SO_4$  3K, but cane yield was the second highest following the  $K_2SO_4$  3K plot because of the greatest number of millable stalks. Although KCl 10K had a similar stalk weight with the other plots, millable stalks were significantly lower, which accounted for the lowest cane yield. As a result, the lowest cane



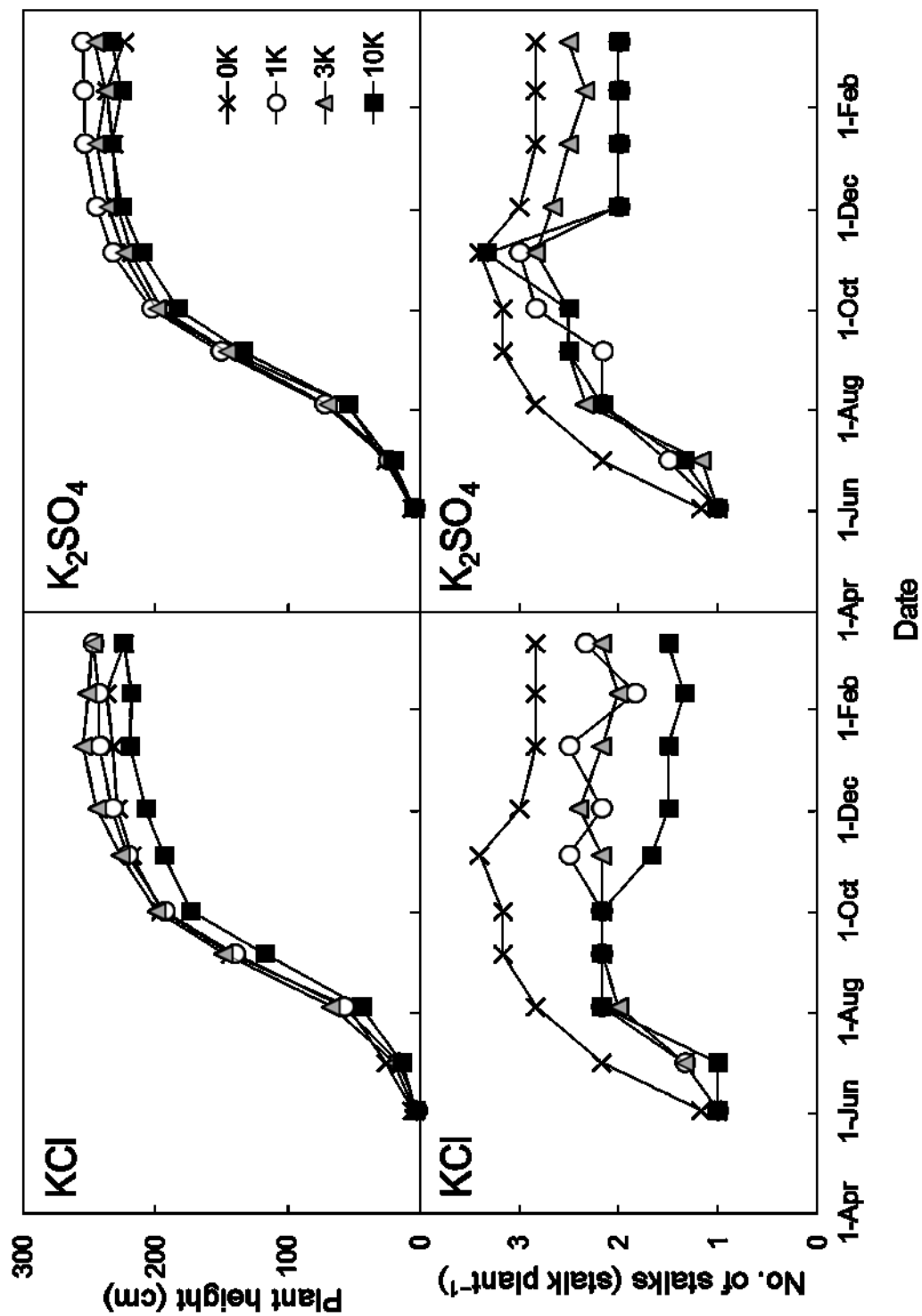


Fig. 4.2-1. Changes of the plant height of the mother stems and the number of stalks plant<sup>-1</sup>.

**Table 4.2-1.** Sugar yield and its components.

Treatment	T <sub>1</sub>		T <sub>2</sub>			
	PIC	Stalk weight	No. of stalks	Yield	PIC	Sugar yield
	%	g stalk <sup>-1</sup>	stalks plant <sup>-1</sup>	kg plant <sup>-1</sup>	%	g plant <sup>-1</sup>
0K	6.1 a	683 b	2.8 a	1.93 a	16.8 a	303 a
1K	7.3 a	753 ab	2.3 a	1.76 a	16.9 a	278 a
KCl 3K	7.0 a	806 ab	2.2 a	1.75 a	16.8 a	273 a
10K	7.3 a	875 ab	1.5 a	1.31 a	15.9 a	197 a
1K	7.2 a	889 ab	2.0 a	1.78 a	16.4 a	272 a
K <sub>2</sub> SO <sub>4</sub> 3K	6.5 a	942 a	2.5 a	2.35 a	16.0 a	352 a
10K	6.9 a	888 ab	2.0 a	1.78 a	16.7 a	276 a

Means with different alphabets are significantly different at the 5% level (Tukey-Kramer method).

yield and PIC led to less sugar yield than any other plots.

Both KCl and K<sub>2</sub>SO<sub>4</sub> applications significantly increased juice K<sup>+</sup> concentration of tillers as well as of mother stems and the 10K plots of KCl and K<sub>2</sub>SO<sub>4</sub> had significantly higher values than 0K and KCl 1K (Table 4.2-2). Similarly, juice Cl<sup>-</sup> concentration was increased by increasing levels of K<sub>2</sub>O applied, especially in the KCl treatment, and the highest value was obtained in KCl 10K. Although increases of K fertilizers led to higher juice K<sup>+</sup> and Cl<sup>-</sup> at T<sub>2</sub> as well, this tendency became unclear than at T<sub>1</sub>; particularly the KCl 10K plot, which had the highest K<sup>+</sup> and Cl<sup>-</sup> at T<sub>1</sub>, showed remarkably lower juice concentrations in mother stems. The result of two-way ANOVA said significant differences in juice ion concentrations were confirmed among mother stems and tillers. On the other hands, at T<sub>1</sub> nor T<sub>2</sub>, PIC was significantly different between treatments. When comparing between stem parts, PIC of mother stems was significantly higher than that of tillers at T<sub>1</sub>, while no significance was obtained at T<sub>2</sub>.

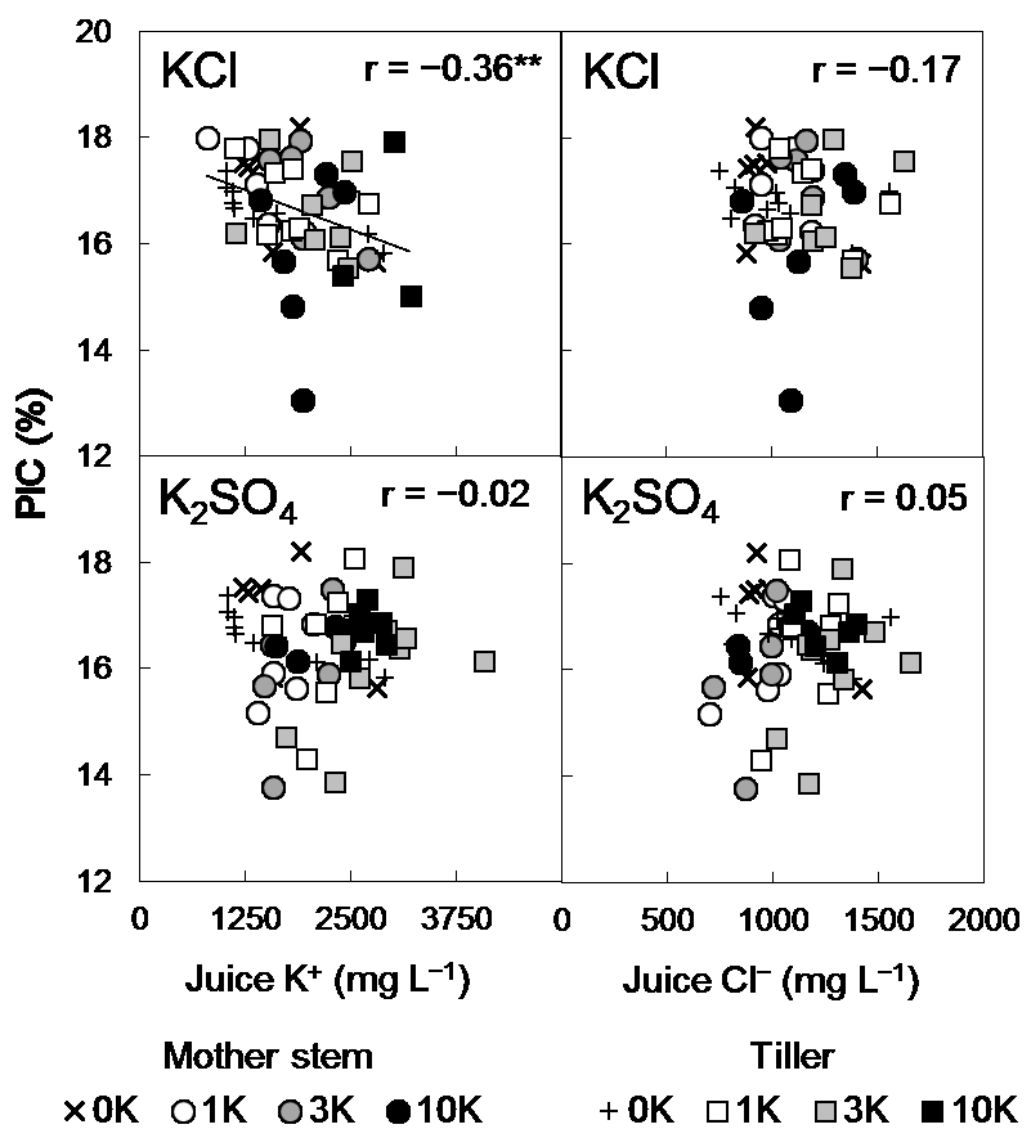
The juice K<sup>+</sup> and Cl<sup>-</sup> concentrations were negatively correlated with PIC in the KCl treatments at T<sub>2</sub>, though the relationships were not very clear and only the one between juice K<sup>+</sup> and PIC in KCl was significant (Fig. 4.2-2). By contrast, the K<sub>2</sub>SO<sub>4</sub> treatments did not have such relations. Variances between samples in each plot were relatively large; for instance, juice K<sup>+</sup> of 0K where no K fertilizer was supplied, ranged from 1000 to 3000 mg L<sup>-1</sup> and Cl<sup>-</sup> 700 to 1400 mg L<sup>-1</sup>. I confirmed the strong relationships between K<sup>+</sup> and Cl<sup>-</sup>, but the slope of the regression line in the KCl treatments was steeper than that in the K<sub>2</sub>SO<sub>4</sub> treatments; yet they were not statistically different (Fig. 4.2-3).

The concentrations of K<sup>+</sup>, Cl<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> in soil fluctuated greatly throughout the experimental period (Fig. 4.2-4). These ions basically followed the supplied amounts, namely higher doses of fertilizer containing ions, higher concentrations of those in soil. After a big typhoon hit the field in the early October, soil ion concentrations, especially in KCl 10K, remarkably increased: K<sup>+</sup> of KCl 10K reached approximately 15 mg 100g<sup>-1</sup> and Cl<sup>-</sup> and

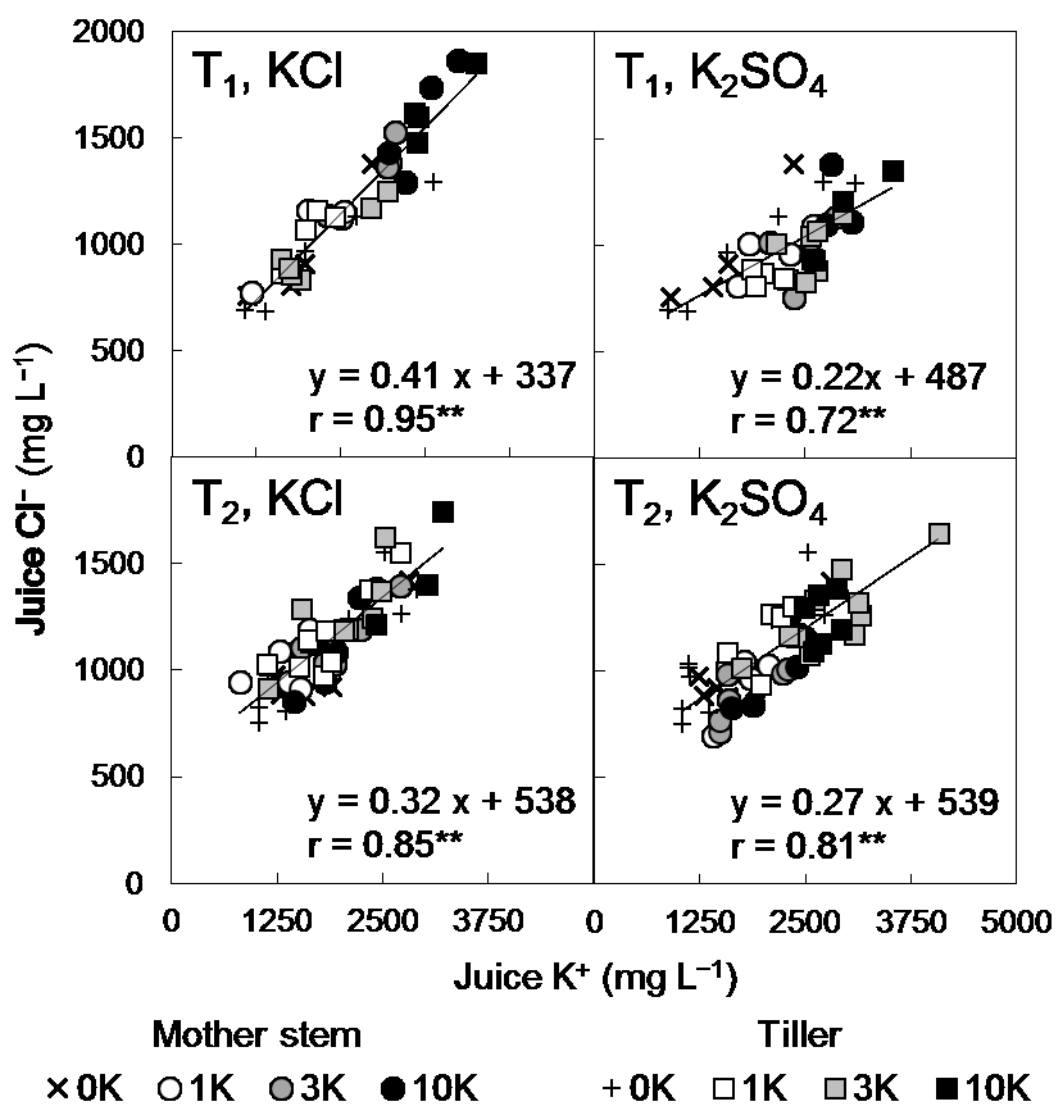
**Table 4.2-2.** Juice ion composition and PIC of mother stems and tillers.

Stem	Treatment	K <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	PIC	
		mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	%	
T <sub>1</sub>	Mother stem	0K	1566 b	962 b	1213 a	6.5 a
		1K	1647 b	1053 b	1040 a	7.6 a
		KCl 3K	2406 ab	1352 ab	1226 a	7.1 a
		10K	2950 a	1580 a	1169 a	7.5 a
		1K	2113 ab	967 b	1078 a	7.4 a
		K <sub>2</sub> SO <sub>4</sub> 3K	2468 ab	964 b	1227 a	7.7 a
		10K	2797 a	1129 ab	1056 a	7.9 a
	Tiller	0K	1926 b	1010 b	1164 a	5.9 a
		1K	1633 b	1056 b	945 a	6.9 a
		KCl 3K	1750 b	991 b	1060 a	6.9 a
		10K	3073 a	1635 a	1062 a	7.2 a
		1K	2059 ab	849 b	1066 a	7.0 a
		K <sub>2</sub> SO <sub>4</sub> 3K	2577 ab	986 b	1008 a	5.8 a
		10K	3016 a	1163 b	983 a	5.6 a
ANOVA	Stem (S)	ns	ns	*	**	
	Treatment (T)	***	***	ns	ns	
	S×T	ns	ns	ns	ns	
T <sub>2</sub>	Mother stem	0K	1709 ab	999 a	1500 a	17.0 a
		1K	1429 b	1045 a	1474 a	17.2 a
		KCl 3K	2021 ab	1153 a	1340 a	17.0 a
		10K	1923 ab	1123 a	1526 a	15.8 a
		1K	1701 ab	961 a	1411 a	16.4 a
		K <sub>2</sub> SO <sub>4</sub> 3K	1768 ab	890 a	1346 a	15.9 a
		10K	2179 a	1051 a	1476 a	16.5 a
	Tiller	0K	1697 c	1084 a	1717 a	16.6 a
		1K	1850 bc	1166 a	1455 a	16.7 a
		KCl 3K	2024 abc	1260 a	1541 a	16.6 a
		10K	2882 ab	1454 a	1659 a	16.1 a
		1K	2116 abc	1156 a	1644 a	16.5 a
		K <sub>2</sub> SO <sub>4</sub> 3K	2816 a	1285 a	1529 a	16.1 a
		10K	2697 ab	1245 a	1720 a	16.8 a
ANOVA	Stem (S)	***	***	***	ns	
	Treatment (T)	***	*	*	ns	
	S×T	*	ns	ns	ns	

Means with different alphabets are significantly different at the 5% level. \*, \*\*, and \*\*\* mean significant difference or interaction at the 5%, 1%, and 0.1% levels, respectively. ns: not significant.



**Fig. 4.2-2.** Relationships of juice K<sup>+</sup> and Cl<sup>-</sup> with PIC. \*\* means significance at the 1% level.



**Fig. 4.2-3.** Relationships between  $\text{K}^+$  and  $\text{Cl}^-$  concentrations in sugarcane juice.  $^{**}$  means significance at the 1% level.

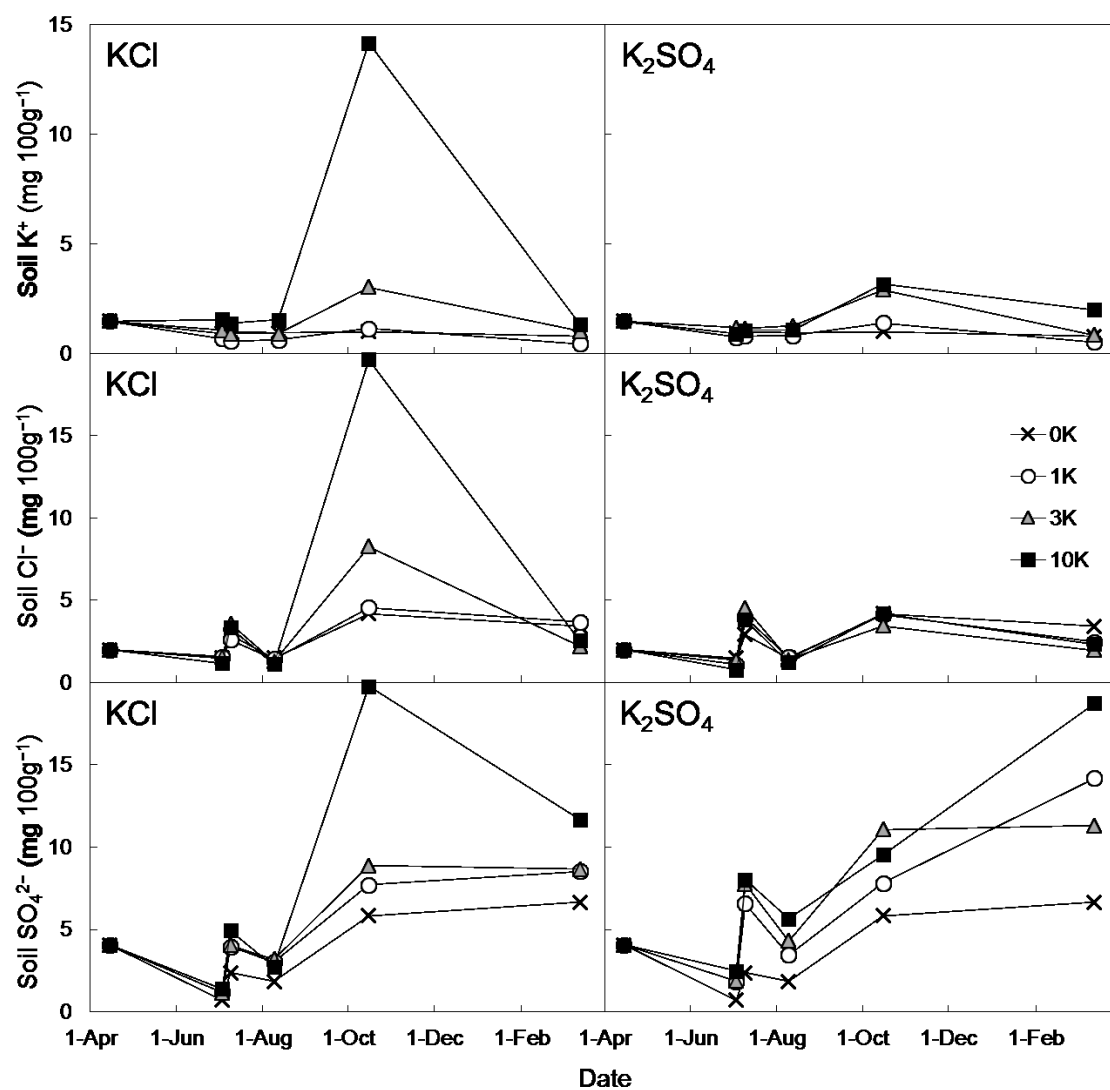


Fig. 4.2-4. Changes of ion concentrations in the field soil.

$\text{SO}_4^{2-}$  20 mg  $100\text{g}^{-1}$ . Except October 15, soil  $\text{K}^+$  and  $\text{Cl}^-$  remained relatively low, while soil  $\text{SO}_4^{2-}$  gradually increased particularly in the  $\text{K}_2\text{SO}_4$  treatments.

## Discussion

This study was conducted to test whether the variance of  $\text{K}^+$  and  $\text{Cl}^-$  in juice is derived from fertilizer application level under a field condition. As being expected, soil and juice  $\text{K}^+$  and  $\text{Cl}^-$  showed increasing trends with applied KCl levels, especially in the early growth period at October, and the highest KCl-treated plot had the lowest PIC value at harvest, meaning that these two ions supplied from KCl were proved to lower sugarcane quality under field conditions as well. Cane yield was lowest also in KCl 10K, Therefore, it can be said that the overdose of KCl impaired cane yield, quality, and sugar yield with increases of both juice  $\text{K}^+$  and  $\text{Cl}^-$ . Outside Japan, not few studies on levels and types of K fertilizers were reported. For example, increasing KCl levels contributed to improved cane yield and quality with increments of  $\text{K}^+$  and  $\text{Cl}^-$  in plants or soils (Akhtar and Akhtar, 2002; Almeida et al., 2015). In comparison with these positive effects of higher KCl doses, Hallmark et al. (2001), Khosa (2002), and Khadr et al. (2004) reported no difference in yield and quality between KCl and  $\text{K}_2\text{SO}_4$ . These reports anyway contained no negative impact by increasing KCl levels such as the reduction of sugar content and different effects of KCl and  $\text{K}_2\text{SO}_4$  application on sugarcane quality, in contradiction to our results. It is probably because of higher doses of  $\text{K}_2\text{O}$  in this study, 60 kg  $10\text{a}^{-1}$  at most, in contrast to those in the previous report less than 20 kg  $10\text{a}^{-1}$ .

In this study, the sampled stems were separated into two groups: mother stem and tiller because tillers, which are thought to be younger than mother stems, could accumulate more nutrients, considering the properties of  $\text{K}^+$  and  $\text{Cl}^-$  moving freely from older to younger parts. This theory could explain widely varying juice ion concentrations within a field and decreasing tendencies of qualitative parameters as their increments (Chapter 4.1). Although I



observed significant differences in juice  $K^+$  and  $Cl^-$  between the two groups at  $T_2$ , PIC of mother stems was not significantly different from that of tillers. In short, tillers had a higher juice  $K^+$  and  $Cl^-$  but a similar sugar content with mother stems; therefore, it can be said that cane maturity is not involved in the sugar reduction.

Irrespective of K fertilizer type, juice  $K^+$  was highly and positively associated with  $Cl^-$  and the coefficients of linear regressions were lower in the  $K_2SO_4$  treatments than in the KCl treatments at both  $T_1$  and  $T_2$ , in accordance with Chapter 3.1, except no significant difference between the treatments found in this study, suggesting a possibility to lower juice  $Cl^-$  concentration under field conditions by substituting  $K_2SO_4$  for KCl in case of increasing K fertilizer dose. This, on the contrary, indicates there would be no great difference between KCl and  $K_2SO_4$  when the amount is small.

Concluding from above all, it was revealed when KCl was overdosed into a field,  $K^+$  and  $Cl^-$  concentrations in soil and juice increased and afterward sugarcane yield and quality were impaired. However, it is required to lower  $K^+$  and  $Cl^-$  present in the whole growth environment in terms of ways other than fertilizer management, e.g. by removing  $K^+$  and  $Cl^-$  remaining in the field with cleaning crops or irrigating with good quality water containing lesser  $K^+$  and  $Cl^-$ . Taken together with the former pot experiments in Chapter 3.1, lessening KCl amount or substituting  $K_2SO_4$  for KCl can be good solutions to reduce  $Cl^-$  that is considered to be mostly involved in the quality impairment, but probably the former one is better and easier, given that  $K_2SO_4$  application may make no difference from KCl application when  $Cl^-$  excessively exists in the field. Taking into account that most of sugarcane fields in Okinawa have been accumulating overabundant  $K_2O$  (Ota et al., 2000) and in this study the 0K plot showed a higher sugar yield than KCl 1K where sugarcanes were conventionally grown, it is worth considering to lessen KCl fertilizer amount, which is also supported by Kuba (1993) from different aspects. To examine the effectiveness of this new method of low KCl application though, further studies should be conducted in fields with various  $K^+$  and  $Cl^-$  levels.

It was unexplainable that, taking the amounts of fertilizers into account, juice  $K^+$  and/or  $Cl^-$  concentrations of the 3K and 10K plots were relatively low. According to Ng Kee Kwong (2003), responses to K fertilizers are not frequently observed in plant cane and even in first and second ratoons, which could elucidate the unclear results of this study. Besides, relationships between the ions and PIC were not as obvious as those in Chapter 4.1. The reason is not exactly known but maybe due to smaller ranges of the juice ion concentrations. Besides, the ion concentrations varied widely within the treatments, indicating the presence of sources of  $K^+$  and  $Cl^-$  other than fertilizer; e.g. soil solution (Goos, 1987; Wiegand et al., 1996; Lingle and Wiegand, 1997), atmosphere (Kafkafi, 2001), and irrigation water (Thomas et al., 1981; Lingle et al., 2000; Golabi et al., 2009); these will be highlighted afterward.

## **Chapter 5**

### **Effects of irrigation water quality on the growth and quality of sugarcane**

#### **5.1. Analysis of irrigation water from sugarcane producing areas and annual changes of rain water quality**

##### **Introduction**

The whole previous chapters revealed that sugarcane quality was impaired when both  $K^+$  and  $Cl^-$  were excessively supplied under field as well as pot conditions and  $Cl^-$  was the key factor accounting for the phenomenon. From Chapter 2.1, a great regional difference in juice ion compositions was found to exist, indicating high amounts of  $K^+$  and  $Cl^-$  present in the fields led to increasing juice  $K^+$  and  $Cl^-$  concentrations. Moreover,  $Cl^-$  ions were thought to have been accumulating in fields, given that in Chapter 4.1 the  $K_2SO_4$ -treated plants, which were given no  $Cl^-$  from fertilizers, absorbed more  $Cl^-$  than those fertilized with  $KCl$ . I assumed the variance in  $Cl^-$  was derived from fertilizers. Then, Chapter 4.2 simulated fields with different levels of  $K^+$  and  $Cl^-$  by applying different kinds and levels of K fertilizer; on the contrary, the results were not as clear as my expectation: it was not unexplainable enough why juice  $K^+$  and  $Cl^-$  differed greatly among areas in terms of fertilizer management practice. From these, knowing the sources of  $Cl^-$  other than fertilizers is of my great interest. As stated before, soil solution (Goos, 1987; Wiegand et al., 1996; Lingle and Wiegand, 1997), irrigation water (Thomas et al., 1981; Lingle et al., 2000; Golabi et al., 2009), and atmosphere (Kafkafi, 2001) are known to be ones of possible sources of  $Cl^-$ . In this chapter, I first investigated the quality of irrigation water collected from several sugarcane producing areas of Nansei Islands in Japan and the annual change of rainwater quality to seek for the sources of  $Cl^-$ .

## Materials and methods

Irrigation water was collected from several sugarcane producing areas; namely three samples from the experimental fields of the University of the Ryukyus on July 19, 2014; three samples from Ie Island on August 15, 2014; four samples from Miyako Island on August 21, 2014; 25 samples from Minamidaito Island on January 1 to 2, 2016; and 15 samples from Tokunoshima Island on January 21 to 25, 2016. Only samples of Minamidaito Island were sorted by sampling site: outside and within the windbreak forest (expressed as coast and inland, respectively) and type of water source: pond, reservoir, and agricultural tank storing irrigation water called marine tank for further analysis. During sampling in Minamidaito Island, sea water was also taken. Rainwater was constantly collected every month for two years from April 2014 to March 2016. To collect rainwater, a 2 L-plastic bottle with a net on the top part to prevent litter from getting inside was fixed at the experimental field of the University of the Ryukyus (No.15; 26°25'N, 127°76'E; 127 m a.s.l.). Both irrigation water and rainwater samples were stored in 50 mL-tubes and kept in the refrigerator until used for analyses. After EC (CM-14P, Toa) measurement, samples were diluted if necessary and filtered with a membrane filter (diameter, 13 mm; pore size, 0.45  $\mu\text{m}$ ; Advantec) and then ion concentrations were determined by ion chromatography method similarly with juice ion analysis previously described in Chapter 2.1.

## Results

$\text{Cl}^-$  was the most dominant ion in most of samples and the highest one was obtained from Minamidaito Island ( $1462 \text{ mg L}^{-1}$ ) (Table 5.1-1).  $\text{Na}^+$  was the second highest followed by  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{SO}_4^{2-}$ , similarly with the order of sea water.  $\text{NO}_3^-$  was close to  $0 \text{ mg L}^{-1}$  except Miyako Island where  $\text{NO}_3^-$  concentration in drinking water is always highlighted as a big concern. Ion composition in irrigation water greatly varied depending on area. Ion concentrations were low in the samples of the University of the Ryukyus, Ie Island, and

**Table 5.1-1. Ion composition and EC of irrigation water from sugarcane producing areas in Japan.**

	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	EC
University of the Ryukyus	6 (0-18)	2 (1-4)	3 (2-4)	11 (9-14)	46 (29-67)	1 (0-4)	3 (0-8)	49 (16-73)
Ie Island	27 (21-36)	3 (3-3)	3 (3-4)	11 (10-13)	43 (33-58)	0 (0-0)	8 (6-10)	25 (19-30)
Miyako Island	58 (25-152)	3 (2-6)	9 (5-19)	69 (52-82)	97 (32-280)	20 (15-25)	31 (20-59)	73 (48-131)
Minamidaito Island	234 (11-641)	10 (1-23)	40 (4-98)	47 (5-120)	546 (22-1462)	2 (0-8)	67 (2-170)	205 (11-527)
Tokunoshima Island	16 (9-22)	1 (0-5)	5 (3-8)	8 (4-18)	29 (15-41)	0 (0-0)	10 (5-32)	22 (13-36)
Sea water	8800	318	1276	1137	19402	0	2411	5200

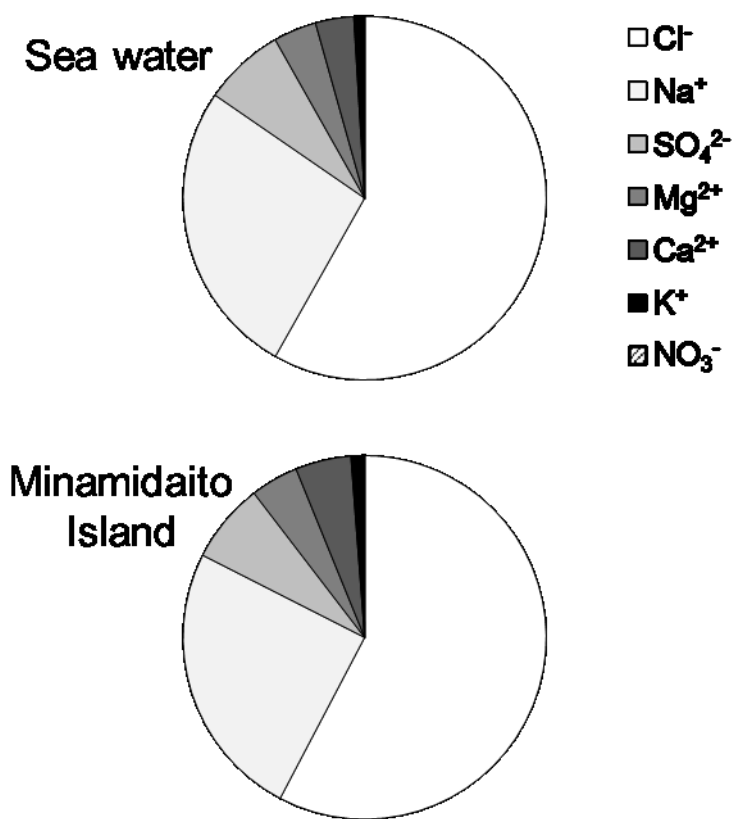
Values are mean and the minimum and the maximum are shown in parenthesis.

Tokunoshima Island. Especially the samples from Tokunoshima Island were all low within a narrow range in spite of the relatively high sample number. In Miyako and Minamidaito islands, meanwhile, the variances in ion composition between samples were large. For example,  $\text{Cl}^-$  concentration ranged from 32 to 280  $\text{mg L}^{-1}$  in Miyako Island and from 22 to 1462  $\text{mg L}^{-1}$  in Minamidaito Island.

Except the University of the Ryukyus, each ion concentration in sea water significantly correlated with those in irrigation water of each area (Fig. 5.1-1). Particularly samples from Minamidaito Island had a strong relationship, indicating ions in those samples were mostly derived from sea water. From this, samples from locations near ocean were thought to have higher ion concentrations, thereby those obtained from Minamidaito Island were sorted by sampling site and type of water source in Table 5.1-2. There was no significance probably due to the small numbers of samples of each area, but  $\text{Cl}^-$  concentration in irrigation water was likely to be higher in the coastal area though inland ponds also had a high value. Categorized by water source type,  $\text{Cl}^-$  concentrations in pond and marine tank exceeded that of reservoir with the significance at the 5% level. Sampling sites were plotted in the map of Minamidaito Island and colored differently depending on their  $\text{Cl}^-$  concentrations, which resulted in no obvious distributing pattern of irrigation  $\text{Cl}^-$ ; yet those located at the central east showed the least concentration (0–100  $\text{mg L}^{-1}$ ) (Fig. 5.1-2).

EC and  $\text{Cl}^-$  concentration in irrigation water were closely correlated and increment of each  $\text{mS m}^{-1}$  in EC contributed to increasing 4.06  $\text{mg L}^{-1}$  of  $\text{Cl}^-$  (Fig. 5.1-3). However, when focusing on comparatively lower EC and  $\text{Cl}^-$ , respectively below 100  $\text{mS m}^{-1}$  and 200  $\text{mg L}^{-1}$ , not few samples scattered apart from the regression line.

$\text{Cl}^-$  concentration in rainwater fluctuated throughout the years and some peaks were seen from 2014 to the early 2015, perhaps with the approach of typhoons, taking account of high precipitation (Fig. 5.1-4). The maximum, 136  $\text{mg L}^{-1}$  of  $\text{Cl}^-$ , was obtained in July, 2014; in comparison, the latter half period had a lower concentration below 20  $\text{mg L}^{-1}$ .



**Fig. 5.1-1.** Ion compositions in sea water and in the irrigation water from Minamidaito Island.

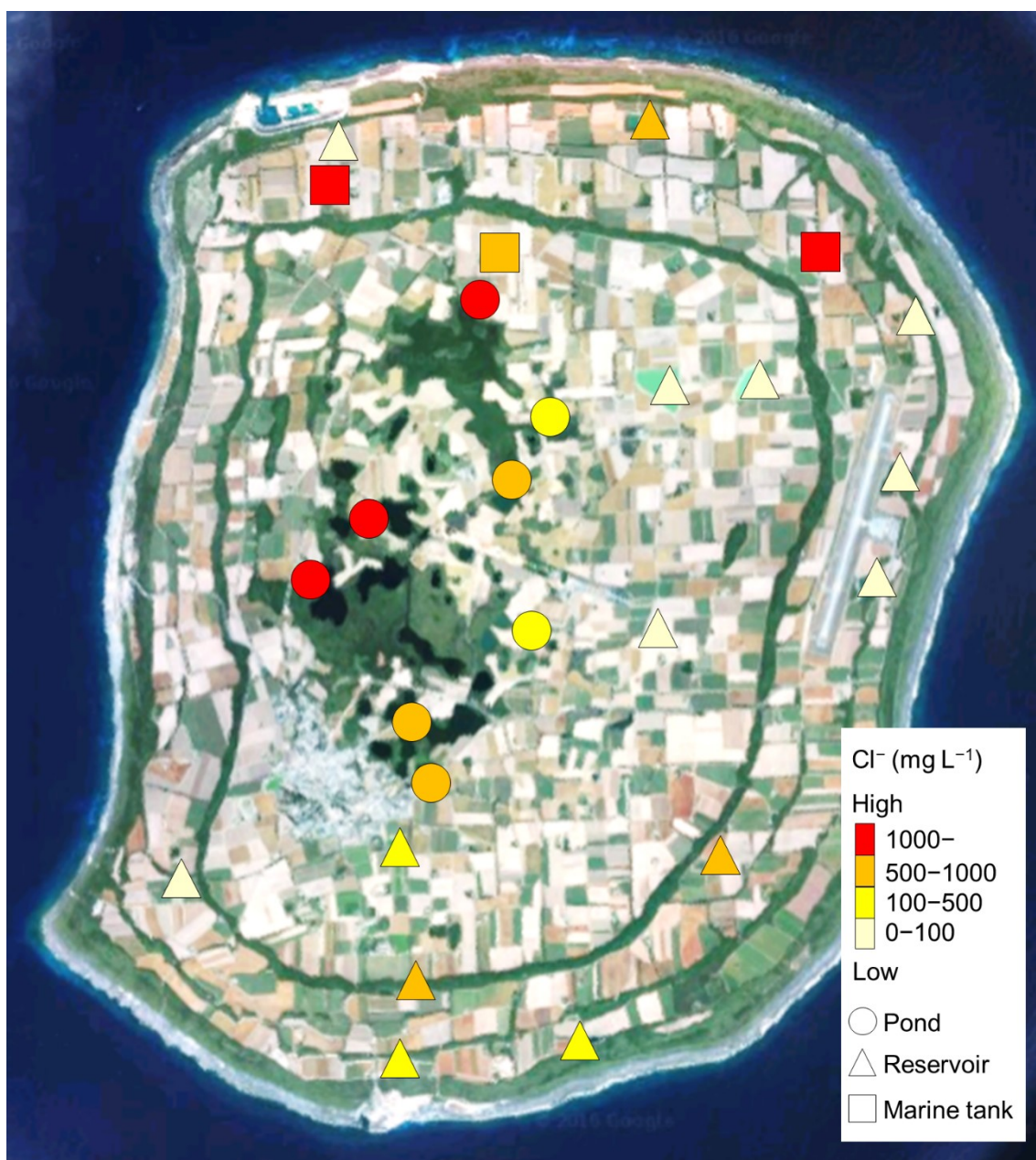
**Table 5.1-2.**  $\text{Cl}^-$  concentration in irrigation water.

Sampling site	Type of water source	No. of sample	Irrigation water $\text{Cl}^-$ concentration
	Pond	0	
Coast	Reservoir	10	$296 \pm 342$
	Marine tank	2	$1291 \pm 186$
	Pond	8	$885 \pm 462$
Inland	Reservoir	4	$112 \pm 53$
	Marine tank	1	570
Sampling site			n.s.
Type of water source			**
Interaction			n.s.

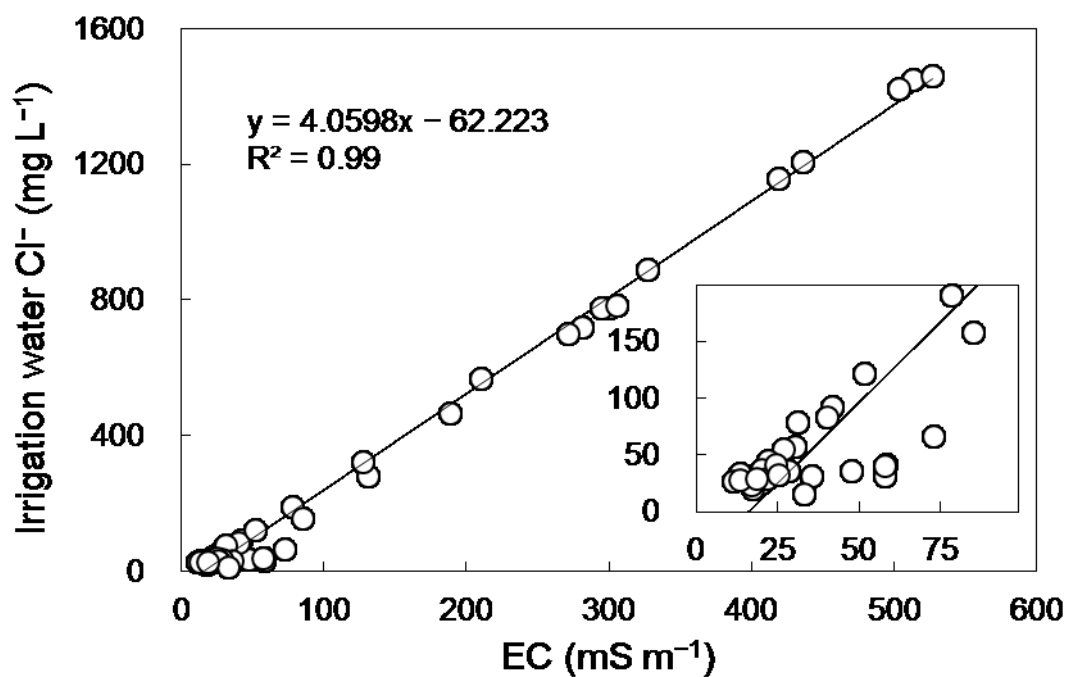
\*\* means significant difference at the 1% level.

ns: not significant.

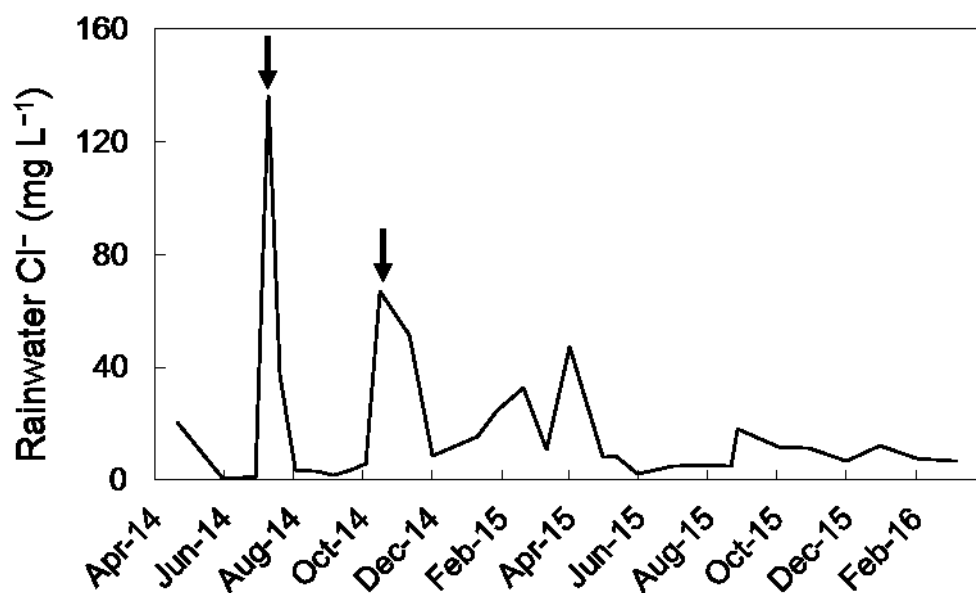




**Fig. 5.1-2.** Sampling sites in Minamidaito Island and the types of water samples: Pond (○), reservoir (△), and marine tank (□). Note that symbols are colored with white, yellow, orange, and red based on the Cl<sup>-</sup> concentrations.



**Fig. 5.1-3.** Relationships between EC and Cl<sup>-</sup> concentration in irrigation water. A figure located at the lower right shows an enlarged part of EC from 0 to 100 ms m<sup>-1</sup> and irrigation water Cl<sup>-</sup> from 0 to 200 mg L<sup>-1</sup>.



**Fig. 5.1-4.** Change of  $\text{Cl}^-$  concentration in rainwater from April 2014 to March 2016. Arrows indicate the dates when typhoons hit the site.

## Discussion

The analysis was performed to examine factors which can affect ion concentrations in sugarcane juice and it revealed that  $\text{Cl}^-$  was the largest ion in both irrigation water and rainwater and some samples contained not little amount of  $\text{Cl}^-$ ; therefore, it is concluded that these two can be also the source of  $\text{Cl}^-$  other than fertilizer. However, the maximum  $\text{Cl}^-$  concentration of rainwater was much lower than that of irrigation water, indicating the greater impact of irrigation water quality on sugarcane juice  $\text{Cl}^-$  concentration even though it was highly dependent on sampling site.

Apart from Main Island Japan, Okinawa is composed of about 160 isolated islands, 40 of which are inhabited, scattering in a vast ocean area approximately with the east to west distance of 1000 km and the north to south of 400 km (Arasaki, 1997). Okinawa Island is the largest of Nansei Islands, however the distance from east to west ranges from about only 4 to 20 km (Koki, 2002). The islands of Okinawa are mostly surrounded by coral reef which makes the fine spray scatter to the atmosphere by breaking waves. Besides, in summer typhoons frequently approach to Okinawa, while in winter the relatively strong monsoon blows, resulting in salt derived from sea spreading widely across the islands through the year (Koki, 2002). In this study, I also confirmed the fluctuation of rainwater  $\text{Cl}^-$  concentration, which is thought to have been influenced by climate and weather such as typhoon and monsoon though some aspects were not explainable: for example, constantly low  $\text{Cl}^-$  concentration in the latter half despite the approach of several typhoons in summer of 2015.

Among irrigation water samples, I especially had a great interest in those from Minamidaito Island because of the wide variation of  $\text{Cl}^-$  concentration. This island has about 80 ponds including the biggest one with the surface area of 47 ha and underground fresh water lens floating on the top of an unconfined aquifer that seawater penetrates into, which is utilized as an irrigation source (Naganuma, 1992). Water sources other than ponds, such as reservoirs, marine tanks, and farm ponds are also available, given that irrigation management

is the most important problem in this island. Nevertheless, in terms of its quality, available water is relatively limited. Actually, the highest  $\text{Cl}^-$  concentration observed was  $1462 \text{ mg L}^{-1}$  concentration, which is equivalent to 6–7% of seawater and thought to be unsuitable for agricultural usage. This point will be focused on in the next chapter.

Fig. 5.1-3 suggested  $\text{Cl}^-$  concentration in irrigation water is expectable by EC, using the following formula:  $y = 4.0598x - 62.223$ . Yet, it is doubtful whether this formula is always applicable because the regression line did not completely fit with samples with EC lower than  $100 \text{ mS m}^{-1}$ . These samples are derived from many areas and ion compositions varied in each area, which is probably one of the reasons accounting for the disagreement. Even so, it is still quite useful in order to briefly know the  $\text{Cl}^-$  concentration because of the easiness of the measurement. The next chapter also reveals the critical EC value to sugarcane growth, based on these results.

## **5.2. Pot experiment with different salinity levels of irrigation water**

### **Introduction**

Salinity stress, which usually occurs in arid and semi-arid regions, is a major environmental constraint to crop productivity (Dasgan et al., 2002). At least 800 ha of land throughout the world are salt affected, which accounts for more than 6% of the world's total land area and 20% of the world's irrigated land area (Metternicht and Zinck, 2003; Munns and Tester, 2008; Zeng et al., 2013). The cost of soil salinization to agriculture is estimated to be approximately US\$ 12 billion a year (Murad et al. 2013). As stated before, salts are derived from many sources. Apart from natural salinity, a significant proportion of recently cultivated agricultural land has become saline owing to irrigation, which causes water tables to rise and concentrate the salts in the root zone (Munns and Tester, 2008). It was found from Chapter 5.1 that agricultural water source for sugarcane contained considerable amounts of salts, whose concentration varied widely depending on sites and reached up to more than 2000 mg L<sup>-1</sup>. Sugarcane is a glycophyte and has been ranked as moderately sensitive to salinity (Rozeff, 1995; Cha-um et al., 2012); therefore, salinity levels of irrigation water probably affects greatly sugarcane ion composition and quality. Many reports have been published to evaluate the effects of salinity stress on sugarcane growth, yield and morphological characters (Thomas et al., 1981; Wahid et al., 1997; Aktar et al., 2001; Hussain et al., 2004; Lingle et al., 2008); physiological parameters such as gas exchange rate, chlorophyll fluorescence, and enzyme activity (Meinzer et al., 1994; Cha-um et al., 2012; Medeiros et al., 2014; Murad et al., 2014; Poonsawat et al., 2015); and nutrient and metabolite levels (Gandonou et al., 2005; Gomathi and Thandapani, 2005; Gandonou et al., 2011; Murad et al., 2014; Poonsawat et al., 2015). Also, some solutions to salinity problem have been suggested; e.g., breeding cultivars with salt tolerant traits (Saxena, 2010; Medeiros et al., 2014; Poonsawat et al., 2015) and amelioration of salinity stress by improving irrigation water quality (Wiedenfield, 2008) and by supplementing other nutrients (Gomathi and Thandapani,

2005; Ashraf et al., 2010). Moreover, Wiegand et al. (1996) demonstrated yield loss by soil salinity using image analysis. In Japan, Koki (2002) and Maeda et al. (2015) reported salinity effects by typhoons and monsoons and importance of water sprinkling to wash out salts on the leaves. Oshiro and Nagatomi (1982) conducted a pot experiment with 10,000 and 20,000 ppm of NaCl water. However, the salt concentrations used in the report are much higher than the ones observed in the previous chapter. Although no evidence has been obtained, it is suggested that water with NaCl concentration up to 3000 mg L<sup>-1</sup> can be used for sugarcane production (personal communication). In short, only few reports related to salinity stress on sugarcane are available in Japan and it has not been fully revealed how relatively moderate salinity levels in irrigation water affect cane yield, quality, and photosynthesis of Japanese sugarcane cultivars. Besides these points, it is also necessary to clear whether Cl<sup>-</sup> derived from irrigation water contributes to influence juice ion composition, given that from the previous chapters it is supposed to be a source of Cl<sup>-</sup> other than fertilizers. In this chapter, thus, changes in ion composition, growth, and quality of sugarcane irrigated with different salinity levels were examined and the mechanism of its negative effects was investigated in terms of photosynthetic parameters.

### **Materials and methods**

A pot experiment was conducted under greenhouse conditions at the University of the Ryukyus, Okinawa, Japan (26°25'N, 127°77'E; 125 m a.s.l.) from November, 2015 to July, 2016. Seedlings of *S. spp.* cv. NiF8 were collected from the fields at the Subtropical Field Science Center of the University of the Ryukyus. One-bud seedlings were immersed in a solution of Benlate-R (5 g L<sup>-1</sup>, Sumitomo Chemical) and in tap water for 24 hours each for sterilization and to improve germination rate. These seedlings were planted and grown in containers from November 1 to December 9, 2015. After the first fully expanded leaves were confirmed, seedlings were transplanted into 1/2000a Wagner pots filled with soil mixture composed of

three materials: dark red soil (Shimajiri mahji), sea sand, and peat moss (1:1:1, v v<sup>-1</sup>). Tillers were immediately removed after emergence. Irrigation was carefully performed with tap water (Na<sup>+</sup>, 19; K<sup>+</sup>, 1; Mg<sup>2+</sup>, 3; Ca<sup>2+</sup>, 9; Cl<sup>-</sup>, 29; SO<sub>4</sub><sup>2-</sup>, 8 mg L<sup>-1</sup>) through daily soil moisture evaluation to prevent water stress. Fertilization was performed once a week after transplantation using a modified Hoagland solution (See Chapter 3.2 for the components).

The treatments were started on May 1 and performed through irrigation. Besides one irrigated with only tap water (Control), five treatments were established by adding different levels of NaCl to tap water: 200, 500, 1000, 2000, and 3000 mg L<sup>-1</sup>. Four plants were prepared for each treatment. Growth characteristics were measured once two weeks following the method of Chapter 3.1. Soil EC was recorded for one month from June 7 to July 6 with 5TE sensors (Decagon).

Photosynthesis and chlorophyll fluorescence measurements were performed on the second visible dewlap leaves before (April) and one and two months after the treatments started (June and July, respectively), using an infrared open gas exchange system (Li-6400, LI-COR).  $A$ ,  $g_s$ , and  $C_i$  were measured at a photosynthetic photon flux density of 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , using a constant flow rate of 400  $\mu\text{mol s}^{-1}$ . The measurements were performed at ambient atmospheric CO<sub>2</sub> concentration, 400  $\mu\text{mol mol}^{-1}$ . Leaf temperature and vapor pressure deficit based on leaf temperature were 33–38°C and 1.7–2.8 kPa, respectively.  $F_v/F_m$  was measured by the method used in Chapter 3.2. Other fluorescence parameters were measured with the same conditions as the gas exchange measurement. Once steady-state photosynthesis was achieved, steady-state fluorescence ( $F_t$ ), maximal fluorescence in light ( $F_m'$ ), and fluorescence removal of actinic light ( $F_o'$ ) were recorded. Then, proportion of open PSII (qP), non-photochemical quenching (NPQ), and quantum yield of PSII ( $\Phi\text{II}$ ) were calculated using the equations below.

$$qP = (F_m' - F_t) / (F_m' - F_o')$$

$$NPQ = (F_m - F_m') / F_m'$$

$$\Phi\text{II} = (F_m' - F_t) / F_m'$$



Plants were harvested on July 14 and leaf area was measured. Stems were pressed to obtain juice. On noon sections of leaf blade used for photosynthesis measurement were taken separately from other parts for leaf ion analysis. Leaf blade and stem were dried to measure dry matter weight. The concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  in leaf and juice and of sucrose, glucose, and fructose were determined by ion chromatography and HPLC, following the previous chapter.

Statistical analysis was performed using the software R (R Core Team, 2015). Data were subjected to one-way ANOVA between the treatments. When significances were found, the Tukey test was conducted, and the significant differences were accepted based on a P value  $< 0.05$ .

## Results

Soil EC measured for one month showed the clear effects of the treatments; namely, it was the lowest in Control and became greater rising up to  $0.4 \text{ mS cm}^{-1}$  as the NaCl levels in irrigation water increased though there was no difference between the 2000 and 3000 plots (Fig. 5.2-1). Stem length was lower in the 2000 and 3000 plots and significant differences were constantly confirmed after the treatments started, but the other four plots had similar values (Fig. 5.2-2). The number of leaves showed an increasing trend through the experimental period. It significantly differed between the plots at the latter period and tended to decrease with the increasing NaCl levels. SPAD value showed a decreasing trend as the treatment period was prolonged. The treatment effects were not distinct enough but were confirmed at the sampling: the higher NaCl levels, the higher SPAD values. Leaf area and dry weight were decreased by the treatments and that of the lowest plot, 3000, was approximately 30% less than that of Control (Fig. 5.2-3). There was no negative effects of the treatments on stem dry weight up to  $500 \text{ mg L}^{-1}$  of NaCl, however, it was linearly decreased with higher levels. As a result, total dry matter weight was the highest in the 200 plot and the 2000 and 3000

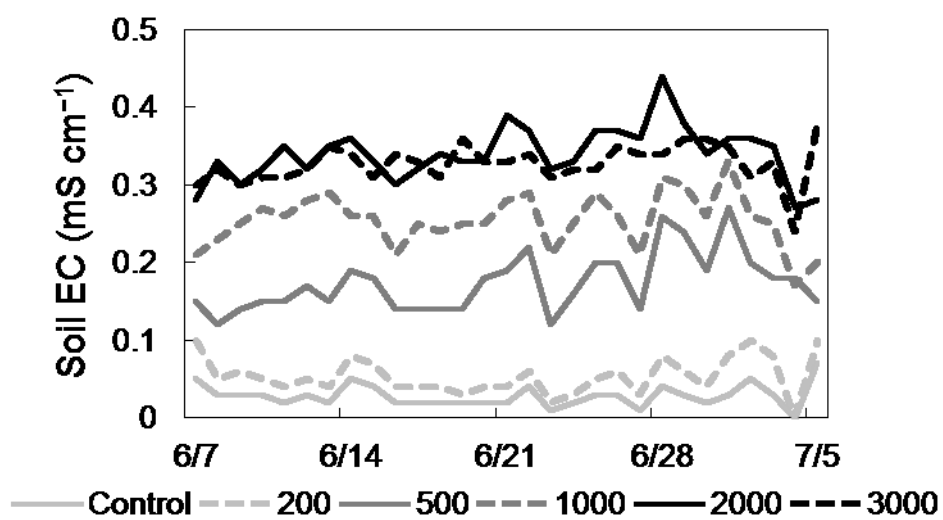
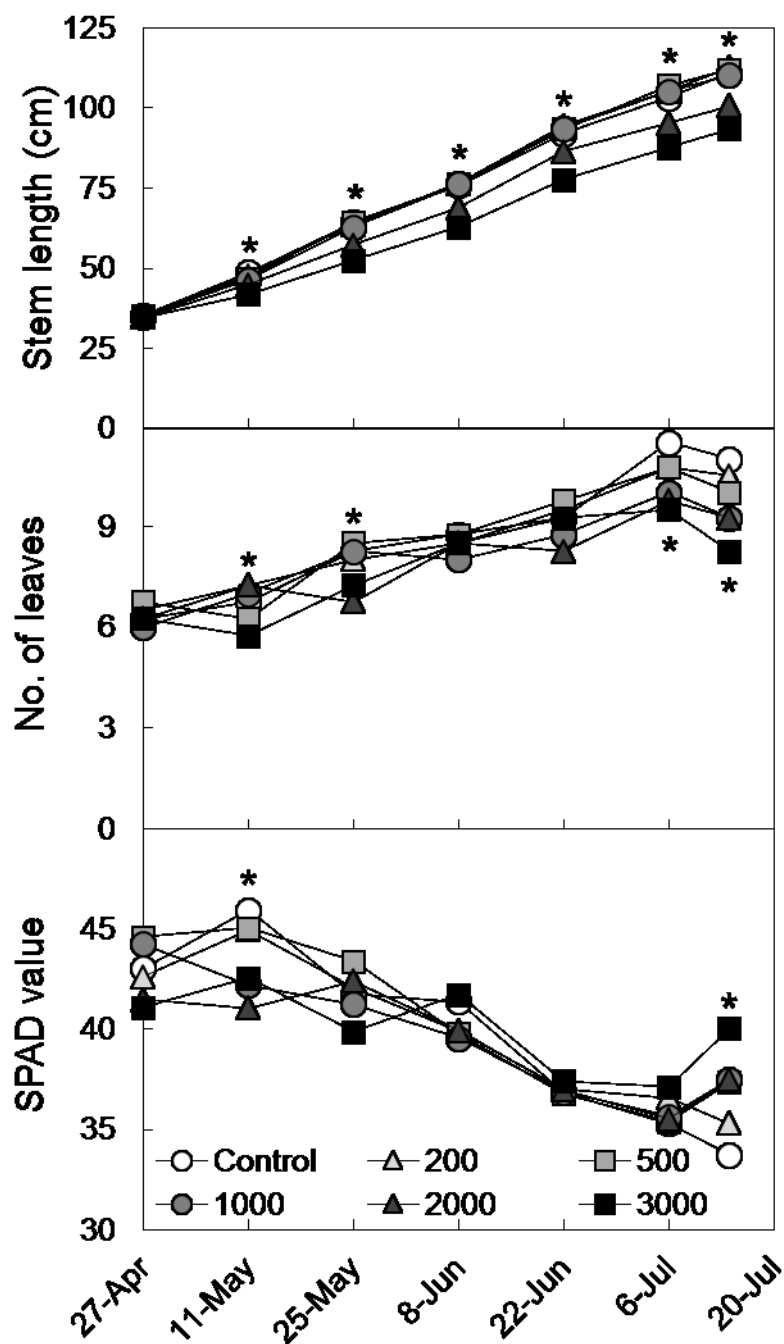
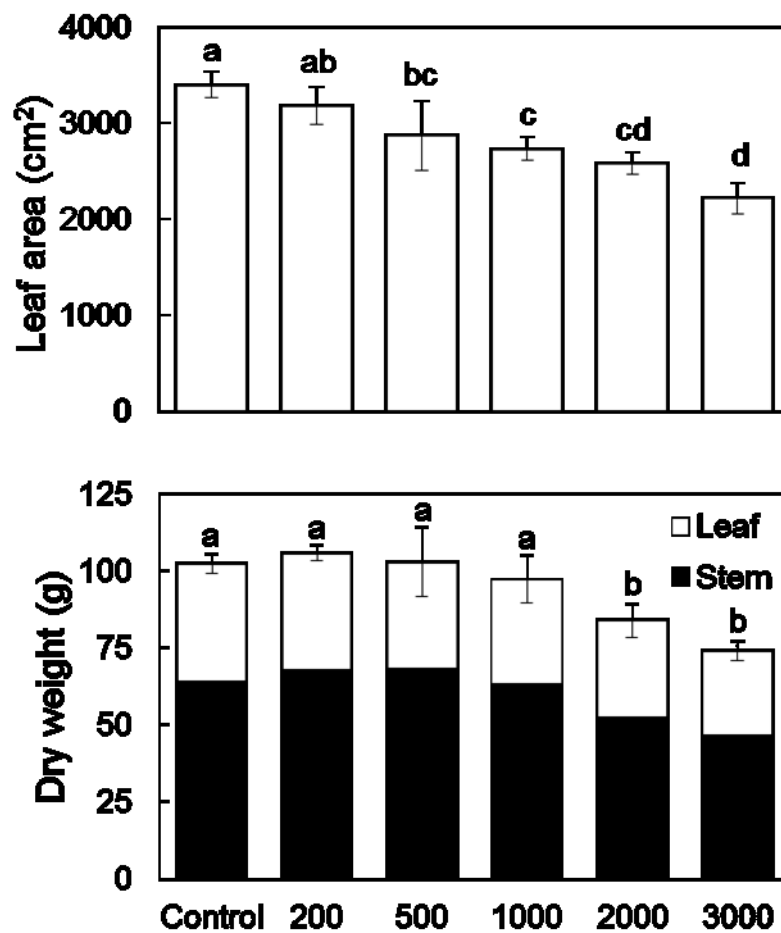


Fig. 5.2-1. Change of soil EC from 7 June to 6 July, 2016.



**Fig. 5.2-2.** Change of stem height, the number of leaves, and SPAD value. \* means a significant difference at the 5% level.



**Fig. 5.2-3.** Effects of the treatments on leaf area and dry weight of leaf and stem. Vertical bars indicate SD. Means with different alphabets are significantly different at the 5% level (Tukey test).

plots had significantly less values than the other plots.

Juice ion composition changed greatly by the NaCl treatments. The concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and surprisingly  $\text{K}^+$  as well dramatically increased with an increasing levels of NaCl (Table 5.2-1). As revealed in the previous chapter,  $\text{K}^+$  and  $\text{Cl}^-$  are the two dominant ions in sugarcane juice, so the concentration of  $\text{Na}^+$  in juice was much lower than those of  $\text{K}^+$  and  $\text{Cl}^-$  however seemed to be more affected by the treatments considering the large difference between those in Control and the 3000 plot. The treatments, on the contrary, did not give a big impact on leaf ion composition. No significant change was seen in leaf  $\text{Na}^+$ . NaCl-treated plots had higher leaf  $\text{Cl}^-$  than Control, but the highest was obtained in the 200 plot and that of Control did not significantly differed with those of 2000 and 3000, while leaf  $\text{K}^+$  tended to increase with an increment of salinity levels even though no more  $\text{K}^+$  was applied through the treatments.

The plots of NaCl 200, 500, and 1000  $\text{mg L}^{-1}$  showed higher juice sucrose concentrations than Control and that of the 500 plot was the highest (Table 5.2-2). Those of the 2000 and 3000 plots were lower than the other NaCl-treated plots but did not significantly differed with that of Control. The NaCl-treated plots all had lower concentrations of reducing sugar than Control. In comparison with juice sucrose, greater differences were observed in leaf sucrose and the NaCl application significantly reduced the concentrations of sucrose and reducing sugar similarly.

Before the treatment started (April),  $A$  was about  $35 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 5.2-4) Then,  $A$  of the 2000 and 3000 plots was decreased in June, whereas  $A$  of the other plots was improved. In July, the NaCl treatments induced lowering  $A$  and its adverse effects were greater when the NaCl levels became higher. Similar results were found in  $g_s$  and  $C_i$  and significances between the treatments were confirmed in all the photosynthetic parameters after the treatments started.  $A$  was closely associated with  $g_s$  and the relationship was well expressed by a fitting curve (Fig. 5.2-5). As compared to gas exchange measurement, chlorophyll fluorescence

**Table 5.2-1.** Ion compositions in juice and leaf.

	Juice (mg L <sup>-1</sup> )			Leaf (mg g <sup>-1</sup> )		
	Na <sup>+</sup>	Cl <sup>-</sup>	K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	K <sup>+</sup>
Control	40 c	1216 e	2591 e	0.22 a	6.9 b	6.8 c
200	91 c	2128 d	3413 d	0.22 a	8.5 a	7.8 bc
500	102 bc	2544 c	3718 cd	0.21 a	8.3 a	8.0 bc
1000	123 bc	2708 b	3900 bc	0.18 a	8.1 a	7.9 bc
2000	273 b	3012 ab	4230 ab	0.25 a	7.3 ab	9.6 ab
3000	490 a	3401 a	4495 a	0.20 a	7.5 ab	9.9 a

Means with different alphabets are significantly different at the 5% level (Tukey test).

**Table 5.2-2.** Sugar compositions in juice and leaf.

	Juice (%)		Leaf (mg g <sup>-1</sup> )	
	Sucrose	Reducing sugar	Sucrose	Reducing sugar
Control	12.4 bc	1.1 a	52.1 a	9.0 a
200	13.5 ab	0.8 ab	48.1 a	7.7 ab
500	14.3 a	0.6 b	45.5 ab	5.3 ab
1000	14.0 a	0.7 b	42.7 ab	5.6 ab
2000	12.4 bc	0.6 b	33.7 b	4.7 b
3000	11.6 c	0.7 b	33.9 b	5.2 ab

Means with different alphabets are significantly different at the 5% level (Tukey test).

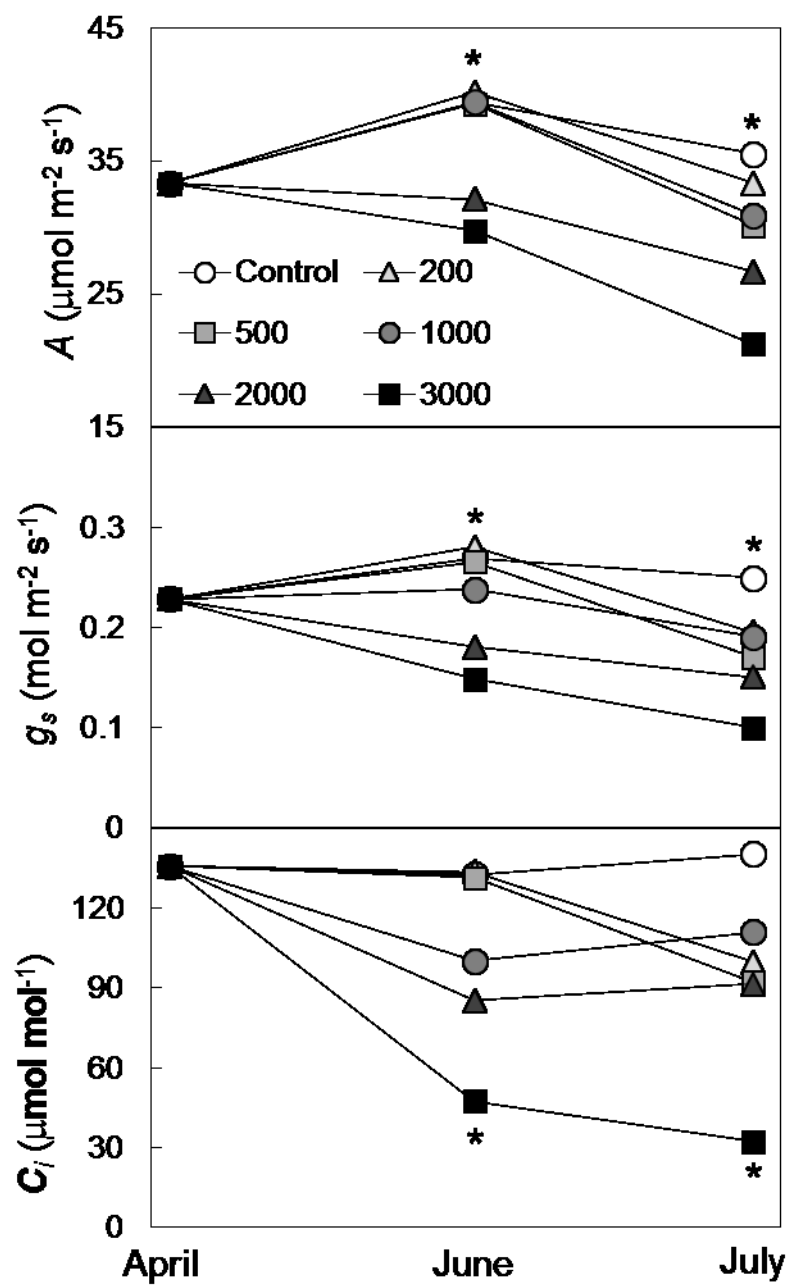


Fig. 5.2-4. Effects of the treatments on gas exchange parameters. \* means a significant difference at the 5% level.



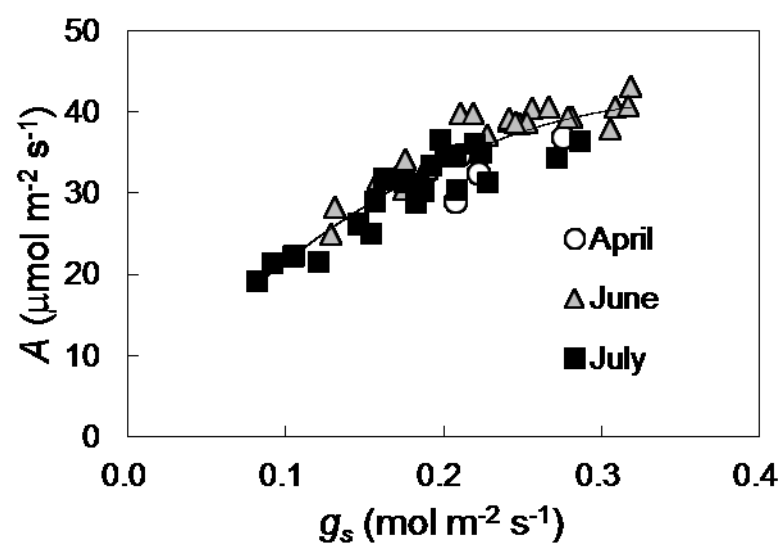


Fig. 5.2-5. Relationship between  $g_s$  and  $A$ .

parameters hardly differed between the treatments; namely no significant difference was obtained in any parameters (data not shown).

The relationship between  $K^+$  and  $Cl^-$  concentrations in juice was also examined (Fig. 5.2-7). They were highly correlated even though given  $K^+$  amount to sugarcane plants were equal among the plots. Fig. 5.2-7 also shows the relationships between juice ion and sucrose concentrations. As mentioned earlier, juice ion concentrations were greatly affected by the treatments. While increasing  $Na^+$  concentration reduced sucrose concentration linearly,  $K^+$  and  $Cl^-$  had parabolic relations with sucrose and the peaks seemed to be existing on about 3500 and 2000  $mg\ L^{-1}$ , respectively.

### **Discussion**

As far as my understanding, this is the first report about the detailed examination of salinity stress to sugarcane in Japan. In terms of growth and quality, more than 2000  $mg\ L^{-1}$  of NaCl in irrigation water could be harmful for sugarcane, but no adverse effects of NaCl levels less than 1000  $mg\ L^{-1}$  was confirmed, which corresponded very well with the results of photosynthesis. Given that the relation between stomatal conductance and gas exchange rate was plotted on a fitting curve and fluorescence parameters were not affected significantly, the growth inhibition in higher NaCl-treated plots was simply explained by lowered photosynthesis ability with stomatal closure and lack of leaf intercellular  $CO_2$ . In addition, leaf expansion was interfered by salinity stress, which led plants to have smaller area for photosynthesis. Taken together, total  $CO_2$  assimilation in whole plants was inhibited due to restricted leaf size and reduced photosynthetic rate. The reduction of photosynthate caused lower stem dry weight, which would eventually result in cane yield loss at harvest. Salinity stress also affected sugarcane juice quality. By contrast with quantitative parameters, no

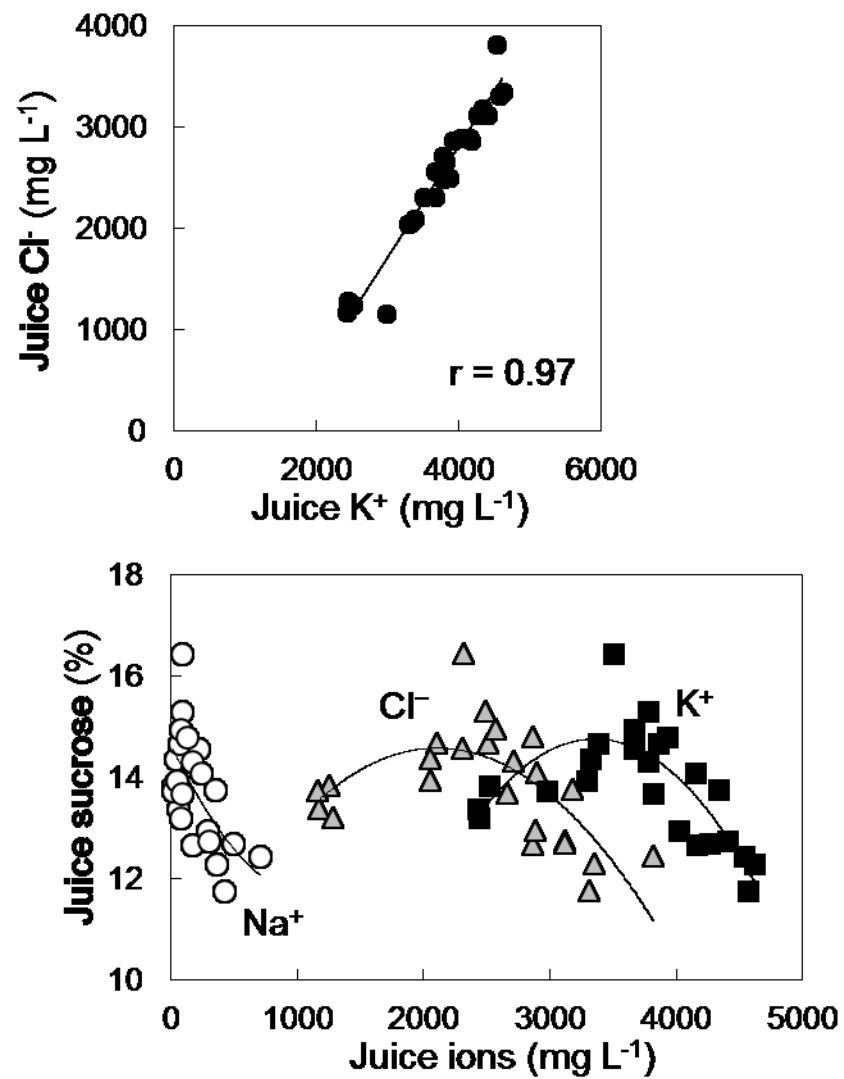


Fig. 5.2-7. Relationship between juice K<sup>+</sup> and Cl<sup>-</sup> concentrations and between juice ions and sucrose concentrations.

significant adverse effects on sugarcane quality was observed at this growth stage, indeed plants with irrigation NaCl levels up to 1000 mg L<sup>-1</sup> had higher juice sucrose concentration than the Control plants. These results contradicted to previous results and my expectation that Cl<sup>-</sup> from irrigation water deteriorates cane quality. One possible reason accounting for this is the seasonal effects on cane maturity. In this experiment, sugarcane plants were relatively small and not fully matured yet. Considering the positive effects at earlier periods and negative effects at latter periods by K<sup>+</sup> and Cl<sup>-</sup> uptake seen in Chapter 3.1, checking its effects on cane quality on practical harvest season is necessary. It is also worthy of conducting experiments combining salinity with drought stress since irrigation water quality is an important concern when plants need water under drought conditions and both types of stress prevent plants from absorbing water (Zhu, 2002).

Juice ion composition was greatly affected by the treatments and Cl<sup>-</sup> was indeed increased up to 3400 mg L<sup>-1</sup> in the highest NaCl-treated plot. Surprisingly, the accumulation of K<sup>+</sup> as well as Na<sup>+</sup> and Cl<sup>-</sup> seemed to be enhanced and its concentration reached 4500 mg L<sup>-1</sup>, which is almost the double of that in Control plants (2591 mg L<sup>-1</sup>), indicating that salinity of irrigation water can be an important source for both K<sup>+</sup> and Cl<sup>-</sup>. This is a new finding which disagrees with a commonly accepted theory that juice or leaf K<sup>+</sup> concentration is reduced by additional Na<sup>+</sup> supply in the form of NaCl (Wahid et al., 1997; Gandonou et al., 2005; Gomathi and Thandapani, 2005; Medeiros et al., 2014) since they are physico-chemically very similar and their uptakes by plants are strongly affected by the competitive interaction (Schachtman and Liu, 1999; Benito et al., 2014). In this study, only the most popular cultivar in Japan, *S. spp.* cv. NiF8, was used, therefore it is unknown if only this cultivar shows these trends or others have similar characteristics. Considering that generally ion accumulating ability differs depending on salt tolerance of each cultivar and ones which accumulate higher K<sup>+</sup> are thought to be salt tolerant, NiF8 may be a salt tolerant cultivar. These points should be further examined using some cultivars with different traits.

In this study, any salt-treated plants had significantly higher juice  $\text{Cl}^-$  than the Control plants. It is notable that even the lowest NaCl level of  $200 \text{ mg L}^{-1}$  significantly increased both  $\text{K}^+$  and  $\text{Cl}^-$  concentrations in juice, which is inconsistent with the results of Chapter 3.2 that weekly application of  $50 \text{ mM NaCl}$  (approximately  $3000 \text{ mg L}^{-1}$ ) did not effectively increase juice  $\text{Cl}^-$  concentration. This indicates that constantly supplied salts through daily irrigation may have stronger impact than given through weekly fertilization and thus an amelioration of irrigation water quality as well as appropriate fertilizer management results in sugarcane quality improvement. However, at least from the results of this study, it is concluded that irrigation water is available with no problem when its NaCl level is less than  $1000 \text{ mg L}^{-1}$  and irrigation is not recommended when it exceeds  $2000 \text{ mg L}^{-1}$ .

## **Chapter 6**

### **Cultivar differences in nutrient and sugar accumulating characteristics**

#### **6.1. Field survey in Minamidaito Island for juice ion and sugar analysis**

##### **Introduction**

Until here, cultivation management practice such as fertilization and irrigation have been discussed against growth conditions surrounded by excessive  $K^+$  and  $Cl^-$ .  $K^+$  and  $Cl^-$  known as ash reduce the efficiency of sugar recovery as stated in Chapter 2.1. This has been a great concern especially in some areas in Australia. Hogarth and Kingston (1983) mentioned ash levels in cane juice can be influenced by conditions associated with the growing environment and genetic differences between varieties of sugarcane and some published papers say sugarcane breeding and cultivar selection can be one of the solutions to the high ash problem recognizing varietal differences in ash levels (Hogarth and Kingston, 1983; Mullins and Roach, 1985; Jackson et al., 2008). By contrast, in Japan, the information about juice ash levels or ion compositions in sugarcane cultivars is scarce. However, Hattori et al. (2016) recently confirmed varietal differences in ash content among Japanese sugarcane cultivars as well, indicating the presence of different ion compositions in juice among cultivars. So far, only one most popular commercial cultivar, NiF8, has been studied in a series of the researches. Yet, it is still unknown whether cultivars with various ion compositions will respond differently to environments comprising high  $K^+$  and  $Cl^-$ . Achieving sugarcane quality improvement through cultivar selection first required to find cultivars which absorb less  $K^+$  and  $Cl^-$  concentrations or which show smaller reduction of sucrose concentration when exposed to high KCl-treated environments. The purpose of this chapter is to examine varietal differences in ion and sugar accumulating ability in order to find cultivars that are expected to show quality impairment to a lesser degree under high KCl dose.

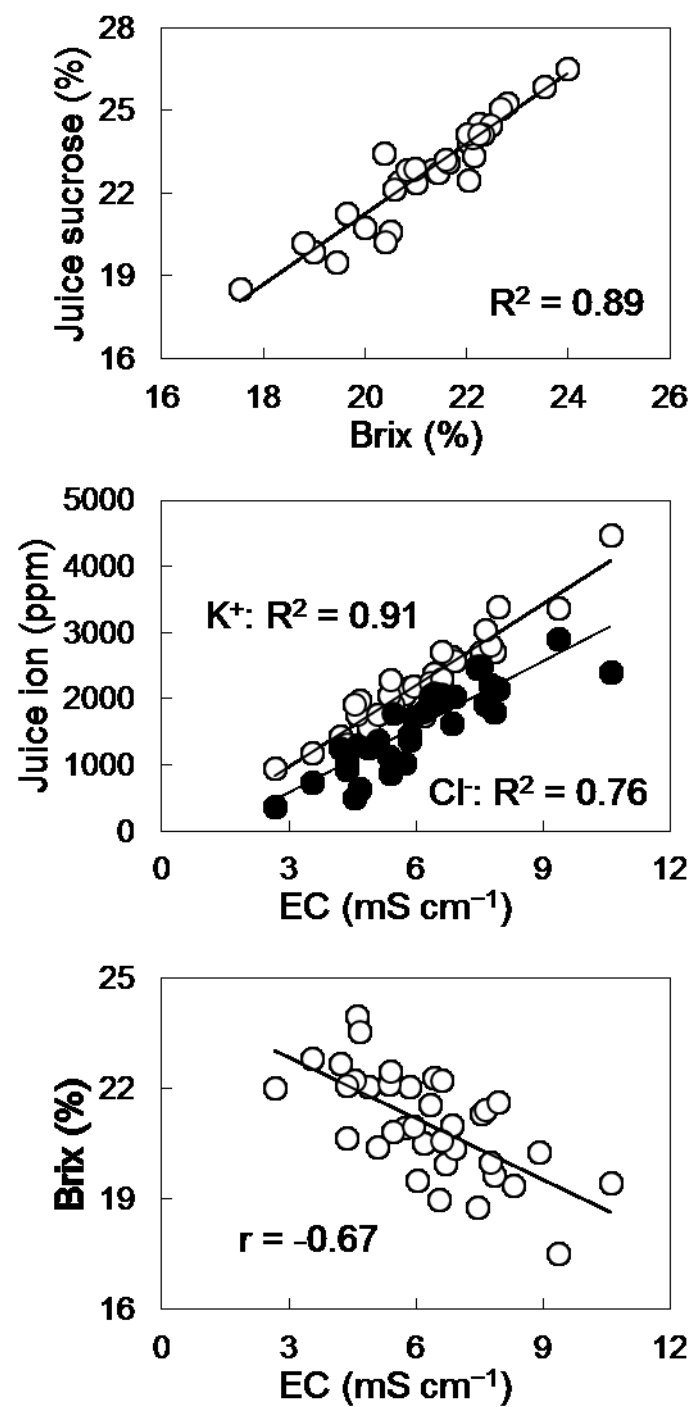
## Materials and methods

The survey was conducted in a sugarcane nursery managed by Daito Sugar Mfg. Co., Ltd. (25°83'N, 131°23'E; 14 m a.s.l.) on March 16 and 17, 2016. Thirty seven cultivars were used for the survey: Ni1, NiN2, NiF3, NiF4, NiF5, Ni6, NiN7, NiF8, Ni9, NiTn10, Ni11, Ni12, Ni13, Ni14, Ni15, Ni16, Ni17, NiTn18, NiTn19, NiTn20, Ni21, Ni22, Ni23, NiN24, NiH25, Ni26, Ni27, Ni28, Ni29, NiN30, Ni31, RK97-14, F161, RK96-6054, NCo310, NCo376, and Badila. Three fully matured stalks for each cultivar were cut at the ground level. After removing the top parts, about 10 cm sections from each cane stalk were cut off and separated into small pieces to squeeze with a garlic press. Obtained juice was immediately used for EC (B-771, Horiba) and Brix (Pal-S, Atago) measurements. Afterward, 1 mL of each juice sample was taken and mixed within a cultivar: 3 mL for each cultivar was prepared. These combined juice samples were frozen until used for further analyses. Unfortunately, four of these samples were lost by careless management: data of NiN2, NiF3, NiF4, and NCo310 were missing. Ion and sugar analyses for juice samples were performed by ion chromatography and HPLC methods, respectively, following the previous chapters.

## Results

Juice EC had a strong relationship with both  $K^+$  and  $Cl^-$  with regression coefficients of 0.91 and 0.76 respectively as confirmed in Chapter 2.1 (Fig. 6.1-1). Similarly, Brix and sucrose concentration in sugarcane juice were closely associated with regression coefficient of 0.89. These indicate it is possible to estimate sucrose,  $K^+$ , and  $Cl^-$  concentrations by easy and rapid measurements. The calculated means of juice EC and Brix for each cultivar were also plotted in Fig. 6.1-1. A negative relationship was confirmed between juice EC and Brix with the correlation coefficient of  $-0.67$  so that cultivars with higher EC had lower Brix.

Juice EC values varied greatly among cultivars ranging from 2.67 to 10.6  $mS\ cm^{-1}$  and the mean of all the cultivars was 6.24  $mS\ cm^{-1}$  (Fig. 6.1-2). The lowest and the highest



**Fig. 6.1-1.** Relationships between Brix and sucrose concentration; between EC and ion concentrations; and between EC and Brix



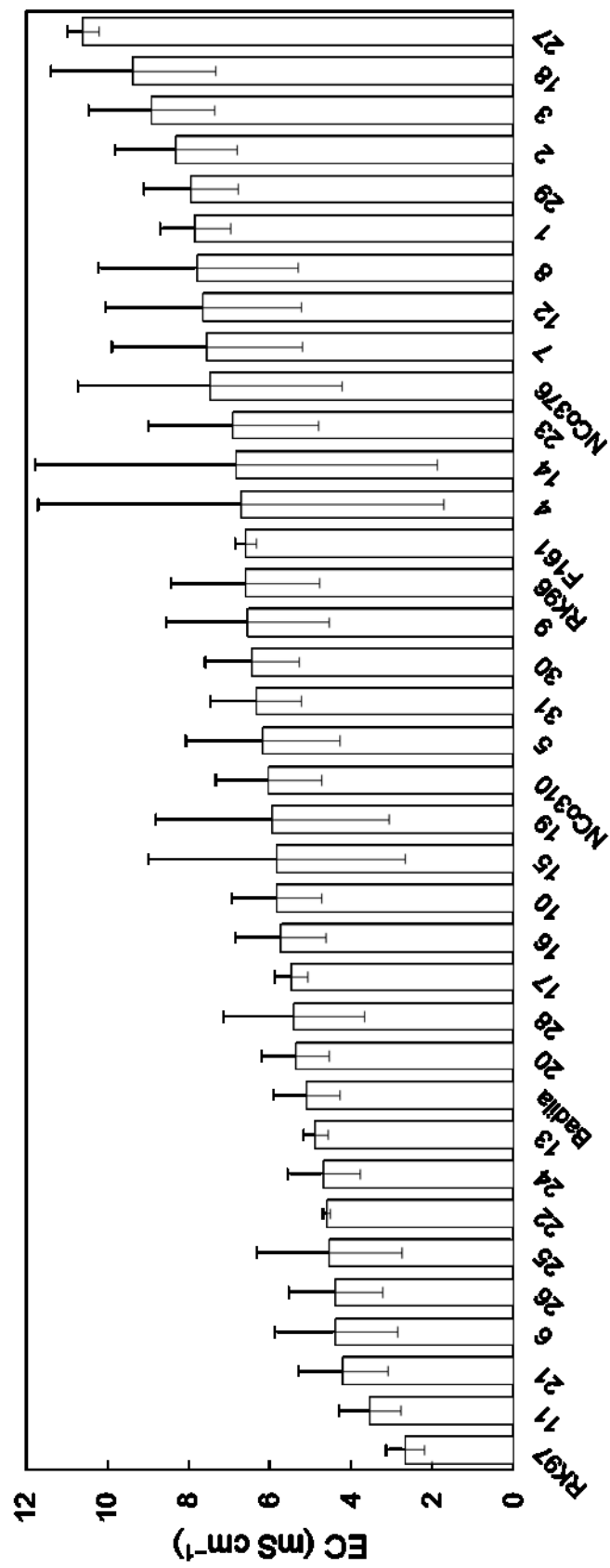


Fig. 6.1-2. Differences in juice EC among cultivars. Vertical bars indicate SD.

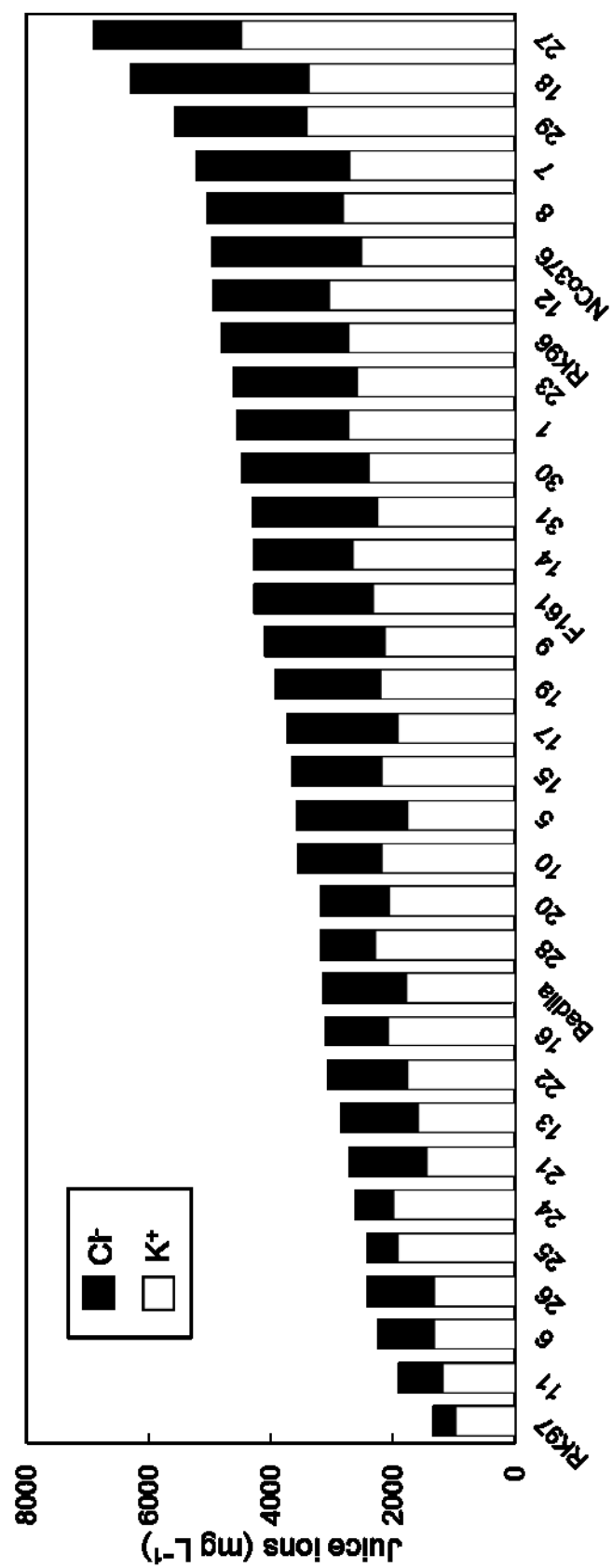


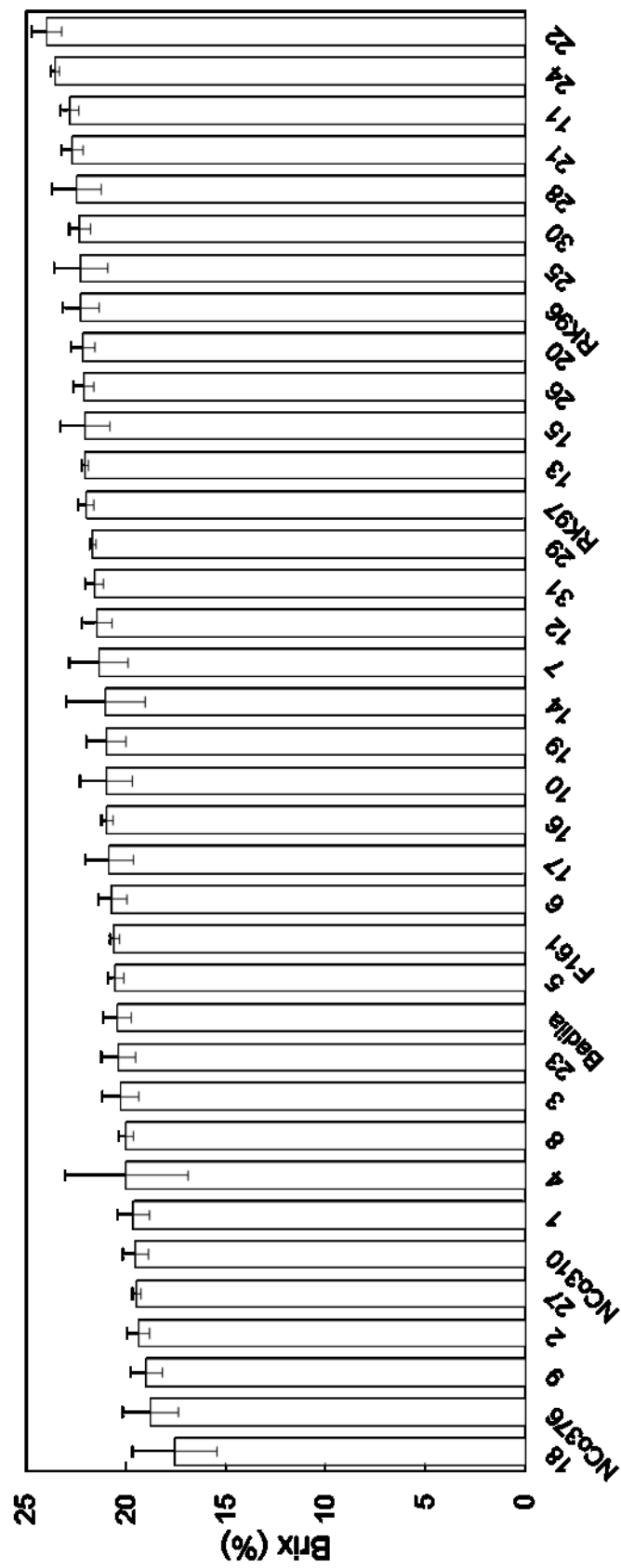
Fig. 6.1-3. Differences in juice ions concentrations among cultivars.

were respectively RK97-14 and NI27. The SDs of some cultivars were large due to the relatively small number of replications. Fig. 6.1-3 demonstrates the sum of juice  $K^+$  and  $Cl^-$  concentrations in ascending order. The order was not necessarily the same as that of EC and the balance of  $K^+$  and  $Cl^-$  also varied a lot among cultivars, e.g., NiH25 and Ni26 had the almost same ion concentrations, but  $K^+:Cl^-$  ratio was much higher in NiH25. Both of the minimum  $K^+$  and  $Cl^-$  were seen in RK97-14, while the cultivar with the maximum  $K^+$  was Ni27 and the maximum  $Cl^-$  was NiTn18.

Brix values ranged from 17.53 to 23.97% showing smaller differences than EC values (Fig. 6.1-4). The mean was 21.06% and NiTn18 was the lowest and Ni22 the highest.

### **Discussion**

The results of this study suggest juice EC and Brix precisely represented  $K^+$  and  $Cl^-$  ions and sucrose concentrations so that they are easily estimated by using EC and Brix meters, respectively. This method is quite useful because it is also applicable for standing canes like this study. As expected, ion and sugar compositions were different depending on cultivars. Juice EC of RK97-14 was the lowest; thus, this cultivar may absorb less  $K^+$  and  $Cl^-$  than others do. Meanwhile, Ni27 had the highest EC and especially its juice  $K^+$  concentration was considerably high, indicating its great ability for ion accumulation. Brix or sucrose concentration in juice was the lowest in NiTn18, whereas Ni22 was the highest. NiTn18 had the second highest EC value and its  $Cl^-$  concentration was higher than any other cultivar. These were likely to be characteristic cultivars in terms of ion and sugar accumulating ability; however NiTn18 is thought to have an undesirable characteristic, given that its Brix and sucrose concentration were quite low. Therefore, in addition to the standard cultivars, the three interesting cultivars, Ni22, Ni27, and RK97-14 were selected for the pot experiment described in the next chapter.



**Fig. 6.1-4. Differences in juice Brix among cultivars. Vertical bars indicate SD.**

## **6.2. Pot experiment by using different cultivars under high KCl condition**

### **Introduction**

In the previous pot experiments in Chapter 3, only one cultivar, NiF8, was tested and, when it was subjected to high KCl treatment,  $K^+$  and  $Cl^-$  contents of that cultivar increased, while sucrose content was significantly reduced. However, it was revealed from Chapter 6.1 that EC and Brix values in juice were greatly different by cultivars, ranging from 2.67 to 10.6  $mS\ cm^{-1}$  in EC and from 17.53 to 23.97% in Brix. It was particularly notable that EC values showed a greater difference and the largest value was four times higher than the least one. The large difference is likely to be even greater when they are subjected to high KCl treatment since sugarcane quickly responds to KCl and absorbs great amounts of  $K^+$  and  $Cl^-$  as previously revealed by using NiF8, and the change will probably cause reductions of juice sucrose content with different extents depending on cultivars. Based on this hypothesis, two types of sugarcane cultivars which have better sugar content under high KCl environments are thought to exist: cultivars with lower  $K^+$  and  $Cl^-$  or ones with lower extent of sucrose reduction. In this chapter, a pot experiment was conducted, using several sugarcane cultivars with different nutrient and sugar accumulating abilities under both normal and high KCl-treated environments to see if the responses to high KCl are different from each other. and which cultivars show better sucrose content in the environments. Chapter 6.1 suggests the cultivars Ni22, Ni27 and RK97-14 were especially characteristic in terms of EC and Brix values. Besides these three cultivars, NiF8, the most popular cultivar in Japan, and NCo310, renowned as one of the major varieties in the world (Nuss and Brett, 1995), were also selected for the test.

### **Materials and methods**

A pot experiment was conducted under a greenhouse condition at the University of the Ryukyus, Okinawa, Japan from March, 2016 to January, 2017. Seedlings of *S. spp.* cv. NiF8, Ni22, Ni27,

RK97-14, and NCo310 were obtained from the nursery of Daito Sugar Mfg. Co., Ltd. The latter procedure was performed following Chapter 3.1.

Fertilization was performed once a week after transplantation using a modified Hoagland solution as described in Chapter 3.2. The treatments were started eight weeks after transplantation. While the *Control* plants were fertilized with only the Hoagland solution, the *KCl* treatment was established by dissolving 50 mM KCl into the Hoagland solution. Except the *KCl* treatment of NiF8 had six pots, four pots for each of the treatments and the cultivars were prepared; however, due to some reasons, one of *Control* of Ni22, two of *KCl* of Ni22, and two of *Control* of NCo310 were died.

Stem height and the number of green leaves were measured monthly following Chapter 3.2. Plants were harvested on January 7, 2017. After the weight of millable stalks was recorded, stalk samples were pressed to obtained juice.

Juice samples were diluted 50 fold with extra-pure water and passed through a membrane filter (diameter, 13 mm; pore size, 0.45  $\mu$ m; Advantec) for ion and sugar analyses. The concentrations of  $K^+$  and  $Cl^-$  were determined by ion chromatography, following Chapter 2.1. Sucrose concentration was determined by HPLC as described in Chapter 2.2.

Statistical analysis was performed using the software R (R Core Team, 2015). Data were subjected to a *t*-test between the treatments and one-way ANOVA between the cultivars. When significances were found, the Tukey-Kramer test was conducted. The significant differences were accepted based on a P value < 0.05.

## Results

Stem height increased through the experimental period irrespective of cultivars and treatments (Fig. 6.2-1). No clear difference in stem height between the treatments was observed till five months after transplanting, but afterward those of *KCl* was more than 10 cm lower than *Control* except NiF8. Eventually stem height reached around 240 to 310 cm and that of Ni22

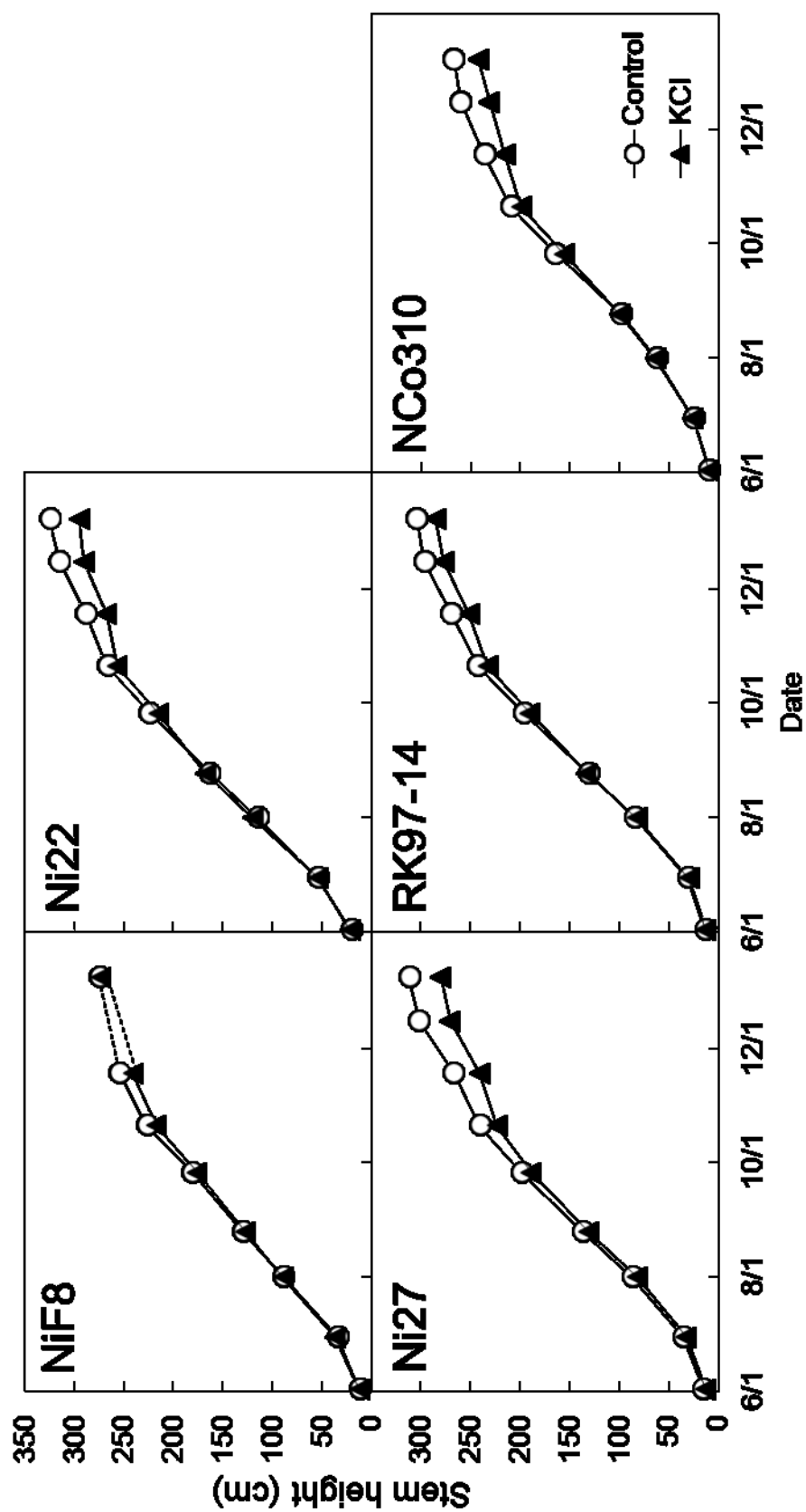


Fig. 6.2-1. Change of stem height. Stem height of NiF8 was not recorded due to flowering one month before harvesting as the broken lines indicate.

was the highest, while that of NCo310 was lowest. Initially the number of green leaves was about 5 and it tended to increase (Fig. 6.2-2). The final values were 10 to 20 depending on treatments and cultivars and Ni22 and NCo310 had relatively higher values. The leaf numbers showed similar changes between the treatments, but sometimes those of the *KCl* treatments showed lower values.

The *Control* treatments had comparably lower  $K^+$  and  $Cl^-$  concentrations below 2000 and 1000  $mg\ L^{-1}$ , respectively (Table 6.2-1). RK97-14 had the lowest  $K^+$  and  $Cl^-$  concentrations and its  $K^+$  concentration was significantly smaller than that of Ni22. Also, the  $Cl^-$  concentration of RK97-14 was significantly lower than those of the other cultivars except Ni27. When plants were subjected to 50 mM KCl treatment,  $K^+$  and  $Cl^-$  concentrations rose up dramatically, but the extents were different depending on cultivars; e.g., NiF8 showed the greatest changes resulting in 4.76 and 6.80 times higher  $K^+$  and  $Cl^-$  concentrations as compared to *Control*, while  $K^+$  and  $Cl^-$  of NCo310 were increased with 3.12 and 4.39 folds, respectively. The maximum  $K^+$  and  $Cl^-$  concentrations were obtained in NiF8 and the minimum in RK97-14. The  $K^+/Cl^-$  ratio ranged from 1.89 to 2.84 under the *Control* condition. That of RK97-14 seemed to be considerably high because of the low  $Cl^-$  concentration of the *Control* treatment. Under high KCl, all the cultivars showed lower  $K^+/Cl^-$  ratios due to the greater increment of  $Cl^-$  by the KCl application. NiF8 had the highest values under both of the *Control* and *KCl* conditions.

Figure 6.2-3 shows the changes in sucrose content by the 50 mM KCl treatment. In *Control*, plants were thought to be matured well since sucrose contents were approximately 20% or more with small variations in each cultivar. RK97-14 was the highest, followed by NiF8 and Ni22. Ni27 and NCo310 had lower values when compared to the other three cultivars. The *KCl* treatment caused a significant reduction of sucrose concentration and, except RK97-14, sucrose content went below 20%. Again, the extents varied depending on cultivars and that of NiF8 was the greatest among the cultivars, resulting in the lowest sucrose



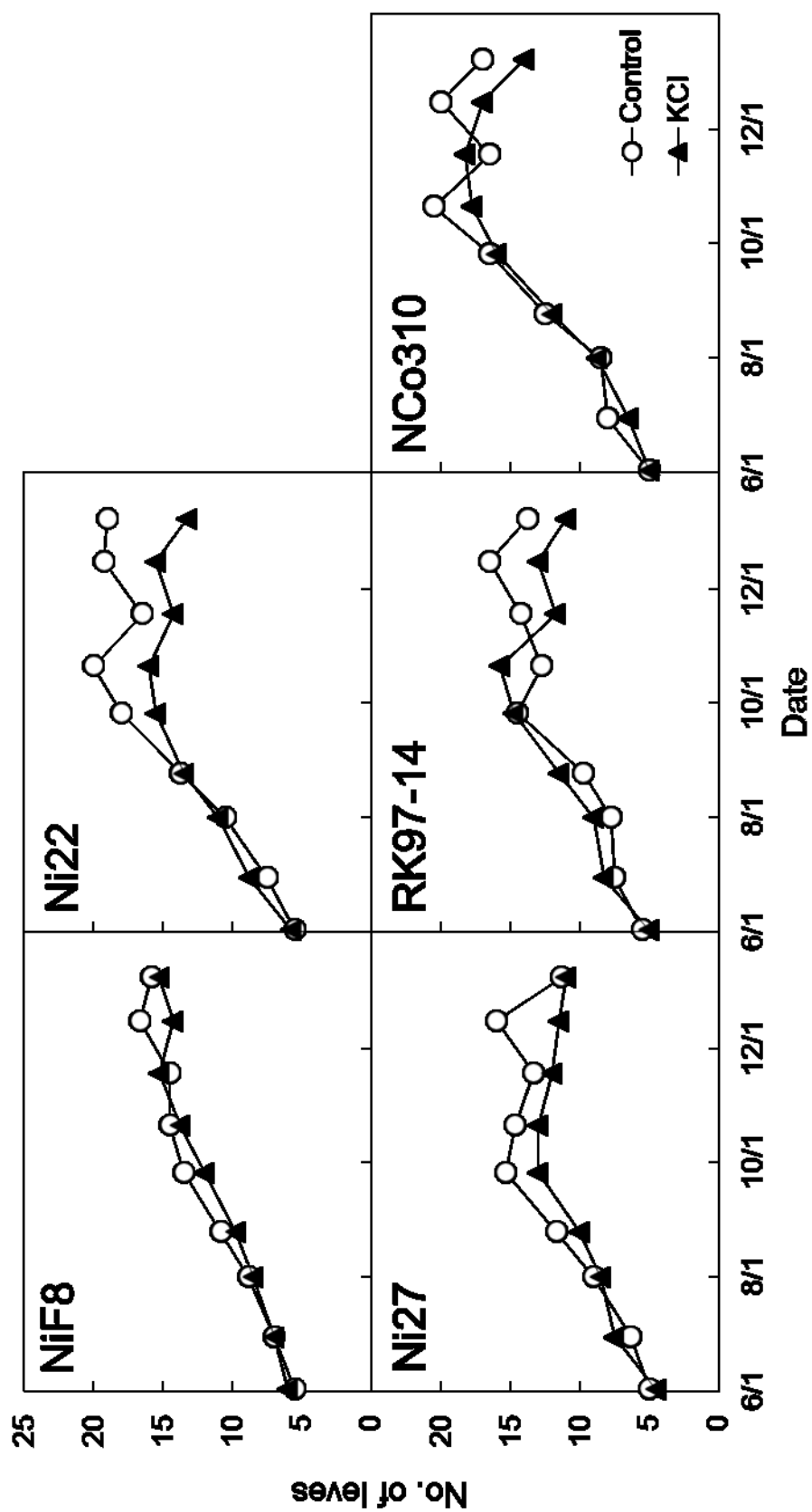
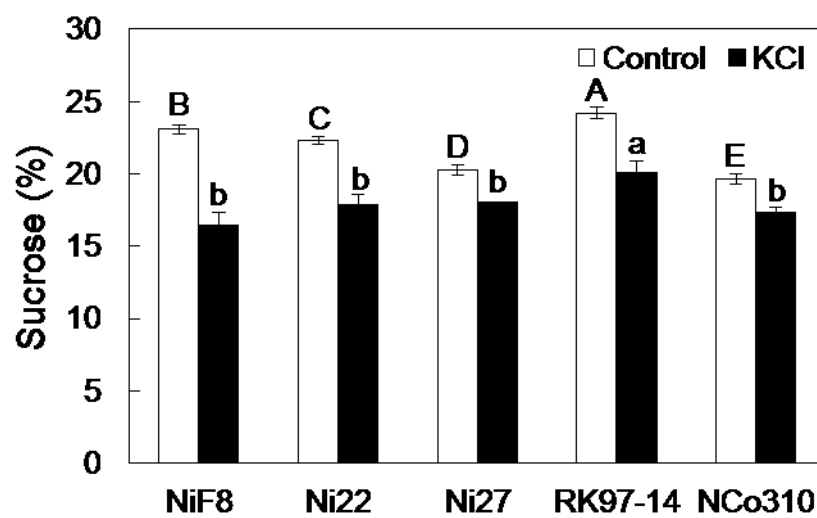


Fig. 6.2-2. Change of the number of green leaves.

**Table. 6.2-1.** Effects of the treatment on juice  $K^+$  and  $Cl^-$  concentrations and the  $K^+:Cl^-$  ratio.

		$K^+$	$Cl^-$	$K^+:Cl^-$
		mg L <sup>-1</sup>		
NiF8	Control	1818 AB	964 A	1.89 C*
	KCl	8662 a*	6550 a*	1.33 b
	KCl/Control (%)	476	680	70
Ni22	Control	1919 A	893 AB	2.15 BC*
	KCl	6199 b*	4556 b*	1.36 b
	KCl/Control (%)	323	510	63
Ni27	Control	1617 AB	714 BC	2.27 B*
	KCl	6456 b*	4603 b*	1.41 ab
	KCl/Control (%)	399	645	67
RK97-14	Control	1392 B	491 C	2.84 A*
	KCl	4957 c*	3214 c*	1.54 a
	KCl/Control (%)	356	654	54
NCo310	Control	1843 AB	914 AB	2.01 BC*
	KCl	5750 bc*	4011 bc*	1.43 ab
	KCl/Control (%)	312	439	80

Means followed by different uppercase letters and lowercase letters mean significant differences at the 5% level among cultivars in *Control* and *KCl*, respectively, by Tukey-Kramer test. \* means a significantly higher value at the 5% level between the *Control* and the *KCl* treatments within a cultivar by *t*-test.



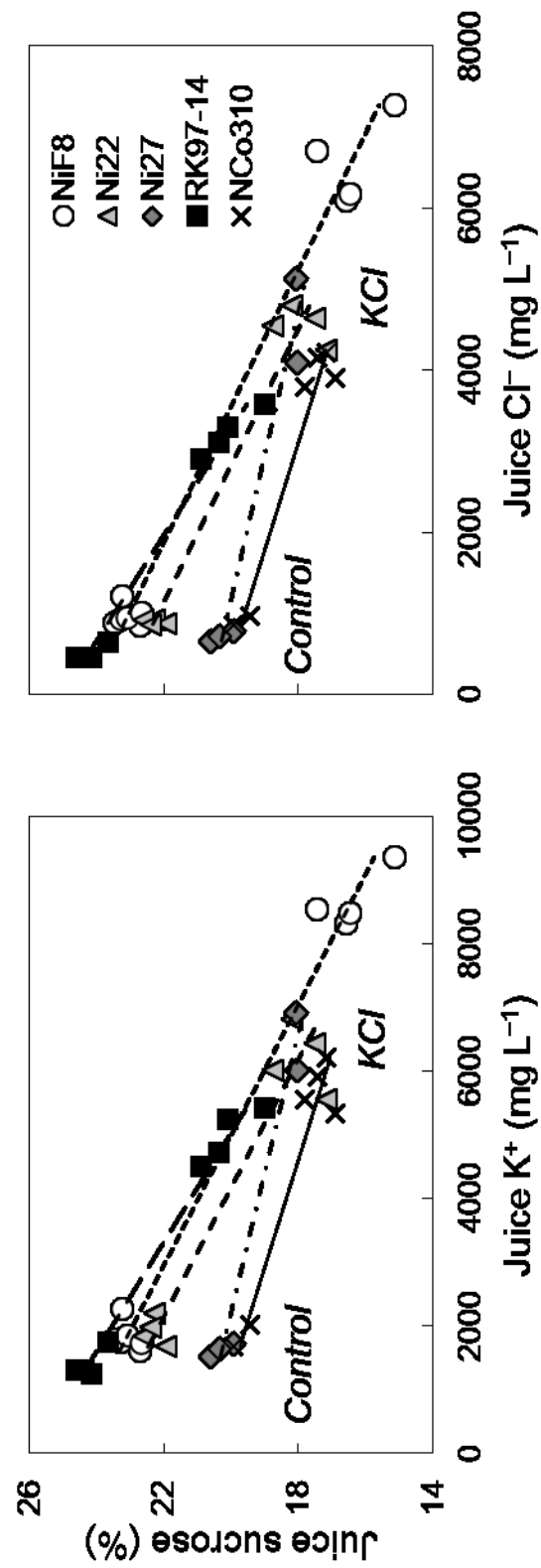
**Fig. 6.2-3.** Effects of the treatment on juice sucrose content. Means followed by different uppercase letters and lowercase letters mean significant differences at the 5% level among cultivars in *Control* and *KCl*, respectively, by Tukey-Kramer test.

content even though that in *Control* was the second highest as mentioned earlier. Between the *KCl* treatments, sucrose of RK97-14 was significantly higher than those of the other varieties. In other words, RK97-14 had the highest values both under the *Control* and *KCl* conditions.

The relationships of  $K^+$  and  $Cl^-$  concentrations with sucrose content were shown in Fig. 6.2-4. While the *Control* plants had less than 3000 and 2000  $mg\ L^{-1}$  of  $K^+$  and  $Cl^-$  concentrations, those of the *KCl* plants ranged widely depending on cultivar from 4000 to 10000  $mg\ L^{-1}$  and 2500 to 8000  $mg\ L^{-1}$ , respectively. There were decreasing trends in sucrose content as juice  $K^+$  and  $Cl^-$  concentrations increased. In *KCl*, as already mentioned in Table 6.2-1, RK97-14 had the lowest  $K^+$  and  $Cl^-$  and the highest sucrose concentrations. On the other hand, the slope of the regression line was the least steep in Ni27, which accounted for the lower reduction of sucrose.

Stalk weight and sugar yield as well as sucrose content were reduced by the *KCl* treatment in any cultivar (Table 6.2-2). The reduction of stalk weight ranged from 5 to 15%. Only NiF8 and RK97-14 had no significant differences in stalk weight between *Control* and *KCl*. Sucrose content was significantly reduced in all the cultivars. As mentioned already, the greatest reduction was observed in NiF8, while Ni27 and NCo310 showed relatively smaller changes. Sugar yield was decreased by 24 to 32%, but which stalk weight or sucrose content more accounted for the reduction of sugar yield depended on the cultivars. For example, in NiF8, the sucrose content more strongly affected sugar yield, but the reduction of stalk weight more contributed to sugar yield loss in NCo310. The reduction was minimum in Ni27 and RK97-14 with 24% loss. Particularly, RK97-14 had the maximum stalk weight and sucrose content and thus sugar yield both in the *Control* and *KCl* treatments.

Fig. 6.2-5 demonstrates the relationships of juice  $K^+$ ,  $Cl^-$ , and  $K^+/Cl^-$  ratio when subjected to 50 mM *KCl* treatment with sugar yield loss calculated by subtracting *KCl/Control* percentage of sugar yield from 100. Sugar yield loss tended to be higher as juice  $K^+$  and  $Cl^-$  concentrations increased or juice  $K^+/Cl^-$  ratio decreased. The  $K^+:Cl^-$  ratio most

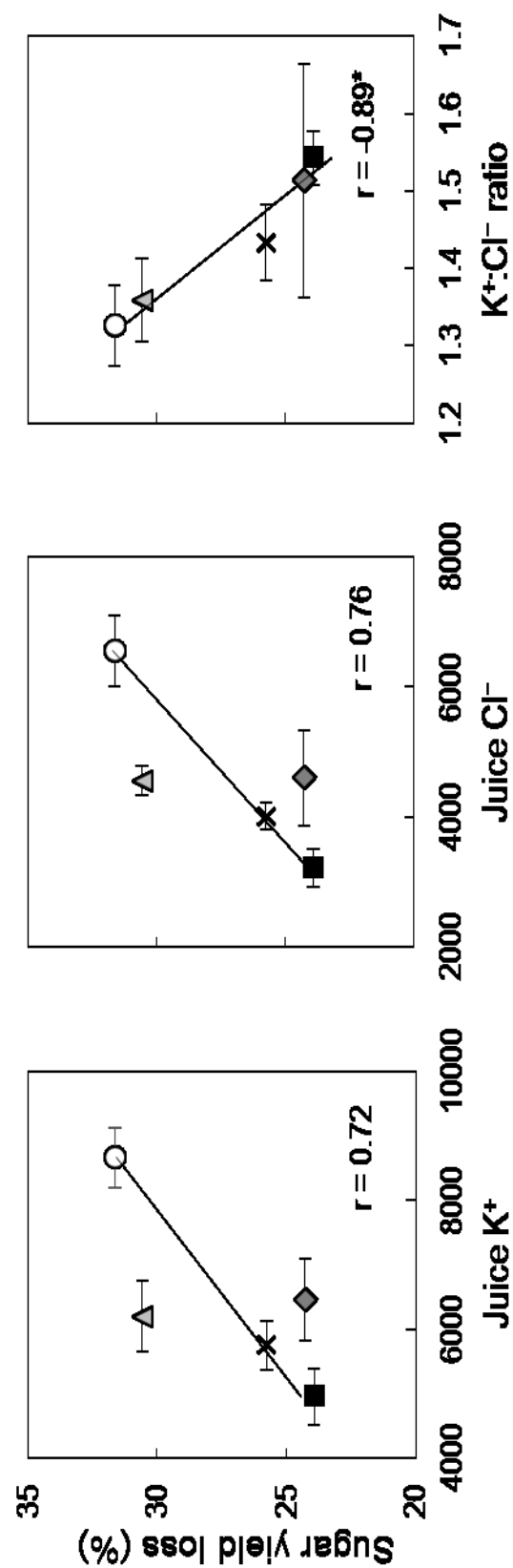


**Fig. 6.2-4.** Relationships of juice K<sup>+</sup> and Cl<sup>-</sup> concentrations **with** sucrose content. As shown in the graphs, data of *Control* and *KCl* are plotted on the **left** and the right sides, respectively.

**Table. 6.2-2.** Effects of the treatment on stalk weight, juice sucrose content, and sugar yield.

		Stalk weight	Sucrose	Sugar yield
		g	%	g
NiF8	Control	1049 B	23.1 B*	146 B*
	KCl	999 bc	16.4 b	100 c
	KCl/Control (%)	95	71	68
Ni22	Control	1074 B*	22.3 C*	137 B*
	KCl	922 c	17.9 b	95 c
	KCl/Control (%)	86	80	69
Ni27	Control	1275 A	20.3 D*	162 B*
	KCl	1106 ab	18.0 b	123 b
	KCl/Control (%)	87	89	76
RK97-14	Control	1334 A	24.2 A*	201 A*
	KCl	1234 a	20.1 a	153 a
	KCl/Control (%)	93	83	76
NCo310	Control	1098 B*	19.7 E*	135 B*
	KCl	932 bc	17.3 b	100 bc
	KCl/Control (%)	85	88	74

Means followed by different uppercase letters and lowercase letters mean significant differences at the 5% level among cultivars in *Control* and *KCl*, respectively, by Tukey-Kramer test. \* means a significantly higher value at the 5% level between the *Control* and the *KCl* treatments within a cultivar by *t*-test.



**Fig. 6.2-5.** Relationships of juice K<sup>+</sup> and Cl<sup>-</sup> concentrations and the K<sup>+</sup>:Cl<sup>-</sup> ratio in the KCl treatments with sugar yield loss.  
 \* means **significance** at the 5% level.

precisely explains the relation with the correlation coefficients of  $-0.89$ .

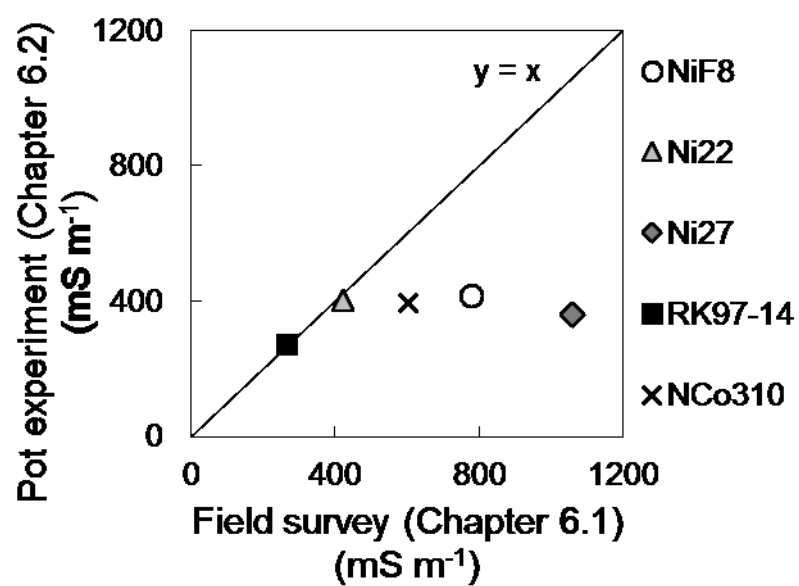
### Discussion

In the present study, weekly applications of 50 mM KCl significantly enhanced accumulations of  $K^+$  and  $Cl^-$  in juice with different extents by cultivars. The greatest accumulation was observed in NiF8, resulting in 4.76 and 6.80 times higher  $K^+$  and  $Cl^-$  than the *Control* plants. It was especially noteworthy that the significant sucrose reduction by the treatment was seen in all the cultivars: as  $K^+$  and  $Cl^-$  were increased, 11 to 29% of reductions compared to *Control* were obtained. Therefore, the hypothesis that responses in nutrient and sugar balance to high KCl differed by cultivars was successfully proved.

The KCl treatment significantly affected yield parameters of some cultivars as well as quality, which had not been observed in the previous experiments in Chapter 3 where only NiF8 was used. In this study, NiF8 showed small reductions of growth relating parameters such as stem height, leaf number, and stalk weight, without any significance but a great reduction of sucrose content. While, in other cultivars, e.g. NCo310, stalk weight was significantly reduced, but sucrose content was less affected. These changes of stalk weight and sucrose content eventually influenced sugar yield, making from 24 to 31% of the loss as compared to *Control*. This indicates cultivars with less sugar yield loss can be considered to be tolerant to high KCl environments. In this study, Ni27 and RK97-14 showed the least reduction in sugar yield and thus it is concluded that these cultivars are recommended to be grown when the field is thought to have been accumulating considerable amounts of  $K^+$  and  $Cl^-$  ions.

Although cultivar differences in juice EC were confirmed, the range from 2.8 to 4.2  $mS\ m^{-1}$  was not as wide as the one obtained in the field survey of Chapter 6.1 and a big difference between the field survey and this pot experiment was observed (Fig. 6.2-6). For example, Ni27 which had the highest EC in the previous chapter showed a relatively smaller





**Fig. 6.2-6.** Effects of the treatment on stalk weight, juice sucrose content, and sugar yield.

EC in the present chapter. The reason for this is not clear, but it may have come from uneven ion distributions in the field because the area of the sampling site was not large enough. It is therefore difficult to evaluate each ion accumulating ability under field conditions, indicating the importance of pot experiments where soil nutrition is uniform and nutrients derived from fertilizers are directly absorbed through the root system. Considering that the juice  $K^+/Cl^-$  ratio in the *KCl* treatment was highly correlated with sugar yield loss, it can be a reliable indicator to predict the reduction of sugar yield when plants are exposed to high KCl. Hattori et al. (2016) has reported that juice EC is determined by cultivars and the values are relatively stable, suggesting that, when evaluating the tolerance for each cultivar against high KCl environments, young sugarcane plants could be used growing the cultivar in a pot culture and applying 50 mM KCl, so that it is unnecessary to keep plants until harvest time.

## Chapter 7

### General discussion

From this study, these points below were revealed.

- $K^+$  and  $Cl^-$  are the most abundant cation and anion present in sugarcane juice (Chapter 2-1)
- $K^+$  and  $Cl^-$  have negative relationships with sugar content (Chapter 2-1, 2, Chapter 4-1)
- Increasing levels of KCl increase both juice  $K^+$  and  $Cl^-$  concentrations and reduce sucrose content under pot (Chapter 3-1, 2) and field conditions (Chapter 4-2)
- $Cl^-$  is the primary factor accounting for sucrose reduction and  $K^+$  is indirectly associated with the reduction by promoting  $Cl^-$  accumulation into stems (Chapter 3-2)
- Sucrose reduction is caused probably not by the inhibition of photosynthesis in leaves but by the change of photosynthate distribution in stems (Chapter 3-2)
- Irrigation water can be the sources of  $K^+$  and  $Cl^-$  (Chapter 5-2)
- Sucrose reduction by high KCl application is caused irrespective of cultivars (Chapter 6-2)
- Increase of juice  $K^+$  and  $Cl^-$  concentrations and decrease of sucrose content by high KCl application differ between cultivars (Chapter 6-2)

Fertilizer management, irrigation water management, and cultivar selection can be solutions against sucrose reduction caused by overdose of KCl.

#### *Fertilizer management*

Roughly speaking, we should reduce  $Cl^-$  application from fertilization. There are two possible ways: reducing KCl amount and substituting K fertilizer not including  $Cl^-$  such as  $K_2SO_4$ . As shown in the results of Chapter 3, using  $K_2SO_4$  can be a solution way because it had no negative effect on sugarcane quality under pot conditions. On the other hand, a field experiment (Chapter

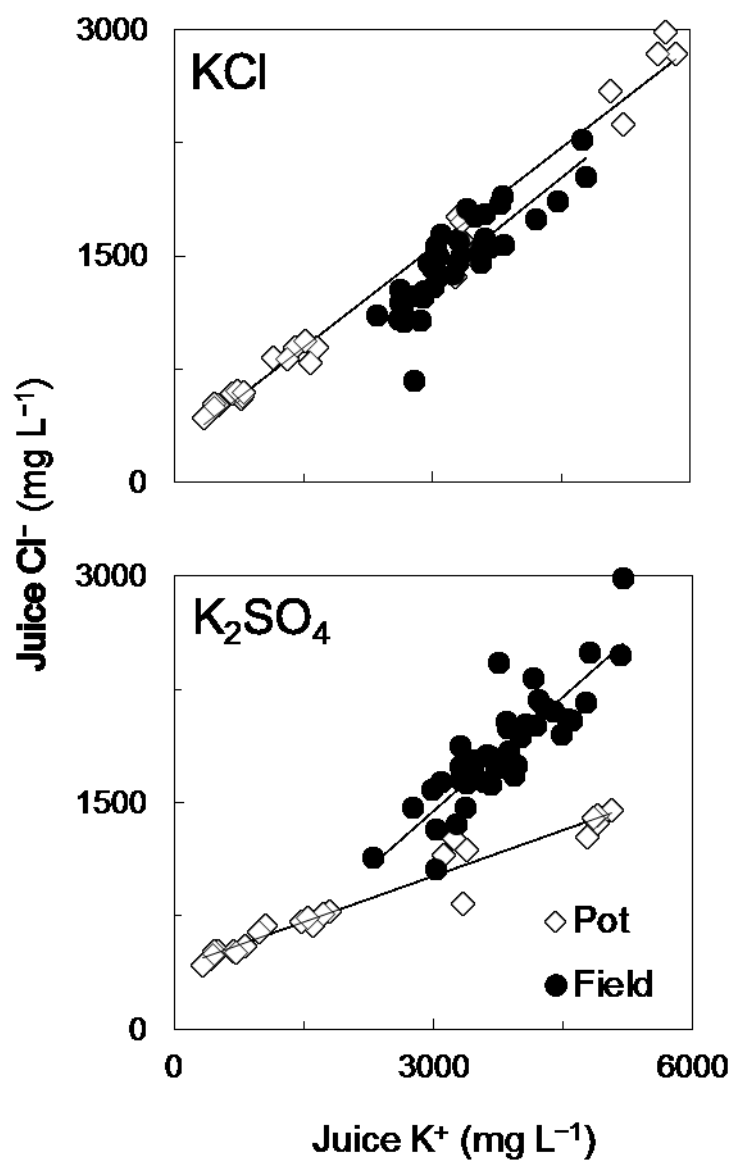
4-1) revealed that sucrose content was decreased with an increase of juice  $K^+$  also in  $K_2SO_4$ -treated plants because, similarly with the KCl-treated plants, juice  $Cl^-$  concentration linearly increased as juice  $K^+$  increased (Fig. 7-1), which was not observed in the pot experiments where the rhizosphere was certainly limited and thus no nutrients other than the applied fertilizers affected sugarcane juice ion composition. In other words,  $Cl^-$  is positively absorbed when it exists in environments in large quantities. Taking it into consideration, lowering KCl input would be a better way to prevent sugarcane from absorbing  $Cl^-$ .

#### *Irrigation water management*

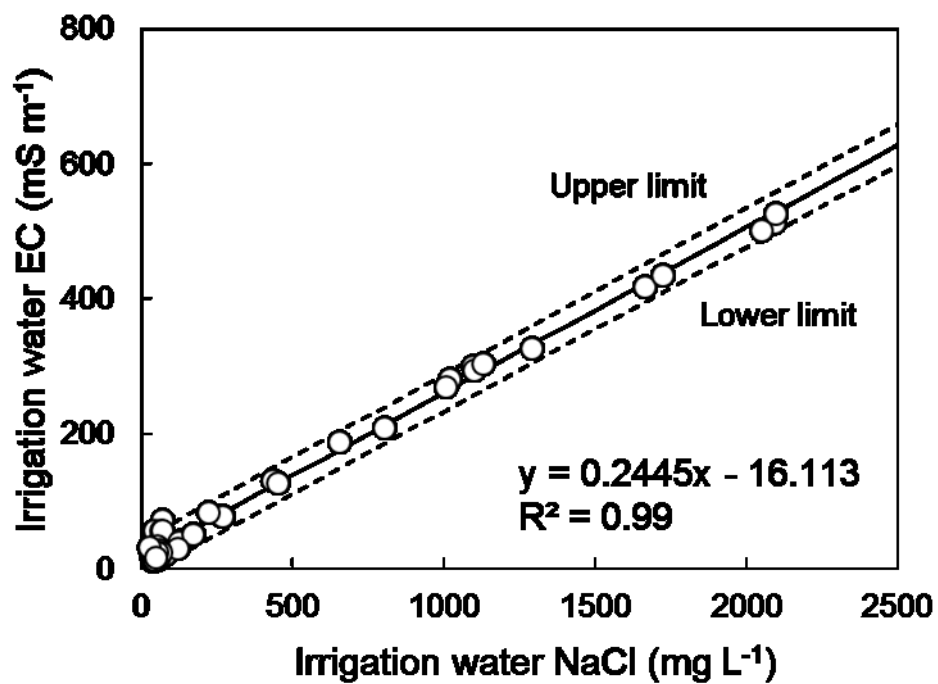
In irrigation water, EC strongly positively correlated with NaCl concentration (Fig. 7-2). Based on regression analysis, the calculated equation was  $y = 0.2445x - 16.113$ . Thus, it is possible to estimate NaCl concentration by EC measurement easily and rapidly. In terms of growth and quality, there was no adverse effect when NaCl concentration in irrigation water was less than  $1000\text{ mg L}^{-1}$  and more than  $2000\text{ mg L}^{-1}$  of NaCl could be harmful for sugarcane. Taking significant level at the 95%, water is available when the EC value is less than  $230\text{ mS m}^{-1}$ ; consideration is required when the EC value is 230 to  $540\text{ mS m}^{-1}$ ; and irrigation should be refrained when the EC value exceeds  $540\text{ mS m}^{-1}$ .

#### *Cultivar selection*

The results of Chapter 6-2 suggest that Ni27 and RK97-14 were thought to be good cultivars suitable for high KCl environments, considering their less loss of sugar yield when plants were exposed to  $50\text{ mM KCl}$  as compared to others. NiF8 had the largest sugar yield loss due to the great reduction of sucrose content, whereas that of Ni22 was affected by both the losses of cane yield and sucrose content. These cultivars should not be used when the amounts of  $K^+$  and  $Cl^-$  are found to be high. Used cultivars were diversified depending on sugarcane producing areas, yet only five cultivars were used in this study. Hence, it is necessary to grasp



**Fig. 7-1.** Relationship between K<sup>+</sup> and Cl<sup>-</sup> concentrations in juice under pot (Chapter 3-1) and field (Chapter 4-1) conditions.

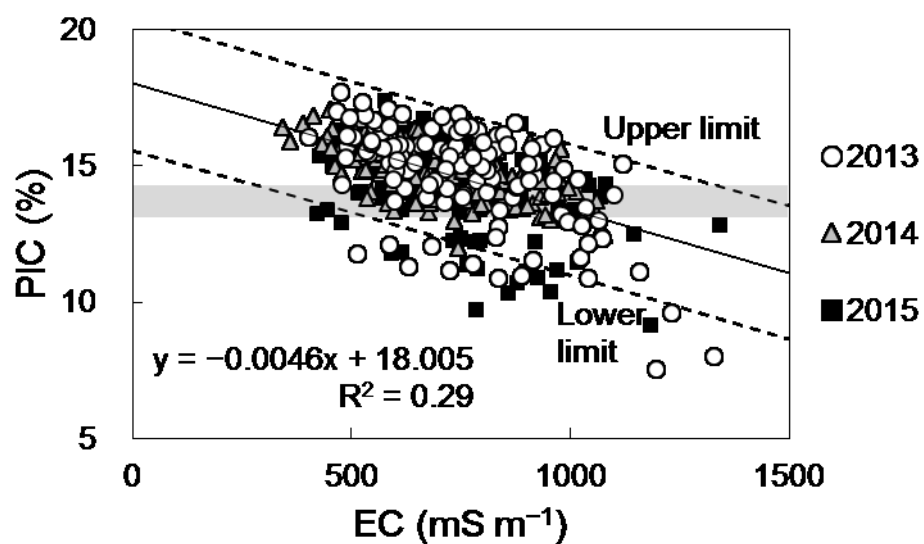


**Fig. 7-2.** Relationship between NaCl concentration and EC in irrigation water. The linear regression line, regarding NaCl and EC as an independent variable and a dependent variable is described by a solid line and the area within the two broken lines indicates the 95% prediction interval.

characteristics of cultivars mainly used in each area, which will enable us to have suggestions of ones suitable for the high KCl environments.

#### *Practical application of EC measurement*

EC of sugarcane juice was highly dependent on both juice  $K^+$  and  $Cl^-$  concentrations and thus represents juice  $K^+$  and  $Cl^-$  well, so that it is useful to estimate juice  $K^+$  and  $Cl^-$  concentrations as a reliable indicator. Because the price of EC meter is low as well as the measurement is easy and rapid, this method was used at a sugar mill in Australia to estimate juice ash composed of inorganic ions like  $K^+$  and  $Cl^-$  (Kingston, 1982b). Hence, it would be beneficial to measure EC of a juice sample in order to evaluate  $K^+$  and  $Cl^-$  concentrations and use the data for fertilizer management. From the results of Chapter 2.1, juice EC as well as  $K^+$  and  $Cl^-$  had a highly negative correlation with PIC (Fig. 7-3). The linear regression equation, regarding EC and PIC as an independent variable and a dependent variable was calculated. The coefficients of determination was relatively low ( $R^2 = 0.28$ ), but it is possible to roughly predict PIC from EC. The equation is expressed as  $y = -0.0046x - 18.005$ . In 2013, 2014, and 2015, respectively, 17.64%, 2.94%, and 12.5% of the total samples were below the standard PIC value used in the quality payment system (13.1–14.3%). The regression equation when inversely estimating EC from PIC, was  $y = -62.2499x + 1632.463$  and the prediction value  $\pm$  the prediction interval width resulted in  $817 \pm 283 \text{ mS m}^{-1}$ , namely 534–1100  $\text{mS m}^{-1}$  of the prediction interval. Therefore, growers should consider reducing KCl application in a year following one in which the EC value of sugarcane juice is found to be over 1100  $\text{mS m}^{-1}$ . Considering that only eight samples had a higher EC than 1100  $\text{mS m}^{-1}$  through the three years, the prediction value of 817  $\text{mS m}^{-1}$  could be a better barometer. Since the loaded canes are mixed before being squeezed, EC monitoring method by EC meter mounted on the mill line (Kingston, 1982b) is not applicable in Japan but would be very beneficial to feedback the results to farmers if the milling system has changed. Moreover, there is a possibility to apply portable EC meter to standing canes and



**Fig. 7-3.** Relationships between EC in sugarcane juice and PIC. The gray zone represents the standard PIC value for the quality payment system. The linear regression line, regarding EC and PIC as an independent variable and a dependent variable is described by a solid line and the area within the two broken lines indicates the 95% prediction interval.



use the data to determine the amount of additional K fertilizer. However, it is still unknown how much of KCl can be applied when it is necessary to reduce the amount. Further study to answer this question and examine the effectiveness of the new practice is needed.

### *Impacts in sugarcane industry*

In the application level, there are still not few problems to be solved, however, it is expected that the results of this study bring a lot of benefits. In terms of fertilizer management practice, Japan has little source of fertilizer and thus relies on oversea countries for fertilizer production. Recently, the fertilizer price increases significantly: in 2008, the price became almost double of that 5 years ago, 2003 (Ministry of Agriculture, Forestry and Fisheries, 2008). Kuba (2011) reported there is a boom to reduce fertilizer amount due to the increasing fertilizer price. To save fertilizer cost, therefore, lowering KCl amount is of great importance. Besides, the improved fertilizer management practices are also good for environments. People are now more interested in sustainable agriculture (Lockeretz, 1988) and the practice can certainly make sugarcane production more environmental friendly as it lowers unnecessary KCl input.

Currently, farmers select cultivars considering many factors such as growth environments and conditions, working efficiency, pest and disease tolerances, yield and quality (Agriculture and Livestock Industries Cooperation, 2017), which is strongly influenced by producing areas. For example, according to the cultivation manual for sugarcane in Okinawa (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries, 2015), some areas are highly dependent on only a few specific cultivars, while the cultivar composition in the southern and central part of Okinawa Island is relatively diversified. By introducing cultivar selection based on their ion accumulating abilities, old cultivars which have not been in use for a long time will be reevaluated or breeding objectives will change to a new way. Those change may eventually result in the

development of cultivar diversification, which is thought to be of importance in terms of the risk reduction (Faraji, 2011).

As mentioned in Chapter 1, the sugarcane price is also influenced by PIC in the quality payment system; therefore, by improving sugarcane quality through these practices, farmers can make more profit from the same unit of sugarcane harvested area. Because of not only higher sucrose concentration but also lower  $K^+$  and  $Cl^-$  concentrations, as they impair efficiency of sugar processing (Hogarth and Kingston, 1983), the practices positively affect sugar production as well in terms of both cultivation and sugar processing aspects. Taking its large production and harvested area into account, this study would have even bigger impacts in the world; therefore, it is worth conducting similar investigations in oversea sugarcane producing areas.

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chance to develop as a researcher.

## Summary

Sugarcane is a sugar accumulating crop grown in most of tropical and subtropical countries. In Japan, it is cultivated in Nansei Islands and the decreasing production and harvested area, low yield, and fluctuated quality are problems currently we are facing. The objective of this study is to improve sugarcane quality through fertilizer management and cultivar selection.

Nutrient diagnosis using leaf is a common practice to manage the mineral nutrition of plants. In sugarcane, however, it can be better to use juice which includes the final product, sucrose. To establish the method, first the key nutrients that affect sugarcane quality were identified.  $K^+$  and  $Cl^-$  were the most abundant cation and anion in juice and had negative relationships with sucrose content, thereby indicating these ions affect sugar production and accumulation in sugarcane. KCl including both  $K^+$  and  $Cl^-$  is the common potassium fertilizer generally used in sugarcane production. KCl application significantly reduced sucrose content under pot conditions, while the  $K_2SO_4$  treatment has no negative effect though it increased  $K^+$  concentration. In the plants given  $Cl^-$  fertilizer with no  $K^+$ ,  $Cl^-$  accumulation was inhibited as compared to the KCl treatment, and thus sucrose did not decrease. Taken together, it was suggested that the primary factor was not  $K^+$  but  $Cl^-$  and  $K^+$  was indirectly associated with this phenomenon by enhancing  $Cl^-$  accumulation.

Next, a field experiment changing types of potassium fertilizer (KCl and  $K_2SO_4$ ) was conducted. Under the field condition, sucrose was reduced as juice  $K^+$  increased in the  $K_2SO_4$  treatment. Juice  $Cl^-$  increased as juice  $K^+$  increased differently from the pot experiments, indicating  $Cl^-$  has sources other than fertilizer. The components in irrigation water also influence sugarcane nutrients. The results of analyzing irrigation water samples from several sugarcane producing areas showed a large regional difference in the components and the maximum NaCl concentration was over  $2000\text{ mg L}^{-1}$ ; thereby it can be the source of  $Cl^-$ .

To reveal the cultivar difference in juice  $K^+$  and  $Cl^-$  concentrations, domestic and

international 37 cultivars were subjected to ion analysis. The lowest and the highest concentrations were obtained in RK97-14 and Ni27. Ni22 had relatively low  $K^+$  and  $Cl^-$  but the highest sucrose. Growing NiF8 and NCo 310 under high KCl conditions resulted in sucrose reduction, but NiF8 was more strongly affected. RK97-14 had the highest yield and sucrose content in both of the control and KCl treatments; therefore, it is expected to introduce the cultivar in high KCl environments. Because juice EC highly correlated with both  $K^+$  and  $Cl^-$ , applying KCl in lower quantities or using cultivars tolerant to high KCl contribute to better sucrose content in a year following one in which EC at harvest is found to be high.

From these, this study certainly suggest a possibility to improve sugarcane quality through fertilizer management and cultivar selection.

## 要旨

サトウキビは熱帯、亜熱帯に属するほぼすべての国で栽培されている甘味資源作物である。日本国内では主に南西諸島で栽培されているが生産量および収穫面積の減少、低単収、品質の変動が著しい問題である。これらの問題解決のために、本研究は施肥管理および品種選択を通じた品質の向上を目的として行われた。

植物の栄養診断には葉身を用いるのが一般的であるが、サトウキビにおいては最終産物であるショ糖を含む搾汁液を用いる方法を検討した。その栄養診断法を確立するため、まず搾汁液中の糖度に影響を与える成分の特定を行った。その結果、 $K^+$ および  $Cl^-$ は搾汁液中に最も多く含まれ、かつ糖度と負の相関関係にあったことから、これらイオンはサトウキビの糖生産・糖蓄積に影響を与えていると考えられた。そこで、これらイオンによる糖度低下を検証するため、 $K^+$ と  $Cl^-$ の両方を含み一般のサトウキビ農家が使用しているカリ肥料 ( $KCl$ ) の施肥量を増加させたところ、対照区に比べ糖度の低下が見られた。ところが、 $Cl^-$ を含まない  $K_2SO_4$  施肥区では施肥量の増加により  $K^+$ が増加しても糖度の低下は確認されなかった。また、 $K^+$ を与えずに  $Cl^-$ を含む塩類を施用した場合、 $Cl^-$ の吸収は抑えられ、糖度の低下は確認されなかった。以上より、 $KCl$  の多量施肥に伴い糖度は低下するが、その主要因は  $K^+$ ではなく  $Cl^-$ であること、並びに  $K^+$ は  $Cl^-$ の吸収を促進することから、この現象に間接的に関与していることが示唆された。

次に、カリの施肥量は固定し種類 ( $KCl$ ,  $K_2SO_4$ ) を変えた圃場試験を実施した。圃場条件下では  $K_2SO_4$  施肥区でも  $K^+$ の増加に伴い糖度は低下した。ポット試験と異なり  $K_2SO_4$  施肥区でも  $K^+$ 含有率の増加に伴い  $Cl^-$ 含有率が増加したことから、 $Cl^-$ は肥料以外の供給源があることが示唆された。灌漑水の含有成分はサトウキビの搾汁液中成分に影響を与える要因の一つである。各サトウキビ生産地の灌漑水を分析したところ、成分には地域間差があり、塩濃度が  $2000\text{ mg L}^{-1}$  を超える貯水タンクもあったことが

ら、灌漑水が  $\text{Cl}^-$  の供給源である可能性が示唆された。

搾汁液中  $\text{K}^+$ 、 $\text{Cl}^-$  含有率の品種間差異を明らかにするため、国内外の 37 品種を調査したところ、RK97-14 で最小、Ni27 で最大となった。Ni22 は比較的  $\text{K}^+$ 、 $\text{Cl}^-$  含有率は低いものの糖度は高かった。標準品種である NiF8、NCo310 を高 KCl 施肥条件で栽培した場合、両品種とも糖度は低下したが、その程度は NiF8 で著しかった。一方、RK97-14 は両条件下で収量、糖度ともに最大であり、高 KCl 圃場への導入が有効であると期待される。搾汁液 EC は  $\text{K}^+$ 、 $\text{Cl}^-$  と高い正の相関を有することから、前年度の搾汁液 EC が高いと判断された場合、KCl の減肥や上述した品種の使用により糖度の低下程度を抑えることが可能であると考えられる。

以上より、本研究は施肥・灌漑管理、品種選択を通じてサトウキビの糖度向上を可能とすることを明らかにした。



## References

- Agriculture and Livestock Industries Cooperation. 2016a. Miyakojima ni okeru satoukibi no genjou to taisaku. [Situation of sugarcane and measures in Miyako Island.] [Online] Available at <https://www.alic.go.jp/content/000112489.pdf>
- Agriculture and Livestock Industries Cooperation. 2016b. Toukei siryou. [Statistical data.] [Online] Available at [http://sugar.alic.go.jp/japan/data/j\\_excel/j\\_1\\_05.xls](http://sugar.alic.go.jp/japan/data/j_excel/j_1_05.xls)
- Akhtar, M. and Akhtar, M. E. 2002. Effect of different levels of potassium on agronomic traits, productivity and quality of sugarcane (*Saccharum officinarum* L.). Asian J. Plant Sci. 1: 349-351.
- Akhtar, S., Wahid, A., Akram, M. and Rasul, E. 2001. Effect of NaCl salinity on yield parameters of some sugarcane genotypes. International Journal of Agriculture and Biology 3: 507-509.
- Almeida, H. J., Cruz, F. J. R., Pancelli, M. A., Flores, R. A., Vasconcelos, R. L. and Prad, R. M. 2015. Decreased potassium fertilization in sugarcane ratoons grown under straw in different soils. Aust. J. Crop Sci. 9: 596-604.
- Anderson, D. L., de Boer, H. G. and Portier, K. M. 1995. Identification of nutritional and environmental factors affecting sugarcane production in Barbados. Commun. Soil Sci. Plant Anal. 26: 2887-2901.
- Arasaki, M. 1997. Profile of Okinawa. Tokyo: Techno Marketing Center.
- Arnon, D. I. and Stout, P. R. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. 14: 371-375.
- Ashraf, M., Rahmatullah, Ahmad, R., Bhatti, A. S., Afzal, M., Sarwar, A., Maqsood, M. A. and Kanwal, S. 2010. Amelioration of salt stress in sugarcane (*Saccharum officinarum* L.) by supplying potassium and silicon in hydroponics. Pedosphere, 20: 153-162.
- Azama, T., Kawamitsu, Y., Fukuzawa, Y., Uehara, N., Taira, E. and Ueno, M. 2007. Effect of

- potassium on growth and sugar accumulation in sugarcane. Jpn. J. Crop Sci. 76: (Extra 1), 130–131.
- Benito, B., Haro, R., Amtmann, A., Cuin, T. A. and Dreyer, I. 2014. The twins K<sup>+</sup> and Na<sup>+</sup> in plants.
- Bloom, A. J. 2010. Chapter 5. Mineral nutrition. *In* Taiz, L. and Zeiger, E. eds., Plant Physiology, Fifth Edition. Sinauer Associates, Inc., Sunderland. 107–130.
- Bokhtiar, S. M. 2004. Interrelation of soil and plant factors with sugar accumulation and maturity of sugarcane and ratoon. Bangl. J. Bot. 33: 63–67.
- Cha-um, S., Chuencharoen, S., Mongkolsiriwatana, C., Ashraf, M. and Kirdmanee, C. 2012. Screening sugarcane (*Saccharum* sp.) genotypes for salt tolerance using multivariate cluster analysis. Plant Cell, Tissue and Organ Culture, 110: 23–33.
- Chen, W., He, Z. L., Yang, X. E., Mishra, S., and Stoffella, P. J. 2010. Chlorine nutrition of higher plants: Progress and perspectives. Journal of Plant Nutrition, 33: 943–952.
- Dasgan, H. Y., Aktas, H., Abak, K. and Cakmak, I. 2002. Determination screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. Plant Science, 163, 695–703.
- Du, Y. C., Kawamitsu, Y., Nose, A., Hiyane, S., Murayama, S., Wasano, K. and Uchida, Y. 1996. Effects of water stress on carbon exchange rate and activities of photosynthetic enzymes in leaves of sugarcane (*Saccharum* Sp.). Australian Journal of Plant Physiology 23: 719–726.
- Du, Y. C., Nose, A., Kondo, A. and Wasano, K. 1998. Responses to water stress of enzyme activities and metabolite levels in relation to sucrose and starch synthesis, the Calvin cycle and the C<sub>4</sub> pathway in sugarcane (*Saccharum* sp.) leaves. Australian Journal of Plant Physiology 25: 253–260.
- Du, Y. C., Nose, A. Kondo, A. and Wasano, K. 2000. Diurnal Changes in Photosynthesis in Sugarcane Leaves: II. Enzyme activities and metabolite levels relating to sucrose and

- starch metabolism. *Plant Production Science* 3: 9–16.
- European Commission's Directorate General for Agriculture and Rural Development. 2016.
- Sugar. [Online] Available at [http://ec.europa.eu/agriculture/sugar/index\\_en.htm](http://ec.europa.eu/agriculture/sugar/index_en.htm)
- Fewkes, D. W., Parker, K. J. and Vlitos, A. J. 1971. Sucrose. *Science Progress* 59: 25–39.
- Food and Agriculture Organization. 2016. FAOSTAT. [Online] Available at <http://faostat3.fao.org/browse/Q/QC/E>
- García, M. and Medina, E. 2013. Effects of salt stress on salt accumulation in roots and leaves of two sugarcane genotypes differing in salinity tolerance. *Journal of Tropical Agriculture* 51: 15–22.
- Gandonou, C., Abrini, J., Idaomar, M. and Skali-Senhaji, N. 2005. Effects of NaCl on growth and ion and proline accumulation in sugarcane (*Saccharum* sp) callus culture. *Belgian Journal of Botany*, 2: 173-180.
- Golabi, M., Naseri, A. A. and Kashkuli, H. A. 2009. Mathematical modeling of the relationship between irrigation water salinity and sugarcane juice quality. *J. Food Agric. Environ.* 7: 600-602.
- Goldschmidt, E. E. and Huber, S.C. 1992. Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars. *Plant Physiology* 99: 1443–1448.
- Gomathi, R. and Thandapani, T. V. 2005. Salt stress in relation to nutrient accumulation and quality of sugarcane genotypes. *Sugar Tech* 7: 39-47.
- Gomathi, R., Vasantha, S. and Thandapani, V. 2010. Mechanism of osmo regulation in response to salinity stress in sugarcane. *Sugar Tech* 12: 305–311.
- Goos, R. J. 1987. Chloride fertilization: the basics. *Crops Soils* 39: 12-13.
- Hallmak, W. B., Williams, G. J., Hawkins, G. L. 2001. Effect of potassium sulfate vs. potassium chloride on sugarcane yields across two years. *Sugarcane research annual progress report* 2001. 173-183.

- Hamid, A. M. A. and Dagash, Y. M. I. 2014. Effect of sulfur on sugarcane yield and quality at the heavy clay soil “Vertisols” of Sudan. *Universal J. Appl. Sci.* 2: 68-71.
- Hartt, C. E. and Burr, G. O. 1967. Factors affecting photosynthesis in sugar cane. *Proceeding of International Society of Sugar Cane Technologists, 12th Congress*: 590–608.
- Hattori, T., Tarumoto, Y., sakaigaichi, T., Tanaka, M. and Hayano, M. 2016. Varietal differences in ash content in sugarcane juice and development of simple estimation technique. *Abstracts of the 241<sup>st</sup> Meeting of the CSSJ*: 230.
- Hogarth, D. M. and Kingston, G. 1983. The inheritance of ash in juice from sugar cane. Bureau of Sugar Experiment Stations Queensland Australia, February 1983.
- Hunsigi, G. 2011. Potassium management strategies to realize high yield and quality of sugarcane. *Karnataka Journal of Agricultural Sciences*. 24: 45-47.
- Hussain, A., Khan, Z. I., Ashraf, M., Rashid, M. H. and Akhtar, M. S. 2004. Effects of salt stress on some growth attributes of sugarcane cultivars CP-77-400 and COJ-84. *International Journal of Agriculture and Biology*, 6: 188-191.
- Iesaka, M. 2001. Okinawaken no nougyouroudouryoku mondai to satoukibi seisan kouzou. [Problem of work force on agriculture and productive structure of sugarcane in Okinawa, Japan.] *Okinawa Kansyatou Nenpou*. [Okinawa Cane Sugar Annual Report.] 32: 21-28.
- Inoue, S. 2006. Present situation and challenges of the sugarcane and sugar industry in Okinawa prefecture. *J. Agric. Policy Res.* 12: 65-84.
- Jackson, P. A., Schroeder, B. L., Rattey, A. R., Wood, A. and O’Shea, M. G. 2008. Management of ash/impurity ration in sugarcane: relative effects of genotypes, and N and K fertilizer rates. *Australian journal of Agricultural Research* 59: 795-801.
- Japan Agriculture, Kagoshima Prefectural Economic Federation of Agricultural Co-operatives. 2016a. BB hiryou, 3. Satoukibi. [BB fertilizer, 3. Sugarcane.] [Online]. Available at <http://www.ks-ja.or.jp/greennet/horticulture/sizai/images/hiryo113.pdf>

- Japan Agriculture, Kagoshima Prefectural Economic Federation of Agricultural Co-operatives. 2016b. BB hiryō, 5. Tuihi yō. [BB fertilizer, 5. For additional fertilizer.] [Online]. Available at <http://www.ks-ja.or.jp/greenet/horticulture/sizai/images/hiryō115.pdf>
- Jones, M. G. K., Outlaw, W. H. and Lowry, O. H. 1977. Enzymic assay of  $10^{-7}$  to  $10^{-14}$  moles of sucrose in plant tissues. *Plant Physiology* 60: 379–383.
- Joshi, G. V. and Nail, G. R. 1980. Response of sugarcane to different types of salt stress. *Plant and Soil* 56: 255–263.
- Kafkafi, U. 2001. Introduction; Potassium and chloride in soils. *In* Johnston, A. E. eds., Potassium and Chloride in Crops and Soils: the Role of Potassium Chloride Fertilizer in Crop Nutrition. International Potash Institute, Basel. 11-15, 29-47.
- Kawamitsu, Y., Azama, T., Fukuzawa, Y., Taira, E. and Ueno, M. 2006. Effects of soil potassium on leaf photosynthesis and sugar accumulation of sugarcane with different sugar content. *Jpn. J. Crop Sci.* 75: (Extra 1), 156-157.
- Khadr, M. S., Negm, A. Y., Khalil, F. A. and Antoun, L. W. 2004. Effect of potassium chloride in comparison with potassium sulfate on sugar cane production and some soil chemical properties under Egyptian conditions. IPI Regional Workshop on Potassium and Fertigation development in West Asia and North Africa, Rabat, Morocco, 24–28 November, 2004. 1-8.
- Khosa, T. M. 2002. Effect of different levels and sources of potassium on growth, yield, and quality of sugarcane. *Better Crops International* 16: 14-15.
- Kikuchi, K. 2009. Sugarcane production in Islands, 'Farm Management and Utilization of Biomass'. Tokyo: Nourin Toukei Press.
- Kingston, G. 1982a. Ash in first expressed cane juice at Rocky Point—I. Factors affecting the inorganic composition of juices. *Proc. Aust. Soc. Sugar Cane Technol.* 1982 Conf. 11-17.

- Kingston, G. 1982b. Ash in first expressed cane juice at Rocky Point–II. Effects of geography and varieties. *Proc. Aust. Soc. Sugar Cane Technol. 1982 Conf.* 19-22.
- Koki, Z. 2002. Hirai enbun ga satoukibi ni oyobosu eikyou (gai). [Harmful effects of atmospheric salt on sugarcane]. *Okinawa Kansyatou Nenpou* 33: 1-11.
- Kuba, M. 1993. Actual condition and soil management of farm land in Okinawa –soil chemical properties and management of fertilization in sugar cane fields–. *Pedologist* 37: 126-137.
- Kumar, V. and Verma, K. S. 1997. Relationship between nutrient element content of the index leaf and cane yield and juice quality of sugarcane genotypes. *Commun. Soil Sci. Plant Anal.* 28: 1021-1032.
- Laties, G. G., MacDonald, I. R., and Dainty, J. 1964. Influence of the counter-ion on the absorption isotherm for chloride at low temperature. *Plant Physiology* 39: 254-262.
- Lingle, S. E. and Wiegand, C. L. 1997. Soil salinity and sugarcane juice quality. *Field Crops Research*, 54: 259-268.
- [Lingle, S. E.](#), Wiedenfeld, R. P. and Irvine, J. E. 2000. Sugarcane response to saline irrigation water. *J. Plant Nutr.* 23: 469-486.
- Long, S. P. and Bernacchi, C. J. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* 54: 2393–2401.
- Lunn, J. E. and Hatch, M. D. 1995. Primary partitioning and storage of photosynthate in sucrose and starch in leaves of C<sub>4</sub> plants. *Planta* 197: 385–391.
- Macklon, A. E. S. and MacDonald, I. R. 1966. The role of transmembrane electrical potential in determining the absorption isotherm for chloride in potato. *J. Exp. Bot.* 17: 703-717.
- Maeda, G., Shimoji, I., Miyagi, K., Tedokon, T., Shimoji, H., Uechi, K., Chinen, J., Ishimine, H. and Sunagawa, M. Changes in growth and quality of sugarcane exposed to salt

- water, the amount of water required removing the salt stuck to the leaf surface. *Journal of Okinawa Agriculture* 47: 3-16.
- Matsuoka, M. 2006. Sugarcane cultivation and sugar industry in Japan. *Sugar Tech.* 8: 3-9.
- Matsuoka, S. and Stolf, R. 2012. Sugarcane tillering and ratooning: key factors for a profitable cropping. *In* Goncalves, J. F. and Correia, K. D. eds., *Sugarcane: Production, Cultivation and Uses*. Nova Science Publishers, Inc., New York. 137-157.
- McGray, J. M., Rice, R. W., Lang, T. A. and Baucum, L. 2010a. Sugarcane plant nutrient diagnosis. [Online] Available at <http://edis.ifas.ufl.edu/sc075>
- McGray, J. M. and Mylavarapu, R. 2010b. Sugarcane nutrient management using leaf analysis. [Online] Available at <http://edis.ifas.ufl.edu/ag345>
- McGray, J. M., Rice, R. W., and Ezenwa, I. V. 2011. Sugarcane leaf tissue sample preparation for diagnostic analysis. [Online] Available at <http://edis.ifas.ufl.edu/sc076>
- Medeiros, C. D., Neto, J. R. C. F., Oliveira, M. T., Rivas, R., Pandolfi, V., Kido, E. A., Baldani, J. I. and Santos, M. G. 2014. Photosynthesis, antioxidant activities and transcriptional responses in two sugarcane (*Saccharum officinarum* L.) cultivars under salt stress. *Acta Physiologiae Plantarum* 36: 447–459.
- Meinzer, F. C., Z. Plaut, and N. Z. Saliendra. 1994. Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. *Plant Physiology* 104: 521–526.
- Metternicht, G. I. and Zinck, J. A. 2003. Remote sensing of soil salinity: potentials and constraints. *Remote Sensing of Environment*, 85: 1-20.
- Meyer, J. H. and Wood, R. A. 2001. The effects of soil fertility and nutrition on sugarcane quality: a review. *Proc. S. Afr. Sug. Technol. Ass.* 75: 242-247.
- Ministry of Agriculture, Forestry and Fisheries. 2003. Dojou kairyou oyobi sehi kaizen shishin, 9. Satoukibi. [Manual for Soil Amelioration and Fertilization Improvement, 9. Sugarcane.] [Online]. Available at [http://www.maff.go.jp/j/seisan/kankyo/hozen\\_type/h\\_sehi\\_kizyun/pdf/08460105chap1\\_159](http://www.maff.go.jp/j/seisan/kankyo/hozen_type/h_sehi_kizyun/pdf/08460105chap1_159)

- Miyazato, K. 1986. Dai 6 shou. Saibai. [Chapter 6. Cultivation.] *In* Satoukibi to sono Saibai. [Sugarcane and its cultivation.] Bunmitsutou Kougyoukai, Naha. 212-312.
- Munns, R. and Tester, M. 2010. Mechanism of Salinity Tolerance.
- Murad, A. M., Molinari, H. B. C., Magalhães, B. S., Franco, A. C., Takahashi, F. S. C., de Oliveira, N. G., Franco, O. L. and Quirino, B. F. 2014. Physiological and proteomic analyses of *Saccharum* spp. grown under salt stress. PLoS One, 9: e98463.
- Nagae, T., Kawamitsu, Y., Ohmi, N., Kawanaka, T., Ueno, M. and Tokashiki, Y. 1997. Improvement of sugar production in sugarcane with crop, soil and system engineering. II. Effects of potassium treatment on sugar content in sugarcane juice. Jpn. J. Crop Sci. 66: (Extra 1), 264-265.
- Naganuma, N. 1992. Hateruma, Minamidaitou ryoutou ni okeru mizukankyou to sono riyou. [Water environment and its utilization in Hateruma and Minamidaitou Islands.] *In* Komazawa Chiri. [Komazawa Geography.] Komazawa Daigaku Bungakubu Chirigaku Kyousitsu, Tokyo. 75-91.
- Ng Kee Kwong, K. E. 2003. The effects of potassium on growth, development, yield and quality of sugarcane. *In* Pasricha, N. S. and Bansal, S. K. eds., Potassium for sustainable production. Proceedings of international symposium on role of potassium in nutrient management for sustainable crop production in India. International Potash Institute, Basel. 430-444.
- Nuss, K. J. and Brett, P. G. C. 1995. The release of variety NCo310 in 1945 and its impact on the sugar industry. Proceedings of The South African Sugar Technologists' Association 69: 1-8.
- Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries. 2015. Satoukibi Saibai Shishin [Cultivation Manual for Sugarcane.] [Online]. Available at [http://www.maff.go.jp/j/seisan/kankyo/hozen\\_type/h\\_sehi\\_kizyun/pdf/satokibi.pdf](http://www.maff.go.jp/j/seisan/kankyo/hozen_type/h_sehi_kizyun/pdf/satokibi.pdf)



- Oliveira, S. R., Neto, J. A. G., Nobrega, J. A. and Jones, B. T. 2010. Determination of macro- and micronutrients in plant leaves by high-resolution continuum source flame atomic absorption spectrometry combining instrumental and sample preparation strategies. *Spectrochim. Acta B.* 65: 316-320.
- Oshiro, S., Ishiki, M., and Takaesu, K. 1994. Satoukibi no eiyoujindan tyousa. [Nutrient diagnosis survey of sugarcane.] Okinawa Kansyatou Nenpou. [Okinawa Cane Sugar Annual Report.] 28: 7-12.
- Oshiro, Y. and Nagatomi, S. 1982. A method for evaluation salt tolerance in sugarcane varieties. *Bulletin of the Okinawa Agricultural Experiment Station* 7: 23-29.
- Oshiro, T. 2001. Satoukibi no seisan shinkou ni muketa torikumi. [Approach toward the advancement of sugarcane production.] [Online]. Available at [http://sugar.alic.go.jp/japan/view/jv\\_0103b.htm](http://sugar.alic.go.jp/japan/view/jv_0103b.htm)
- Ota, M., Kuba, M. and Yara, C. 2000. Nutritional and soil diagnosis of sugarcane in Okinawa. *Rep. Kyusyu Br. Crop. Sci. Jpn.* 66: 56-59.
- Paul, M. J. and Pellny, T. K. 2003. Carbon metabolite feedback regulation of leaf photosynthesis and development. *Journal of Experimental Botany* 54: 539–547.
- Peele, T. C., Webb, H. J. and Bullock, J. F. 1960. Chemical composition of irrigation waters in the South Carolina coastal plain and effects of chloride in irrigation water on the quality of flue-cured tobacco. *Agronomy journal* 52: 464-467.
- Poole, R. J. 2010. Chapter 6. Solute transport. *In* Taiz, L. and Zeiger, E. eds., *Plant Physiology*, Fifth Edition. Sinauer Associates, Inc., Sunderland. 131-159.
- Poonsawat, W., Theerawitaya, C., Suwan, T., Mongkolsiriwatana, C., Samphumphuang, T., Cha-um, S. and Kirdmanee, C. 2015. Regulation of some salt defense-related genes in relation to physiological and biochemical changes in three sugarcane genotypes subjected to salt stress. *Protoplasma*, 252: 231-243.
- Prajapati, K., and Modi, H. A. 2012. The importance of potassium in plant growth—a review.

- Indian Journal of Plant Sciences 1: 177–186.
- R Core Team. 2015. R: A language and environment for statistical computing. Austria: R Foundation for Statistical Computing Vienna.
- Ribeiro, R. V., Machado, R. S., Machado, E. C., Machado, D. F. S. P., Filho, J. R. M. and Landell, M. G. A. 2013. Revealing drought-resistance and productive patterns in sugarcane genotypes by evaluating both physiological responses and stalk yield. *Experimental Agriculture* 49: 212–224.
- Rozeff, N. 1995. Sugarcane and salinity – a review paper. *Sugar Cane*, 5: 8-19.
- Ryukyu Fertilizer Co., Ltd. 2015. Seihin ichiran. [List of the products.] [Online]. Available at <http://ryuhi.sakura.ne.jp/seihinsyoukai.html>
- Samuels, G., Landrau, P., Alers, S. A. 1955. Part I: taking the sugarcane-leaf samples. In *The method of foliar diagnosis as applied to sugarcane*. University of Puerto Rico, San Juan. 8-22.
- Saxena, P., Srivastava, R. P. and Sharma, M. L. 2010. Studies on salinity stress tolerance in sugarcane varieties. *Sugar Tech*, 12: 59-63.
- Schachtman, D. and Liu, W. 1999. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends in Plant Science* 4: 281-287.
- Schnabl, H. and Raschke, K. 1980. Potassium chloride as stomatal osmoticum in *Allium cepa* L., a species devoid of starch in guard cells. *Plant Physiology*, 65, 88-93.
- Stewart, C. M., Melvin, J. F., Diteburne, N., Tham, S. H. and Zerdoner, E. 1973. The effect of season of growth on the chemical composition of cambial saps of *Eucalyptus regnans* trees. *Oecologia* 29: 349-372.
- Stuart, D. A., and Jones, R. L. 1978. Role of cation and anion uptake in salt-stimulated elongation of lettuce hypocotyl sections. *Plant Physiology*, 61, 180-183.
- Sugimoto, A. and Terajima, Y. 2006. Breeding high yield sugarcane for new biomass industry of sugar and another product. *J. Jpn. Soc. Agric. Mach.* 68: 4-8.

- Sümer, A., Zörb, C., Feng, Y. and Schubert, S. 2004. Evidence of sodium toxicity for the vegetative growth of maize (*Zea mays* L.) during the first phase of salt stress. *Journal of Applied Botany and Food Quality* 78: 135–139.
- Stevenson, D. M., McGrath, G. J. and Statham, M. K. 1970. A potash survey in the Pioneer mill area. *Proc. Qd. Soc. Sugar Cane Technol.*, thirty-seventh Conf. 39-49.
- Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P. and McDonald, G. K. 2011. Additive effects of Na<sup>+</sup> and Cl<sup>-</sup> ions on barley growth under salinity stress. *Journal of Experimental Botany* 62: 2189–2203.
- Thomas, J. R., Salinas, F. G. and Oerther, G. F. 1981. Use of saline water for supplemental irrigation of sugarcane. *Agronomy Journal* 73: 1011-1017.
- Uehara, N., Sasaki, H., Kawamitsu, Y. and Osugi, R. 2004. Effect of excess potassium on sugar-yield in sugarcane. *Jpn. J. Crop Sci.* 73: (Extra 1), 262-263.
- Ueno, M., Kawamitsu, Y., Sun, L., Taira, E. and Maeda, K. 2005. Combined applications of NIR, RS, and GIS for sustainable sugarcane production. *Proceedings of ISSCT* 25: 204-210.
- Vasantha, S., Venkataramana, S., Rao, P. N. G. and Gomathi, R. 2010. Long term salinity on growth, photosynthesis and osmotic characteristics in sugarcane. *Sugar Tech* 12: 5–8.
- Wahid, A., Rao, A. R. and Rasul, E. 1997. Identification of salt tolerance traits in sugarcane lines. *Field Crops Research*, 54: 9-17.
- Wahid, A. 2004. Toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane. *Botanical Bulletin of Academia Sinica* 45: 133–141.
- White, P. J. and Broadley, M. R. 2001. Chloride in soils and its uptake and movement within the plant: a review. *Annals of Botany*. 88: 967-988.
- Wiedenfield, B. 2008. Effects of irrigation water salinity and electrostatic water treatment for sugarcane production. *Agricultural Water Management* 95: 85-88.
- Wiegand, C., Anderson, G., Lingle, S and Escobar, D. 1996. Soil salinity effects on crop

- growth and yield – Illustration of an analysis and mapping methodology for sugarcane. *Journal of Plant Physiology*. 148: 418-424.
- Zapała, E., Kuklis, I., Fabjańska-Świeca, G., and Tarnowska, J. 2013. Application of ion chromatography for determination of chlorine content in solid biomass for power sector. *CHEMIK*. 67: 1217-1226.
- Zeng, J., Chen, A., Li, D., Yi, B. and Wu, W. 2013. Effects of salt stress on the growth, physiological responses, and glycoside contents of *Stevia rebaudiana* Berton. *Journal of Agricultural and Food Chemistry*, 61: 5720-5726.
- Zhu, J. K. 2002. Salt and drought stress signal transduction in plants. *Annual review of Plant Biology* 53: 247-273.