Studies on Sustainable Sugarcane Production through Effective Use of Varietal Diversity

品種多様性の有効活用による持続可能なサトウキビ生産に関する研究

Doctoral Thesis

学位論文

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Number in parenthesis means the percentage of root mass in each layer to total root mass.

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TR: tiller regulation

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Note: * means significant difference between treatments at 5% level (t-test, n=3 pots).

Biomass and leaf area of tillers were not tested statistically.

TR: tiller regulation, RDI: root depth index, S/R: shoot/root mass, LA: leaf area, RGR: relative growth rate, NAR: net assimilation rate, LAR: leaf area ratio, SLA: specific leaf area

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Table 3.3.6 Dry matter production for each treatment in Exp. 2.

Note: No significant difference between treatments at 1% level (n=3 pots).

RDI: root depth index, S/R: shoot/root mass, RGR: relative growth rate, NAR: net assimilation rate, LAR: leaf area ratio.

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Abbreviations

CLES, conventional leaf erectness score.
DAP, days after planting.
DAT, days after transplanting.
DI, diversity index.
DW, dry weight.
EC, electric conductivity.
EI, evenness index.
F, accumulated LAI from the top of canopy.
GIS, geographic information system.
GPS, global positioning system.
K, light extinction coefficient.
LAD, leaf area density.
LAI, leaf area index.
LAR, leaf area ratio.
LEI, leaf erectness index.
LSI, leaf shape index.
MI, mixture index.
NAR, net assimilation rate.
N_{LA}, leaf nitrogen content per unit leaf area
PAR, photosynthetic active radiation.
PFD, photon flux density.
RDI, root depth index.
RGR, relative growth rate.
RLI, relative light intensity.
RUE, radiation use efficiency.
SLA, specific leaf area.
S/R, shoot mass / root mass ratio
SSL, severity score of lodging.
TR, tiller regulation.
VR, vegetation rate.

Chapter 1: General introduction

1.1 Sugarcane production and role of breeding in Japan

Sugarcane (*Saccharum* spp.) is an economically important crop in subtropical regions, especially in many isolated Japanese islands, such as the Nansei Islands (Inoue 2006). Even though its importance has long been recognized, sugarcane production in Japan has decreased since the 1990s even while world production has continued to increase (Fig. 1.1). There will be no significant improvements in terms of the number of growers and the area under cultivation as the sugarcane growers in Japan get older, so the importance of improving sugarcane yield per unit land area is increasing (Matsuoka 2006; Kikuchi 2009; Takaragawa et al. 2018e). Sugarcane yield in Japan, however, is low and unstable due to climatic disasters, such as typhoons and drought (Fig. 1.1). Such a situation causes a negative spiral: decreases in sugarcane yield per unit land area negatively affect the growers' enthusiasm, resulting in further yield reduction. Based on these imperatives, growing practices are needed to produce higher and more stable yields, but with ecofriendly and labor-saving techniques, to achieve sustainable sugarcane production (Sugimoto 1996; Shinzato 2015; Maesato 2016).

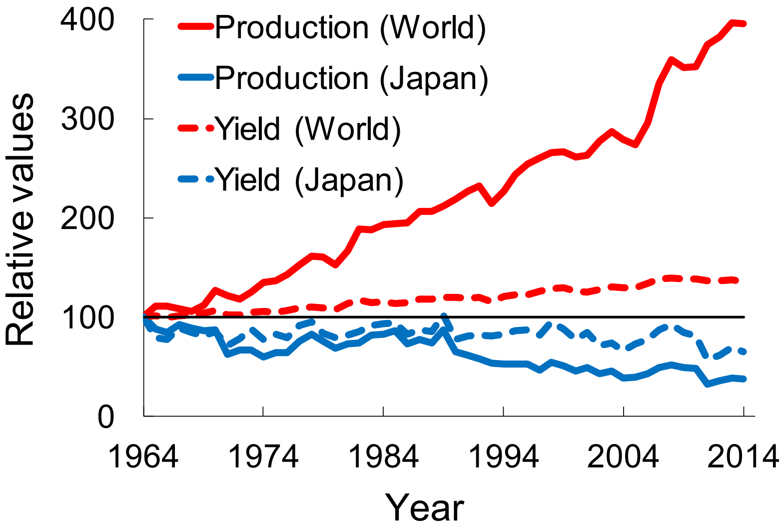


Fig. 1.1 Sugarcane production in Japan and the world.
Note: Data was collected from FAOSTAT (2016). Data was shown as relative value with that of 1964 being 100 %.

Selecting sugarcane varieties with a greater adaptability to a range of environmental conditions is one of the most effective methods for increasing sugarcane yield (Sugimoto 1994; Sugimoto 2004; Inoue 2017). According to the concept of selecting varieties suitable for a specific region or area, which has been adopted in both breeding programs and in variety use (Inman-bamber and Stead 1990; Satoukibi zousan project kaigi 2005; Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2015), geo-ecological characteristics have been taken into account from the early days of sugarcane breeding in Japan (Nagatomi et al. 1982b; Irei 2011; Takaragawa et al. 2018e). To date, 37 varieties have been developed (Ni1 – Ni33 etc.) and there are currently (December 2018) 21 varieties registered in Japan (2018/12). It takes at least 12 years to select and register varieties and a further few years to propagate and release sufficient planting stock to growers as part of the breeding process (Sato et al. 2018). Therefore, there exists a time lag from the grower's demand to release of the new variety to growers, during which much labor and cost are consumed. As a consequence, it has been suggested that not only breeding new varieties, but also utilizing and maintaining the existing range of varieties efficiently is required to achieve sustainable sugarcane production (Takaragawa et al. 2015). In addition, the effective use of existing varieties may contribute to improving the cost-benefit of sugarcane breeding, a concept that will be increasingly important for the sustainable development of breeding in the future.

1.2 Biodiversity and agriculture

The concepts concerning biodiversity in an agricultural ecosystem (crop diversity, agrobiodiversity, agroecosystem, agroforestry, etc.) and their application are important to achieve sustainable agricultural production (Thrupp 2000). One of the most important arguments is that a monoculture of one variety of one species over-simplifies the agroecosystem and damages its biodiversity and its ecosystem services in the field (Karp et al. 2012). Many of these concepts with respect to the interspecific relationship suggested that cultivation of several crop species either at temporal and spatial scales such as rotation and intercropping was considered as one main method to conserve the agrobiodiversity (Thrupp 2000; Karp et al. 2012).

Unfortunately, in the Nansei Islands, the cultivation of crop species other than sugarcane, such as the economically important horticultural crops, requires high levels of skill and cost (Terauchi 2013) for these crops to be grown under the prevailing adverse conditions such as typhoons, drought, and infertile soils (Takaragawa et al. 2018e). Increasing the biodiversity in a sugarcane field by using several different species will not necessarily be effective at attaining sustainable production in Japan. On the other hand, the intraspecific crop diversity could also positively affect the agricultural ecosystem (Newton et al. 2008; Suzuki et al. 2017). Intraspecific crop diversity and/or diversity of existing varieties is described as 'varietal diversity' (Fig. 1.2). Diverse definitions have been developed to define and measure biodiversity (Anderson et al. 2011; Mori 2018). Varietal diversity as defined here is divided into three parts: i) richness diversity, of which the indicators are numerous, ii) genetic diversity, of which the indicator is variation at the gene level, and iii) functional diversity, the indicator of which is a functional trait in the present study.

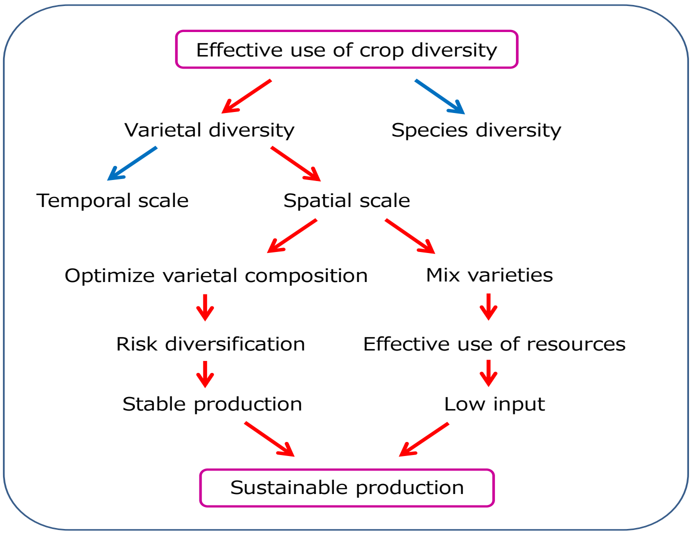


Fig. 1.2 Assumed connection between crop diversity and agricultural production.
Note: Red arrows show the direction of the present study.

1.3 Evaluation of varietal diversity in Japan.

Traditionally in Japan, sugarcane production was dependent on only one specific variety, such as the local variety ‘Yomitanzan’ (*Saccharum sinense* Roxb.) until the 1930s, when the imported varieties were introduced, such as ‘POJ2725’ between the 1930s and the 1960s, and ‘NCo310’ between the 1960 and the 1990s, while various varieties have recently been used that were developed through the process of geo-ecological breeding (Miyazato 1986; Takaragawa et al. 2018e). Among the released varieties, unrecommended ones remain in cultivation in many regions, resulting in increased varietal diversity. In sugarcane, the facts that many genetic markers have been developed (Hoarau et al. 2001; Casu et al. 2005) and that the sugarcane genome has been fully sequenced (Garsmeur et al. 2018) suggests that the highly advanced breeding of this crop at the gene level will be carried out in the near future. In Japan, sugarcane genetic diversity has not yet been assessed by next-generation sequencing, but current estimates are low because many of the varieties are related to each other since they share parents such as ‘NCo310’ and ‘CP29-116’ (Tarumoto et al. 2016). On the other hand, all varieties exhibit ‘distinctness’ from other varieties as part of the Registration process, so that all varieties should be identifiable on the grounds of morphological or ecological traits (functional traits) (Plant Variety Protection and Seed Act 3-1-1, Murabayashi et al. 2013), a fact which indicates that some functional diversity exists among existing varieties. Therefore, richness and functional diversities have been maintained in recent breeding programs, while genetic diversity is still low among Japanese varieties due to the narrow genetic basis of the parental material available. To date, only lip service has been paid to the current status of varietal diversity and its maintenance and utilization by the Japanese sugar industry.

1.4 Maintenance and management of varietal diversity.

An understanding of the current status of variety use after release to the growers is considered to be central to developing an effective method by which to spread and manage varieties. The issues concerning varietal diversity and its use are as below:

1.4.1 Is varietal diversity truly increasing? Specific varieties still dominate specific regions.

Although many varieties have been released and recommended, one or two specific varieties still occupy 60%–80% of the cultivation area in some regions (2017/2018). To evaluate varietal diversity, it is important to consider not only the number of varieties grown, but also the cultivation area under each variety in individual regions. Unfortunately, indicators for this are lacking.

1.4.2 How to know functional diversity?

Distinctness is necessary to register varieties, which means that all varieties should be identifiable by using one or more different traits (Plant Variety Protection and Seed Act 3-1-1, Murabayashi et al. 2013). However, many of these traits are qualitatively evaluated using visual observation according to guidelines (Terauchi 2013a), which makes it unclear how much functional diversity exists among existing varieties. The quantitative evaluation of functional diversity may contribute to revealing a genetic mechanism and a physiological role for each trait through genetic analysis, such as quantitative trait locus analysis. The diversity of several traits, such as maturity (Jackson and Morgan 2003; Sugimoto 1999), root elongation ability (Fukuzawa et al. 2009), leaf characteristics (Nagatomi et al. 1982a; Shimabuku 1984; Terauchi and Matsuoka 2000), and photosynthetic ability (Nose and Nakama 1990) have been evaluated among intraspecific and interspecific germplasm. However, many of these reports used germplasm, such as breeding lines, and wild relatives used under pre-breeding activities, rather than existing varieties which have been registered and released to growers. Unlike these previously reported studies, the present study attempted to quantitatively evaluate functional diversity among Japanese sugarcane varieties, taking plant type for example.

1.4.3 In practice, how are varieties used after being released to growers?

The breeder develops promising clones with traits required by the growers, while sugar mill workers also select varieties according to evaluation after use for 6–7 generations. Then, varieties are registered and recommended for cultivation in specific regions (Takaragawa et al. 2018e). Nevertheless, some of these varieties have not spread to a wider area and replaced other, older and outdated varieties. The current status of the use of each variety, which reflected grower's decision making, can be understood only through using statistical varietal area data compiled by each prefecture. Ultimately, neither the government nor the sugar mill companies have the power to force growers to select specific varieties. On the other hand, a belief has spread to the entire sugarcane industry that it is not necessary to understand the current picture of variety use or to optimize the varietal composition. Generally, crop varieties are selected based on their management requirements and site preference (Ooe et al. 1993; Nakamura et al, 2005; Wakabayashi 2014). In my opinion, the long-term monitoring of the status of variety use should enable us to obtain much important information, and to promote public awareness: how to use several varieties and control the issue of unintentional varietal mixtures. Of course, it should also contribute to improvements in identifying breeding targets and appropriate methods.

1.4.4 Does varietal performance change?

In addition to Distinctness, which has already been mentioned, the Uniformity and Stability of traits are required to register varieties (Plant Variety Protection and Seed Act 3-1-2, Murabayashi et al. 2013). Therefore, traits are carefully evaluated by breeders through more than ten generations of the breeding process (Sato et al. 2018). From interviews with people involved in the sugarcane industry, around 30% of the respondents answered that they recognized the trait changes in a single variety over time, such as thinner stalk from the seedling field after multiple ratooning and increased asynchrony of bud sprouting (Takaragawa, unpublished data). These types of changes are known as 'varietal deterioration' (Suge 1987). In the 1950s, yield decline and varietal deterioration in sugarcane were discussed (King 1950; King 1959; Mangelsdorf 1959; Martin et al. 1959). So far, there is a consensus that varieties cannot show their true performance if pathogens accumulate in the planting material and soil. In Japan, although sugarcane varietal yield decline caused by disease was indicated in the era of 'NCo310' (Nagatomi 1980), little attention has been paid to this topic to date, probably because it is difficult to distinguish varietal decline from variations in yield due to changeable climate conditions year-on-year.

1.4.5 Can varietal diversity contribute to improved sugarcane production?

Traditionally in Japan, sugarcane production tended to depend on only one superior variety in each region. However, in the early 1970s, smut disease decreased the productivity of 'NCo310' and rapidly decreased the cultivation area of this variety. Based on this negative experience, varietal diversification was accelerated in the late 1980s (Miyahira 2000; Sato 2017). Varietal diversification may have potential to lessen the risk presented by disease spread or climatic disaster (Miyahira 2000) and to increase the choice for growers, although it may also have the potential to reduce yield and quality if the regional adaptability of each variety is misjudged (Nakamori and Kawamura 1997).

Although such types of discussion had been reported before, no study has been conducted on the effect of varietal diversity on sugarcane productivity and the industry in practice.

Japanese sugarcane research has paid a great deal of attention to breeding new varieties, but two subsequent processes are lacking: a 'feedback' process from the growers to achieve efficient breeding through evaluation of richness and functional diversity, in the form of varietal diversification, and the subsequent 'follow-up' process to support growers in selecting appropriate varieties and suitable growing methods for each variety through evaluation of the current status and long-term performance after release.

1.5 Effective use of varietal diversity.

There are two scales for the use of varietal diversity: temporal and spatial scales (Fig. 1.2). The former includes introducing new varieties to replace previous varieties, which have some problems such as varietal deterioration, and rotation cropping of different varieties when renewing a field after multiple ratooning. Replacing varieties is one of the most important roles of plant breeding (Nagatomi 1980). Experienced growers practice the rotation of varieties and its potential effect on the biological control of harmful nematode, using nematode-tolerant varieties, was demonstrated in the case of the sweet potato (Suzuki et al. 2017).

In the present study, two strategies to use varietal diversity at the spatial scale will be discussed (Fig. 1.3). One is the use of different varieties in different fields or in different regions. This strategy will be discussed in terms of the optimization of variety composition as the main subject of Chapter 2. Another is the use of varietal diversity at the within-field level, namely deliberate and systematic mixed-planting of several varieties. Although only a few examples exist regarding variety mixtures in sugarcane, it has been reported to be successful in small-grain crops such as rice and wheat, this method should improve disease control (Zhu et al. 2000) as well as yield and grain quality (Newton and Swanson 1999). It was suggested that the yield and quality effects were due to variations in phenological and architectural characteristics among the varieties, which improved resource-use efficiency (Newton et al. 2008). Therefore, a mixture of varieties is considered to be one of the most sustainable cultivation methods that could reduce the use of agrochemicals, fertilizers, and irrigation by improving disease control and resource-use efficiency, resulting in a high and stable yield (Faraji 2011; Barot et al. 2017).

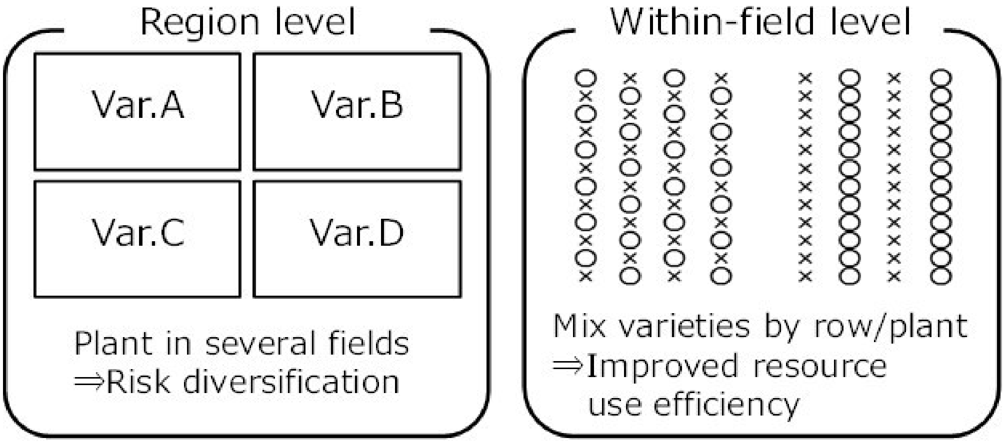


Fig. 1.3 Directions of use of varietal diversity at spatial scale.

In Japan, in practice sugarcane varieties are often mix-planted carelessly, or deliberately, with a lodging-resistant variety being planted in the headland of fields or along the roadside, to act as a windbreak; see Chapter 2.3 for the current status of the planting of variety mixtures by growers. A number of attempts have been made to grow a mixture of either randomly selected sugarcane varieties (Kodama and Nagatomi 1969; Brown et al. 1972; Kapur et al. 1988) or varieties selected for complementary targeted traits, particularly disease and/or pest tolerance (Srinivasan 1968; Cadet et al. 2007). On the other hand, the effects of sugarcane variety mixtures on above- and below-ground biomass, and the mode-of-action of any beneficial effects, have never been reported. In addition, information on the optimum combination of varieties for sugarcane mixtures is also lacking.

Sugarcane yield is determined more by the number rather than by the weight of millable stalks (Miller and James 1974; Shimabuku 1997). Therefore, maintaining and/or improving the number of millable stalks has been considered to be an important goal for sugarcane production (Sato and Yoshida 2001; Satoukibi zousan project kaigi 2005; Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2015). The number of stalks per unit land area increases rapidly during the active tillering stage (i.e., the early growth stage, for about 3 months or 700°C-days at a base temperature of 16°C after planting), reaches a peak stalk density, which then decreases gradually because of competition for resources such as light and nutrients during the yield formation stage (i.e. the later growth stage) (Nose et al. 1989; Singels and Smit 2009; Zhou and Shoko 2011). The present study will focus on the improvement of the above-ground canopy and the below-ground structure to achieve high numbers of millable stalks per unit land area. The hypothesized relationship between varietal mixtures and the number of millable stalks in terms of above-ground and below-ground parts will be discussed in the following subsections.

1.5.1 Optimum combination of varieties for mixture to improve canopy structure.

The number of millable stalks is dependent on light conditions within the canopy, while light transmittance to the canopy is controlled by the canopy structure (Shimabuku 1997; Zhou et al. 2003; Marchiori et al. 2010). Generally, canopy structure depends on genetic traits, such as the degree of leaf erectness (plant type) (Hikosaka and Hirose 1997; Shimabuku 1997; Monsi and Saeki 2005; Marchiori et al. 2010), plant size (i.e. stem length and plant height) (Tsuchiya and Kinoshita 1984; Tominaga et al. 2015), and tiller number (Zhou et al. 2003), and on agronomic features such as row spacing and planting density (Nose et al. 1989; Singels and Smit 2009). Optimal plant density for sugarcane is determined by local factors (Irvine et al. 1980). For example, optimal ridge width depends on machinery size (e.g. planter, cultivator, and harvester width) in Japan (Hiyane et al. 2005; Akachi et al. 2017). In addition, as typhoons and strong winds disturb the formation of the canopy, lodging and wind resistance are also important traits to factor into the design of canopy structure and to attain the optimal number of millable stalks in sugarcane fields in the Nansei Islands (Sugimoto 1994).

Generally, universal or generalist varieties have never existed, where one variety combines all the advantageous traits (Barot et al. 2017). It will take a longer time for universal varieties to be developed in sugarcane than in other crops because the sugarcane genome has only recently been sequenced (Garsmeur et al. 2018). With respect to plant type, desirable traits in sugarcane differ depending on the growth stage (e.g. horizontal leaf at the early growth stage and erect leaf at the later growth stage (Shimabuku 1997)), which makes it difficult to design the ideal variety (i.e. ideotype). From these facts, the author has hypothesized that a mixture of varieties with different plant types, such as tillering ability, ratooning ability, and lodging tolerance, where the varieties complement each other, could achieve greater growth and yield as proposed below:

Hypothesis 1: a varietal mixture with different plant types would improve canopy light-use efficiency. A horizontal-leafed ("planophile") variety would capture radiation transmitted through erect-leafed variety, resulting in a reduction of light yield loss during the early growth stage, while an erect-leafed variety ("erectophile") would improve the light conditions at the lower canopy layer later in the season, suppressing the reduction in millable stalk numbers later in crop development due to mutual shading. Such habitat segregation could improve radiation-use efficiency and create a canopy that has adaptability at each growth stage.

Hypothesis 2: a varietal mixture with different tillering and ratooning abilities would compensate for reductions in bud sprouting and stalk production, resulting in a greater number of millable stalks at harvest.

Hypothesis 3: a varietal mixture with different sensitivities to lodging could achieve increased yield by the lodging-resistant variety supporting the sensitive variety as supporter or windbreaks, resulting in less damage to and less reduction in millable stalk numbers in the lodging-sensitive variety.

1.5.2 Optimum combination of varieties in a mixture to improve underground architecture.

The importance of root architecture to plant productivity comes from the fact that many soil resources are either unevenly distributed or subject to localized depletion (Lynch 1995). Water resource is the main limiting factor for sugarcane growth (Du et al. 1996; Jaiphong et al. 2016; Dinh et al. 2017) and the number of millable stalks is reduced under drought stress conditions (Robertson et al. 1999; Smit and Singels 2006). Root plasticity is induced under conditions of soil water deficit and contributes to plant survival under suboptimal conditions (Kano et al. 2011; Tran et al. 2015; Suralta et al. 2016). This plasticity was also reported in an intercropping system between sugarcane and other crop species, such as soybean (Li et al. 2012). In addition, many studies have shown that there were differences in root-growth patterns between varieties (Negi et al. 1971; Spaull 1980 Smith et al. 1999; Fukuzawa et al. 2009). Therefore, interactions between individual plants of different genotypes in terms of root growth and pattern should also occur in varietal mixtures, particularly when they have different root traits. In sugarcane, mixed varieties could also decrease the population size of underground pathogens, such as root rot *Pythium* (Srinivasan 1968), and harmful nematodes (Cadet et al. 2007); however, little research has been carried out on the effectiveness of sugarcane varietal mixtures with respect to biomass production, particularly root formation.

Hypothesis 4: a varietal mixture with different root elongation abilities could induce habitat segregation where a mixture of roots can penetrate the space less occupied under a single-variety crop population, resulting in denser root architecture. This type of root plasticity would improve soil resource-use efficiency.

1.6 Structure of the dissertation and general objectives.

In Chapter 2, four sections are arranged to answer five questions concerning the current status and further improvement of varietal diversity as described above in subsection 1.4.

Section 1: Quantitative evaluation of varietal diversity through monitoring long-term changes in variety use (see subsection 1.4.1)

Section 2: Functional diversity of varieties and its evaluation with reference to plant type (see

subsection 1.4.2)
Section 3: Questionnaire survey on current status of variety use (see subsection 1.4.3)
Section 4: Long-term evaluation of varietal performance following variety release and its effect on varietal diversity in the sugar industry (see subsection 1.4.4-5)

From these sections, the current status of varietal diversity in Japan will be explained, followed by a discussion of the effective distribution of varieties and management practices to maintain varietal diversity.

In Chapter 3, four sections are arranged as described below to investigate the four hypotheses proposed in subsection 1.5, concerning the morphological/physiological bases of varietal mixtures.

Section 1: a varietal mixture with different plant types (response to Hypothesis 1)
Section 2: a varietal mixture with different patterns of yield formation (response to Hypothesis 2)
Section 3: a varietal mixture with different rooting abilities (response to Hypothesis 4)
Section 4: a varietal mixture with different lodging resistances (response to Hypothesis 3)

From these sections, the effects of varietal mixtures on above- and below-ground biomass will be revealed, providing information on the optimum combination of varieties for individual mixtures for different conditions.

Finally, the objective of the present study is to reveal the current status of varietal diversity in Japan, to evaluate the potential use of varietal mixtures as a novel sugarcane cultivation method, and to discuss sustainable sugarcane production through the effective use of varietal diversity.

Chapter 2: Current status of varietal diversity and use after variety release in Japan

2.1 Quantitative evaluation of varietal diversity through monitoring long-term change in variety use.

2.1.1 Introduction

“Species richness”, a number of species which exist in a community, is the most general indicator of species diversity in plant communities (Sasaki et al. 2015). However, the corresponding parameter “variety richness” cannot fully explain diversity information concerning quantitative data, such as the number of individuals, coverage, cultivated area, and what kind of varieties are included. For example, consider two regions (A, B), which are assumed to contain five varieties: one variety occupies 90% of cultivation area in A while each variety occupies 20% of the cultivation area in B, meaning that varietal diversity is greater in B than in A. In this example, area composition is known as relative dominance. A higher evenness index occurs when relative dominance is equal among the varieties. Diversity index, which considers both richness and evenness, was developed for the quantitative evaluation of diversity in ecological studies (Simpson 1949; Sasaki et al. 2015). In addition, the concepts of α, β, and γ diversities are useful to express the diversity of varietal composition at regional scales, such as differences in diversity among regions (Whittaker 1965; Sasaki et al. 2015). Varietal diversity, diversification, and their importance in sugarcane have been recognized from the era of ‘NCo310’ (Nagatomi 1980; Miyahira 2000; Sato 2017). The present study is the first evaluation of sugarcane varietal diversity, taking into account both its richness and evenness, although a number of reports have focused on the cultivation area composition of each variety in each region.

The objective of the present study was to understand the current status of varietal diversity of sugarcane in Japan and to discuss how to operate, maintain, and optimize varietal diversity through monitoring long-term changes in varietal diversity using the diversity index, the evenness index, and α-β-γ diversities.

2.1.2 Materials and Methods

2.1.2.1 *Variety area data*

Variety area data were derived from annual statistical reports from each prefecture, named ‘Annual report of sugarcane and cane sugar (Satoukibi oyobi Kansyatou Seisanjisseki)’. These data were originally from receipts of harvested cane for each grower that were published by the sugar mill factories and compiled by each city, town, and village. ‘Others’ in these data contain the cases of unrecommended varieties or genotypes, variety mixtures, and “unknown” in each prefecture, (see Chapter 2.3 for details). For simplicity, ‘Others’ was considered to be one variety because this category should include at least one variety. Targeted regions and islands were northern Okinawa, central Okinawa, southern Okinawa, Iheya, Izena, Ie, Aguni, Kume, Minami Daito, Kita Daito, Ishigaki, Taketomi, Yonaguni, making up a total of 13 regions in Okinawa prefecture, and Tanegashima, Kikai, Amami Oshima, Tokunoshima, Okinoerabu, Yoron, a total of six regions in Kagoshima prefecture (Fig. 2.1.1).

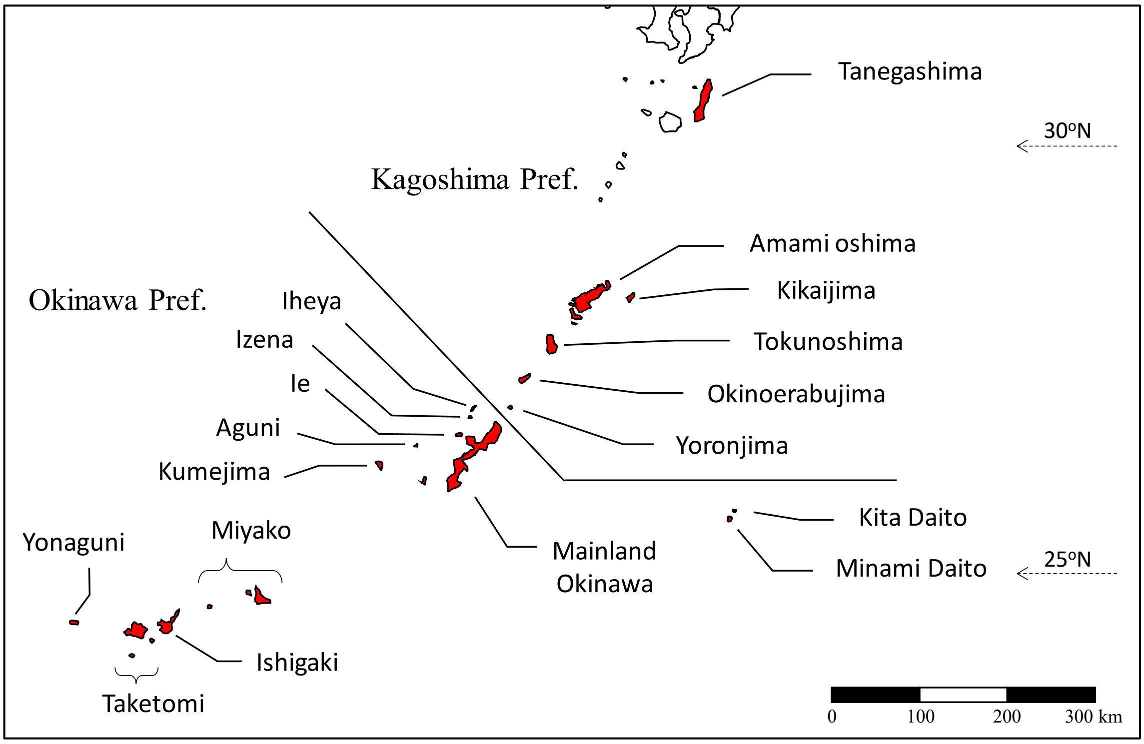


Fig. 2.1.1. Map of Nansei islands and component islands.
Note: red drawing means sugarcane cultivated region.

2.1.2.2 *Calculation of diversity parameters*

Simpson's diversity index (DI) and evenness index (EI) of sugarcane varieties in each region were calculated according to equations (2.1.1)–(2.2.2) as below (Simpson 1949; Sasaki et al. 2015):

$$\mathrm{DI} = 1/\sum_{i}^{S} Pi^{2} \qquad (2.1.1)$$

$$\mathrm{EI} = \mathrm{DI}/\mathrm{S} \qquad (2.1.2)$$

where S and Pi are the number of varieties harvested in each region and area percentage of i_{th} variety in each region, respectively.

The α, β, and γ diversities of sugarcane varieties in Okinawa prefecture, Kagoshima prefecture, and Japan were calculated according to equations (2.1.3)–(2.1.6) as below (Sasaki et al. 2015):

$$\alpha = \sum_{i}^{N} S_{i}/\mathrm{N} \qquad (2.1.3)$$

$$\gamma = \text{total number of varieties in each prefecture} \qquad (2.1.4)$$

where N and S_i are the numbers of regions and the number of varieties in i_{th} region, respectively. α and γ diversities indicate the mean number of varieties using values of all regions in each prefecture and the total number of varieties in each prefecture, respectively.

β diversity is an indicator of change and/or difference of varietal composition between regions, which is calculated by additive (equation (2.1.5)) and multiplicative equations (equation (2.1.6)) as shown below (Anderson et al. 2010; Sasaki et al. 2015):

$$\beta_{\mathrm{add}} = \sum_{i}^{N}(\gamma - \alpha_{i})/\mathrm{N} \qquad (2.1.5)$$

$$\beta_{\mathrm{w}} = \gamma/\alpha \qquad (2.1.6)$$

where β_{add} indicates the absolute number of varieties that exist only in the i_{th} region while β_{w} indicates the ratio of turnover of varieties between regions in each prefecture.

Results and Discussion

Variety richness, expressed as number of varieties grown or harvested, was different between regions and tended to increase in many regions from the late 2000s in Okinawa prefecture, while it showed a consistent tendency at 3–5 varieties in Kagoshima prefecture (Fig. 2.1.2a, b). EI was different between regions and fluctuated year-on-year (Fig. 2.1.2c, d). In Okinawa, many regions tended to show lower values below 0.4, although some islands, such as Iheya and Ie, showed greater EI in some seasons (Fig. 2.1.2c). On the other hand, many regions in Kagoshima tended to exhibit increased EI values greater than 0.4 from the late 2000s (Fig. 2.1.2d). Three varieties, each an equal area composition of 30%, are often suggested to optimize variety composition in the literature (Miyahira 2000; Takaragawa et al. 2018d). When the remaining 10% is assumed to be occupied by another variety, EI would be 0.89, which suggests that both prefectures should improve varietal bias to attain greater evenness. DI, which takes into account both richness and evenness of varieties, tended to differ between the regions in Okinawa, while it was stable and low in Kagoshima (Fig. 2.1.2e, f).

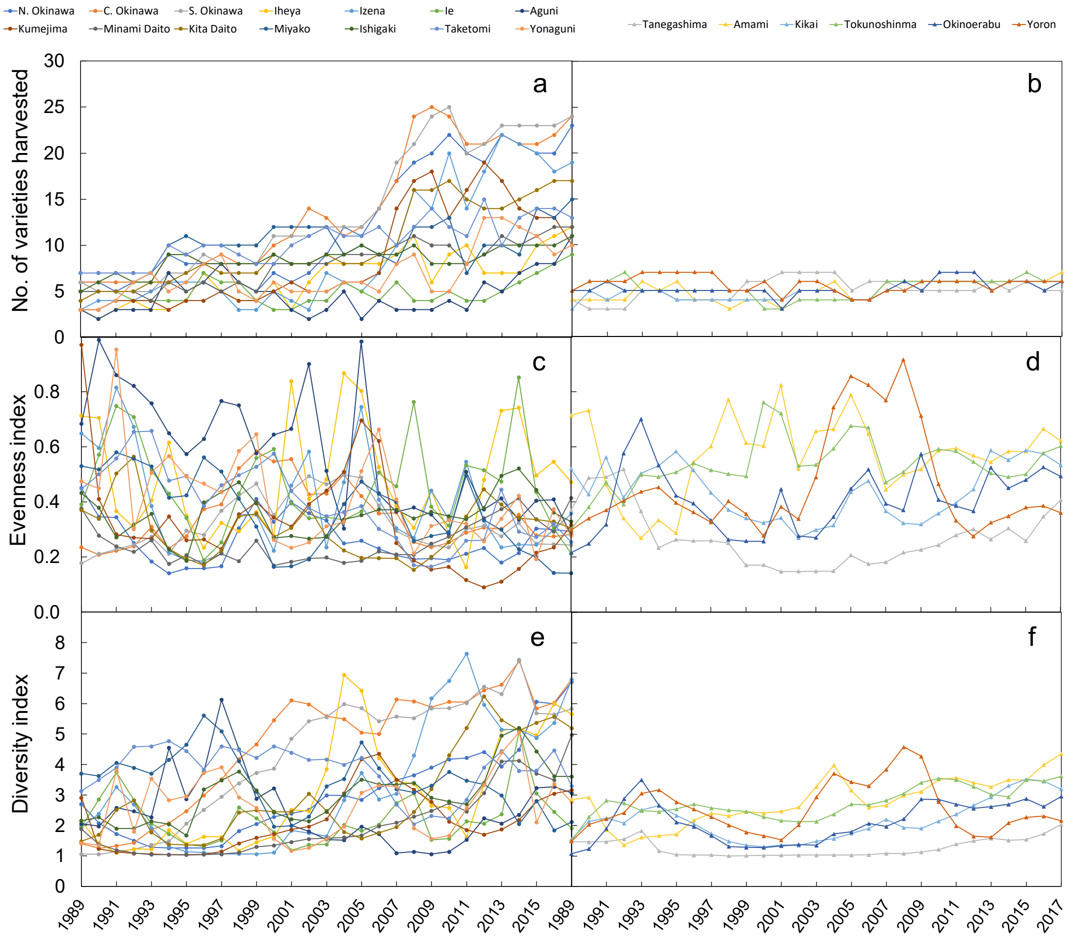


Fig. 2.1.2 Indicators of varietal diversity in each region.

Note: a, c, and e indicate number of varieties harvested, evenness index, and diversity index in Okinawa regions while b, d, and f indicate those in Kagoshima, respectively.

These results suggested that varietal diversity was very different between the two prefectures. To compare the trends in the two prefectures, richness, EI, and DI were compiled per unit of prefecture (Fig. 2.1.3). It was clear that the richness index tended to increase in Okinawa, while the evenness tended to increase in Kagoshima. DI was fluctuating, but tended to increase in Okinawa while the index in Kagoshima tended to keep on increasing until it finally approached the value in Okinawa.

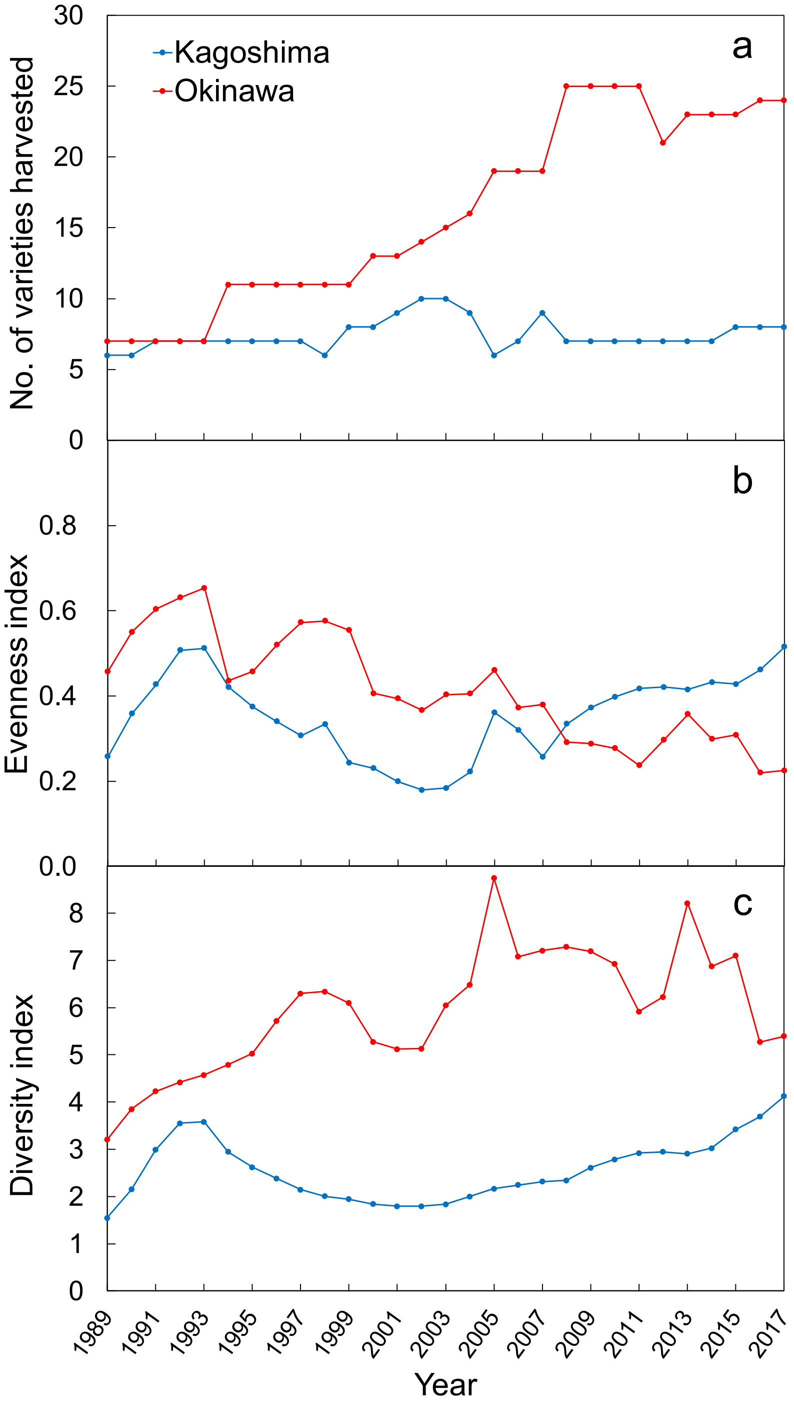


Fig. 2.1.3. Indicators of varietal diversity in each prefecture.
Note: a, b, and c show number of varieties harvested, evenness index, and diversity index in two prefectures.

DI was positively correlated with richness in Okinawa (r=0.698, *P*<0.01) and with evenness in Kagoshima (r=0.919, *P*<0.01) (Fig. 2.1.4). This difference appeared to be due to different policies operating in the different prefectures, where varieties were separately registered and propagated in each prefecture. Specifically, Okinawa prefecture preferred to register many varieties to supply them to growers for different environmental conditions, while Kagoshima prefecture carefully considered the possible alternative varieties to register, resulting in greater evenness of variety composition in Kagoshima.

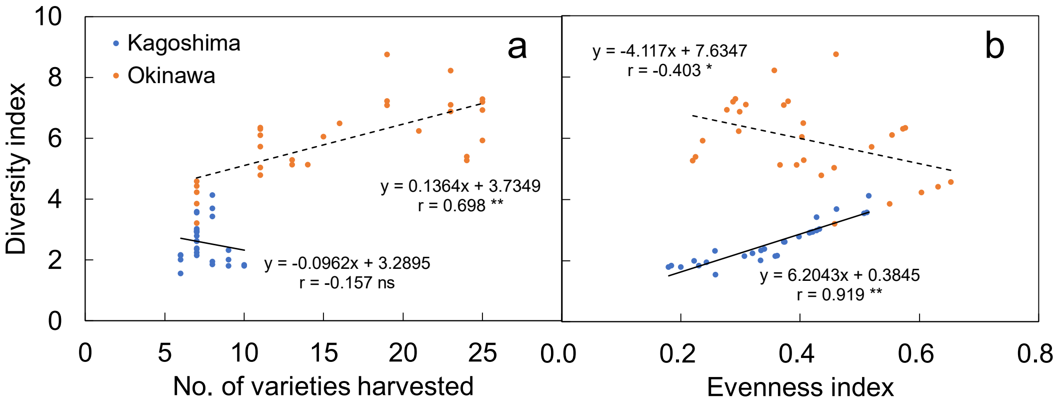


Fig. 2.1.4 Correlation of diversity index with number of varieties (a) and evenness index (b). Note: **, *, and ns mean significance at 1%, 5%, and no significance, respectively.

Although simple comparisons should not usually be accepted because the number of component regions was different between the two prefectures, Okinawa provided many options, which placed a heavy burden onto the growers to select suitable varieties and on the prefecture itself to propagate and maintain the many varieties. On the contrary, Kagoshima forced growers to select specific varieties, which could make the growers feel uneasy, but the prefecture itself and the sugar mill companies were able to control varietal selection and its composition.

In my opinion, a management system should be established to monitor and maintain varietal performance after release, with this system being necessary before many varieties are released and recommended. It should also be important, through extension work and the development of a guidebook, for growers to be made more aware of the concept of “optimum variety for optimum region”. To reduce the burden on the growers, which would be heavier for the management of different varieties after release, such as varietal selection and propagation, the number of varieties should be restricted carefully before release to control variety use after released to growers.

β diversity, varietal diversity at the regional scale i.e. diversity based on the difference of variety composition between regions, showed totally different trends between additive and multiplicative equations (Fig. 2.1.5c, d). β_{add} showed a clear difference between prefectures (Fig. 2.1.5c), while the difference of β_w between the prefectures was unclear (Fig. 2.1.5d). Greater β_{add} in Okinawa prefecture indicated greater diversity in the region. However, lower β_w in Okinawa indicated that the varieties cultivated and the varietal compositions were similar between regions in each prefecture despite the varietal option being much greater in Okinawa than in Kagoshima.

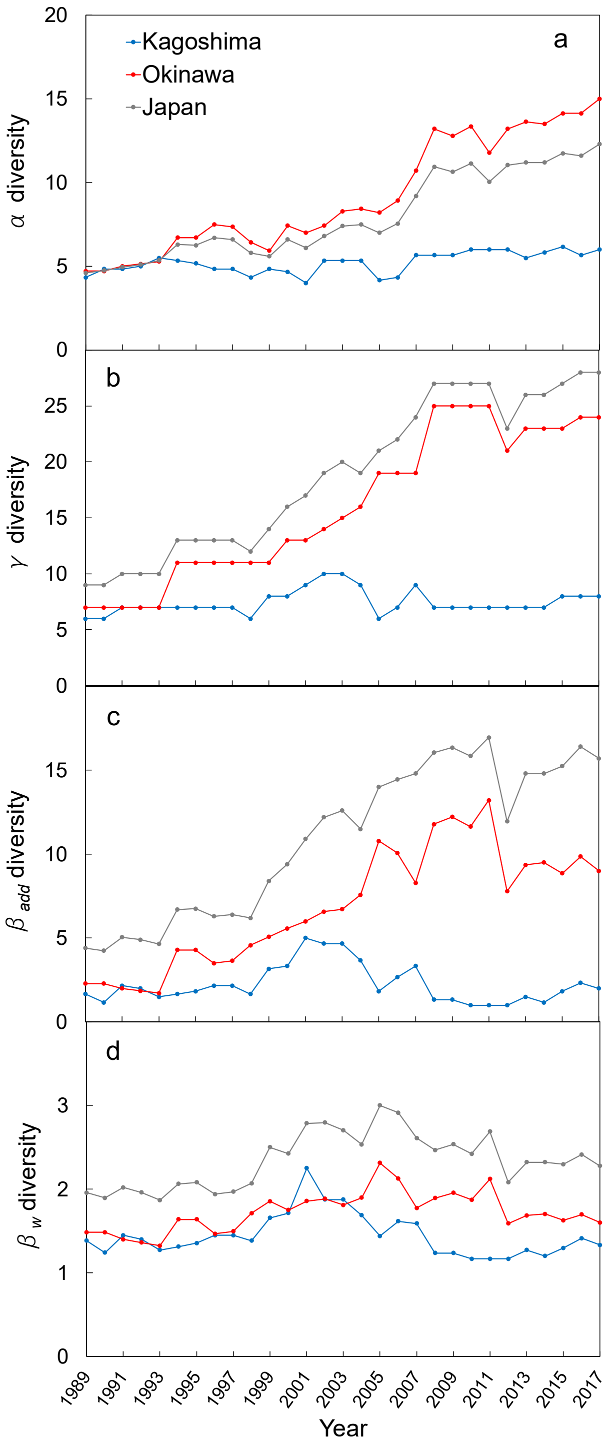


Fig. 2.1.5. Indicators of varietal diversity at regional scale in each prefecture.

Note: a, b, c, and d show α diversity, γ diversity, β_{add} diversity, and β_{w} diversity, respectively.

In the present study, varietal diversity, which had up to this point only been vaguely explained, was quantitatively evaluated using ecological indexes. In Japan, especially in Okinawa, many superior varieties for diverse regions have been developed through geo-ecological breeding (Irei 2011; Takaragawa et al. 2018e). However, the present study suggested that, although the breeding succeeded in providing many options and to contribute, to a certain extent, to the rich varietal diversity (Fig.

2.1.5a, b, c), only a proportion of these varieties would be acceptable to increase regional varietal diversity (Fig. 2.1.5d). This indicated that there was a limitation to increase regional varietal diversity only by increasing number of varieties. It was suggested that political direction with respect to variety registration and propagation, in order to control variety use, and to increase awareness among growers of the concept of an optimum variety for an optimum area and/or region, is essential for the efficient spread and maintenance of different varieties. Further follow-up education for growers as well as methods to effectively use such varieties are also required.

2.2 Functional diversity of varieties and its evaluation with example of plant type.

2.2.1 Introduction

Sugarcane yield is determined more by number than by the weight per stalks (Miller and James 1974; Shimabuku 1997). The number of stalks increases rapidly during the active-tillering stage and, then, decreases gradually because of light and nutrient competition. Light transmittance into the canopy is controlled by its structure (Shimabuku 1997; Zhou et al. 2003; Marchiori et al. 2010). Generally, the canopy structure depends on genetic traits, such as the degree of leaf erectness (Shimabuku 1997; Monsi and Saeki 2005) and tiller number (Takaragawa et al. 2016), and also on cultivation methods such as row spacing and planting density (Singels and Smit 2009). Optimal planting density is determined by local circumstances (Irvine et al. 1980). For example, the ridge width depends on machinery size (e.g., planter, cultivator, and harvester). However, the canopy structure could be optimized by the use of other methods. For example, mixing two varieties with different canopy structure improved the canopy growth by optimizing light interception (Takaragawa et al. 2016). Thus, there is a possibility that, by optimizing canopy structure using a diversity of leaf erectness and planting methods, the yield of sugarcane can be increased further.

Previous studies have examined the varietal diversity and the inheritance of leaf erectness (Shimabuku 1984), the relationship between the leaf traits and the yield components (Shimabuku and Kudo 1979; Nagatomi et al. 1982a, b), and the correlations between the light extinction coefficients and the sugarcane yield (Shimabuku 1997). Summarizing these studies comprehensively, Shimabuku (1997) suggested that breeding programs should focus on developing erect-leaf varieties in sugarcane. However, the evaluation of leaf erectness during the breeding process depends on visual observation by breeders (Terauchi 2013) (Fig. 2.2.1). Visual observation is convenient for quick evaluation but is less available for beginner because it is highly dependent on the experience of observer. To our knowledge, leaf erectness has not been considered as an important criterion for the selection of the best line likely because of the complex nature of the trait. Therefore, the clarification of the physiological mechanism controlling leaf erectness, as for rice (Sakamoto et al. 2006), has not been attempted for sugarcane. If methods can be developed to analyze leaf erectness quantitatively to produce an index using leaf morphological features, such an index would facilitate the breeding process and also contribute to the elucidation of the physiological and/or genetic mechanism.

The objective of the present study was to provide a new evaluation method for quantifying sugarcane leaf erectness using the leaf features.

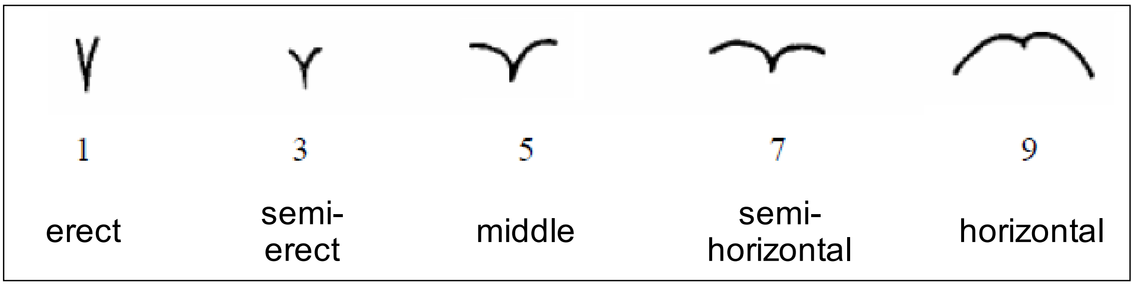


Fig. 2.2.1. Conventional sugarcane leaf erectness score through direct observation.
Note: Extracted from Terauchi (2013).

2.2.2 Materials and Methods

This study was conducted at the University of the Ryukyus, Japan (26°15′N, 127°45′E; altitude 127 m). Within the experimental field at this location, 37 sugarcane varieties and *Saccharum* species, including 33 Japanese varieties and one noble cane (*S. officinarum* L. cv. Badilla), were cultivated. Table 1 shows information on experimental materials. A leaf erectness scoring system using a visual-observation procedure (nine-grade evaluation, Fig. 2.2.1), was obtained from a bibliographic survey of scoring systems, such as the "Manual for sugarcane cultivation in Okinawa" (Okinawa Prefectural Government, Development of Agriculture, Forestry and Fisheries 2015). The varieties were selected from our collection which has information about conventional leaf erectness score observed by breeders. Single-bud sugarcane setts were soaked in a mixture of water and fungicide (5 g L^{-1} of Benlate-R, Sumitomo Chemical) for one day and, then, were planted in seedling trays in a greenhouse on 21 March 2016. The germinated setts were transplanted in the field after 40 days at a planting density of 3.33 plants m^{-2} (spacing between germinated setts was 0.3 m, row spacing was 1.0 m). The size of experimental plots for each variety was not fixed and with no replicate plot due to varying number of setts, but minimum plot size was 6.7 m^2. Fertilizer was applied on 24 May and 6 July; total amounts of N, P_2O_5, and K_2O were 134, 50, and 50 kg ha^{-1}, respectively. Morphological measurements were taken on 10 August (102 days after transplanting) on four stalks from each variety under a windless condition. No serious damage was observed on the leaves before these morphological measurements were made. The following measurements were taken on the first (upper, younger), third (middle), and fifth (lower, older) fully expanded leaves: leaf length, leaf width, acute angle between stalk and basal part of leaf, horizontal distances from the stalk to leaf peak and leaf tip, and heights from ground level to dewlap, leaf peak, and leaf tip. Total number of leaves per stalk was also measured. Based on these measurements, a leaf silhouette of each variety was drawn according to Udagawa et al. (1968) (Fig. 2.2.2). After the leaf morphological measurements were completed, leaves were collected to measure leaf area (LI3100, LI-COR) and dry weight. The results of the prior measurements were used to calculate the leaf shape index (length/width) and the specific leaf area (SLA).

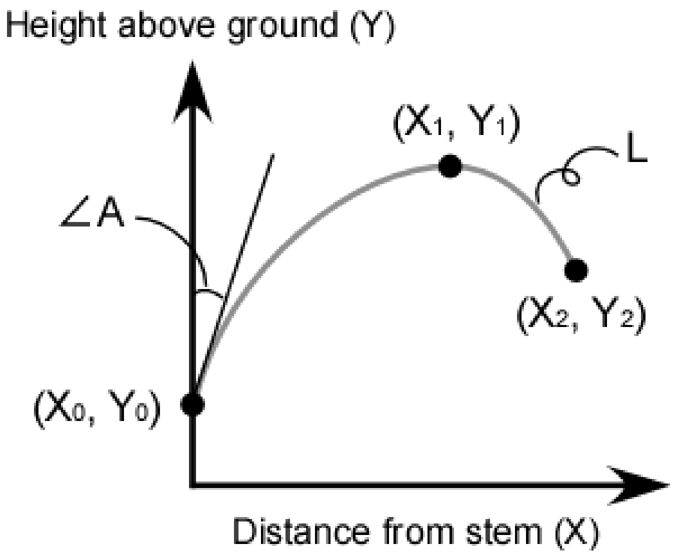


Fig. 2.2.2. The parameters of leaf to draw the silhouette of its curve.
Note: According to the method of Udagawa et al. (1968).
Coordinate (X_0, Y_0), (X_1, Y_1), and (X_2, Y_2) mean leaf dewlap, leaf peak, and leaf tip, respectively. A is an acute angle between stem and basal part of leaf. L is a length of leaf.

To evaluate differences that may occur among growth stages and environmental condition, a pot experiment was conducted using the plant propagation materials of varieties NCo310, NiF4, NiF8, NiTn10, Ni12, Ni22, Ni27, and RK97-14. Four single-bud germinated setts per variety were transplanted (on the same date as the field experiment) in each 1/5000a Wagner's pot filled with mixed soil (dark red soil, sea sand, and peat moss with the ratio of 1:1:1, v v^{-1}). Plants were well watered and fertilized with Hoagland solution containing 6 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 100 µM $C_{10}H_{12}FeN_2NaO_8$, 25 µM H_3BO_3, 10 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, and 0.5 µM H_2MoO_4. Leaf morphological measurements were made on 24 June (85 days after transplanting) using the same methods as were used in the field experiment.

Correlation analyses were done with statistical analysis software (Excell-Tokei 2010, Social Survey Research Information Co., Ltd.) using the average values of upper leaf features measured.

2.2.3 Results and Discussion

Sugarcane had varietal diversity in leaf erectness as shown by photographs (Fig. 2.2.3). Total leaf number was ranged from 7 to 13 in all varieties when measurement was done. The diversity was shown clearly also by leaf silhouettes of upper (first), middle (third), and lower (fifth) leaves (Fig. 2.2.4). When all the varietal leaf silhouettes for each leaf position were placed on the same figure (positioned so that leaf dewlaps were aligned), a wide variation in leaf silhouettes was observed especially for upper leaves, while smaller variations occurred among middle and lower leaves which were oriented more horizontally (Fig. 2.2.5). Of the leaf features shown in Fig. 2.2.2, leaf tip height was the feature that seemed to be well related with leaf erectness (upper leaf silhouette of Fig. 2.2.5). Furthermore, leaf erectness score significantly correlated with the height from dewlap to the tip of upper leaf ($r = -0.62$, $P < 0.01$) (Table 2.2.2). Correlation coefficients were also significant between leaf erectness score and some parameters based on the height of leaf tip (Table 2.2.2). For eight of the sugarcane varieties used in pot experiment, almost similar correlation was obtained. Among several parameters based on the height of leaf tip, the difference between the parameters derived from field and pot experiments was smaller in the ratios using leaf tip height while the subtraction of leaf tip height to aboveground height of its dewlap of upper leaf overestimated under pot condition (data not shown). In addition, an index for our study is better if the plant growth using stem height (i.e. height of its dewlap of upper leaf), the general indicator of growth, were considered and index were derived from a simple ratio of two traits that were conveniently measured in the field. From these results, a derived sugarcane leaf erectness index (LEI) was calculated and was defined as the ratio of leaf tip height to aboveground height of its dewlap of upper leaf. The LEIs of the sugarcane varieties examined in our study ranged from 0.36 to 2.55, with higher values associated with more erect-leaf varieties (Table 2.2.1).

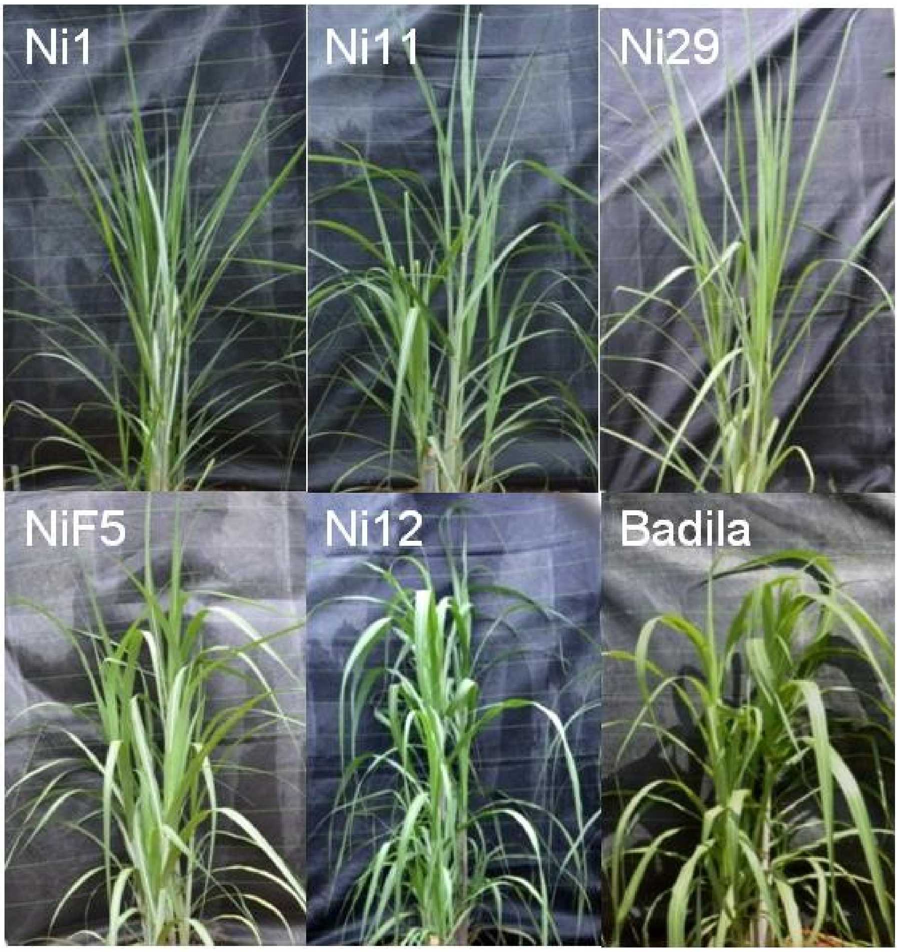


Fig. 2.2.3. Varietal differences of sugarcane leaf erectness.

Note: Pictures were taken on 8 August in the field experiment.

A short, erect leaf could give the same LEI value as that of a long, horizontal leaf. Even though various leaf silhouette patterns can produce the same LEI value (Fig. 2.2.6), leaf tip height can still be important for leaf erectness evaluation (upper leaf silhouette in Fig. 2.2.5 and Table 2.2.2). Silhouettes A, D, and H in Fig. 2.2.6 are similar; however, they have different heights of leaf tips, which could be discriminated by LEI. Additionally, silhouettes A and E in Fig. 2.2.6 were unlikely from our result of each varietal leaf silhouette. Consequently, it may be possible to adequately evaluate and differentiate plant types having the same LEI value based on various leaf silhouette patterns. That may be why there existed the discrepancy between leaf erectness score and LEI ($r = -0.61$, $P < 0.01$) (Table 2.2.2). Therefore, we should develop another sub-trait or sub-index to classify varieties having similar LEI values into more specific groups. This will require further research using many more varieties.

Table 2.2.1. Information of varieties used in this study.

Variety	CLES	LEI$_{field}$	LEI$_{pot}$
Ni1	1	2.18	-
NiN2	5	0.97	-
NiF3	5	1.04	-
NiF4	3	1.36	1.51
NiF5	7	0.73	-
Ni6	5	0.83	-
NiN7	5	1.32	-
NiF8	4	0.90	0.93
Ni9	5	0.91	-
NiTn10	1	1.19	1.77
Ni11	1	2.55	-
Ni12	7	0.69	0.90
Ni13	3	1.45	-
Ni14	5	1.12	-
Ni15	3	1.20	-
Ni16	5	1.28	-
Ni17	6	0.83	-
NiTn18	2	0.71	-
NiTn19	1	1.35	-
NiTn20	5	0.85	-
Ni21	4	0.86	-
Ni22	5	0.76	0.97
Ni23	5	0.94	-
NiN24	2	0.89	-
NiH25	5	0.75	-
Ni26	4	1.07	-
Ni27	3	0.83	1.30
Ni28	5	0.86	-
Ni29	1	2.21	-
KN00-114	3	0.98	-
KY99-176	3	1.43	-
RK97-14	5	0.83	1.20
RK96-6054	1	1.63	-
NCo310	5	1.01	1.31
NCo376	5	1.35	-
F161	1	0.90	-
Badila	5	0.36	-
Average	3.81	1.11	1.24
CV	0.47	0.40	0.24

Note: CLES, conventional leaf erectness score; LEI$_{field}$, leaf erectness index under field conditions; LEI$_{pot}$, leaf erectness index under pot conditions.

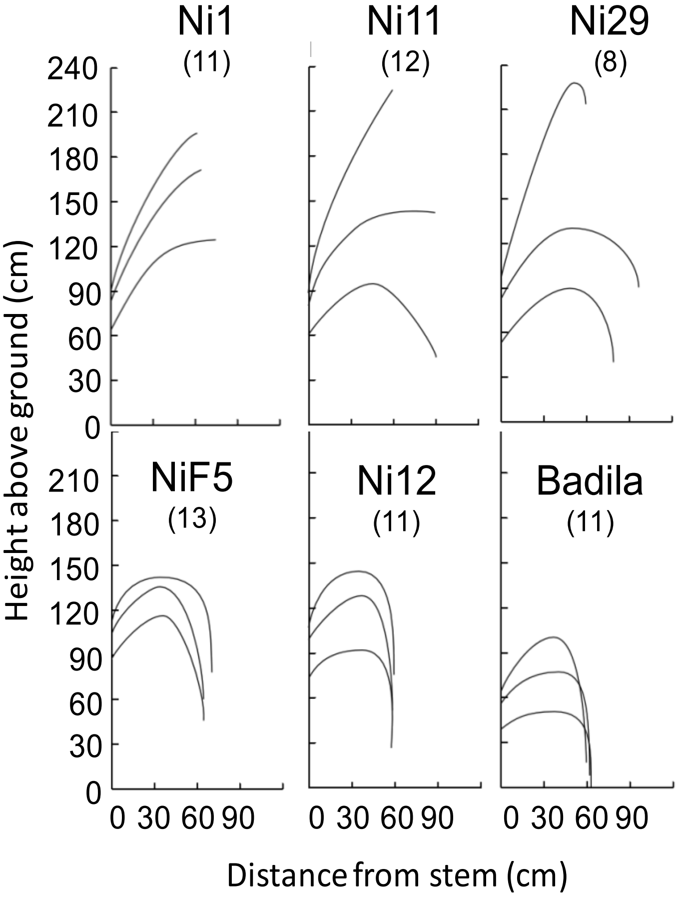


Fig. 2.2.4. Varietal differences of leaf silhouette.

Note: Three curves indicate the silhouettes of upper, middle, and lower leaves. Number in parenthesis means total leaf number of each variety.

Table 2.2.2. Correlations between conventional leaf erectness score and some leaf features.

Condition	Angle	Length	Width	Y_0	Y_1	Y_2	X_1	X_2	Y_1-Y_0	Y_2-Y_0	(Y_2-Y_0) /X_2	Y_2/Y_0	Y_2/L
Field	0.25	0.10	0.22	0.32	-0.02	-0.46 **	-0.20	-0.20	-0.41 *	-0.62 **	-0.58 **	-0.61 **	-0.62 **
Pot	-0.11	-0.07	0.46	0.48	0.05	-0.53	-0.31	0.12	-0.58	-0.83 *	-0.85 **	-0.83 *	-0.50

Note: X_1 and X_2 indicate horizontal distances from main stem to leaf peak and tip, respectively. Y_0, Y_1 and Y_2 indicate heights from ground level to dewlap, leaf peak and leaf tip, respectively. L means leaf length. * and ** mean significant correlation at 5 and 1%, respectively.

For eight of the sugarcane varieties used both in our pot and field experiments, the LEIs obtained from the pot experiment ($\mathrm{LEI}_{\mathrm{pot}}$) and the indices from the field experiment ($\mathrm{LEI}_{\mathrm{field}}$) were significantly correlated ($P < 0.05$), but $\mathrm{LEI}_{\mathrm{pot}}$ indices were higher than the $\mathrm{LEI}_{\mathrm{field}}$ indices (Fig. 2.2.7). This discrepancy was likely due to differences in the date on which measurements were made and the fact that younger stalks in the pot experiment may have had shorter inter-node lengths, which resulted

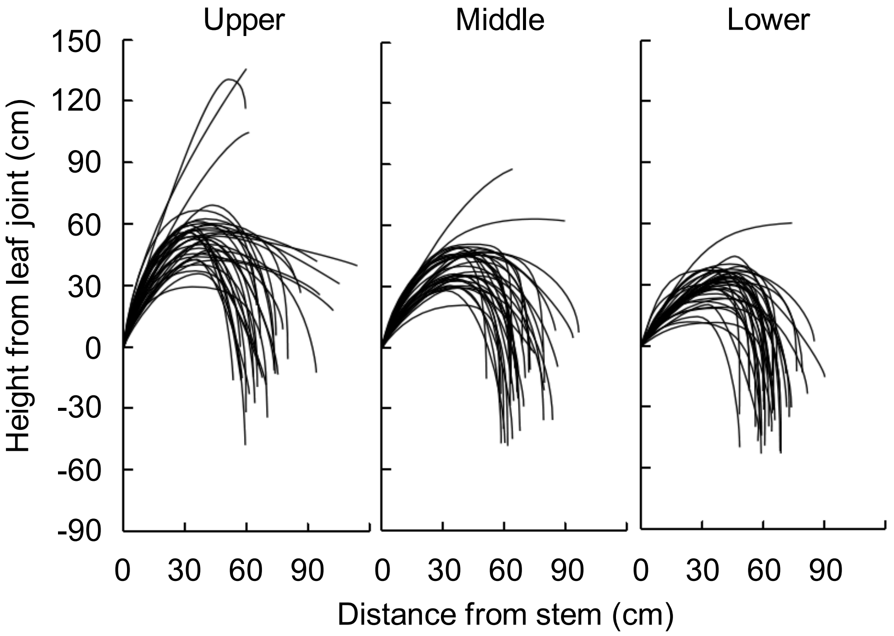


Fig. 2.2.5. Varietal leaf silhouette of each leaf position where aboveground heights of dewlaps were aligned as zero.

Note: Each curve shows the leaf silhouette of each variety.

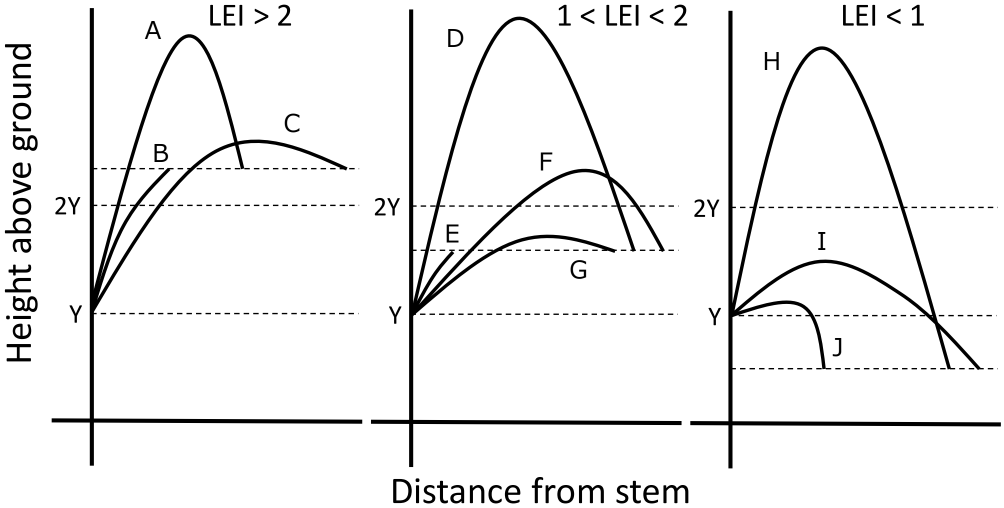


Fig. 2.2.6. Various pattern of leaf silhouette with the same value of LEI.

Note: Each curve shows the leaf silhouette assumed. LEI>2: A, higher peak height; B, short leaf; C, lower peak height and longer distance of leaf tip from stem. 1<LEI<2: D, higher peak height; E, short leaf; F, lower peak height and longer distance of leaf tip from stem; G, lower peak height and short leaf. LEI<1: H, higher peak height; I, lower peak height and longer distance of leaf tip from stem; J, short leaf.

in shorter dewlap heights (data not shown). Using the sugarcane variety “NCo310” as the standard and calculating LEIs as relative values with LEI of NCo310 being 1.0, the difference in index values between LEI_{pot} and LEI_{field} was minimized (Fig. 2.2.8). Therefore, relating the LEIs of varieties of interest to the LEI of a standard variety allows the evaluation of LEI more precisely as the growth stage and the environmental conditions change over time.

Correlation analyses were conducted between the LEI and a variety of leaf features to understand which features were related to LEI better (Table 2.2.3). The results showed that the LEI correlated with leaf angle ($r = -0.33$, $P < 0.05$), which may indicate a relationship between leaf erectness and the rigidity of the basal part of leaves (Table 2.2.3). Previous studies have reported that wider leaves do not always have thicker midribs, and that erect leaves often have thick midribs with a high proportion of midrib weight and area to total leaf weight and area (Shimabuku and Kudo 1979). The rigidity of a basal part of a leaf may reflect the rigidity and thickness of its midrib. Even though midrib characteristics were not a focus of our study, we speculate that leaves with strong and thick midribs are more erect and that their tips are higher.

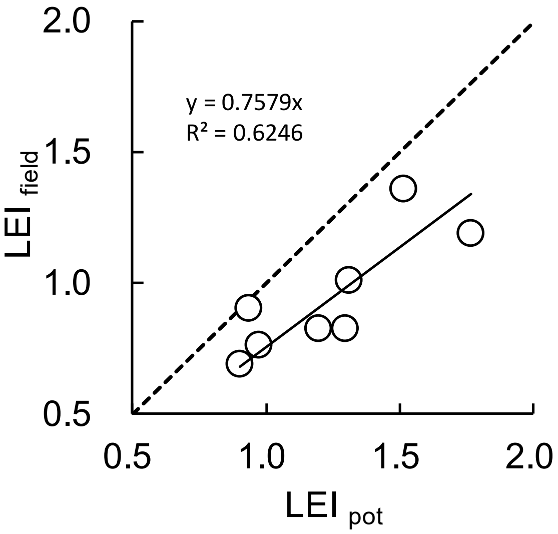


Fig. 2.2.7. Correlation between LEIs measured in field and pot experiments.
Note: Dotted line means the linear equation Y=X.

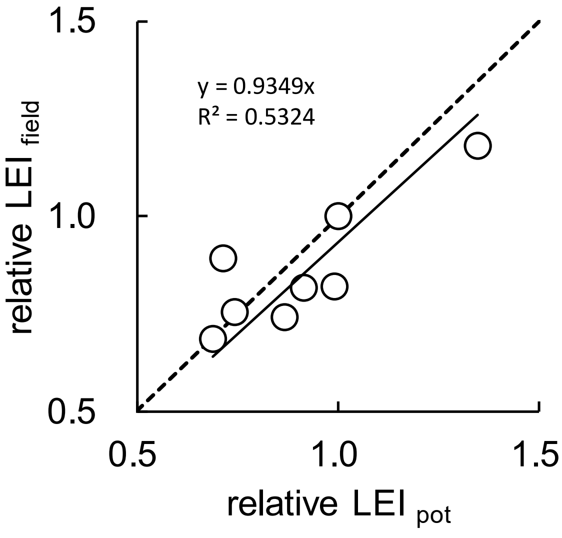


Fig. 2.2.8. Correlation between relative LEIs measured in field and pot experiments.
Note: LEIs in this figure were calculated as relative values with LEIs of NCo310 being 1.0. Dotted line means the linear equation Y=X.

Values of LEI were also significantly correlated with the leaf shape index ($r = 0.43$, $P < 0.01$) and SLA ($r = –0.45$, $P < 0.01$) (Table 2.2.3), indicating that erect leaves tend to be narrow, long, and thick. Terauchi and Matsuoka (2000) suggested that low SLA was a limiting factor for the early growth of sugarcane. They concluded that shorter and thinner leaves were ideal for optimizing early growth. Our results showed erect-leaf varieties of sugarcane have high leaf shape index and low SLA (Table 2.2.3) which may be disadvantageous during early growth stages. Shimabuku (1997) suggested that horizontal-leaf varieties of sugarcane show high leaf area index and are ideal for early growth because they develop leaves faster to capture radiation, while erect-leaf varieties show high net assimilation rates (the indicator of canopy photosynthesis) and are ideal for later growth periods after the canopy has developed so that radiation more easily penetrates to lower canopy leaves. Unfortunately, Shimabuku (1997) concluded that it was difficult to develop a sugarcane variety with ideal leaf features for both early and later growth. The cultivation of mixtures of varieties with varying canopy structure may lead to better light interception (Takaragawa et al. 2016b), at specific growth stages for the overall field population, but when it comes to individual varieties this will not serve the purpose. The LEI value could also be useful to select varieties to be mixed from many varieties with diverse leaf erectness as the present study showed.

Table 2.2.3. Correlations between LEI and some features of upper leaves.

Angle	Length	Width	LSI	LA	SLA
-0.33	-0.03	-0.46	0.43	-0.39	-0.45
*		**	**	*	**

Note: LSI, leaf shape index; LA, one leaf area; SLA, specific leaf area. * and ** mean significant at 5 and 1%, respectively. Data was obtained from field survey.

2.3 Questionnaire survey on current status of variety use

2.3.1 Introduction

Establishing growing practices and varieties suitable for specific regions/areas is important to achieve increased sugarcane productivity (Satoukibi Zousan Project Kaigi 2005; Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2015). Breeders develop promising clones selected according to the demands of growers while varieties are selected according to evaluation by sugar mill workers during process of 6-7th generations. Then, varieties are registered considering their role in the recommended regions (Takaragawa et al. 2018e). It takes at least 12 years to select and register a new sugarcane variety, and a further few years to propagate sufficient planting material stock to distribute to growers in breeding program (Sato et al. 2018). Therefore, there is a time lag from the grower's demand to distribution of planting stocks of the new variety to growers, associated with spending an enormous amount of time, labor, and money. A previous study had suggested that not only breeding new varieties, but also utilizing and maintaining the existing diverse varieties was required for sustainable sugarcane production (Takaragawa et al. 2015). In addition, the effective use of existing varieties may contribute to improvements in the cost-benefit of plant breeding, and ultimately contribute to the future sugarcane sustainable breeding program in Japan.

While many superior varieties have been released and recommended in Japan, there is plenty of information available on varieties before or at the time of release, but little after the distribution of stocks to the growers. Therefore, the current status of sugarcane variety use in practice in Japan has never been revealed. The status of variety use at grower level and growers' demand for new varieties are considered to be key factors in the breeding cycle. Therefore, a better understanding of the situation after variety release should contribute to promoting an efficient sugarcane breeding program.

Generally, growers select crop varieties on the grounds of personal preferences and their suitability to the prevailing conditions at each grower's site (Ooe et al. 1993; Nakamura et al. 2005; Wakabayashi 2014). While varieties in other crops such as rice and fruit are considered to be brand products, consumers (i.e. sugar mill companies and public consumers) are not interested in the variety name in sugarcane, as the final product is purified sugar. On the contrary, sugarcane growers pay much attention to varieties and select them with regard to their scale of business (Arai and Nagata 2009; Imai and Nakajima 2014). Nevertheless, based on personal observations and on interviews with extension workers, growers often cannot avoid to mixed-plant different varieties due to complementary planting (no data reported). As growers and even sugar mill companies are only interested in sugar extraction from sugarcane, there is no strict policy and/or direction to control or manage the use by growers of particular varieties at present, singly, or in mixtures. Some regions and sugar mill companies have attempted to control and diversify the varieties grown, but potential benefits and problems from the cultivation of several different varieties have never been publicly discussed. To grasp the current status of variety use such as several varieties use and inactive mixture is required prior to make an efficient plan to manage variety use.

The objective of this study was to reveal the current status of variety use in Japan, using the analysis of statistical data from both prefectures and an analysis of responses to a questionnaire survey with growers and extension workers.

2.3.2 Materials and Methods

2.3.2.1. Evaluation of conventional statistical varietal area data.

Variety area composition data were derived from the annual statistical report named 'Annual Report of Sugarcane and Cane Sugar (Satoukibi oyobi Kansyatou Seisanjisseki)' from each prefecture, and those from each region for the season 2016/2017 were analyzed. These data were originally from receipts of harvested cane for each grower, which are published by each sugar mill factory and compiled by each city, town, and village. Current recommended varieties are shown in Table 2.3.1 (December 2018). Note that varieties 'RK97-14' ('Ni33') for Okinawa and 'Ni27' for Kagoshima were registered only in 2016 and were included in 'Others' in data from the season 2016/2017. 'Others' in these data include varieties occupying less than 1% of the cultivated area, non-recommended varieties or strains, mixture and "unknown" in each prefecture. There is no clear definition of 'Others' and the detail in this category was not available in the published statistical records. The details of and reasons for using 'Others' were investigated through interviews with sugar mill workers using phone calls and e-mail, but only for regions where 'Others' occupied a marked proportion of the cultivated area composition. In addition, the areas associated with ratoon cropping, which means areas under long-term monoculture, were calculated using the data of the total and ratooning areas in 2016/2017.

Table 2.3.1. Recommended varieties in Japan (2018/12).

Prefecture	Variety	Former line	Registered year	Reccomended area
Okinawa	F161	Imported	1979	Miyako, Yaeyama, Daitoh
	Ni9	RK80-1010	1991	Whole area
	NiF8	KF81-11	1994	Whole area
	Ni15	RK90-0039	2002	Whole area
	Ni17	RK91-1004	2003	Kumejima
	Miyako 1	-	2005	Miyako
	NiTn20	KF92T-519	2006	Southern mainland, Yaeyama
	Ni21	RK94-4035	2006	Kumejima
	Ni22	KY96-189	2008	Yaeyama
	NiN24	KN91-49	2008	Southern mainland
	NiH25	RH86-410	2008	Miyako
	Ni26	RK95-1	2008	Daitoh
	Ni27	KR96-93	2010	Miyako, Yaeyama
	Ni28	RK96-6049	2010	Daitoh
	Ni29	RK97-7020	2010	Daitoh
	Ni31	KY99-176	2013	Miyako
	Ni33	RK97-14	2016	Whole area
Kagoshima	NiF8	KF81-11	1990	Whole area
	Ni17	RK91-1004	2001	Amami
	NiTn18	KF92-93	2003	Tanegashima
	Ni22	KY96-189	2005	Whole area
	Ni23	KY96T-537	2007	Amami
	NiN30	KN00-114	2011	Amami
	NiTn32	KTn03-54	2013	Tanegashima
	Ni27	KR96-93	2016	Amami

Note: RK97-14 in Okinawa and Ni27 in Kagoshima have just been registered in 2016 currently under propagation which were included in ‘Others’ in 2016/2017.

2.3.2.2. Questionnaire survey on variety use of workers and researchers involved in sugarcane.

The questionnaire survey was conducted at the annual meeting for sugarcane production and involved researchers at Okinawa prefectural Agricultural Research Center in Itoman city, Okinawa on October 6th, 2016. This meeting place was considered to be the most useful forum at which to compile many opinions because all sugarcane workers, in the areas of research, extension work, sugar mill, and administration participated in the meeting. Questionnaire sheets were distributed to all participants and completed sheets were returned by a total of 64 persons. Of the respondents, 25, 31, 10, and 34% were sugar mill workers, researchers, administrators, and others, respectively.

2.3.2.3. Interview survey with growers on variety use.

The interview survey on use of different varieties, singly, or in mixtures, was conducted with 18 growers in Minami Daito on 16–17th March 2016 and with 12 growers in Tokunoshima on 22–25th March 2016. Minami Daito and Tokunoshima are among the most productive regions in Japan with annual production rates of approximately 100,000 and 220,000 ton, respectively. Growers for interview were introduced by each sugar mill worker because the time period for survey was limited and this format was used to mitigate the burden on the interviewed growers, such as complicated explanations about varietal mixtures. Finally, the valid answers were collected from them and the data collated.

2.3.3. Results and Discussion

2.3.3.1. Current status of varietal composition and details of composition of category 'Others' in each region.

The number of harvested varieties that occupied at least 1% of the cultivated area numbered only three in Miyako and Tanegashima compared to between four and 11 varieties in other regions (Table 2.3.2). Regions where one variety occupied more than 30% of the cultivation area were Irabu ('Ni27', 86%), Miyako ('Ni27', 71%), Tanegashima ('NiF8', 74%), Hateruma ('Ni27', 63%), Ie ('Ni27', 63%), Yoron ('Ni23', 61%), Tarama ('Ni27', 56%), Kume ('Ni21', 50%), Yonaguni ('Ni15', 48%), Aguni ('Ni27', 46%), Okinoerabu ('NiF8', 45%; 'Ni23', 41%), Iriomote ('Ni27', 44%; 'NiF'8, 37%), Ishigaki ('NiH25', 42%), Kohama ('NiH2'5, 41%; 'Ni27', 40%), Tokunoshima ('NiF8', 38%; 'Ni23', 34%), Izena ('NiH25', 35%), and northern Okinawa ('NiF8', 33%), where sugarcane production depended heavily on one or sometimes two specific varieties. In particular, two varieties were dominant, making up a total proportion of around 80% in Iriomote and Kohama. Regions where one variety and ratoon cropping were dominant such as Kume, Miyako, Tanegashima, and Yoron may have the potential risk of long-term monoculture of one variety in one field. Such monocultures would accelerate disease spread due to a simplification of soil microorganism flora and the accumulation of pathogens. The risk associated with a limited varietal profile was recognized as a problem almost 20 years ago (Miyahira 2000), but the situation has still yet to be improved in some regions where greater diversification of varieties is required. To avoid dependence on one variety in these regions,, three or four varieties at same composition rates exhibiting biotic and abiotic stress tolerance are needed to optimize varietal area composition (Miyahira 2000). Interestingly, regions where one variety occupied >30% of the cultivation area also had a higher composition of 'Others', especially in Okinawa. The percentage of 'Others' was region-dependent from 3% in Ishigaki to 44% in Minami Daito. Interview surveys were conducted with sugar mill workers in regions with high proportions of 'Others', such as central and southern Okinawa, Minami, and Kita Daito, Iheya, Amami Oshima, Kikai, and Yoron, in an attempt to understand the reason for the cultivation of such a large area to 'Others'. According to the results of the interviews, in Minami Daito, where 'Others' was the highest at 44%, there were unrecommended varieties or genotypes such as 'RK96-6054' and 'RK97-14' and some mixtures in 'Others'. In Kita Daito, unrecommended varieties such as 'KN00-114' and 'Ni23', imported by growers and with high regional adaptabilities, were present at a high frequency in 'Others'. No specific varieties were dominant in 'Others' in central and southern Okinawa. It was estimated that the diverse preferences in each region and the high frequencies of variety mixtures were mainly due to the greater ratooning area

Table 2.3.2. Information of variety use in each region.

Prefecture	Region	No. of varieties with <1% area composition	Varieties with <30% area composition		Varieties as 'Others' (%)	Ratoon area (%)
			No.	Name (Area, %)		
Okinawa	Norther Okinawa	11	1	NiF8 (33)	11	77
	Southern Okinawa	10	0	-	32	74
	Central Okinawa	9	0	-	30	70
	Izena	10	1	NiH25 (35)	7	66
	Kume	7	1	Ni21 (50)	6	66
	Kita Daito	10	0	-	25	68
	Minami Daito	6	0	-	44	78
	Miyako	3	1	Ni27 (71)	9	55
	Irabu	5	1	Ni27 (86)	6	34
	Ishigaki	7	1	NiH25 (42)	3	41
	Ie	5	1	Ni27 (61)	1	12
	Aguni	4	1	Ni27 (46)	5	3
	Yonaguni	7	1	Ni15 (48)	1	61
	Iriomote	6	2	Ni27 (44), NiF8 (37)	5	22
	Iheya	7	0	-	28	54
	Tarama	7	1	Ni27 (56)	0	19
	Kohama	5	2	NiH25 (41), Ni27 (40)	1	40
	Hateruma	5	1	Ni27 (63)	3	19
Kagoshima	Tanegashima	3	1	NiF8 (74)	1	67
	Amami Oshima	4	1	Ni22 (33)	24	68
	Kikai	4	1	Ni23 (35)	32	63
	Tokunoshima	5	2	NiF8 (38), Ni23 (34)	14	73
	Okinoerabu	4	2	NiF8 (45), Ni23 (41)	8	67
	Yoron	5	1	Ni23 (61)	19	82

Note: 2016/2017.

(<70%) and multiple ratooning. In addition, these regions may contain too many growers and fields than the other isolated island areas to survey for details of 'Others', resulting in the vagueness of the information. In Iheya, unrecommended varieties were not used as much and the high frequency of 'Others' was attributed mainly to mixtures. The inability of many growers to prepare enough seedling area in a nursery field for the targets variety resulted in the inactive use of mixtures.

In the 2016/2017 season, the sugar mill company in Iheya started to supply cane top after detrashing process, including unspecified varieties, as seedlings that promoted the proportion of mixtures which were planted. In Amami Oshima, 'Others' were represented mainly by unrecommended varieties such as 'NiTn20' and 'Ni21' and partly by mixtures. In Kikai, 'Others' mainly included unrecommended varieties such as 'Ni9' and 'F177' and partly mixtures. In Yoron, the composition of 'Others' was mainly mixtures and, to a lesser extent, unrecommended varieties such as 'Ni21' and 'F177'. Therefore, the composition of the 'Others' category was region-dependent, being either mainly unrecommended varieties or varietal mixtures. Although 'Others' is not recommended to estimate a sugarcane production and to prepare official seedling field in each prefecture, it included at least one variety, which suggests that high proportions of 'Others' could reflect diverse varieties selected by growers. There is no consensus for the definition or classification of 'Others', even in some guidelines used by municipalities and sugar mill companies (e.g. 'Guideline for Production

Estimation Survey (Satoukibi oyobi Kansyatou Seisan Mikomi Chousa Youryou)' (Okinawa Prefectural Government, Department of Development of Agriculture, Forestry and Fisheries 2017); to my knowledge, each institute has adopted different definitions (sugar mill company, municipality, prefecture, etc.). With respect to varietal mixtures, there are many cases where a worker records a situation where one variety is clearly dominant in a mixed-variety crop as not a mixture, or where another worker records the crop to be a mixture regardless of the relative proportions of the mixture. In order to establish an effective system to monitor variety use, guidelines should be modified to clearly specify the consensus for 'Others' and mixtures.

Unrecommended varieties such as 'KN00-114' and 'Ni23' in Kita Daito and 'Ni27' in Kagoshima (note that this variety has already been registered and recommended in Kagoshima in December, 2017, although it was classified as "unrecommended" during the 2016/2017 survey period) were grown in prefectures different from those that registered them as recommended ones. This observation was a good example of the effective use of existing varieties on a regional scale. However, the potential problem with unrecommended varieties may be the propagation of seedlings that are not disease-free. The propagation of varieties that are not recommended in a particular prefecture by the *National Center for Seeds and Seedlings* in the Okinawa or Kagoshima branches, will not be supported financially. Growers who wish to cultivate an unrecommended variety need to import seedlings of such varieties by themselves without using certified disease-free planting material in most cases, which poses a threat to disease control measures (Degi and Yonaha 2013). Therefore, unrecommended varieties should be registered before they spread to be dominant varieties, such as 'Ni27' in Kagoshima, which is propagated at national or local levels, such as by the private sugar mill company named 'Nansei Togyo Co., Ltd.', which has a micro propagation facility using meristem culture to generate certified "disease-free" planting material. In the latter case, the private company should sign a contract agreement with the breeder who has the Plant Breeders' Rights of the variety in question. When policy in Japan for variety use will be changed, examples from other countries and crop species may be helpful. In Australia, it is very interesting that biosecurity zones are arranged where sugarcane budded shoots (even ones which remain in machinery after harvesting) are banned from being moved between the biosecurity zones without the permission of the biosecurity center (Sugar Research Australia 2018).

2.3.3.2. Current status of varietal use from the viewpoint of workers and researchers involved in sugarcane.

The respondents involved in sugarcane to this survey understood the advantages of the use of diverse varieties, such as being able to select the variety best-suited to a particular region, risk diversification in terms of climate disaster or disease spread, extension of the harvest season, and the suggested use of diverse varieties by growers (Table 2.3.3). It also suggested current and potential issues from the use of diverse varieties, such as difficulties in managing and harvesting seedling fields, the management of factory data, and the selection of varieties. Varietal diversification in Japan has been accelerated by the breeding of Japanese varieties in the early 1990s after the era of 'NCo310' (Miyahira 2000; Sato, 2017). Such variety diversification may have the potential to lessen the risk from disease spread or climate disaster (Miyahira 2000) and to increase the opportunity for selection by growers; however, it may also have the potential to reduce yield and quality should misjudgments be made as to the regional adaptability of each variety (Nakamori and Kawamura 1997). In Japan, guidelines for varietal selection were published in ‘Selection from Varietal Characteristics’ (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2015), ‘Identification key for regional adaptability of sugarcane varieties’ (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 1992), and ‘Guidebook for variety use’ (Daito Sugar Mfg. Co., Ltd. 2014). In interviews with many workers, they declared that they often did not know how to distinguish varieties and how to tell growers varietal characteristics even after they attended the meeting for breeding and saw the report on the performance of a new variety. A new guidebook that covers all varieties (unrecommended and recommended) cultivated in all regions would be particularly helpful for workers and, by extension, growers, to distinguish and select varieties. Ideally, it should contain a key for identification of all sugarcane varieties, as well as a growing manual for each variety. For this, some examples of guidebooks in Louisiana and Australia (SRA 2018) are available.

Table 2.3.3. Result of questionnaire survey with workers about use of several varieties.

Question	Answer	No. of answerers	Percentage (%)
1. Do you recommend to grow several varieties ?	Yes	52	81
	No	3	5
	Neither	9	14
2. Advantage for use of various varieties （multiple choices）	Suitable variety for suitable region	45	70
	Risk diversification for climate disasters	44	69
	Control of disease spread	40	63
	Extension of harvest period	29	45
	Diversification of cultivation work periods	9	14
	None	1	2
	Others	1	2
3. Issue for use of various varieties （multiple choices）	Mixture from seedling fields	28	44
	Management of seedling field	27	42
	Management of factory data	20	31
	Difficult selection	18	28
	Limited option of varieties	5	8
	Others	6	9

Note: n=64.

Varietal mixtures, regardless of the proportions of individual varieties in the field, were well recognized by 80% of respondents (Table 2.3.4). In addition, 50% of respondents estimated that mixtures would exist in at least 10% of cultivated fields. The reasons for using mixtures were mainly “filling in gaps where a plant was missing” (i.e. complementary planting), limitation of available individual varietal seedlings, and ratoon from a previous year’s cropping. Prior to this survey, the author had received many negative opinions in regard to varietal mixtures. However, the number of respondents who recognized it to be a poor development was low, while many of the respondents answered that variety mixtures were neither beneficial nor disadvantageous. Some respondents stated that the development of varietal mixtures was an inevitable move for growers. On the other hand, some workers had attempted to prevent the use of varietal mixtures by teaching growers not to mix-plant or take seedlings from mixed-variety fields, and by providing single-variety seedlings or managing seedling fields for growers.

Table 2.3.4. Result of questionnaire survey with workers about varietal mixture.

Question	Answer	No. of answerers	Percentage (%)
1. Do you recognize mixture ?	Yes	52	81
	No	12	19
2 Estimation of occupation of mixture fields	Common (≥50%)	6	9
	Medium (≥10%, <50%)	26	41
	Rare (<10%)	13	20
	No idea	18	28
	No answer	1	0
3 Reason for mixture （multiple choices）	Complementary planting	48	75
	Lack of seedlings	32	50
	Unintentional ratoon	20	31
	No idea	6	9
	Others	11	17
4 Implession for mixture	Good	9	14
	Bad	16	25
	Neither	36	56
	No answer	1	2
	Others	2	3
5. Approach against mixture （for answerers who selected 'Bad' in Question 4）	Yes	5	31
	No	10	63
	No answer	1	6
6. Examples of approach against mixture （for answerers who selected 'Yes' in Question 5） （multiple choices）	Provide seedlings	4	6
	Guide to avoid mixed-planting	4	6
	Guide to avoid taking seedling from mixture field	4	6
	Prepare enough seedling fields	3	5
	Carefull manage of variety information in each field	1	2
	Others	2	3

Note: n=64.

2.3.3.3. Current status of varietal use from the viewpoint of the growers.

Many growers used different varieties, indicating that the concept of a suitable variety for a suitable field has become common (Table 2.3.5). Some growers suggested that one reason for the use of different varieties was a selection trial that they had carried out themselves. Such local trials on released varieties are interesting and suggestive for a future perspective on the breeding process. round 70% of growers answered that they had fields in which different varieties were mix-planted (Table 2.3.6). Of these growers, around 80% stated that the reasons for growing a mixture was to make up for missing plants in the field, a finding which was the same as that stated by workers (Table 2.3.4). Although the effects of varietal mixtures on growth and yield in sugarcane should be examined (Takaragawa et al. 2016b, 2018b), from the results of interviews, mixtures would be unnecessary if improvements could be achieved for germination and ratooning abilities, well-planned seedling fields, and raising seedlings of individual varieties for complementary planting. Appropriate fallow periods after soil management could be helpful in avoiding the unintentional mixture of varieties naturally grown from the last ratoon field (Tew 1987). Complementary planting is an essential growing method to attain high and stable yield, especially for ratoon cropping, where mixtures of different varieties with high elongation ability at the early growth stage were suggested to be used (Shinzato et al. 2010). Although it is true that the complementary planting of suitable varieties could contribute to high productivity (Shinzato et al. 2010), taking seedlings from such a variety mixture field should be avoided from the viewpoint of stable production and variety management, in particular when the rate of mixture is high. Small-scale growers may use the seedlings from variety mixture fields because they could not prepare seedling fields well. However, the present study did not consider the size of growers' fields, their financial scale, or the presence of seedling fields. In addition, only two regions were targeted in these surveys. Further research concerning these points should be carried out on a larger scale using annual survey of growers' information from the OCR survey (Satoukibi Uetuke Hojou Chousa).

Table 2.3.5. Result of questionnaire survey with growers about use of several varieties.

Question	Answer	No. of answerers	Percen-tage (%)
1. Do you grow several varieties ?	Yes	23	77
	No	7	23
2. Reason for growing seeral varieties （for answerers who selected 'Yes' in Question 1）（multiple choices）	Selection trial	8	35
	Suitable variety for suitable field	4	17
	None	3	13
	Differenciate harvest time	2	9
	Risk diversification	2	9
	Others	1	4
	No answer	3	13

Note: n=30.

Table 2.3.6. Result of questionnaire survey with growers about varietal mixture.

Question	Answer	No. of answerers	Percen-tage (%)
1. Do you have a mixture field ?	Yes	20	67
	No	9	30
	No idea	1	3
2. Reason for mixture （for answerers who selected 'Yes' in Question 1）（multiple choices）	Complementary planting	16	80
	Trial	3	15
	Limitation of seedlings	1	5

Note: n=30.

2.3.3.4. Effective variety use and management.

In conclusion, the use of diverse varieties was enabled because of the technical developments in sugarcane breeding and the considerable efforts of the institutes involved in producing large numbers of sugarcane varieties. The advantages of this wide range of varieties were well recognized by growers and workers. However, they also reported the negative issues associated with diversification of varieties, such as difficulties in selecting complementary varieties from many varieties, and that varietal mixtures could be avoided by well-planned and intensive management of the crop. Decision making for growers and extension workers should be supported by a guidebook that explains each varietal characteristic and provides a management plan for each variety as well as the best combination of varieties for risk diversification. Such an enlightened approach would result in the optimum varietal composition with minimum bias.

2.4 Long-term evaluation of varietal performance after release.

2.4.1 Introduction

In Japan, several superior sugarcane cultivars have been released from breeding programs (Takaragawa et al. 2018e). However, following release, the subsequent yield performance of these cultivars has not been systematically evaluated, despite the need to assess the effective use of these cultivars and the cost-benefit analysis of sugarcane breeding programs. This may come from the experimental limitation that it is difficult to study long-term varietal performance because it needs to be carried out over a period of years using a specific variety in the same multiple sites under the same environmental conditions (Coleman 1974). There are two current types of data sources available for the long-term evaluation of varietal productivity: namely, the productivity of a standard (reference) cultivar in breeding trials (Miyagi et al. 2001; Takaragawa et al. 2016a), and sugar mill data that contain information on the cultivar, cane productivity and area harvested (Taira 2005; Taira et al. 2005; Degi 2009; Degi et al. 2011). The former data are derived from experimental fields without extensive management and contain data not only on yield, but also yield components, such as stalk length and the number of millable stalks. On the other hand, the latter data shows the real status of varietal performance after release in the form of big data, though the figures for area and variety are less reliable because this information is usually obtained from interviews with growers. In addition, the latter data have only yield and sugar content. Therefore, each data source has both advantages and disadvantages. In this chapter, the author focused on the latter data source, i.e. from sugar mills.

The sugar mill in the Daitoh Islands has collected precise data from each harvested field on-site since the 1980s. Sugar mill workers have manually recorded precise area data for a long time before the 2000s, using global positioning system (GPS) and geographical information system (GIS) techniques from the early 2000s (Ueno et al. 2004; Fig. 2.4.1). Using the loading records of the cane harvested from each harvested area, the precise evaluation of cane yields became possible.

The present study attempted to evaluate the long-term performances of individual sugarcane cultivars using such factory data (1989–2017), particularly in ratooning crops, the dominant sugarcane cultivation practiced in the Daitoh Islands (Fig. 2.4.2) and having continuous data over more than five years for many cultivars introduced into this district.

2.4.2 Materials and Methods

Factory data (1989–2017) were collected from Daito Sugar Mfg. Co., Ltd. (located in Minami Daito Island, 25°83′N, 131°20′E) and Kita-daito Sugar Mfg. Co., Ltd. (located in Kita Daito Island, 25°95′N, 131°30′E). There are 1300–1500 sugarcane fields (covering an area of 1000–1200 ha in total) and 500–600 sugarcane fields (400–500 ha in total) in Minami Daito and Kita Daito Islands, respectively (Fig.2.4.1).

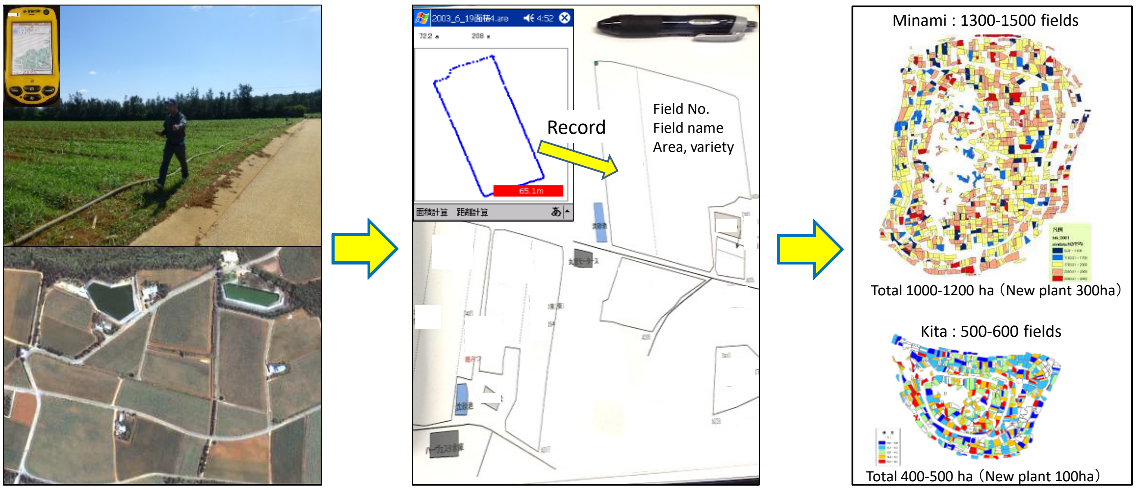


Fig. 2.4.1 Access to record area of each field using GPS and GIS technique.
Note: 1. Walking around field with mobile GPS (left) → 2. Recording area using mobile GPS and checking data in field work book (center) → 3. Mapping on GIS system (right).

Yield was calculated by the division of the total harvested cane amount by the total harvested area for each variety. The number of fields and yield from each field were not considered in the present study. Relative yield value was calculated as equation (2.4.1):

$$\text{Relative yield} = \text{Yield of test variety} / \text{Yield of standard variety} \qquad (2.4.1)$$

The standard variety was selected from long-term yield trends as shown below in Results. With this index, a value of 1 indicates that the observed test variety yield was equal to that of standard variety, whereas values of less than or greater than 1 indicate under-yielding and over-yielding, respectively. To evaluate the contribution of varietal diversity to sugarcane production in each island, relative production value was also calculated as equation (2.4.2):

$$\text{Relative production} = \text{Real status of production} / \text{Production of monoculture of standard variety} \qquad (2.4.2)$$

The production of monoculture of the standard variety was calculated, assuming that the standard variety covered all cultivation areas. With this index, values of 1 indicate that the production of diverse varieties was equal to that of the standard variety monoculture, whereas the values of less than or greater than 1 indicate under-yielding and over-yielding, respectively.

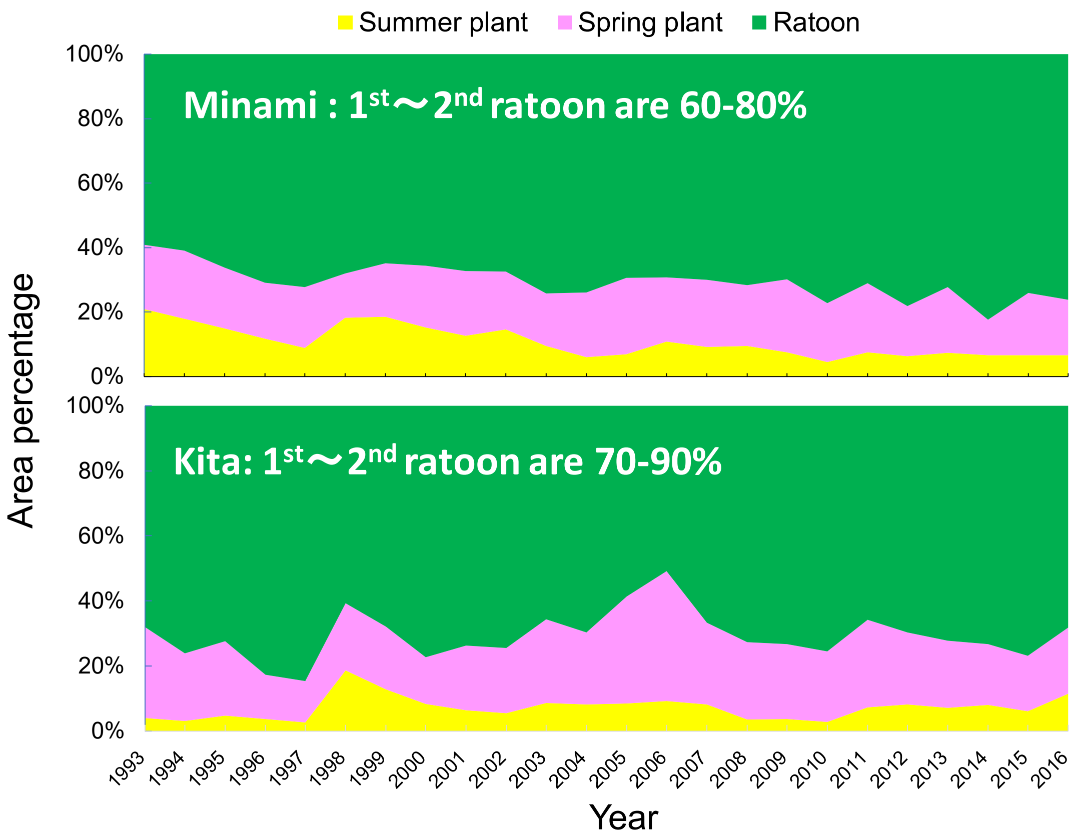


Fig. 2.4.2 Area percentage of three cropping types.

Note: Ratoon cropping includes ratoons after spring and summer plantings and any times ratoons.

2.4.3 Results

From the early 2000s, the varietal compositions in Daito Islands diversified after releases of several varieties (Fig. 2.4.3). The variety ‘F 161’, imported from Taiwan in the early 1970s, has been the dominant variety for a long period in this area (Fig. 2.4.3). Yield of ‘F161’ has shown a similar trend to the total average yield in these islands (Fig. 2.4.4). This variety also exhibited the highest yield in the recent high-yielding year, 2016/2017, which suggested its continuing potential for high yields, with no signs of varietal deterioration. From these results, the yield of each variety, relative to that of ‘F 161’ as the standard cultivar, was analyzed to minimize the effects of climate change and/or social background of the grower on the varietal yield evaluation. Ratoon yields in excess of 1.0, relative to ‘F 161’, were identified for several cultivars introduced since late 2000s, such as ‘Ni15’, ‘NiTn20’, and ‘Ni26’ in Minami Daito and ‘NiTn19’, ‘Ni26’, and ‘Ni28’ in Kita Daito, while some other varieties recently showed relative yields of less than 1.0, such as ‘Ni9’ in Minami Daito and ‘NiH25’ in Kita Daito (Fig. 2.4.5). Mean relative yields of varieties, except ‘F161’, are shown in Fig. 2.4.6. It was clear that these values were constantly higher in ratoon cropping than 1.0in recent years on both islands. Although the number of sampling years was different between varieties, the mean relative yield in each variety showed higher ratoon yield in recent varieties compared with ‘F161’ (Fig. 2.4.7). The values of cane production, relative to monoculture, were increased in recent years, especially in 2013 with over 10% increments (Fig. 2.4.8).

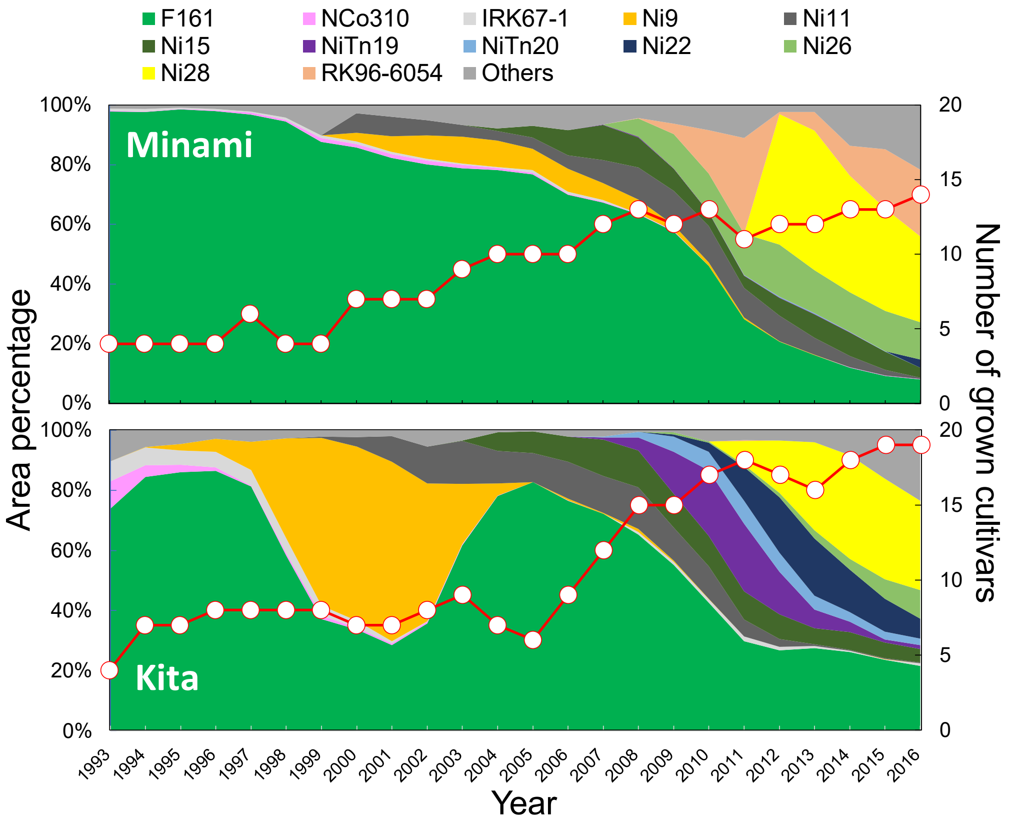


Fig. 2.4.3 Area percentage and number of varieties grown in Daito islands.

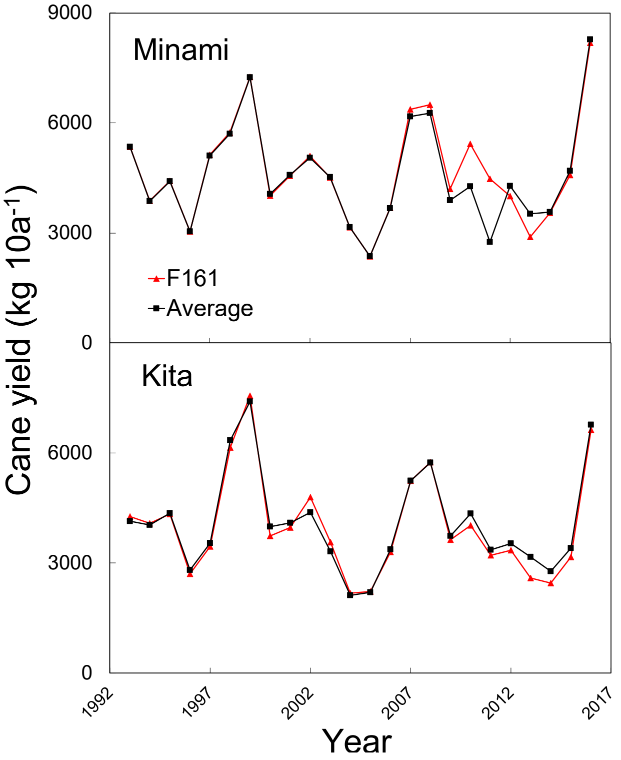


Fig. 2.4.4 Change of ratoon yield of average and F161 in Daito islands.

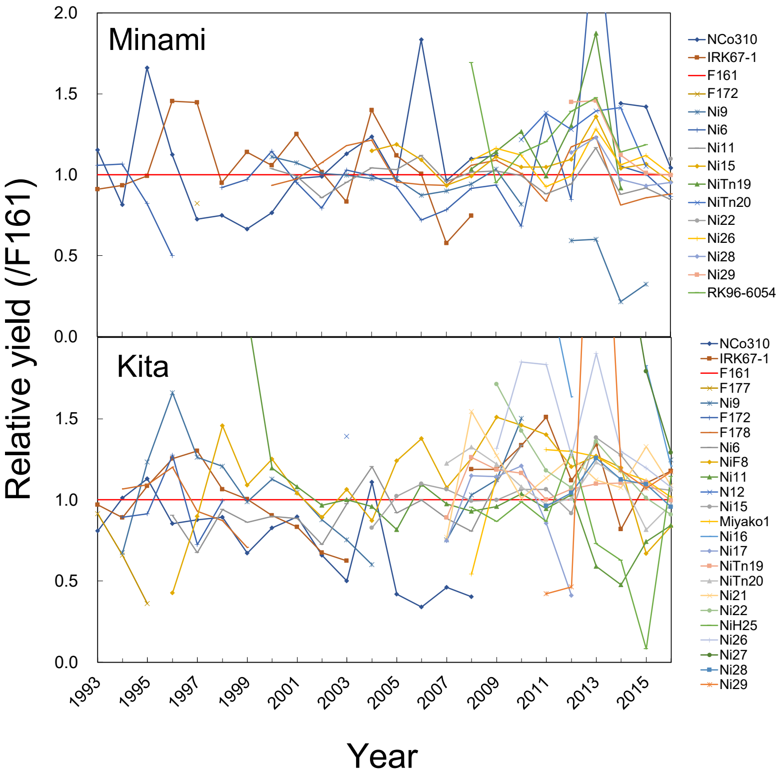


Fig. 2.4.5 Change of ratoon yield of each variety relative to that of F161 in Daito islands.

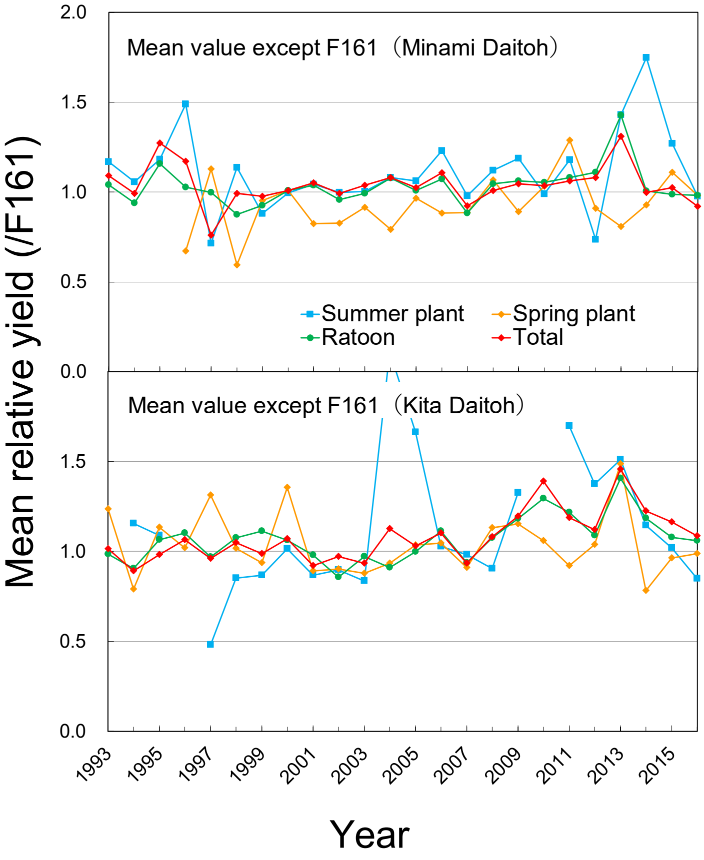


Fig. 2.4.6 Change of mean relative yield of all varieties except F161 in Daito islands.

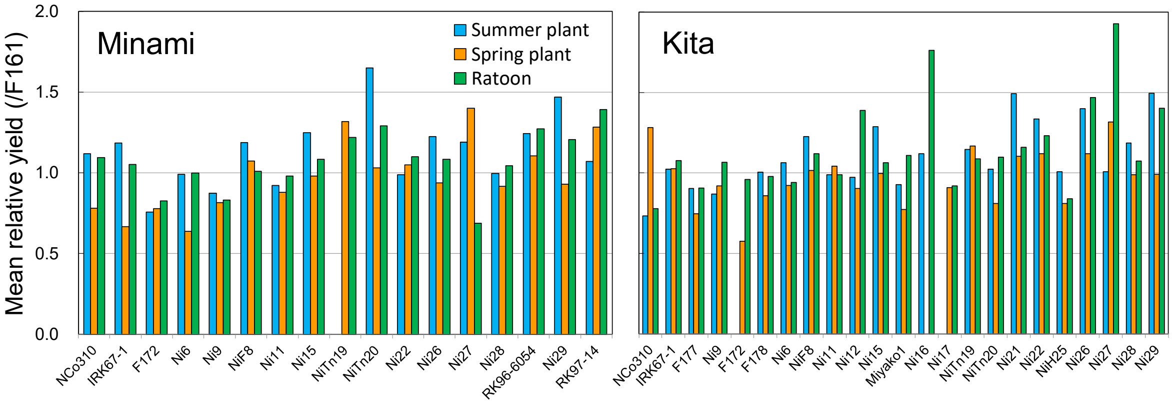


Fig. 2.4.7 Mean relative yield of each variety in Daito islands.
Note: Number of years for each variety was not fixed.

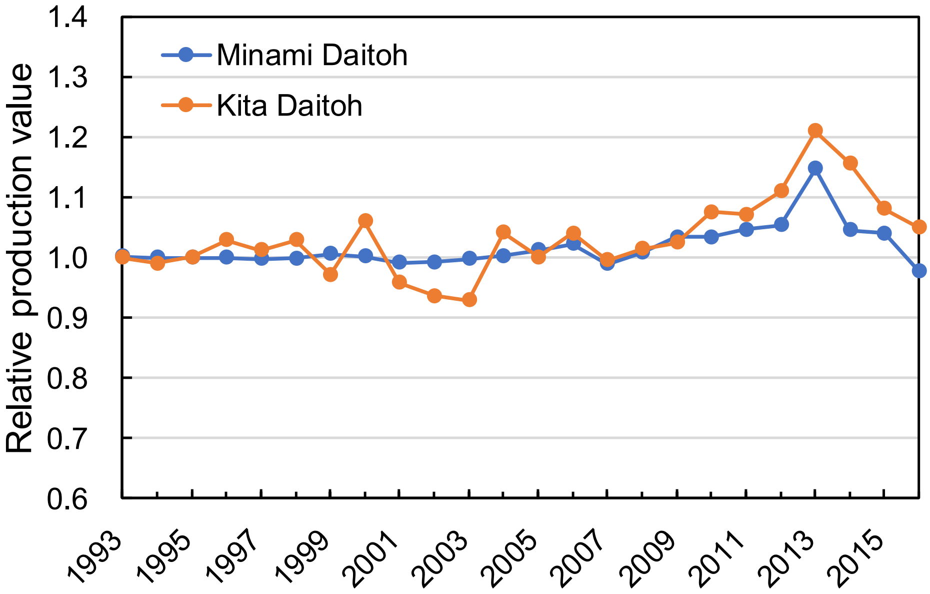


Fig. 2.4.8 Change of relative production value to that of F161 monoculture in Daito islands.

2.4.4. Discussion

2.4.4.1. Varietal deterioration

The old variety ‘F161’, which had been cultivated in the islands for a long time, still exhibited high-yielding ability in the recent favorable season which saw increased yields, thus mitigating against the possibility of varietal decline in terms of performance (Fig. 2.4.4). However, when the long-term data were divided into some parts of the periods, the yield of ‘F 161’ seemed to decrease from 1999 to 2005, which suggested that apparent varietal deterioration due to seedling quality and/or climate condition could not be denied. In addition, the degree of change of varietal performance may be region-dependent (Davidson 1968). Warner (1968) suggested that the factors responsible for a decline in varietal performance affect a variety that has been grown continually in a given area to a greater degree than new or different varieties. The present study suggested that varietal deterioration was not exhibited in one specific ability (yield), in one specific variety (‘F161’), and only in one specific region (Daito), but could not deny the presence of varietal deterioration of other traits in other varieties in other regions. Especially in Japan, meta climatic factors, such as typhoons and severe drought, can affect yield performance and make it difficult to see the year-on-year yield change of the variety itself. There are some other cases where the variety could not exhibit their true potential, with the ability of the variety seeming decrease as a result of pathogen accumulation and changes in cultivation practice. The yield of ‘NCo310’ gradually decreased due to the spread of smut, while the yield of ‘NiF8’, a variety sensitive to low temperatures, also tended to decrease due to the low application of plastic mulching in Tanegashima (Takaragawa et al. 2018a). As shown above, it would be possible to monitor the performance of other varieties, comparing them to that of the standard variety, which showed no trend of deterioration ((Fig. 2.4.6-7). For this purpose, close monitoring may need to continue for 10–20 years.

In other countries, the reasons for varietal deterioration seemed to be pathogen accumulation, such as ratoon stunt disease and phytopathogenic nematodes (King 1950; King 1959; Mangelsdorf 1959; Martin et al. 1959; Viswanathan 2016). In many of these reports, the importance of pathogen-free cane seedling setts was emphasized. In Japan, pathogen-free sugarcane is propagated by the *National Center for Seeds and Seedlings* and a private facility to propagate pathogen-free meristem clones (Maezono 1999; Matsumoto 2000; Suzuki 2012). Although the positive effects of pathogen-free canes on growth and yield have been demonstrated at the experimental field level (Degi et al. 2013), such effects in a grower’s field level over longer periods of time have not yet been reported.

2.3.4.2. Contribution of varietal diversity to sugarcane production.

Relative yields in excess of 1.0, relative to ‘F161’, have been identified for several cultivars introduced since 2008 (Fig. 2.4.5), which indicated that recent breeding efforts were effective for increasing ratoon yield. In addition, the relative values of cane production, relative to monocultures, were increased in recent years, especially in 2013 with more than 10% increases (Fig. 2.4.8). Therefore, it was suggested that the diversified varieties truly contributed to the increase in cane production in these islands. This may be because varieties that could yield well even in low-yielding fields and under multiple ratoon cropping, such as ‘NiTn20’, with high ratooning ability and drought tolerance (Hokama et al. 2005; Irei et al. 2009), and ‘Ni26’ with high ratooning ability (Miyagi et al. 2009), were selected and recommended here in recent years. In the 2013/2014 season when long-term drought followed the rainy season and frequent typhoons after October caused severe yield reductions in all regions, the yield reduction of drought- and typhoon-sensitive ‘F161’ may have also enhanced the superior abilities of other recent varieties (Fig. 2.4.4). Additionally, it is suggested that varietal yield data should be made available in the planned guidelines to select superior varieties for cultivation under biotic and abiotic stresses and to improve the efficiency of breeding trials in real fields through continuous feedbacks to the breeders (Degi 2009).

In conclusion, long-term variety records could be useful not only in monitoring cultivar deterioration, but also in evaluating the performance of breeding programs. To my knowledge, there are no specific and concrete data that show that the diversification of varieties would mitigate against risk from disease pandemic or climate disaster. Further research concerning this topic needs to be conducted analyzing the type of data generated in the present study.

Chapter 3: Effects of varietal mixtures on sugarcane growth and yield

3.1. Varietal mixture with different plant types.

3.1.1 Introduction

Sugarcane yield is determined to a greater extent by the number than by the weight of millable stalks (Miller and James 1974; Shimabuku 1997). The number of stalks per unit land area increases rapidly during the active-tillering stage (i.e., the early growth stage, for about 3 months or 700 ºC days with base temperature being 16 ºC), reaches a peak stalk density, which then decreases gradually because of competition for light and nutrients during the yield formation stage (i.e., later growth stage) (Singels and Smit 2009; Zhou and Shoko 2011). Light transmittance to the canopy is controlled by the canopy structure (Shimabuku 1997; Zhou et al. 2003; Marchiori et al. 2010). Generally, canopy structure depends on genetic traits, such as the degree of leaf erectness (plant type) (Hikosaka and Hirose 1997; Shimabuku 1997; Monsi and Saeki 2005; Marchiori et al. 2010), plant size (i.e., stem length and plant height) (Tsuchiya and Kinoshita 1984; Tominaga et al. 2015), and tiller number (Zhou et al. 2003; Takaragawa et al. 2016b), and on agronomic features such as row spacing and planting density (Singels and Smit 2009). Optimal plant density for sugarcane is determined by local circumstances (Irvine et al. 1980). For example, the ridge width depends on machinery size (e.g., planter, cultivator, and harvester) in Japan (Hiyane et al. 2005; Akachi et al. 2017). However, the canopy structure can also be optimized using other means. For example, mixing two varieties with different canopy structures increased plant community growth by optimizing light interception (Takaragawa et al. 2016b). Shimabuku (1997) suggested that horizontal-leafed varieties of sugarcane demonstrate high leaf area index (LAI) and that they are ideal for early growth because they develop leaves faster to capture radiation early in the season, while erect-leafed varieties show high net assimilation rates (NAR, the indicator of canopy photosynthesis) and are ideal for later growth periods after the canopy has developed, so that radiation more easily penetrates through the erect foliage to the lower canopy leaves. Sugarcane varieties with a diverse plant types have been developed by breeders in Japan (Takaragawa et al. 2018c). Unfortunately, Shimabuku (1997) concluded that it was difficult to develop a sugarcane variety with ideal leaf traits for both early and later growth stages. Thus, there is a possibility that, by optimizing community structure using a diversity of plant types and planting methods, the yield of sugarcane can be increased further.

It has been reported that varietal mixtures improved canopy structure and light use efficiency by combining varieties with different plant heights in rice (Tsuchiya and Kinoshita 1984) or different tillering abilities in sugarcane (Takaragawa et al. 2016b); however, this has not been reported with different plant types in any other species. In present study, it was hypothesized that varietal mixture with different plant types improves within-canopy light use through habitat segregation: horizontal-leafed variety would capture light penetrated through the canopy of erect-leafed variety.

The objective of the present study was to examine the effect of mixing two sugarcane varieties with different plant types on their canopy light use and growth at early growth stage under glasshouse condition (Experiment 1) and until harvest under field condition (Experiment 2).

3.1.2 Materials and Methods

3.1.2.1 Glasshouse experiment

3.1.2.1.1 Plant material

The experiment was conducted in the glasshouse (light interception rate 0.5) at the University of Ryukyus, Japan (26° 15′ N, 127° 45′ E; altitude 127 m), creating simulated crop canopies to mimic growth following spring planting (Matsuoka 2006). The reason for investigating the simulated canopies in greenhouse conditions was to avoid the influence of the typhoon and tillering on the potential varietal canopy growth. The mean air temperature (VP3; Decagon, Pullman, WA, USA) and the mean daily accumulated radiation (SE-SP215; Apogee, Logan, UT, USA) during the experimental period were 29 °C and 9.1 MJ m^{-2} day^{-1}, respectively. Single-bud setts were planted on 17 May 2017 (14 plants per container) to 80-L containers (52 × 82 cm of soil surface, 10-cm soil depth) filled with a soil mixture (soil : peat : sand = 1:1:1, v/v/v). The 14 plants per container were planted in a double row with 25 cm inter-row distance and 10 cm inter-plant distance as a simulated canopy (Fig. 3.1.1). Monocropped Ni12 (*Saccharum* spp.; horizontal-leaf type) (*mono Ni12*) and Ni29 (erect-leaf type) (*mono Ni29*), and a mixture of these within a container (one row Ni12; *mix Ni12* + one row Ni29; *mix Ni29*) (*Mix*), were arranged in a replicated randomized block design with four replicates. Containers were arranged with the distance of 1 m from other containers and interference between containers was minimum. Plants were well watered and fertilized weekly with 2 L per container of Hoagland solution containing 6 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 100 μM $C_{10}H_{12}FeN_2NaO_8$, 25 μM H_3BO_3, 10 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, and 0.5 μM H_2MoO_4. Tillers were removed immediately after their emergence because we avoided the influence of different tillering ability of each variety on canopy growth, and dead leaves were also removed.

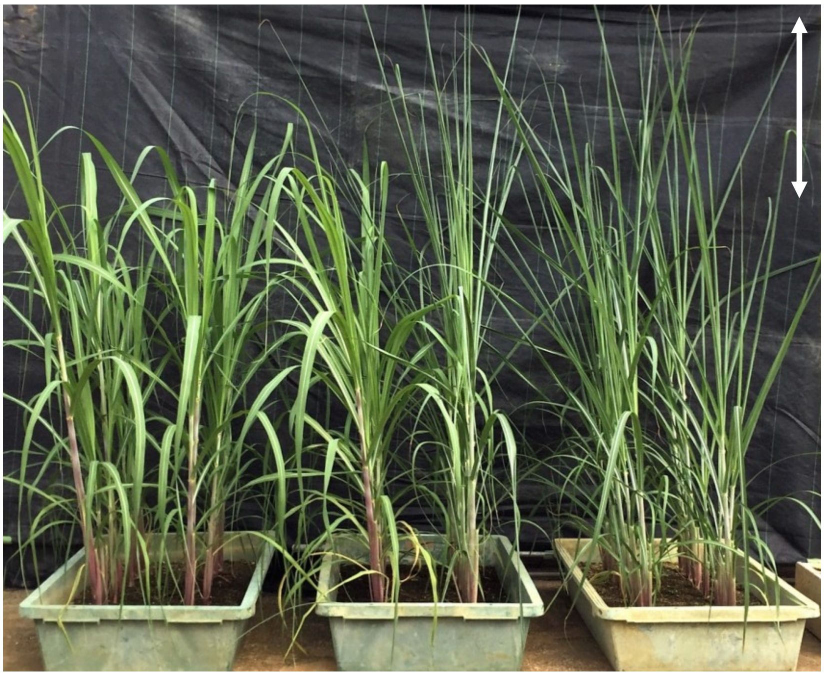


Fig. 3.1.1 Outline of the simulated canopy on 15 August, 2017 (90 DAP).

Note: mono-cropped Ni12 (left), mixture (middle), mono-cropped Ni29 (right). Mixture contains Ni12 and Ni29 at left and right rows, respectively. White arrow means 50-cm length.

3.1.2.1.2 Data collection

Measurements were conducted every 3 days for relative light intensity at ground level (RLI; I/I_0)

and vegetation rate (VR), and weekly for stem (culm) length (length from ground to the visible dewlap of the first fully expanded leaf) and plant height (the natural height of the highest part of the plant) of all plants from 3 June [17 days after planting (DAP)] onwards. Photon flux density (PFD; μmol m^{-2} s^{-1}) on a horizontal level above the canopy (I_0) and at a given depth (I) within the canopy was recorded at noon, when the light penetration from the side of canopy on the measurement of RLI was minimum, using a one-point light sensor (LI-250A; LI-COR, Lincoln, Nebraska, USA) and line PFD sensors bars (#s366816; Spectrum Technologies Inc., Aurora, IL, USA) inserted between rows within the canopy, respectively. A photograph of the canopy viewed from above was taken at 2-m height for a record of VR using image analysis software (ImageJ, National Institutes of Health, USA), which separates the areas of vegetation and soil.

Leaf gas exchange measurements were conducted using a portable system (LI-6400XT, LI-COR, Lincoln, Nebraska, USA) with red and blue LEDs as light source. Photosynthetic light response curves were measured on 11 August for four plants per variety, which had been grown as a monocrop, by reducing the PFD in six steps from 2000 μmol m^{-2} s^{-1} (followed by 1000, 500, 200, 100, 50 and 0 μmol m^{-2} s^{-1}). A non-rectangular hyperbola was used to formulate the light response curve of individual leaf net photosynthesis (P_n):

$$P_n = [(P_{max} + \varphi I_L) - \{(P_{max} + \varphi I_L)^2 - 4\, P_{max}\, \varphi \theta I_L\,\}^{1/2}]/2\theta - R_d \qquad (3.1.1)$$

where P_{max}, I_L, R_d, φ, and θ are light-saturated photosynthesis, PFD on a leaf surface, dark respiration, curvature factor, and quantum yield, respectively (Hirose and Werger 1987a). P_{max} was measured on 16 leaves per variety from different, randomly selected heights within the canopies of monocropped varieties (*mono Ni12* and *mono Ni29*). Leaf photosynthesis is related to leaf nitrogen content per unit leaf area (N_{LA}) by assuming that P_{max} increases linearly as N_{LA} increases (Hirose and Werger 1987a):

$$P_{max} = \mathrm{a}\, N_{LA} + \mathrm{b} \qquad (3.1.2)$$

where a and b are the slope and y-intercept of the P_{max} vs. N_{LA} relationship. R_d, φ, and θ were assumed to be independent of N_{LA} and a constant value for each variety in this study. The canopy photosynthesis (P_c) is an integration of the instantaneous leaf photosynthesis (P_n) throughout the canopy at a given I_0. Using the stratified clip method (Monsi and Saeki 2005), RLI was recorded at every 30-cm height and the plants were harvested at every 30-cm layer to measure leaf area (LI-3100; LI-COR, Lincoln, Nebraska, USA) and dry weight (constant value after drying at 80 °C) determined on 14 and 15 August (90 DAP). Although the tillers were removed in this study, the growth duration was assumed to be from planting until saturation of tillering (early growth). Following Beer–Lambert law, the light extinction coefficient (K) was calculated (Monsi and Saeki 2005):

$$I = I_0\, \mathrm{e}^{-K F} \qquad (3.1.3)$$

where F is the cumulative LAI from the top of the canopy. Leaf area per unit of layer volume [i.e., leaf area density (LAD)] was calculated at each layer using F. Peak height of LAD was calculated from the following equation:

$$\text{Peak height of LAD (cm)} = \Sigma\{(\text{LAD \% at each layer}) \times (\text{median of each layer height})\}/100 \tag{3.1.4}$$

Light interception was calculated using the data of daily accumulated radiation and RLI and was integrated to attain accumulated light interception. Radiation use efficiency (RUE) was calculated from accumulated light interception and above-ground biomass (Keating and Carberry 1993):

$$\text{RUE} = \text{Above-ground biomass/Accumulated light interception} \tag{3.1.5}$$

To compare the growth parameter of the mixture to the one predicted on the basis of the two-component monocropped ones, a mixture index (MI) was calculated for the mixture treatment (Trenbath 1974):

$$MI = Y_{mix}\times 2/(Y_{monoNi12}+Y_{monoNi29}) \tag{3.1.6}$$

where Y_{mix} is the observed parameter for the mixture, and $Y_{monoNi12}$ and $Y_{monoNi29}$ are the parameters of monocropped Ni12 and Ni29, respectively. With this index, values of 1 indicate that the observed parameter was equal to the predicted one, whereas the values of less than or greater than 1 indicate under-yielding and over-yielding, respectively (Trenbath 1974).

3.1.2.1.3 Statistical analysis

ANOVA was used to determine whether statistically significant differences existed between the samples. If significant differences existed, the significance of differences between mean values for each treatment were assessed using the pairwise multiple comparison Tukey test, with statistical significance assumed at $P < 0.01$.

3.1.2.2 Field experiment

3.1.2.2.1 Plant material

The field experiment was conducted at a grower's field in Tokuwase, Tokunoshima-cho, Kagoshima prefecture (27°45'40.5"N 129°01'57.0"E; altitude 40 m) (soil: dark red soil, pH 5.4, EC 7.0 mS m^{-1}, 5 a, east-west directed row). Single-bud sugarcane setts after one day soaking in water were planted on 24 March, 2016 with a planting density of 2.78 plants m^{-2} (within-row 0.3 m, inter-row 1.2 m). The semi-erect-leafed variety NiF8 and the semi-horizontal-leafed variety Ni17 were selected from recommended varieties in the region. The two varieties were mono-cropped (*mono* NiF8 and *mono* Ni17) or mixed 1:1 by row (*mix* NiF8 and *mix* Ni17; *Mix*) (see Fig. 3.2.2) with two replicate plots (7.2 m×10.8 m× 2 plots per treatment). Plants were well irrigated, protected by applying pesticides, and fertilized with $N : P_2O_5 : K_2O = 11 : 8 : 5$ kg 10 a^{-1} as basal fertilizer and top-dressed with 11 : 0 : 5 kg 10 a^{-1} on 15 August 2016 according to growing manual (Kagoshima Sugar Industry Development Association 2015).

3.1.2.2.2 Data collection

The stem length and number of stalks were measured for eight plants (4 plants × 2 rows) per plot on 77, 143, 231, and 339 days after planting (DAP). On 11–13 November (231 DAP), when canopy formation was completed, six plants within each subplot (2.4 m×0.9 m) were harvested according to the stratified clip method (Monsi and Saeki 2005). RLI was measured at each 40 cm height using the same devices and methods as Exp.1. Plants were cut and divided into leaf and stem parts to measure leaf area and partial dry weight. Leaves were cut into 20–30 cm length and laid on a white plastic sheet to take a photograph from above at 2 m height with a scale bar. The images were analyzed using image analysis software 'Image J' (https://imagej.nih.gov/ij/index.html) to measure leaf area. Using leaf area and RLI, the light-extinction coefficient was calculated according to same equation (3) as in Exp. 1. The peak height of leaf area duration (LAD) was also calculated according to the same equation (4) as Exp. 1.

On 27 February 2017 (339 DAP), eight plants within each subplot (2.4 m×1.2 m) were harvested to determine the yield and yield components. The number of millable stalks, stem length, stalk length, stalk diameter, number of nodes, and stalk weight were determined. A subsample of the harvested stalks was brought back to the laboratory in the University of the Ryukyus and then squeezed. The sucrose concentration in the sugarcane juice was analyzed using HPLC (RID-10A, SCR101-H, Shimadzu) (Watanabe et al. 2016).

3.1.3 Results

3.1.3.1 Glasshouse experiment

Under the simulated canopy conditions, plants grew well to show the expected characteristics for each variety, as Ni29 had the more erect and shorter leaves while Ni12 had the more horizontal and wider leaves (no data recorded; see Fig. 3.1.1). RLI at the ground level decreased as VR increased (Fig. 3.1.2). The RLI for *mono Ni29* was higher than for the other two treatments during the growth period while the RLI for *mono Ni12* decreased rapidly and maintained this lower value after 40 DAT (Fig. 3.1.2a). Mix tended to exhibit an RLI value similar to that of *mono Ni12* until 60 DAT, after which it maintained an RLI value slightly higher than that of *mono Ni12* until the end of the experiment. VR showed a trend opposite to that of RLI and reached 70%–80% coverage of the soil surface (Fig. 3.1.2b).

Plant height was significantly higher in *mono Ni29* than in *mono Ni12* due to greater leaf erectness (Fig. 3.1.3a). Finally, in the mixed planting, the plant height of Ni29 tended to increase while that of Ni12 decreased. This tendency was apparent with respect to stem length in that *mono Ni12* showed the highest stem length value followed by *mix Ni29*, *mono Ni29*, and *mix Ni12* (Fig. 3.1.3b).

Mean P_{max} was not significantly higher in Ni12 (27.5 μmol m^{-2} s^{-1}) than that in Ni29 (22.6 μmol m^{-2} s^{-1}) (data not shown). The correlation of P_{max} with N_{LA} was significant only for Ni12 but the correlation between P_{max} and height was significant for both varieties ($r = 0.795$, $P < 0.01$ for Ni12; $r = -0.505$, $P < 0.05$ for Ni29) (data not shown). P_{max} and P_n expressed per unit leaf area (P_{leaf}) were higher in the higher canopy layer (Fig. 3.1.4a, b). Each mixed variety showed higher values of P_{max} and P_{leaf} than those of the corresponding monocropped variety, especially in the higher canopy layers. The vertical gradient of P_n expressed per unit land area (P_{land}) had the peak at the middle layer (Fig. 3.1.4c). P_{land} peak was highest in *mono Ni12* followed by *Mix* and *mono Ni29*. The components of *Mix* had peaks at different layers of the canopy: *mix Ni29* at the higher layer and *mix Ni12* at the lower layer.

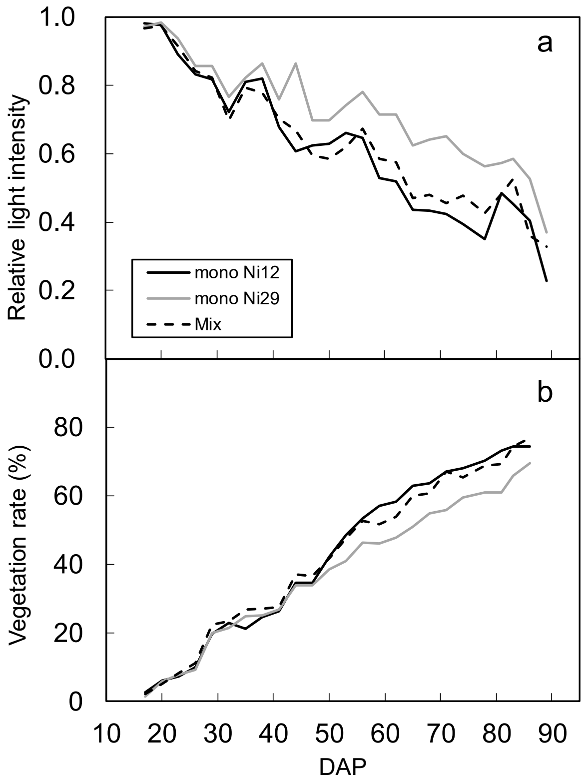


Fig. 3.1.2 Changes of relative light intensity at ground level (a) and vegetation rate (b).
Note: n=4. DAP, days after planting,

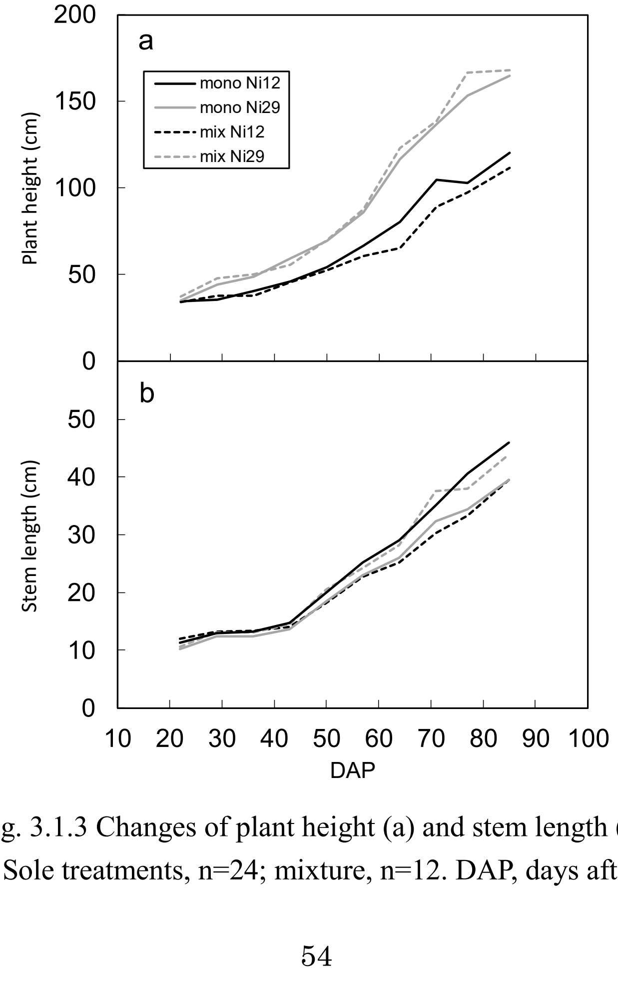


Fig. 3.1.3 Changes of plant height (a) and stem length (b).
Note: Sole treatments, n=24; mixture, n=12. DAP, days after planting.

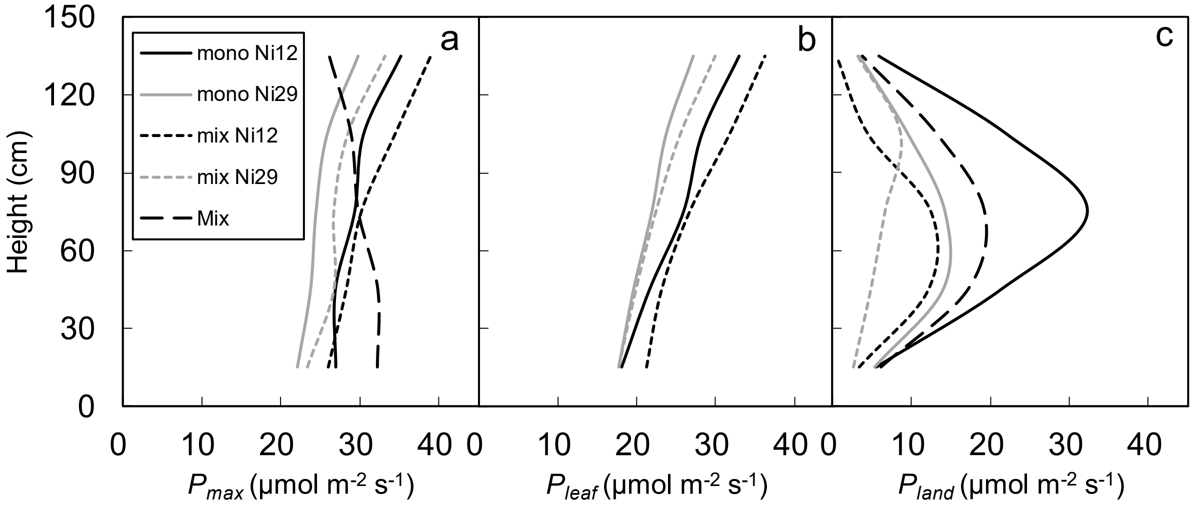


Fig. 3.1.4 Vertical profiles of P_{max} (a), P_{leaf} (b), and P_{land} (c) under mono-cropped and mixed varieties. Note: n=4. P_{max}, light saturated photosynthetic rate; P_{leaf}, P_n expressed as per unit leaf area; P_{land}, P_n expressed as per unit land area; P_n, instantaneous photosynthetic rate at a layer in the canopy where the PFD above the canopy (I_0) = 2000 μmol m^{-2} s^{-1}. "mix Ni12" and "mix Ni29" show each value of each row (P_{land}; land area=26×82 cm^2) while "Mix" shows value of total canopy of mixture (P_{land}; land area=52×82 cm^2).

The RLI within the canopy of *mono Ni12* started to decrease at 150 cm and kept decreasing slightly until reaching ground level (Fig. 3.1.5a). On the other hand, the RLI in *mono Ni29* started to decrease at 90–120 cm, and lessened the rate of decreasing at 30 cm. The RLI in *Mix* started to decrease at 120 cm and lessened the rate of decreasing at 60 cm. LAD showed a trend similar to that of P_{land} (Figs. 3.1.4c and 3.1.5b). The peak heights of LAD were higher in *mono Ni12* (71.0 cm) than in *mono Ni29* (67.1 cm). The LADs were higher at a higher layer in *mix Ni29* (76.5-cm peak height) and at a lower layer in *mix Ni12* (58.6 cm) than in each monocropped variety. N_{LA} was higher in the higher layer of the canopy (Fig. 3.1.5c). The N_{LA} of *mono Ni12* was lower than those of *Mix* and *mono Ni29* at any layer. *Mix* showed a similar trend with respect to N_{LA} to that of *mono Ni29*. In the components of *Mix*, N_{LA} of *mix Ni29* was higher at any layer than that of *mono Ni29* and decreased rapidly until it reached ground level. Specific leaf area (SLA) decreased as layer height increased in all treatments (Fig. 3.1.5d). SLA of *mono Ni12* was higher than that of *mono Ni29* over all layers. The vertical profile of SLA showed that *mix Ni12* and *mix Ni29* had lower values than those of *mono Ni12* and *mono Ni29*, respectively. Consequently, *Mix* tended to show an SLA profile similar to that of *mono Ni29*. N_{LA} increased as SLA decreased (Fig. 3.1.6), and the N_{LA} – SLA relationship fitted a power approximation ($y = 216.78x^{-1.145}$, $R^2 = 0.860$, $P < 0.01$).

The *K* was lower in *Mix* with no significance (Table 3.1.1). The *F* and above-ground biomass were both highest in *mono Ni12* followed by *Mix* and *mono Ni29*, with and without significance, respectively. Accumulated intercepted PAR was significantly higher in *mono Ni12* and *Mix* than in *mono Ni29*, while the opposite trend was shown with respect to RUE. P_c at 2000 μmol m^{-2} s^{-1} of I_0 (P_{cmax}) was significantly higher in *mono Ni12* than in *Mix* and *mono Ni29*. *MI*s for *K*, *F*, RUE, and P_c were below 1.0 (indicating a negative effect of varietal mixtures, compared with varietal monocrops), while the indices for above-ground biomass and accumulated intercepted PAR were greater than 1.0 (indicating a positive effect of varietal mixtures, compared with varietal monocrops).

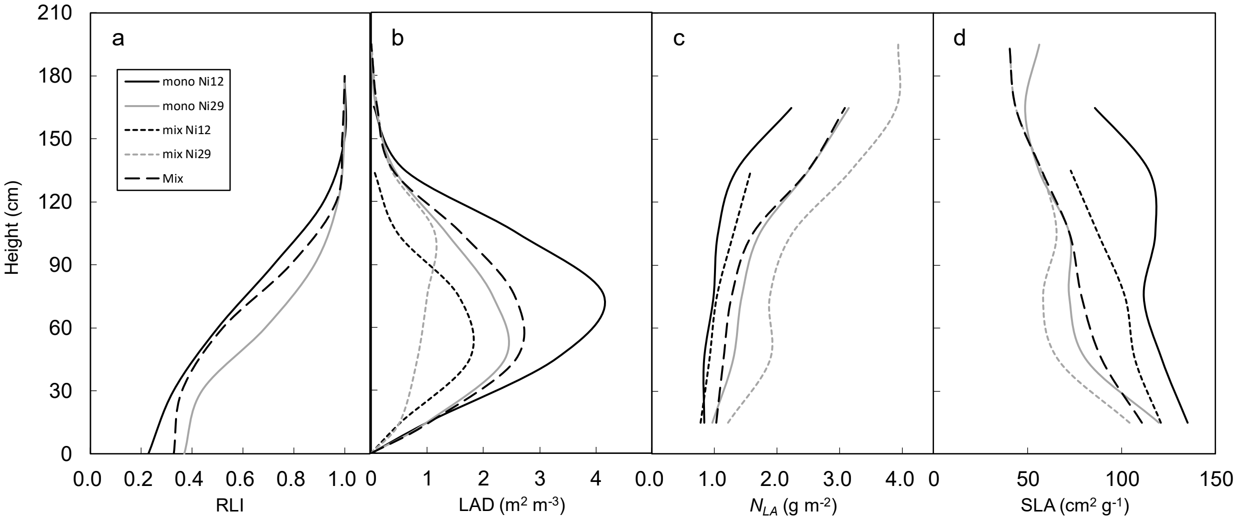


Fig. 3.1.5 Vertical profiles of *RLI* (a), *LAD* (b), N_{LA} (c), and *SLA* (d) under mono-cropped and mixed varieties.

Note: n=4. *RLI*, relative light intensity; *LAD*; leaf area density; N_{LA}, leaf nitrogen content per unit leaf area; *SLA*, specific leaf area. “mix Ni12” and “mix Ni29” show each value of each row (*LAD*; land area=26×82 cm^2) while “Mix” shows value of total canopy of mixture (*LAD*; land area=52×82 cm^2).

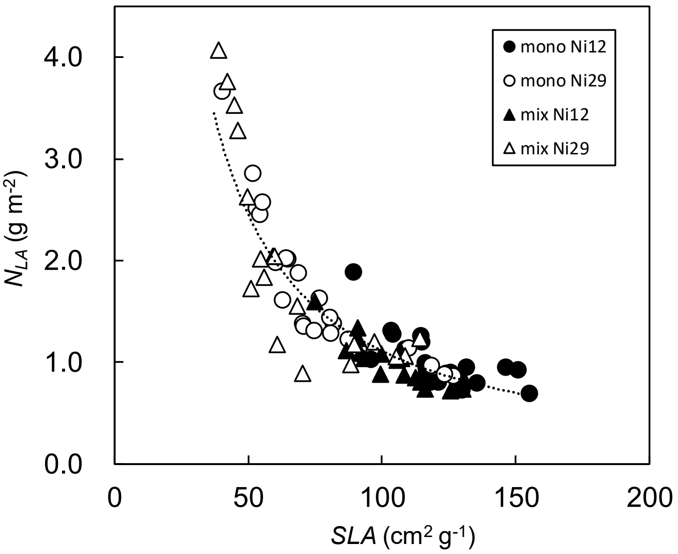


Fig. 3.1.6 Correlation between N_{LA} and *SLA*.

Note: N_{LA}, leaf nitrogen content per unit leaf area; *SLA*, specific leaf area.

Table 3.1.1 Canopy growth and light use under mixed varieties.

Treatment	K	F (m^2 m^{-2})	Above ground biomass (gDW pot^{-1})	Accumulative intercepted PAR (MJ pot^{-1})	RUE (gDW MJ^{-1})	P_{cmax} (μmol $m^{-2}s^{-1}$)
mono Ni12	0.426 a	3.598 b	299.1 a	59.0 b	5.07 a	83.3 c
mono NI29	0.465 a	2.156 a	267.2 a	42.8 a	6.27 b	46.3 a
Mix	0.369 a	2.546 a	289.4 a	56.6 b	5.12 a	58.8 b
MI	0.828	0.869	1.022	1.113	0.902	0.907

Note: *K*, light extinction coefficient; *F*, accumulated LAI from the top of the canopy; *RUE*, radiation use efficiency; P_{cmax}, crop photosynthesis at 2000 μmol $m^{-2}s^{-1}$ of I_0; *MI*, mixture index. Different alphabets mean significant difference between treatments at 1% level (Tukey test, n=4).

3.1.3.2 Field experiment

Climatic conditions during the experiment in Tokunoshima are shown as Fig.3.1.7. Mean temperatures tended to be similar to the average value and rainfall was affected by the rainy season and typhoons, but without drought. Two typhoons approached the island in early September but were not so damaging as to cause severe lodging. Therefore, the disturbance of canopy structure by strong wind was minimal.

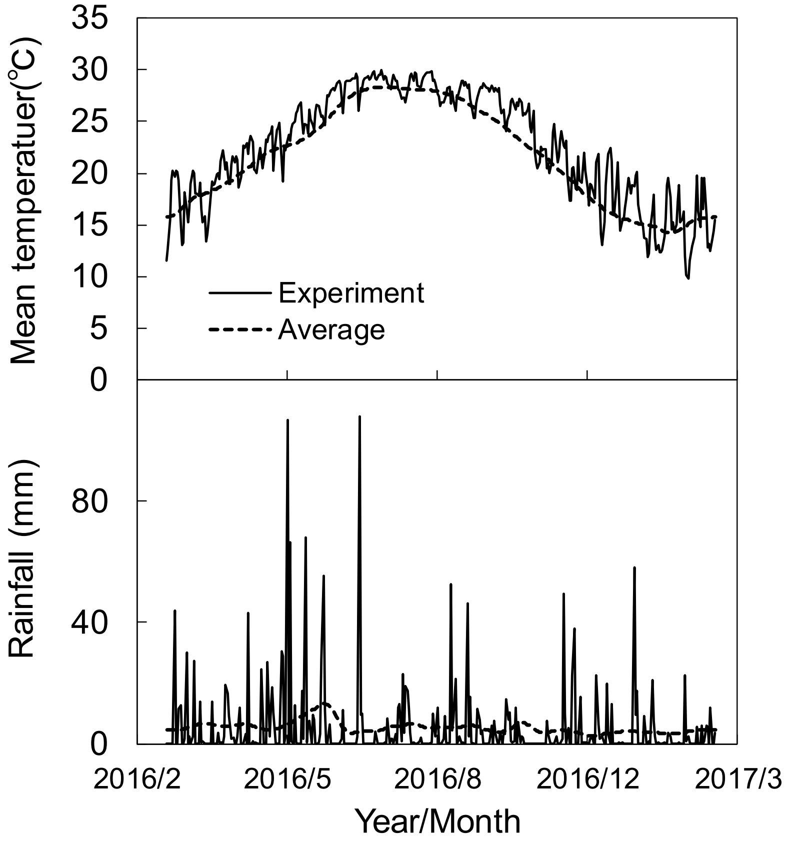


Fig.3.1.7 Climate condition during experimental period in Tokunoshima (Exp. 2).

Note: Data from Isen weather station (JMA 2018). Average values were derived from data between 1981-2010.

Stem length tended to increase until 231 DAP and plateaued without significant differences between treatments (Fig.3.1.8). The number of tillers tended to increase rapidly from 77 DAP to 143 DAP and then gradually decreased, following a trend which was similar to the typical one (Sato and Yoshida 2001). The number of tillers was not significantly different between treatments and delayed-tillered young tillers were also affected to an extent, but the tiller number of the mixture tended to be greater than that of each monoculture at harvest (339 DAP).

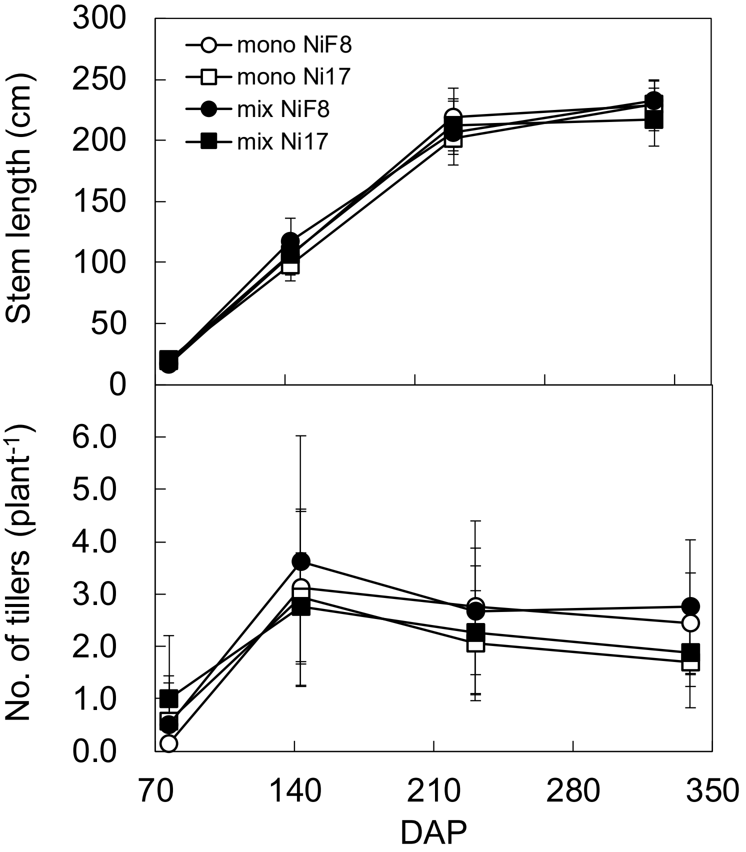


Fig. 3.1.8 Changes of stem length and number of tillers (Exp. 2).
Note: Monocultures, n=16; Mixture, n=8.

Canopy light use and vertical leaf area distribution at the later growth stage, when canopy formation seemed to be completed, were variety- and treatment-specific (Fig.3.1.9). Namely, RLI tended to decrease rapidly from above the canopy to 200-cm height and then not to decrease as much until ground level in *mono* NiF8, while RLI tended to keep gradually decreasing until ground level in *mono* Ni17. Therefore, RLI at ground level tended to be greater in *mono* NiF8 than in *mono* Ni17. In the mixture, RLI tended to keep decreasing slightly until 120-cm height and then to decrease more gradually until ground level, resulting in greater RLI at ground level than that of *mono* Ni17. Light-extinction coefficients (K) were 0.646 in *mono* NiF8 and 0.830 in *mono* Ni17. The K of the mixture was the lowest as 0.594 (Table3.1.2). The above-ground biomass per unit land area and LAI at 231 DAP tended to be greater in the mixture than those of either monoculture. Peak LAD occurred at heights of 202.3 cm in *mono* NiF8, 195.7 cm in *mono* Ni17, and 198.8 cm in the mixture, tending to be higher in the erect-leafed variety, with the mixture exhibiting a value in the mixture intermediate between those of the two monocultures.

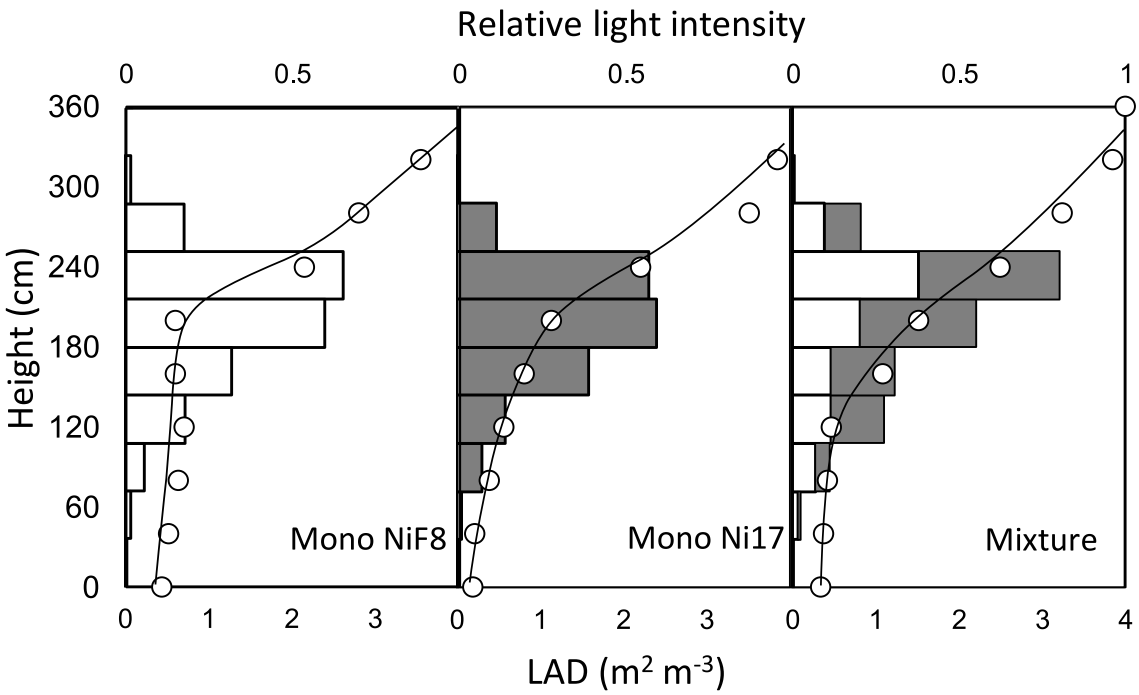


Fig. 3.1.9 Vertical profiles of RLI and LAD at 231 DAP (Exp. 2).
Note: n=2. Open circle and bar mean RLI and LAD, respectively. White and Black bars mean LAD of NiF8 and Ni17, respectively.

Table 3.1.2 Canopy growth and light use under mixed varieties (Exp. 2).

Treatment	K	F	Above ground biomass (gDW m^{-2})	Peak height of LAD (cm)
mono NiF8	0.646	3.24	2004	202.3
mono Ni17	0.830	3.09	2054	195.7
Mix	0.594	3.68	2199	198.8
MI	0.804	1.161	1.084	0.999

Note: n=2.

Stalk parameters at harvest were not significantly different between the various treatments (Table 3.1.3). One stalk weight tended to be heavier in ’Ni17’ than that of ‘NiF8’. The number of millable stalks was highest in *mono* NiF8 (about 96,000 stalks per ha) followed by the mixture (about 92,000) and then *mono* Ni17 (about 75,000). In the mixture, both component varieties tended to exhibit increases in not only one stalk weights, but also the number of millable stalks compared to when grown as a monoculture. Finally, cane yields were highest in the mixture (7.87 ton 10 a^{-1}) followed by *mono* NiF8 (6.95) and then *mono* Ni17 (6.64). Sugar yield showed a tendency similar to that of cane yield.

Table 3.1.2 Yield and yield components under mixed varieties (Exp. 2).

Treatment	Variety	Stalk length (cm)	Stalk diameter (mm)	Weight of 1 stalk (gFW)	No. of millable stalks (ha^{-1})	Cane yield (t $10a^{-1}$)	Sugar content (%)	Sugar yield (t $10a^{-1}$)
Monoculture	NiF8	195.2	23.8	762	95486	6.95	22.2	9.23
	Ni17	196.4	25.7	913	74653	6.64	21.8	8.69
Mixture	NiF8	195.0	24.5	795	104167	8.19	21.1	10.35
	Ni17	182.3	25.2	926	79861	7.55	21.4	9.65
	Total	188.6	24.9	860	92014	7.87	21.2	10.03

Note: No significant difference in stalk length, diameter, and weight between treatments (monoculture, n=16; mixture, n=8). Other variables were not tested (n=2).

3.1.4 Discussion

3.1.4.1 Strategy of each variety for achieving canopy growth under monocrop

RLI decreases from the top of the canopy because the leaves intercept the light within the canopy. The orientation and carbon allocation of leaves (i.e., canopy structure) are the main determinants of the canopy light conditions (Monsi and Saeki 2005), whereas leaf erectness (Hikosaka and Hirose 1997; Shimabuku 1997; Monsi and Saeki 2005; Marchiori et al. 2010) and planting methods (Singels and Smit 2009; Takaragawa et al. 2016b) characterize canopy structure and its light use. Our results showed that these canopy characteristics were different between the two varieties and between the two planting methods (monocrop and mixture). The canopy of *mono Ni12* was characterized by its lower canopy height because of its horizontal leaves, and lower RLI during the growth period because of high VR and *F*, resulting in higher accumulated light interception and biomass production (Figs 3.1.2 and 3.1.3a and Table 3.1.1). The canopy of *mono Ni12* captured more light at the higher layer (Fig. 3.1.5a) because it produced wider and more horizontal leaves at the higher layer, which was reflected by the higher peak height for LAD (Fig. 3.1.5b). This higher peak height for LAD was suggested to be associated with the greater stem length (Fig. 3.1.3b) and larger number of dead leaves (no data obtained) due to the reduced incident light at the lower canopy layer (Fig. 3.1.5a).

Generally, horizontal leaves are more advantageous for canopy growth under light-competitive conditions than are erect leaves (Hikosaka and Hirose 1997). One of the reasons for this is the higher SLA, resulting in greater early growth rate in sugarcane (Terauchi and Matsuoka 2000). In fact, horizontal-leaf Ni12, with a higher SLA, produced the greater biomass (Fig. 3.1.5d and Table 3.1.1). From the results, it could be concluded that horizontal leaves were optimal for early growth of sugarcane before tiller growth accelerated because they captured much more radiation than erect leaves, as many studies have reported (Irvine 1975; Shimabuku 1997). However, the optimal sugarcane canopy structure and *K* change as tiller growth accelerates during the later growth stage (Shimabuku 1997) because the LAI is increased over the optimal value. Thus, artificial modification of the canopy structure of a horizontal-leaf variety monocrop after its early growth would be needed to yield the higher number of millable stalks at harvest. For example, removal of the older leaves, which have lower photosynthetic ability and lower photosynthetic water use efficiency, at the lower canopy layer may be effective at keeping P_c high (Kawamitsu and Uehara 2002; Maekawa et al. 2017) through high water use efficiency and improved light condition at the lower canopy layer.

On the other hand, the canopy of *mono Ni29* was characterized by its greater canopy height and good penetration of light within the canopy during the growth period as a result of its erect leaves and small F (Figs. 3.1.2 and 3.1.3 and Table 3.1.1). Therefore, the RUE for *mono Ni29* was significantly higher than that for *mono Ni12* due to the lower accumulative light interception (Table 3.1.1). Final RLI at ground level was higher in *mono Ni29* than in *mono Ni12* but the K for this canopy tended to be higher due to the lower F value (Fig. 3.1.5a and Table 3.1.1), indicating that the loss of light yield by canopy was greater. Irvine (1975) investigated the canopy structures in several sugarcane varieties and suggested that the higher LAI associated with erect leaves was suitable for maximum growth. These results suggest that *mono Ni29* could yield more when grown at higher plant density, including under freely tillered conditions, or in an intercropping (or varietal mixture) system to reduce such light loss (Keating and Carberry 1993).

From the results of individual leaf photosynthesis measurements, canopy photosynthesis was calculated for the evaluation of canopy growth. Although the varieties showed different photosynthetic features per unit leaf area (Fig. 3.1.4a, b), it appeared that canopy photosynthesis was highly dependent on leaf distribution (Figs 3.1.4c and 3.1.5b). P_c and P_{land} were higher in *mono Ni12* because *mono Ni12* exhibited not only higher P_{max} and P_{leaf} but also higher SLA and LAD than *mono Ni29*, and higher canopy photosynthesis resulted in higher above-ground biomass production. Plants have two strategies to achieve higher growth rate (i.e., photosynthesis): higher SLA to capture much light (Evans and Poorter 2001) and higher N_{LA} to increase photosynthetic ability (Hirose and Werger 1987a; Tominaga et al. 2015). However, these parameters are negatively correlated, as a reduction in light intensity increases SLA but decreases N_{LA} (Reich and Walters 1994) (Fig. 3.1.6). As horizontal-leaf Ni12 had higher SLA and erect-leaf Ni29 had higher N_{LA}, the former was considered to be the more appropriate strategy for biomass production at the early growth stage.

3.1.4.2 Phenotypic plasticity of canopy growth induced by mixed varieties

Considering the sugarcane growth cycle, optimal light conditions and/or canopy structure to achieve higher numbers of millable stalks vary depending on the growth stage (Shimabuku 1997). Previous studies had revealed the potential of varietal mixtures to improve the canopy structure and its light use (Tsuchiya and Kinoshita 1984; Takaragawa et al. 2016b). The present study attempted to mix erect-leaf and horizontal-leaf varieties for improving the within-canopy light condition and canopy growth. It was also revealed that the mixture reduced K and yielded increased biomass through increased light interception (Table 3.1.1). *Mix* yielded higher biomass production than *mono Ni29* while not surpassing the yield of *mono Ni12* (Table 3.1.1). Additionally, the *MI* for biomass excessed 1.0 (Table 3.1.1), indicating that the mixture may have exhibited coexistence of the two varieties within the community through habitat segregation (Takaragawa et al. 2016b) rather than destruction of one variety due to competition. The habitat segregation under the varietal mixture is considered to come from phenological plasticity (Newton et al. 2008; Faraji 2011; Takaragawa et al. 2018b). In the present study, there was plasticity of the mixed varieties in terms of plant size (stem length and plant height) (Fig. 3.1.3), leaf biomass allocation (SLA and the vertical profile of LAD) (Fig. 3.1.5b,d and

Table 3.1.1), and leaf nitrogen distribution (N_{LA}) (Fig. 3.1.5c).

Hikosaka and Hirose (1997) suggested that a horizontal-leaf habit was a more advantageous strategy for survival because a horizontal-leafed plant surrounded by clonal erect-leafed plants could capture the light that penetrated through the erect-leaf plants, resulting in higher photosynthesis. In our results, however, when the two varieties were mix-planted by row, plant size was suppressed in the horizontal-leaf Ni12 while it was increased in the erect-leaf Ni29 (Fig. 3.1.3). Our results may have disagreed with the suggestion of Hikosaka and Hirose (1997), because they assumed to ignore the other canopy traits, except for leaf erectness, such as shoot height and leaf orientation. Erect-leaf Ni29 may have elongated the stem and allocated leaves at higher canopy layers to capture much light and to overcome such disadvantageous conditions in mixed culture with a horizontal-leafed variety, resulting in higher biomass than in *mono Ni29* (Figs. 3.1.3 and 3.1.5b). Therefore, these results indicated that *mix Ni29* may have compensated for the reduction of *mix Ni12* growth (i.e., a compensation effect) through habitat segregation for light use within the community.

Canopy leaf area (*F*) was lower in *Mix* than in *mono Ni12* but higher vegetation ability and light interception of *mix Ni12* resulted in the lower *K* (Figs. 3.1.2 and 3.1.5 and Table 3.1.1). The *K* with moderate LAI could contribute to light use to stimulate the emergence and growth of tillers, although they were removed in the present study. From the monocrop study, erect-leaf Ni29 exhibited higher RUE than other treatments (Table 3.1.1). That may have explained why *mix Ni29* could maintain growth under light-competitive conditions with *mix Ni12*. According to Equation 3, the lower *K* value of the mixture means higher light availability at lower canopy layers even if tillers had emerged and increased the whole canopy LAI. The change of within-canopy light conditions caused the plasticity of SLA: the leaves of Ni12 became thicker in the mixture than in the monocrop (Fig. 3.1.5d). Such light-induced plasticity in SLA was reported in other species as lower light with higher SLA while higher light with lower SLA at a common leaf weight (Rice and Bazzaz 1989). The main strategies for thin-wide (high SLA) and thick-narrow (low SLA) leaves are to capture as much light as possible under light-competitive condition and to increase photosynthetic ability under strong light conditions, respectively (Evans and Poorter 2001). That is, habitat segregation for light use decreased the light competition, resulting in thicker leaves (Fig. 3.1.5d), which may have compensated for the lower LAI (Fig. 3.1.5b and Table 3.1.1) to maintain high growth under conditions where light penetrated into the canopy well (Fig. 3.1.5a). Higher SLA or LAI with horizontal leaves is needed for the early growth stage, whereas higher NAR with erect leaves is needed for the later growth stage (Shimabuku 1997; Terauchi and Matsuoka 2000). In addition, a single variety could meet only one of these requirements under a monocrop situation (Shimabuku 1997). *Mix*, however, combined the lower SLA, moderate LAI, and lower *K* with higher radiation capture. Therefore, a mixture of erect- and horizontal-leaf varieties could build up the canopy, which has the adaptability to a variation in the optimal pattern of canopy light use during the tillering stage.

From the results from the monocrops, Ni29 was considered to be the higher-N_{LA} and lower-SLA type of variety while Ni12 was the lower-N_{LA} and higher-SLA type of variety (Figs. 3.1.5c,d and 3.1.6); under monoculture conditions, higher SLA was the more appropriate strategy for the early growth stage. Under a variety mixture, on the other hand, higher N_{LA} may have also been one of the appropriate strategies for variety growth as *mix Ni29*, with higher N_{LA}, grew better than *mix Ni12* in the mixture because of the less light-competitive conditions (Figs. 3.1.4 and 3.1.5). After the early growth stage, canopy closure would be accelerated as the tillers expanded their leaves. During the later growth stage, higher NAR, an indicator of canopy photosynthesis, contributed to increase and/or maintenance of the growth rate (Shimabuku 1997). A steeper N_{LA} profile is more suitable for canopy photosynthesis than a uniform profile as reported by other studies (Hirose and Werger 1987b; Tominaga et al. 2015). *Mix* had as steep an N_{LA} profile as did *mono Ni29*, due to the steeper profile of *mix Ni29* (Fig. 3.1.5c), indicating potential greater growth during the later growth period. Thus, the plasticity of the N_{LA} profile in the mixture could be also one of the reasons for its potential to adapt to changes in canopy structure and light use from before to after the peak of tillering.

Irvine (1975) showed that the effect of erect leaves on final yield depended on the climate during that growing year. Thus, it was suggested that the optimal plant type may vary, depending also on the climatic conditions during a given growth stage. The optimal plant type may change at different growth stages and in different years because of the effects of varying climatic conditions, especially radiation both within and outside the canopy. If so, habitat segregation for light use caused by a mixture of varieties with different plant types could allow the canopy to maintain the growth under such fluctuating light conditions over the entire growth period, as explained by moderate LAI with lower K (Table 3.1.1).

3.1.4.3 Effect of varietal mixture under field condition

In the canopy of the mixture, LAD at 120–160 cm tended to be greater, especially due to 'Ni17' (Fig. 3.1.9), which may have resulted in increased capture of radiation at 120–200 cm height below the peak height of LAD (Table 3.1.2). This was suggested to be the reason for the lower K despite the higher LAI (Fig. 3.1.9 and Table 3.1.2). Plasticity induced in the mixture appeared to be less under field conditions than under glasshouse conditions (Fig. 3.1.8, Table 3.1.2–3). The reason for this may be the difference in experimental conditions between glasshouse and field experiments: different choice of varieties, experimental period, and planting density. However, it was clear that the changes in LAD profile, F, and K induced by mixed-planting indicated habitat segregation of leaf distribution and its benefit for canopy light use and biomass production (Fig. 3.1.9 and Table 3.1.2).

Sugarcane yield is determined to a greater extent by the number than by the weight of millable stalks, especially in spring planting (Miller and James 1974; Shimabuku 1997). Generally, in sugarcane, the correlation between the number and the weight of millable stalks is negative, namely a greater number of stalks are associated with thinner and lighter stalks (Miller and James 1974; Shimabuku 1997; Sato and Yoshida 2001). However, in the mixture, the greater number of millable stalks was achieved in conjunction with the maintenance of one-stalk weight, resulting in greater yield (Table 3.1.3). Both two varieties examined in the present study were stalk-weight type varieties that have relatively low tillering ability, but can yield high weight per stalk. A varietal mixture with different canopy structures could improve within-canopy light conditions, which could enhance the yield expression of each variety, with a tiller-number type variety yielding much greater number of stalks than monocrops (Takaragawa et al. 2016b). Combining these facts, it is expected that a varietal mixture with different plant types and high tillering abilities would enhance the positive effect of the mixture on the number of millable stalks. With respect to tiller germination and the establishment of millable stalks, it was reported that tillers that germinated before the early rainy season on late May to late June are represented in the millable stalks at harvest (Sato and Yoshida 2001). In other words, the maintenance of the growth of tillers that sprouted at the early growth stage is necessary to yield an increased number of millable stalks. Shimabuku (1997) suggested that a canopy with lower K could provide a greater number of millable stalks because the more radiation penetrating into the lower canopy layer could reduce the number of dead leaves and tillers there.

In conclusion, the increased yield and biomass exhibited by the varietal mixture with different plant types, compared to the corresponding monocrops appear to be as follows: the mixture formed a canopy where radiation penetrated better into the lower canopy layer (Fig. 3.1.9), maintaining the number of tillers (Fig. 3.1.8), improving the growth of tillers, especially those which germinated in the early growth stage, increasing the weight per stalk (Table 3.1.3), and finally yielding a greater number of millable stalks with increased yield but without a negative correlation between stalk number and mean stalk weight (Table 3.1.3).

3.2 Varietal mixture with different types of yield formation.

3.2.1 Introduction

Sugarcane has a longer growing period than many other agricultural crops. In addition, it is also grown over so-called ‘crop cycles’ covering a number of ratoons. A monoculture consisting of several sugarcane cycles grown continuously without a break crop has the potential to destroy the biodiversity of farmland (Karp et al. 2012). Nevertheless, ratoon cropping is considered as essential method to achieve labor-saving and financially stable farming while growers are aging and their numbers are decreasing (Matsuoka 2006; Kikuchi 2009; Takaragawa et al. 2018e). In addition, multiple ratoon cropping is expected to increase in the future (Sato 2017). In the present study, the author would like to provide one option to achieve both maintenance and to increase of biodiversity within agricultural fields and ratoon cropping: a varietal mixture.

While a monoculture of one variety is not desirable, the use of a number of varieties could help increase the biodiversity and decrease the risk of losses in productivity (Faraji 2011). Examples exist where cultivars of the same species have been planted as mixed crops (Zhu et al. 2000; Newton and Swanson 1999). Mixed-planting of varieties of small-grain cereal crops, such as rice, wheat, and barley, have been reported to enhance disease control by causing cultural breaks in the field (Zhu et al. 2000). This type of strategy has also increased yields and the quality of crops through the competition and compensation effects between cultivars (Newton and Swanson 1999). Unfortunately, in cereal crops, hand harvesting is often needed to separate cultivars depending on the characteristics of grain quality (Wolfe 2000). However, this is not necessary with sugarcane because different varieties do not have to be separated prior to milling and sugar extraction. In sugarcane, some positive yield effects have been reported with mixed-variety stands. Cadet et al. (2007) found that damage from harmful nematodes was reduced through growing nematode-tolerant and nematode-sensitive sugarcane varieties together as a mixture. Kapur et al. (1988) showed that some mixtures of sugarcane varieties increased yield and added to yield stability compared with pure stands. Although the reason for this yield improvement was not identified, it was believed that it could relate to a change in crop canopy structure (Tsuchiya and Kinoshita 1984).

In addition, in the real fields, there are many cases where the complementary planting of different varieties together in a mixture was considered to be an effective technique to yield enough stalks at harvest (Shinzato et al. 2010; Takaragawa et al. 2018d). However, complementary planting is one of the farm techniques that needs to be more labor-saving due to difficulties in mechanizing (Shinzato 2015).

From this information, it is hypothesized that mixed-planting of varieties with different tillering and/or ratooning abilities could induce a compensatory effect to maintain the number of stalks when that was decreased due to missing plants or some biotic or abiotic stress. In this study, we investigated the effects of mixing cultivars on growth and yields of new-planted and ratoon-cropped sugarcane by assessing effects on canopy structure and yield expression.

3.2.2 Materials and methods

Sugarcane was grown at an experimental field of the Faculty of Agriculture, University of the Ryukyus, Okinawa (dark red soil, pH 5.9, EC 8.8mS m^{-1}). Sugarcane varieties NiF8 and Ni22 were planted. Variety 'NiF8' is high yielding because of its increased stalk weight, while 'Ni22' combines high yield with high tiller number and ratooning ability.

The experiments were conducted in two cropping seasons: the first was a new planting in season 2015/2016 and its ratoon cropping in 2016/2017, while the second was new planting in 2016/2017 and its ratoon cropping in 2017/2018. Climatic conditions during the experimental periods are shown in Fig.3.2.1. Temperature tended to be higher than that of the 30-year average, especially in 2016. Rainfall tended to be lower than the average in April to May before the rainy season and in August to September after the rainy season in 2015 and 2017 while it rained annually enough in 2016. Typhoons approached Okinawa 6 times in 2015/2016, 7 times in 2016/2017 and 7 times in 2016/2017, which broke the canopy structures due to lodging and broken stalks.

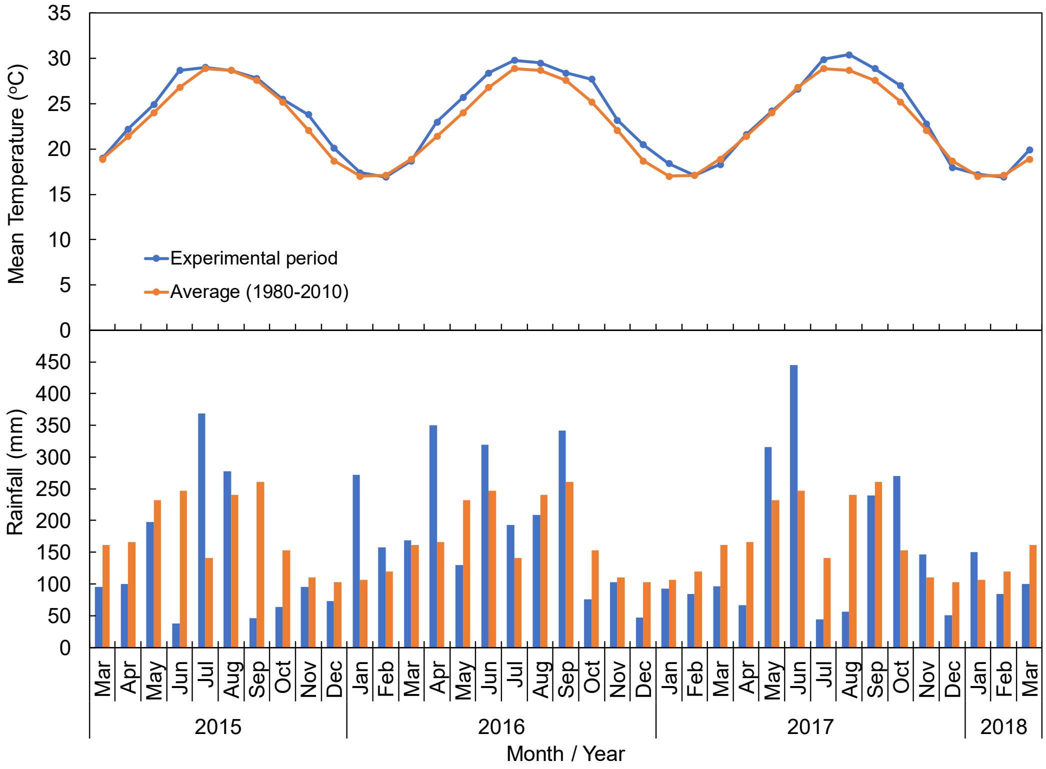


Fig. 3.2.1 Climate condition during experimental periods.

Note: Data from Naha meteorological station (Japan Meteorological Agency 2018). Average values are calculated by data from 1980 to 2010.

The two varieties were mono-cropped and mixed by row and by plant (Fig. 3.2.2). Each treatment had two replicates. The plot size of each replicate was 42.84 m^2, consisting of seven 5.1-m-long rows. Single-bud sugarcane setts were planted in seedling trays in a greenhouse on 13 March 2015 and 10 March 2016. The germinated setts were planted in the field trial on 3 April 2015 and 29 April 2016 with a planting density of 2.78 plants m^{-2} (within-row 0.3 m, inter-row 1.2 m). Ratoon treatment was started from 29 April 2016 and 26 March 2017. Fertilizer was applied three times for each cropping type according to the cultivation manual (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2015); total amounts of N, P_2O_5 and K_2O were 200, 60 and 60 kg ha^{-1}, respectively.

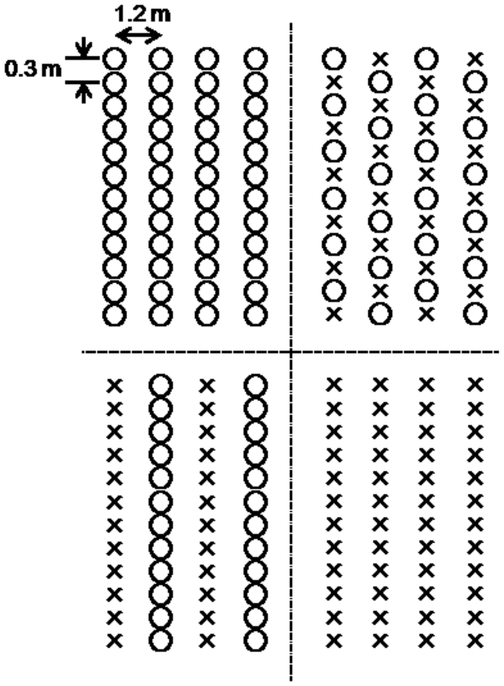


Fig. 3.2.2 Patterns of mixing varieties.

Note: Open circle and cross indicate different varieties. Lower left part and upper right part indicate mixture-by-row and mixture-by-plant, respectively.

Stalk length and the number of tillers were recorded monthly for each of six plants from each treatment plot. Unfortunately, the varieties in mixture-by-plant were not identified in ratoon cropping. Sugarcane canopy was determined in two sub-plots (2.4 m × 0.9 m) for each treatment according to the stratified clip method (Monsi and Saeki 2005), and light interception within each canopy was measured using a light sensor on 22–24 September 2015 to determine the canopy structure and light distribution for spring planting in 2015/2016. Light-extinction coefficient (K) was calculated according to equation (3.2.1) as below (Monsi and Saeki 2005):

$$I = I_0 \, e^{-KF} \qquad (3.2.1)$$

where F is the cumulative LAI from the top of the canopy. I_0 and I are light intensity on a horizontal level above the canopy and at a given depth within the canopy, respectively.

Sugarcane was harvested from two sub-plots (2.4 × 1.2 m) within each treatment on 24 February

2016, 22 February 2017, and 1 March 2018. The number and weight of millable stalks were recorded. Sucrose content was determined on the first-expressed juice samples from the millable stalks of each cultivar using HPLC (RID-10A, SCR101-H, Shimadzu) (Watanabe et al. 2016).

To compare the growth parameters of the mixture to the one predicted on the basis of the two-component monocultured ones, a mixture index (*MI*) was calculated for the mixture treatment (Trenbath 1974):

$$MI = Y_{mix} \times 2/(Y_{monoNi12}+Y_{monoNi29}) \quad (3.2.2)$$

where Y_{mix} is the observed parameter for the mixture, and $Y_{monoNi12}$ and $Y_{monoNi29}$ are the parameters of monocultured 'Ni12' and 'Ni29', respectively. With this index, values of 1 indicate that the observed parameter was equal to the predicted one, whereas the values of less than or greater than 1 indicate under-yielding and over-yielding, respectively (Trenbath 1974).

Three-way analysis of variance was conducted for yield and its components using statistical software (BellCurve for Excel, Social Survey Research Information Co., Ltd.).

3.2.3 Results

3.2.3.1 Plant cane

The culm length of both varieties tended to be higher in mixed plots than in monocultured plots during the later growth stage of new planting (Fig. 3.2.3). The change in the numbers of stalks was dependent on variety: 'NiF8' had more tillers when mono-cropped while 'Ni22' had more tillers when cultivars were mixed by row in 2015/2016 (Fig. 3.2.4). In 2016/2017, the number of stalks including that of 'Ni22' in mixture-by-row was decreased during mid-term growth period because of damage from a severe typhoon.

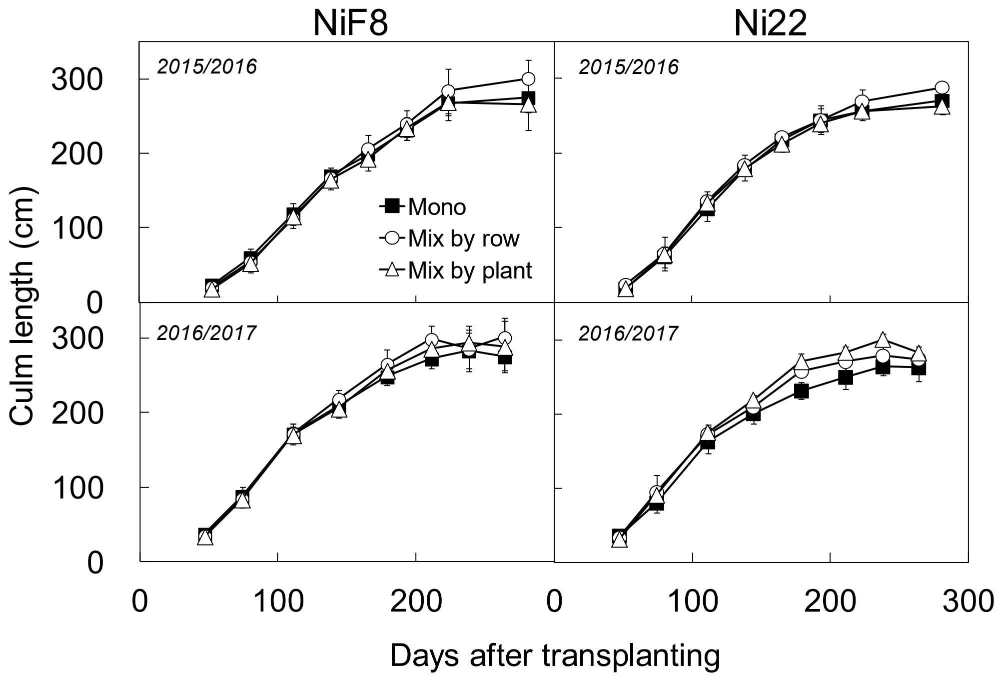


Fig. 3.2.3 Culm length during cultivation in spring planting.

Note: Closed square, open circle and open triangle indicate monoculture, mixture-by-row and mixture-by-plant, respectively.

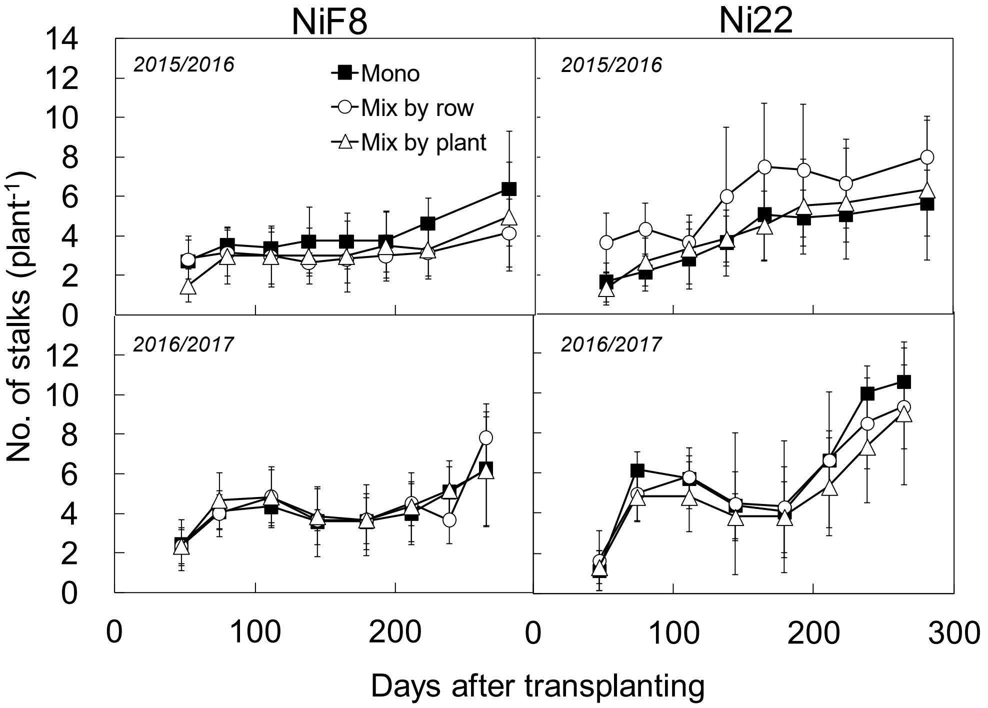


Fig. 3.2.4 Number of stalks during cultivation in spring planting.

Note: Closed square, open circle and open triangle indicate monoculture, mixture-by-row and mixture-by-plant, respectively.

The difference in the canopy structure of the two sugarcane varieties is shown in Fig. 3.2.5. Mono-cropped 'Ni22' tended to have more leaves in the upper layer than did mono-cropped 'NiF8'. The canopies of mixture-by-row and mixture-by-plant tended to capture more radiation in the upper layer, while the light-extinction coefficient (K) of the mixture-by-row tended to be lower, indicating better light conditions in this canopy than other canopies.

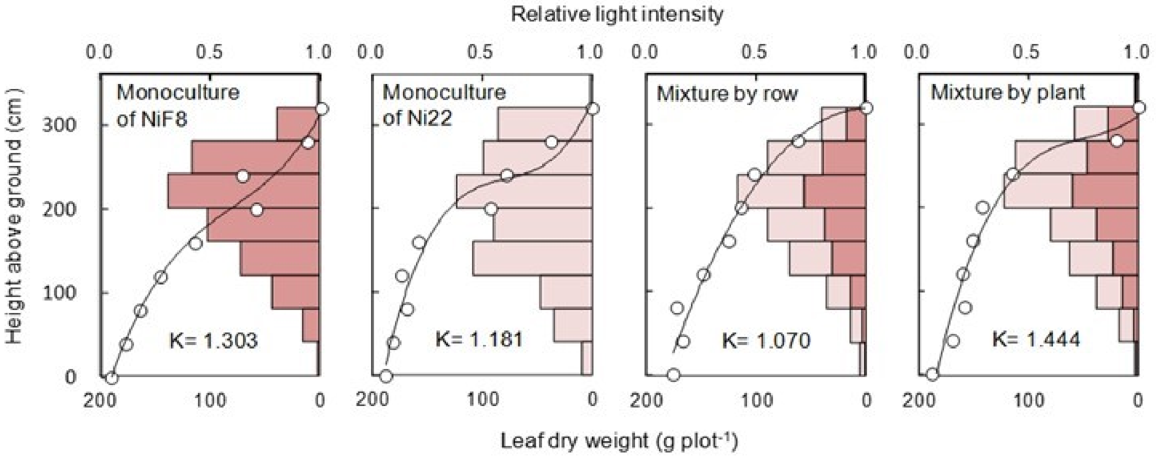


Fig. 3.2.5 Productive structure diagram of sugarcane monoculture and mixture on 173 days after transplanting in spring planting of Exp 1 on 2015/2016.

Note: Closed bar, open bar and open circle indicate leaf weight of NiF8, that of Ni22 and relative light intensity, respectively. K is the light extinction coefficient.

The yield and yield component data are shown in Table 3.2.1. The average stalk weight of ‘NiF8’ was significantly greater than that of ‘Ni22’, but with no significant differences between treatments in 2015/2016. While the stalk weight was affected by variety, the number of millable stalks was treatment dependent. The number of stalks of ‘NiF8’ tended to be lower for the mixture (by-row and by-plant) than for the monoculture in new plantings. The number of stalks of ‘Ni22’ tended to increase in the mixture-by-row treatment, especially in 2015/2016. As a result, the yield of the mixture-by-row tended to be higher than that of the monocultured ‘Ni22’ and lower than that of monocultured ‘NiF8’. In the mixture-by-plant, both cultivars showed a tendency toward decreased stalk numbers with reduced yields. The mixture index was greater than 1.00 in the mixture-by-row in both new planting seasons, while the index was year dependent in mixture-by-plant (Table 3.2.2).

Table 3.2.1 Yield and yield components of sugarcane.

Year	Treat-ment	Cultivar	Weight of 1 stalk (gFW)	No. of millable stalks (ha^{-1})	Cane yield (t ha^{-1})	Sucrose content (%)	Sugar yield (t ha^{-1})
1st plant (2015/2016)	Monoculture	NiF8	936	79861	74.5	23.6	10.6
		Ni22	752	79861	59.8	23.9	8.6
	Mixture by row	NiF8	914	69444	62.9	23.8	9.0
		Ni22	699	107639	74.8	24.0	10.8
		Total	792	86806	68.8	23.9	9.9
	Mixture by plant	NiF8	989	55556	54.2	23.7	7.7
		Ni22	740	79861	59.2	23.1	8.3
		Total	839	67708	56.7	23.4	8.0
2nd plant (2016/2017)	Monoculture	NiF8	736	81597	60.3	21.1	9.3
		Ni22	657	83333	54.8	21.8	7.6
	Mixture by row	NiF8	956	65972	63.2	20.1	7.6
		Ni22	656	86806	56.9	21.3	7.3
		Total	786	76389	60.1	20.7	7.4
	Mixture by plant	NiF8	992	62500	62.1	21.0	7.8
		Ni22	773	72917	55.4	21.0	7.1
		Total	872	67708	58.8	21.0	7.5
Ratoon after 1st plant (2016/2017)	Monoculture	NiF8	953	81597	75.0	21.1	10.4
		Ni22	1018	92014	89.2	21.3	11.0
	Mixture by row	NiF8	908	83333	75.6	20.1	9.3
		Ni22	964	97222	93.6	20.7	12.2
		Total	885	90278	84.6	20.4	10.4
	Mixture by plant	NiF8	—	—	—	—	—
		Ni22	—	—	—	—	—
		Total	825	112847	93.2	23.7	14.8
Ratoon after 2nd plant (2017/2018)	Monoculture	NiF8	1079	69444	74.5	16.3	7.3
		Ni22	819	97222	79.2	17.7	8.8
	Mixture by row	NiF8	820	93750	76.6	15.2	5.9
		Ni22	929	72917	67.5	15.6	6.3
		Total	868	83333	72.0	15.4	6.4
	Mixture by plant	NiF8	—	—	—	—	—
		Ni22	—	—	—	—	—
		Total	747	105903	75.8	15.3	8.5
ANOVA	Season (S)				**	**	**
	Cropping type (C)		*	**	**	**	
	Treatment (T)						
	S × C					**	
	S × T						
	C × T			*	*		
	S × C × T						

Note: Juice extraction rate of 60 % is used. For ratoon cropping, the data of each cultivar in mixture by plant was not available as identification of cultivars was difficult in this treatment. * and ** mean significance at 5 and 1% levels, respectively (No. of millable canes, cane yield, and sugar yield, n=2; Others, n=16).

Table 3.2.2. Comparison of cane yield in mixture with the expected one when mono-cropped.

Treatment	1st planting	ratoon after 1st planting	1st + ratoon	2nd planting	ratoon after 2nd planting	2nd + ratoon
Mix by row	1.03	1.01	1.02	1.04	0.94	0.98
Mix by plant	0.84	1.24	1.06	1.02	0.99	1.00

3.2.3.2 Ratoon cane

The effects of treatments on monthly-measured culm length and the number of stalks per plant were unclear during ratoon cropping (Fig. 3.2.6).

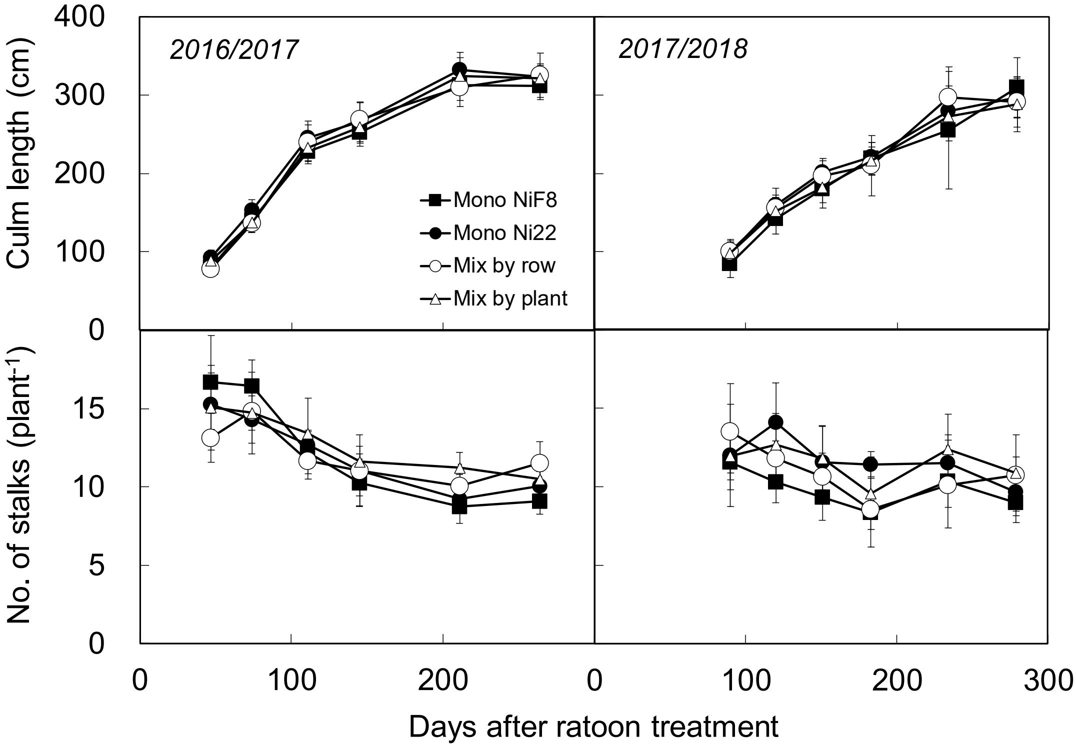


Fig. 3.2.6 Culm length and number of stalks during cultivation in ratoon cropping.
Note: Closed square, closed circle, open circle and open triangle indicate monoculture of NiF8, monoculture of Ni22, mixture-by-row and mixture-by-plant, respectively.

Yield components of mixtures were changed when ratoon-cropped and season-dependent (Table 3.2.1). While the stalk weight of each variety tended to be lower for the mixtures than that of the monoculture, the number of stalks and yield tended to increase not only in the mixture-by-row but also mixture-by-plant treatments in 2016/2017. In 2017/2018, monoculture of ‘Ni22’ exhibited lower stalk weight than that of monoculture of ‘NiF8’ but had higher number of stalks than that of ‘NiF8’ in the monoculture. On the other hand, the component ‘Ni22’ in mixture-by-row showed the opposite trends, with a decreased number of stalks and stalk yield. Mixture-by-plant exhibited a higher number of stalks but did not over-yield compared to both monocultures due to the lower weight of stalk in 2017/2018.

3.2.4 Discussion

In spite of having the same plant density of mixed varieties, the effect of mixture on the yield was different between the patterns of mixing (Table 3.2.1 and 3.2.2). This indicated that the distance between individual stools of the varieties and their growth stage was the key factor in understanding the effect of mixture on growth and yield. The interaction of varieties in the mixture-by-plant occurred early, while that of the mixture-by-row occurred later due to the increased distance between plants of the different varieties. Habitat segregation of the varieties was possible in the mixture-by-row treatment in new planting. In ratoon cropping, on the other hand, ‘Ni22’ of the mixture-by-plant treatment could grow dominant at an earlier stage as ‘NiF8’ had fewer tillers and lower ratoon ability, resulting in yield increases in mixture-by-plant (Table 3.2.1 and 3.2.2). However, in 2017/2018 when the weight of the ‘Ni22’ stalk was not as high as in the other ratoon, the yield of mixture-by-plant did not surpass yields of either monocultures. Therefore, as ‘Ni22’ has high ratooning ability, the number of stalks of ‘Ni22’ may have been the key factor in determining the yield difference between treatments under ratoon cropping.

In this study, the differences in yield were mainly dependent on the number of stalks. These results are consistent with the differences in light conditions in the sugarcane canopy (Fig.3.2.5). Mixture-by-plant could promote competition for light during the earlier growth stage and reduce the number of stalks in new planting, while mixture-by-row may improve the light conditions in the canopy during later growth stages and increase the number of stalks. In terms of ratoon cropping, the mixture of varieties has a higher yield potential than that of the separately monocultured varieties.

Compared to a monoculture of one variety, a mixture of sugarcane varieties with different canopy structures resulted in differences in the growth and yield that were dependent on the patterns of mixing (by row or by plant), by choice of variety (‘Ni22’ and ‘NiF8’), and by cropping type (new planting or rattoon cropping). Mixing varieties by row improved light conditions in the canopy and increased the stalk number of a so-called ‘tiller-number’ type variety, but reduced yield compared to the monocultured ‘stem-weight’ type variety in new planting. On the other hand, mixing varieties by plant resulted in decreased light conditions in the canopy, decreased stalk number of a ‘stem-weight’ type variety, and decreased yield compared to either monoculture in new planting. These results indicate that habitat segregation occurred during the later stage of growth as the interaction between the varieties was delayed when varieties were mixed by row. However, the yields of mixtures were higher than those of the monocultures of single varieties when cropping was continued on to ratoon cropping, but only when the ‘tiller-number’ type variety exhibited the higher ratoon ability. As tiller-number type variety was suggested to have high compensatory ability when mix-planted (Marquard et al. 2009; Barot et al. 2017), the positive effect of the mixture could be gained especially in a season when this type of variety was more vigorous. Further research including the effects on multiple ratoon cropping and different combination of variety types is required to evaluate the use of mixtures of varieties as a new technique to achieve sustainable sugarcane production.

3.3. Varietal mixture with different rooting abilities.

3.3.1 Introduction

The importance of root architecture in plant productivity comes from the fact that many soil resources are either unevenly distributed or subject to localized depletion (Lynch 1995). Therefore, roots grow with the plasticity necessary to adapt to diverse changes in the soil environment (Smith et al. 2005). Despite the importance of root architecture and plasticity, those in sugarcane have been studied less than many other crops because of experimental limitations, such as its longer growth period and larger plant size (Matsuoka and Garcia 2011). It is important to better understand sugarcane root plasticity by dividing root interaction mechanisms into two parts: internal (between the main stem and tillers) and inter-individual (between individuals).

As many other grass species, sugarcane has tillers, and there arise competition between the main stem and tillers for photoassimilate, particularly during the early growth stage of the tillers. Main stem and tillers interact to utilize soil resources because of soil capacity limitations. While the emergence and aboveground growth of tillers has been well studied (Sato and Yoshida 2001), the interaction between main stem and tiller root growth has not received attention. Therefore, we attempted to show the effect of tiller emergence and growth on sugarcane root architecture by regulating number of tillers. Based on these results, the effect of internal interactions between the main stem and tillers on root formation was evaluated.

The effect of inter-individual interactions on root formation was reported in an intercropping system of sugarcane with other crop species such as soybean (Li et al. 2012). In addition, many studies showed that there were differences in root-growth patterns between varieties (Fukuzawa et al. 2009; Smith et al. 1999; Negi et al. 1971; Spaull 1980). Inter-individual interactions in root growth and formation should also occur between varieties that are mix-planted, particularly when they have different root traits. In sugarcane, mixed varieties could decrease the number of underground pathogens such as root rot *Pythium* (Srinivasan 1968) and harmful nematodes (Cadet et al. 2007); however, few researches were concerned with biomass production, particularly root formation under mixed varieties. In the present study, the author hypothesized that root plasticity would be induced under mixed varieties with different root traits because a mixture of roots can penetrate the space less occupied under a mono-crop system, resulting in a denser root architecture. Based on this premise, the effect of inter-individual interactions on root formation was evaluated using sole and mixed varieties.

The author conducted experiments to reveal the effect of tiller regulation (experiment 1) and mixed varieties (experiment 2) on sugarcane root architecture. The objective of the present study was to discuss root plasticity in sugarcane based on these results.

3.3.2 Materials and Methods

The two experiments were conducted in a greenhouse at the University of Ryukyus, Japan (26°15′N, 127°45′E; altitude 127 m). The in-greenhouse mean air temperature (VP3, Decagon) increased from 24°C at the beginning of the experiments to 33°C at the end of experiments, and the mean daily accumulated radiation (SE-SP215, Apogee) was 15.2 kW m^{-2} day^{-1}.

3.3.2.1 Effect of tiller regulation on root and shoot growth (Exp. 1).

A Japanese commercial sugarcane variety (*Saccharum* spp. cv. NiF8) was used. Single bud setts were soaked in water and fungicide for one day each and raised in seedling trays in the greenhouse from March 10, 2016. The germinated seedlings were transplanted to a root box (50-cm width, 86-cm height, 9-cm depth, n = 1) and long cylindrical pots (20-cm bore, 100-cm height, n = 3) (Fig. 3.3.1) were filled with well-mixed and sieved (<2 mm) soil that was composed of soil, sand, and peat (1:1:1, v/v) on May 5, 2016. The root boxes and pots were placed vertically. And the acrylic glass side of root box was covered with black sheet to block the light penetration. The soil levels were 80 and 90 cm for the root box and cylindrical pots, respectively (Fig. 3.3.1). Soil weight was calculated using the soil bulk density of 0.9 g cm^{-3}. The two treatments were designed: to regulate tillers after they emerged (TR), and to allow tillers to grow (control). The plants were well irrigated using a drip irrigation system and fertilized weekly with 500 mL of modified Hoagland solution [6 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 100 µM $C_{10}H_{12}FeN_2NaO_8$, 25 µM H_3BO_3, 10 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, and 0.5 µM H_2MoO_4]. Water was drained from the holes via a mesh at the bottom of the root box and cylindrical pots. The main stem length and number of tillers were measured weekly for all plants after transplanting. Three plants were sampled before transplanting to measure

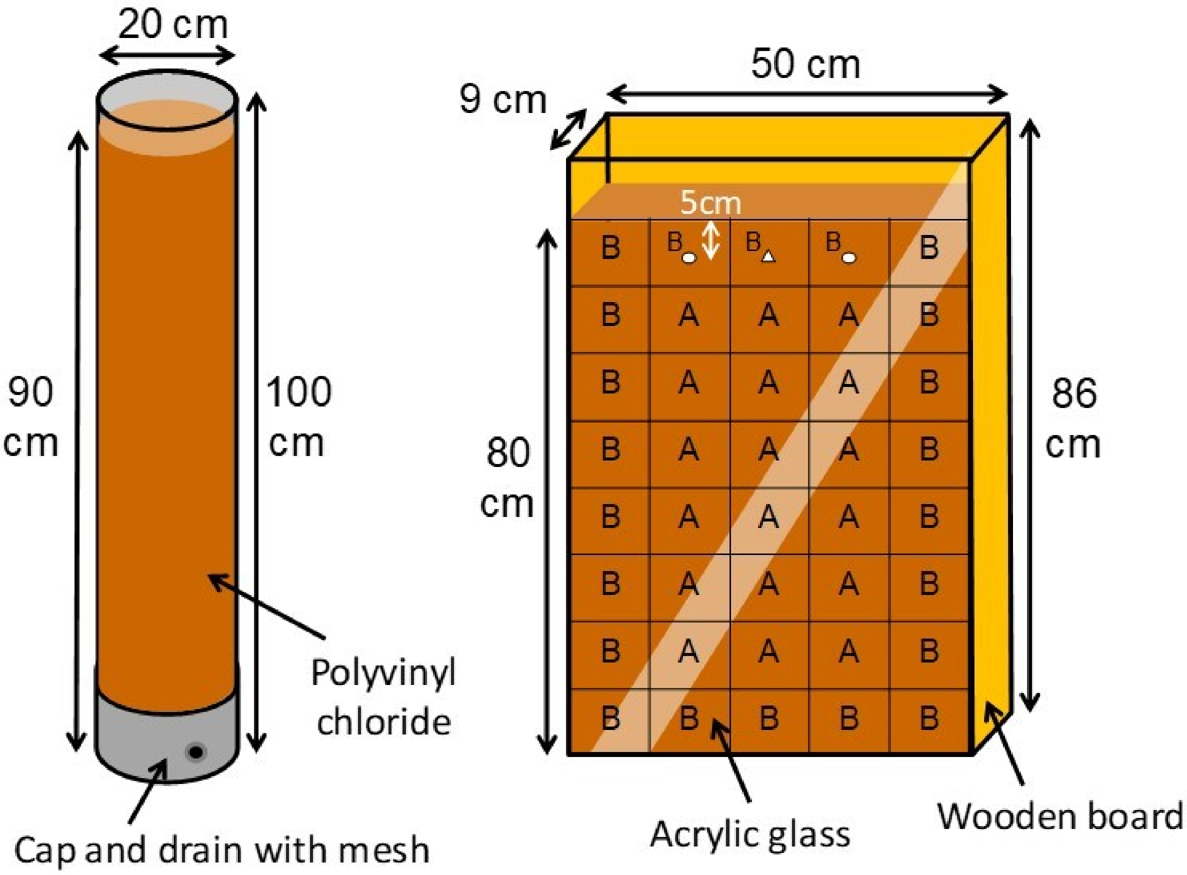


Fig. 3.3.1 Outline of the cylindrical long pot and root box used in the present study.

Note: Open triangle and circles in the root box mean the positions of seedlings transplanted for Exp. 1 and Exp. 2, respectively. Planting depth was 5 cm. Seedlings were transplanted at the center of soil surface (Exp. 1). Distances between plants were 20 and 10 cm for root box and cylindrical pot, respectively (Exp. 2). Plot A and B in the root box mean the center plots and border plots, respectively.

the leaf area, dry weight of each organ, and root number. One plant from each of the three pots was harvested on July 26 (82 days after transplanting; DAT) to measure the main stem length, main stem diameter, leaf area, and dry weight. The roots in the pots were washed and cut at depths of 10-cm increments to measure root number and root dry weight in each layer. The roots in the root box were washed and cut into 10 cm × 10 cm squares to measure the dry weight of each square. Root density (mg DW cm^{-3}) was calculated using the result of root dry weight, and its profile was divided into three soil layers: 0–20 cm (top), 20–50 cm (middle), and >50-cm depth (bottom) because it has been reported that sugarcane roots mostly accumulate in the top 0–20 cm of soil (Ball-Coelho et al. 1992) and the effective farmland soil depth for plant growth in Okinawa is 40–60 cm (Yoshinaga and Sakai 2004). The underground shoot weight was included in that of aboveground shoots. The root depth index (RDI), an indicator of the center of gravity of root depth, was calculated using data from the cylindrical pots according to the equation of Oyanagi et al. (1993) as follows:

$$\text{RDI (cm)} = \sum\{(\text{median of each soil-depth layer}) \times (\text{percentage of root mass in each layer})\} / 100 \qquad (3.3.1)$$

3.3.2.2 Effect of mixed varieties with a different drought tolerance on root-growth plasticity (Exp. 2).

Two Japanese commercial varieties, drought susceptible “NiF8” and tolerant “Ni22”, were used (Okinawa Prefectural Government, Development of Agriculture, Forestry and Fisheries 2015). Seedlings of both the varieties were raised by the same method and on the same date as Exp. 1. Three treatments were designed: mono-cropped NiF8, mono-cropped Ni22, and a mixture of both. Two plants for each treatment were transplanted into the same root box (n = 1) and cylindrical pots (n = 3) as Exp. 1. Distance between the plants was 20 cm and 10 cm for the root box and cylindrical pots, respectively. Planting was done to reduce light competition between plants by considering their phyllotaxy. Tillers were immediately removed after emergence during the growth period. Irrigation and fertilization were followed as Exp. 1. The three plants of each variety were sampled before transplanting to measure leaf area, dry weight of each organ, and root number. Plants grown in the pots were harvested on July 26 (82 DAT) to measure main stem length, main stem diameter, leaf area, and dry weight. Plant roots in the cylindrical pots were directly cut at each 10 cm soil depth in round slices and washed to measure root number, root dry weight in each layer, and RDI. Roots in the root box were washed and cut into 10 cm × 10 cm squares to measure the dry weight of each square. Root density was calculated and its profile was divided into three soil layers: 0–20 cm (top), 20–50 cm (middle), >50-cm depth (bottom). The plots of the root box were divided into center parts (A) and borders (B) (Fig. 3.3.1) because roots in a root box tend to accumulate at the borders (Yamauchi et al. 1987). Coefficients of variance (CV) for all plots (total of 40 plots) or A plots (18 plots) were calculated as an indicator of root densification using the following equation:

$$\text{CV (\%)} = (\text{Standard deviation of root densities of plots}) / (\text{Mean root density of plots}) \text{ X } 100 \qquad (3.3.2)$$

3.3.3 Results

3.3.3.1 Exp.1

Tillers appeared at around 30 DAT and were removed in the TR treatment (Fig. 3.3.2). The average tiller number in the control was 2.0 at the end of experiment (Fig. 3.3.2 & Table 3.3.1). The main stem length tended to be higher in the TR than in the control from around 60 DAT (Fig. 3.3.2). By the end of the experiment, the main stem length and diameter also tended to be higher in the TR than in the control (Table 3.3.1). The number of shoot roots tended to be higher in the TR (Table 3.3.1) and the ratio of shoot roots to total number of roots was significantly higher in the TR than in the control. However, the total number of roots and the ratios of shoot roots plus tiller roots to the total number of roots were not significantly different between treatments. The vertical root distribution showed that the roots were distributed more in the top to middle soil layers (0–50 cm depth) in the control, while root density and the percentage of root biomass to total biomass in the bottom layer (>50 cm depth) were higher in the TR (Fig. 3.3.3). Results from the root box revealed that roots in the control were more distributed in the top layer, particularly around seedlings, while roots in the TR tended to be more distributed in the bottom layer (Fig. 3.3.4). The root density of each layer was higher in the root box than in cylindrical pots (Figs. 3.3.3 & 3.3.4). The percentage root density in the TR tended to be higher in the middle and bottom layers than in the control (Fig. 3.3.4). Therefore, RDI tended to be deeper in the TR (Table 3.3.2). The main shoot biomass was significantly higher in the TR than in the control; however, the total shoot biomass, including the main stem and tillers, was not significantly different between treatments. Root biomass tended to be higher and the shoot/root mass (S/R) ratio was lower in the control. The leaf area of the main shoot tended to be higher in the TR; however, the total leaf area, including the leaf areas of the main stem and tillers, and leaf area ratio (LAR) were higher in the control. The specific leaf area (SLA) was significantly higher in the control. The net assimilation rate (NAR) was higher in the TR while the relative growth rate (RGR) was not different between treatments.

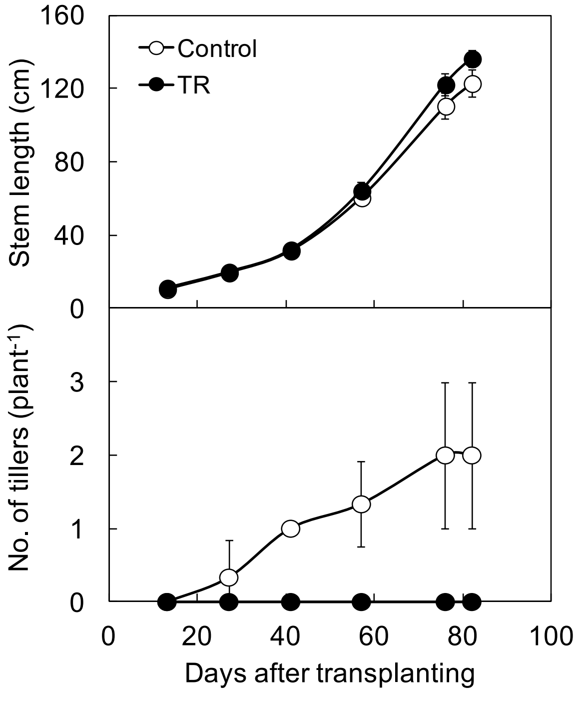
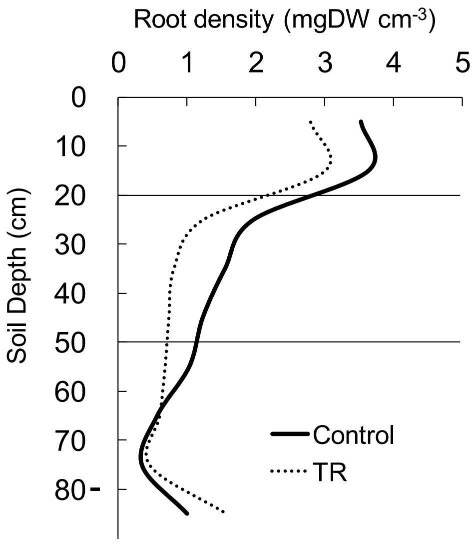


Fig. 3.3.2 Changes of stem length and number of tillers for each treatment in Exp. 1 (n=3 pots). Note: Vertical bar shows standard deviation of each value. TR: tiller regulation

Table 3.3.1 Numbers of primary roots for each treatment in Exp. 1.

Treatment	Main Stem Length (cm)	Main Stem Diameter (mm)	No. of Tillers (plant^{-1})	Shoot root (no. plant^{-1})	Sett root (no. plant^{-1})	Tiller root (no. plant^{-1})	Total (no. plant^{-1})	Shoot root /Total root	(Shoot root + Tiller root) /Total root
Control	123	19.0	2.0	87	6	22	115	0.77	0.96
TR	137	21.0	0.0	101	8	0	109	0.92 *	0.92

Note: * means significant difference between treatments at 5% level (t-test, n=3 pots). Number of tillers and tiller roots were not tested statistically. TR: tiller regulation



Soil depth (cm)	Accumulated root density (mgDW cm^{-3}) Control	Accumulated root density (mgDW cm^{-3}) TR
Top 0-20	7.2 (47.3)	5.8 (48.7)
Middle 20-50	4.7 (31.4)	2.8 (23.2)
Bottom 50-	2.9 (21.3)	3.3 (28.1)

Fig. 3.3.3 Vertical root distribution for each treatment in Exp. 1 (n=3 pots).

Note: Number in parenthesis means the percentage of root mass in each layer to total root mass.

TR: tiller regulation

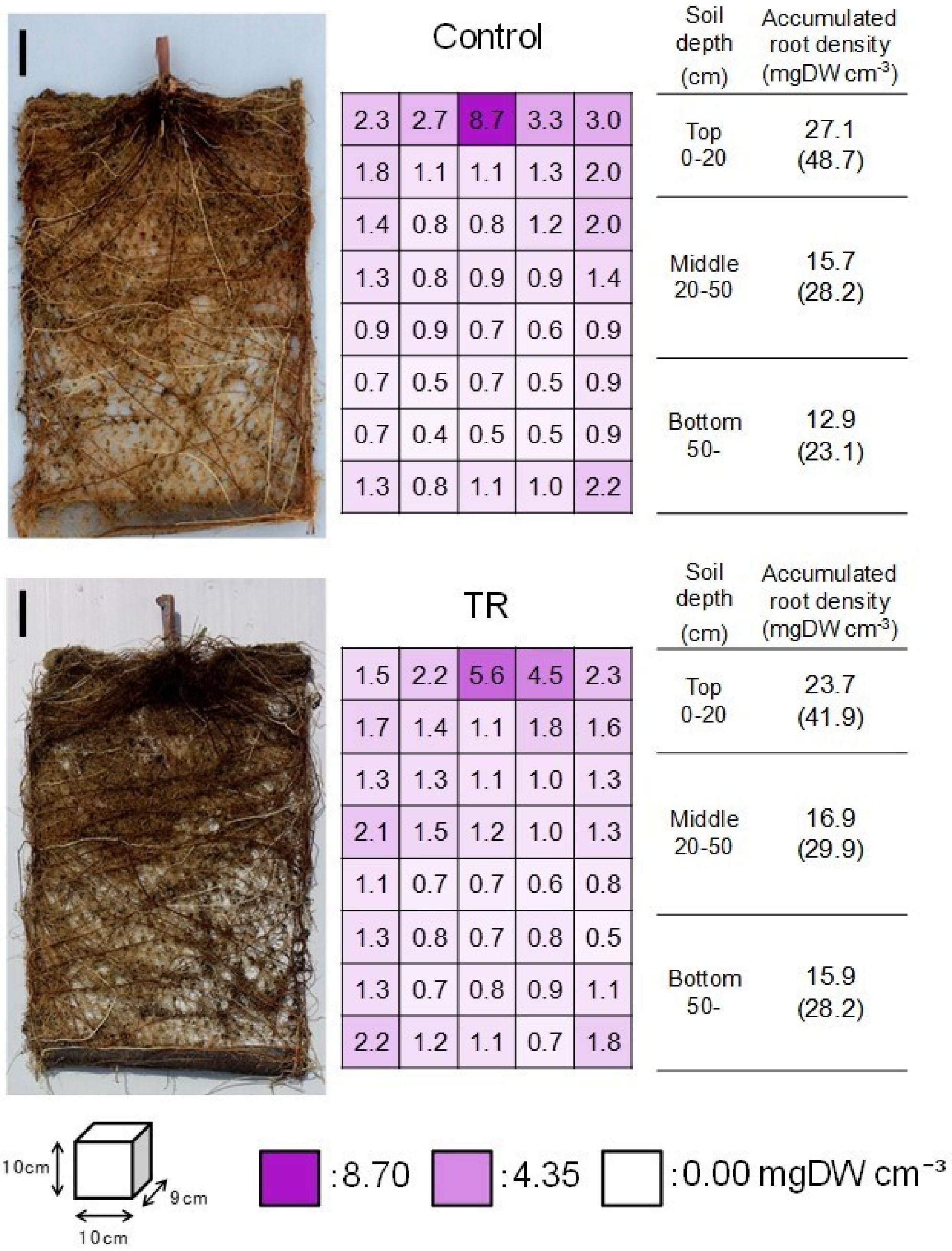
Table 3.3.2 Dry matter production for each treatment in Exp. 1.

Treatment	RDI	Biomass (gDW plant^{-1})				S/R ratio	LA (cm^2 plant^{-1})			RGR	NAR	LAR	SLA
	(cm)	Main shoot	Tiller	Root	Total		Main shoot	Tiller	Total	(g g^{-1} day^{-1})	(g m^2 day^{-1})	(m^2 g^{-1})	(cm^2 gDW^{-1})
Control	32.0	101	23	47	170	2.7	3979	1227	5206	0.056	17.9	0.0031	129
TR	36.6	122 *	0	37	159	3.4	4268	0	4268	0.055	19.6	0.0028	116 *

Note: * means significant difference between treatments at 5% level (t-test, n=3 pots).

Biomass and leaf area of tillers were not tested statistically.

TR: tiller regulation, RDI: root depth index, S/R: shoot/root mass, LA: leaf area, RGR: relative growth rate, NAR: net assimilation rate, LAR: leaf area ratio, SLA: specific leaf area



Control

2.3	2.7	8.7	3.3	3.0
1.8	1.1	1.1	1.3	2.0
1.4	0.8	0.8	1.2	2.0
1.3	0.8	0.9	0.9	1.4
0.9	0.9	0.7	0.6	0.9
0.7	0.5	0.7	0.5	0.9
0.7	0.4	0.5	0.5	0.9
1.3	0.8	1.1	1.0	2.2

Soil depth (cm)	Accumulated root density (mgDW cm^{-3})
Top 0-20	27.1 (48.7)
Middle 20-50	15.7 (28.2)
Bottom 50-	12.9 (23.1)

TR

1.5	2.2	5.6	4.5	2.3
1.7	1.4	1.1	1.8	1.6
1.3	1.3	1.1	1.0	1.3
2.1	1.5	1.2	1.0	1.3
1.1	0.7	0.7	0.6	0.8
1.3	0.8	0.7	0.8	0.5
1.3	0.7	0.8	0.9	1.1
2.2	1.2	1.1	0.7	1.8

Soil depth (cm)	Accumulated root density (mgDW cm^{-3})
Top 0-20	23.7 (41.9)
Middle 20-50	16.9 (29.9)
Bottom 50-	15.9 (28.2)

Fig. 3.3.4 Spatial root distribution of sugarcane under root box condition for each treatment in Exp. 1 (n=1 box).

Note: Black Bars mean 10-cm length. Number in each square (10×10cm) means root density (mgDW cm^{-3}) of each plot. Number in parenthesis means the percentage of root mass in each layer to total root mass. TR: tiller regulation

3.3.3.2 Exp. 2

The number of shoot roots was significantly higher in NiF8 while the number of sett roots was higher in Ni22 at transplanting (Table 3.3.3). On 82 DAT, the number of shoot roots tended to be higher in NiF8 than in Ni22; in addition, they decreased in NiF8 and increased in Ni22 under a mixed cropping compared with mono-cropped treatments. The difference in vertical root distribution between treatments was small (Fig. 3.3.5). Roots of mono-cropped NiF8 tended to distribute more in the top layer (0–20 cm), while those of mono-cropped Ni22 tended to distribute more in the bottom layer (>50 cm) (Fig. 3.3.5). Mixed varieties tended to distribute more roots in the middle layer (20–50 cm) than the other treatments, showing the medium value of root density in top and bottom soil layers of monocultures. The results from the root box showed a spatial root distribution (Fig. 3.3.6); roots were more distributed in the root box border (Figs. 3.3.1 & 3.3.6). Mono-cropped NiF8 tended to distribute its roots more around seedling setts and in the top layer while mono-cropped Ni22 tended to distribute its roots more in the bottom layer. The percentages of root density under a mixed cropping tended to be higher in the middle layer than under other treatments. The total root density of all plots was also higher under a mixed cropping than mono-cropped cultivars under the root box condition (Table 3.3.4). Total root densities of all plots or plots without border plots were higher under a mixed cropping and the CV for plots without borders was smaller under a mixed cropping than those under other treatments (Fig. 3.3.1 & Table 3.3.4). Ni22 showed longer and thinner stems, higher SLA, and higher shoot biomass than NiF8; however, there was no significant difference between treatments for each variety (Table 3.3.5). A mixed cropping yielded medium RDI, shoot biomass, total biomass, RGR, and NAR values compared to both mono-cropped varieties (Table 3.3.6). Root biomass showed no significant difference between treatments, although its distribution pattern was varied (Fig. 3.3.5 & Table 3.3.6). The S/R ratio tended to be higher and leaf area tended to be lower in mono-cropped Ni22, although no drastic changes in these parameters occurred under a mixed crop compared with the mean values of each mono-cropped cultivar.

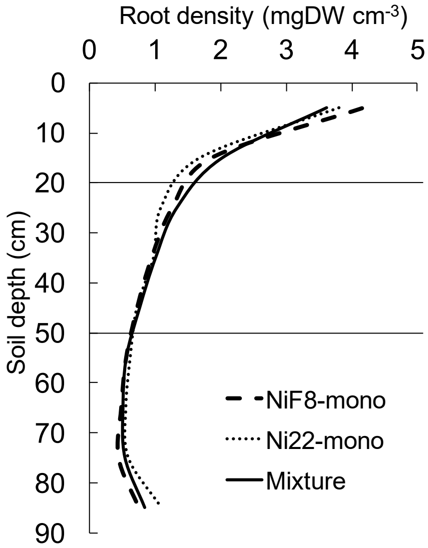
Table 3.3.3 Numbers of primary roots for each treatment in Exp. 2.

Date	Treat-ment	Variety	Shoot root (no. plant^{-1})	Sett root (no. plant^{-1})	Total root (no. plant^{-1})	Shoot root/ Total root
5 May (0 DAT)	— (n=3)	NiF8	9	24	33	0.29
		Ni22	3 **	31	34	0.09
26 July (82 DAT)	Mono (n=6)	NiF8	90	17	106	0.85
		Ni22	68	14	82	0.83
	Mix (n=3)	NiF8	78	18	96	0.81
		Ni22	74	11	85	0.87

Note: Only primary root was counted but secondary branch root was not.

** means significant difference between cultivars at 1% level (t-test).

No significant difference between treatments at 1% level on 82 DAT.



Soil depth (cm)	Accumulated root density (mgDW cm^{-3}) NiF8-mono	Ni22-mono	Mixture
Top 0-20	6.0 (53.5)	5.5 (49.3)	5.6 (50.4)
Middle 20-50	2.9 (26.3)	2.8 (25.0)	3.1 (27.7)
Bottom 50-	2.3 (20.2)	2.8 (25.7)	2.5 (21.9)

Fig. 3.3.5 Vertical root distribution for each treatment in Exp. 2 (n=3 pots).
Note: Number in parenthesis means the percentage of root mass in each layer to total root mass.

Table 3.3.4. Coefficient of variation of root density in all plots or plots without border plots for each treatment in Exp. 2 (n=1 box).

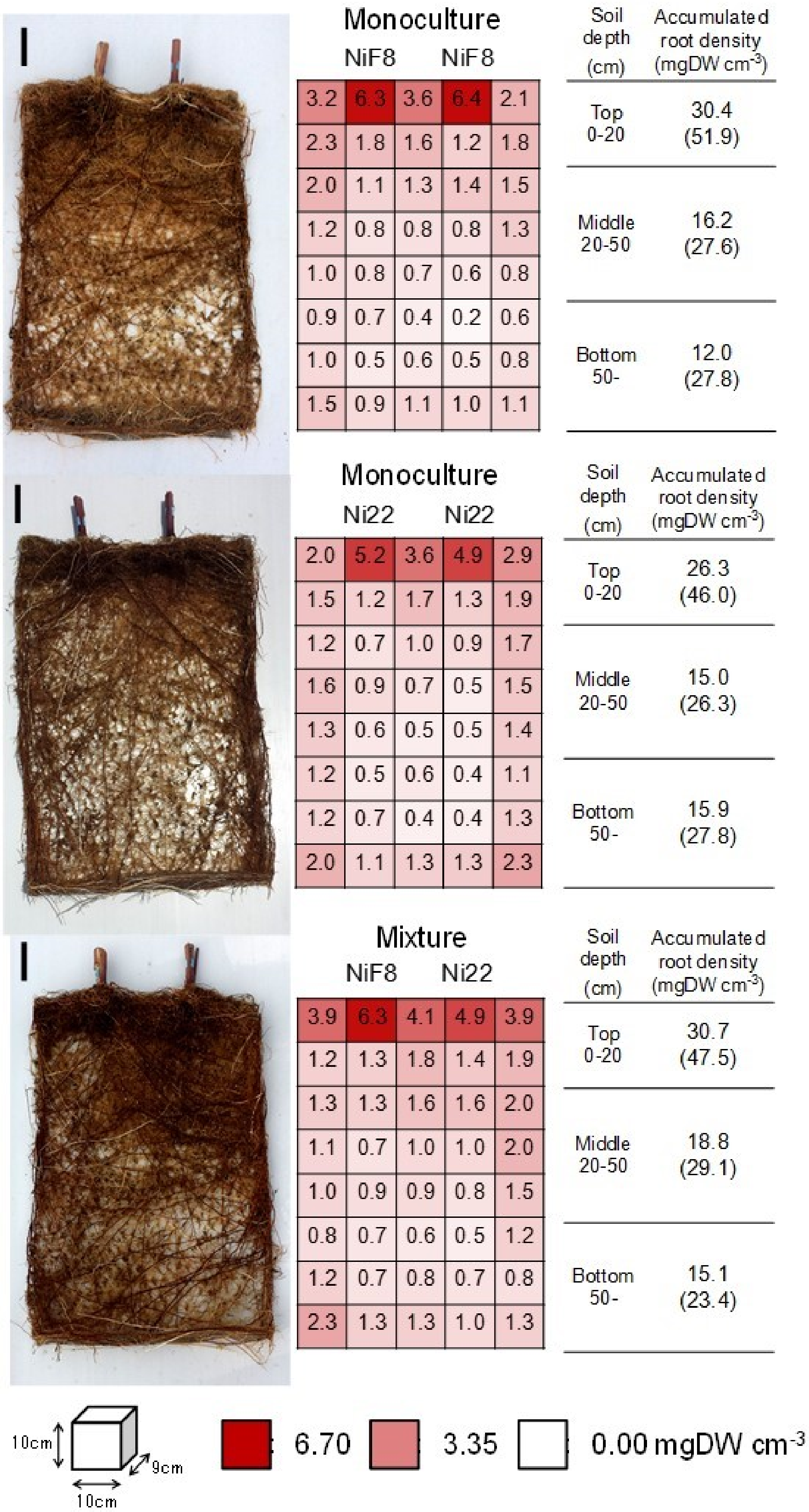
Variable	Total root density (mgDW cm^{-3})			Coefficient of variation		
	NiF8-mono	Ni22-mono	Mixture	NiF8-mono	Ni22-mono	Mixture
All plots	58.6	57.2	64.6	0.91	0.76	0.78
Plots without border	16.1	13.7	18.4	0.49	0.47	0.39

Note: Plot means 10×10 cm of square of root box. All plots include center plots (A) and border plots (B) (Fig. 1).

Table 3.3.5 Above-ground growth for each treatment in Exp. 2.

Treat -ment	Variety	Stem length (cm)	Stem diameter (mm)	Leaf area (cm^2 plant^{-1})	SLA (cm^2 gDW^{-1})	Shoot biomass (gDW plant^{-1})
Mono (n=6)	NiF8	103 a	18.0 a	2920 a	127 ab	90 a
	Ni22	121 a	16.4 a	2818 a	114 a	103 a
Mix (n=3)	NiF8	100 a	19.0 a	2957 a	138 b	86 a
	Ni22	123 a	17.3 a	2927 a	106 a	109 a

Note: Different alphabets mean significant difference between treatments at 1% level (Tukey test). SLA: specific leaf area.



Monoculture

	NiF8		NiF8	
3.2	6.3	3.6	6.4	2.1
2.3	1.8	1.6	1.2	1.8
2.0	1.1	1.3	1.4	1.5
1.2	0.8	0.8	0.8	1.3
1.0	0.8	0.7	0.6	0.8
0.9	0.7	0.4	0.2	0.6
1.0	0.5	0.6	0.5	0.8
1.5	0.9	1.1	1.0	1.1

Soil depth (cm)	Accumulated root density (mgDW cm^{-3})
Top 0-20	30.4 (51.9)
Middle 20-50	16.2 (27.6)
Bottom 50-	12.0 (27.8)

Monoculture

	Ni22		Ni22	
2.0	5.2	3.6	4.9	2.9
1.5	1.2	1.7	1.3	1.9
1.2	0.7	1.0	0.9	1.7
1.6	0.9	0.7	0.5	1.5
1.3	0.6	0.5	0.5	1.4
1.2	0.5	0.6	0.4	1.1
1.2	0.7	0.4	0.4	1.3
2.0	1.1	1.3	1.3	2.3

Soil depth (cm)	Accumulated root density (mgDW cm^{-3})
Top 0-20	26.3 (46.0)
Middle 20-50	15.0 (26.3)
Bottom 50-	15.9 (27.8)

Mixture

	NiF8		Ni22	
3.9	6.3	4.1	4.9	3.9
1.2	1.3	1.8	1.4	1.9
1.3	1.3	1.6	1.6	2.0
1.1	0.7	1.0	1.0	2.0
1.0	0.9	0.9	0.8	1.5
0.8	0.7	0.6	0.5	1.2
1.2	0.7	0.8	0.7	0.8
2.3	1.3	1.3	1.0	1.3

Soil depth (cm)	Accumulated root density (mgDW cm^{-3})
Top 0-20	30.7 (47.5)
Middle 20-50	18.8 (29.1)
Bottom 50-	15.1 (23.4)

Fig. 3.3.6 Spatial root distribution for each treatment in Exp. 2 (n=1 box).

Note: Black Bars mean 10-cm length.

Number in each square (10×10cm) means root density (mgDW cm^{-3}) of each plot.

Number in parenthesis means the percentage of root mass in each layer to total root mass.

Table 3.3.6 Dry matter production for each treatment in Exp. 2.

Treatment	RDI	Biomass (gDW pot^{-1})			S/R ratio	Leaf area	RGR	NAR	LAR
	(cm)	Shoot	Root	Total		(cm^2 pot^{-1})	(g g^{-1} day^{-1})	(g m^{-2} day^{-1})	(m^2 g^{-1})
NiF8-mono (①)	28.2 a	186.3 a	33.8 a	220.1 a	5.9 a	5840 a	0.051	18.0	0.0028
Ni22-mono (②)	30.9 a	208.1 a	33.0 a	241.1 a	6.3 a	5635 a	0.054	21.6	0.0025
Mixture (③)	29.2 a	194.1 a	33.8 a	227.9 a	5.9 a	5884 a	0.053	19.1	0.0028
③×2/(①+②)	0.99	0.98	1.01	0.99	0.97	1.03	1.01	0.96	1.04

Note: No significant difference between treatments at 1% level (n=3 pots). RDI: root depth index, S/R: shoot/root mass, RGR: relative growth rate, NAR: net assimilation rate, LAR: leaf area ratio.

3.3.4 Discussion

3.3.4.1 Root plasticity under tiller regulation

The results showed that removing tillers increased the main stem growth (Fig. 3.3.2 & Table 3.3.2). However, the total shoot biomass was not different between treatments (Table 3.3.2). RGR was not different between treatments; however, the components of RGR, which was calculated by multiplying NAR by LAR, were different. Tiller regulation increased NAR while it decreased LAR and SLA. It was suggested that tiller regulation (i.e., defoliation) increased the thickness and photosynthetic ability of the remaining leaves (Kawamitsu and Uehara 2000). Therefore, the plasticity of shoot growth and its carbon allocation was induced by tiller regulation. On the other hand, removing tillers decreased root biomass, resulting in the increment of S/R ratio (Table 3.3.2). Smith et al. (1999) showed that the phenotypic plasticity of the sugarcane S/R ratio converged on a fixed value, even after regulation of shoot or root biomass. They suggested that sugarcane, at least during its early growth, appeared to have more roots than were required to meet the demand of shoots. Although they did not consider tillers and their root growth, such a disagreement of demand with supply may have occurred in the present study because the lower S/R ratio of the tillers (no data obtained) may have resulted in a lower S/R ratio of the whole plant in the control (Table 3.3.2). Moreover, internal competition toward photosynthetic assimilates between the main stem and tillers could also contribute to a reduction in main stem growth and a lower S/R ratio in the control (Kim et al. 2010). Therefore, tillering (or tiller regulation) induced not only shoot growth plasticity but also root growth plasticity, suggesting a plasticity of the internal root-shoot relationship between the main stem and tillers in sugarcane.

Roots were distributed more in the superficial layer if tillers were allowed to grow (Figs. 3.3.3 & 3.3.4), which indicates that tillering may cause a disadvantage in water uptake in deeper soil layers, at least during the early growth of tillers. Therefore, the root biomass under tillering may contribute less to the deeper root system and be less tolerant to severe drought stress, at least until tiller roots are well developed in deeper soil layers. Moreover, there could be another water use disadvantage because tillers increase the plant leaf area, which could increase the plant water demand, although plants with tillers have a shallow root system. This may be the same in field conditions where plants have tillers. Kim et al. (2010) suggested that a higher-tillering cultivar of sorghum would utilize much more water than a lower-tillering cultivar under lower-tillering conditions (high temperature and strong radiation). They concluded that the effect of tillering on water use and drought tolerance was highly dependent on the environmental condition. Although further research needs to be conducted on the contribution of tillering to root growth and a deeper root distribution, drought tolerance, or adaptability during later growth stages in sugarcane, it was clearly shown that tillering (or tiller regulation) changed the root distribution pattern of the whole plant.

Pot experiments of sugarcane growth and nutrient uptake have been conducted during early growth before tillers emerged (Fukuzawa et al. 2009) or under tiller regulation (Watanabe et al. 2016) because of the difficulty in fixing the number of tillers. So far, the effect of removing tillers on plant growth has been less focused on under pot conditions, but our results suggest that it may be important to consider changes in root formation and plasticity of the root-shoot relationship under pot experiments when the results of pot experiments are applied to field-grown sugarcane.

3.3.4.2 Root plasticity under mixed varieties

It is known that there is a difference in the numeric balance between sett roots and shoot roots between varieties during early growth in sugarcane (Fukuzawa et al. 2009; Spaull 1980). Two varieties with different numbers of sett roots were transplanted when sett roots were dominant (Table 3.3.3). NiF8 (with lower sett roots) may have decreased its shoot roots and Ni22 (with many sett roots) may have been dominant and increased its shoot roots when they were mix-planted (Table 3.3.3). This indicates that each cultivar may have escaped from competition for soil space which occurred in the top soil after transplanting (habitat segregation, compensation effect). Therefore, while mono-cropped NiF8 with a higher number of shoot roots distributed their roots in the shallow layer and mono-cropped Ni22 with a lower number of shoot roots had deeper roots, a mixed crop distributed more roots in the middle soil layer than those of mono-cropped varieties (Figs. 3.3.5 & 3.3.6). In general, fertilizer is applied to the top soil layer so nutrients are higher there while water is higher in deeper layers. Therefore, the optimum soil layer for both water and nutrients is highly limited (Ozawa et al. 1989). Roots of mixed varieties with different root-elongation abilities may be neutral for both water and nutrients, and it could be one desirable root trait to stem the buffering effect of shoot growth under stress conditions such as drought and nutrient deficiency (Smith et al. 2005). Moreover, the results of root spatial distribution showed that roots under a mixed cropping tended to be dense in central parts although there are no significant differences (Fig. 3.3.6 & Table 3.3.4). On the other hand, a mixed cropping had less effect on shoot growth because water and fertilizer were well applied in the present study (Table 3.3.6). Root plasticity is particularly induced by a soil water deficit and contributes to plant survival under suboptimal conditions (Tran et al. 2015; Kano et al. 2011; Suralta et al. 2016). Many authors reported the phenomenon called “hydraulic rift,” where plants with shallower roots could use water that plants with deeper roots absorb and supply from a deeper to a shallow soil layer (Caldwell et al. 1998; Sekiya and Yano 2004). Further research is needed to reveal the effects of root densification in mixed varieties on growth and water use under water stress conditions.

Vertical root distribution was less affected by a mixed cropping in Exp. 2 than tiller regulation in Exp. 1 (Figs. 3.3.3 & 3.3.5). However, there were some changes in root growth, such as root number and RDI, that allowed us to interpret the effect of a mixed crop on root formation (Tables 3.3.3 & 3.3.6). In Exp. 2, it was impossible to separate the distribution of roots from each stem of each variety, and the interaction of their roots remained unclear. The development of a method to identify the roots of each variety or plant could help our understanding of root plasticity and internal competition of root formation. In both experiments, irrespective of treatment, roots tended to distribute much more in the bottom (>80-cm depth) of the cylindrical pot and in the border of the root box (Figs. 3.3.3–6). It was suggested that roots penetrated into the more aerobic part of the soil and that the root-growth chamber should be improved with a higher soil volume for longer sugarcane growth.

3.4 Varietal mixture with different lodging resistances (case study)

3.4.1 Introduction

In Tanegashima, a southwestern island of Japan, where sugarcane production is limited by low temperatures and frost (Hayashi 2011), cane growers have traditionally relied on specific superior varieties such as NCo310 or NiF8, and are currently obliged to choose from a limited selection, including NiF8, NiTn18, and Ni22 (Fig. 3.4.1). NiF8 is a dominant variety with poor adaptability to low temperatures but high tolerance to the main sugarcane diseases. Although the area cultivated for NiF8 is gradually reducing, that for NiTn18 is increasing because it tolerates low temperatures and yields are high even without mulching; however, due to its high elongation ability, disorderly lodging often occurs in strong winds (Terajima et al. 2010). The growing area of Ni22 is also increasing slightly because the variety has a high ratooning ability, tiller number, and sugar content, although it is not so vigorous in a new planting (Irei et al. 2010). Thus, each variety has both advantages and disadvantages.

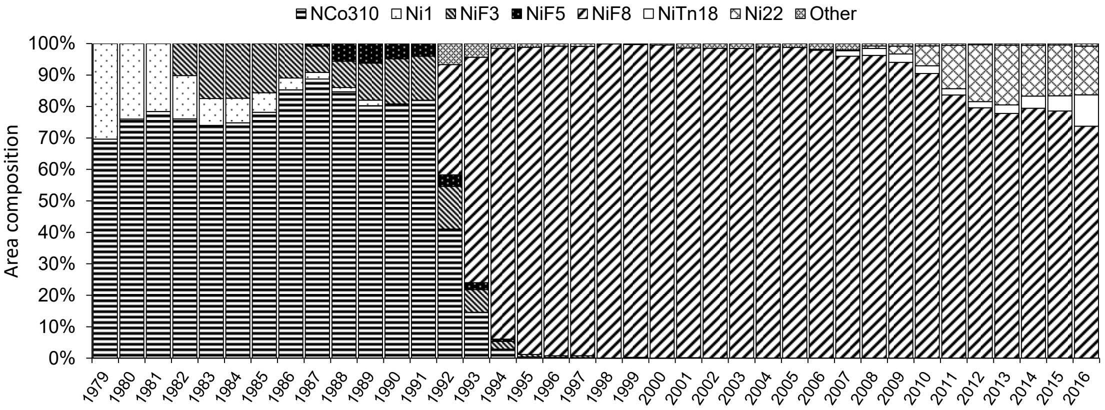


Fig. 3.4.1 Change in the varietal composition of area harvested in Tanegashima.
Note: Data from sugar mill factory.

The author found an innovative grower in Tanegashima who, over many years and through trial and error, has planted a mixture of two varieties in one field to best exploit the characteristics of each. The grower focuses on lodging and mechanical harvest by alternately planting two to three rows of Ni22 and one row of NiTn18. Japanese growers are reluctant to use mixed planting using two or three varieties because of the shortage of seedling setts and supplementary planting (Takaragawa et al. 2018d). However, based on the potential advantages of systematic varietal mixtures, several field trials have been attempted (Takaragawa et al. 2016b). To my knowledge, the present trial in Tanegashima is the first case worldwide wherein a grower systematically planted a mixture of two varieties before the publication of any research suggesting this as an option.

In this study the author investigated the potential advantages of varietal mixtures by evaluating the effects of mixed planting on their lodging level and yield using field survey and interview survey with the grower as a case study.

3.4.2 Materials and Methods

The growing area studied is located in Nakatane-cho, Tanegashima, Kagoshima, Japan. Ni22 and NiTn18 were mix-planted in a ratio of 3:1 centres (*Mix* field). For comparison purposes, a mono-planted NiTn18 field with a similar cultivation method but a different grower was selected near the *Mix* field (*Mono* field). The planting dates for the *Mix* and *Mono* fields were February 24, 2017 and April 20, 2017, respectively. Two-bud setts were planted in dual rows as pairs of rows 0.2 m apart with 1.6 m between centres (Figs. 3.4.2 and 3.4.4). A hand-made planter was used to plant dual rows with a plant density, 4,125 setts $10a^{-1}$. Inter-tillage ridging was conducted three times after fertilization by the same operator with the same machine and completed before the typhoon season. Other cultural conditions are presented in Table 3.4.1.

Sugarcane was harvested from four plots ($14.4\ m^2 = 1.6\ m \times 3$ centres $\times\ 3\ m$) within each field (Fig. 3.4.2) on December 5, 2017 to record severity score of lodging (*SSL*), number of broken stalks, number of millable stalks (*NMS*), and six-stalk weight. Three centres in the *Mix* field included mixed NiTn18, Ni22 next to mixed NiTn18 (*Ni22 next*), and Ni22 between two centres of Ni22 (*Ni22 center*). *SSL* was scored by visual evaluation with five levels of stalk erectness (0, no lodging; 1, a few; 2, medium; 3, much; and 4, severe). The juice from the harvested millable stalks was analyzed for sugar content using a modular circular polarimeter (MCP500, Anton-Paar). Time taken to harvest the crop was measured for each 15 m of centre to calculate the harvesting speed as an indicator of lodging effect. The mechanical harvester (MCH-15WE2, Matsumoto-kiko) was operated by the same operator in both fields.

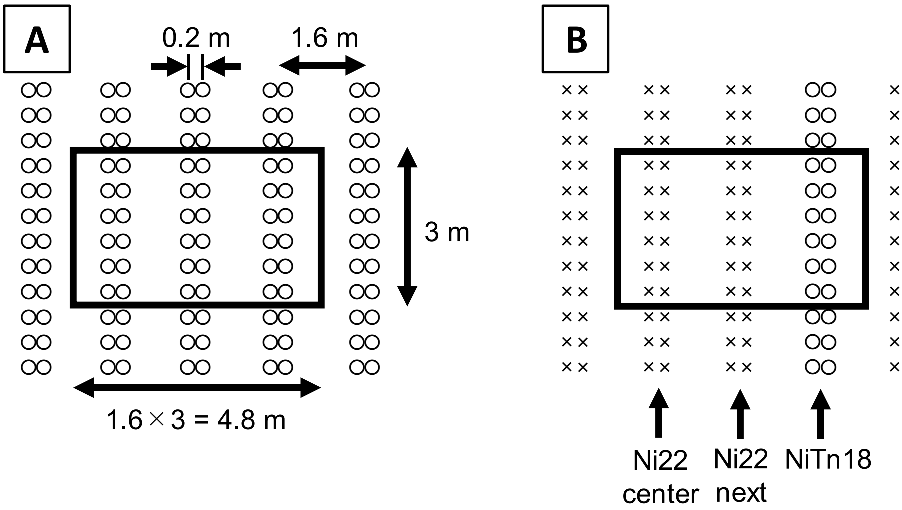


Fig. 3.4.2. Harvested plot (4.8m×3.0m) in the present study.
Note: A, monoculture of NiTn18; B, mixture. Open circle and cross mean NiTn18 and Ni22, respectively. Plant density within centres is not correct.

Table 3.4.1 Information on the cultivation method used in this study.

Condition	Mixture	Mono cropping
Address	Yuku, Nakatane-cho 30°30'10.1"N, 130°57'17.1"E	Noma, Nakatane-cho 30°30'50.2"N, 130°56'19.0"E
Variety	Ni22:NiTn18=3 rows :1 row	NiTn18
Planting date	Feb. 24, 2017 Dual rows planting with 2-bud setts	Apr. 20, 2017 Dual rows planting with 2-bud setts
Planting Density	1.6m between centres, 0.2m between rows 4125 setts /10a	1.6m between centres, 0.2m between rows 4125 setts /10a
Area	23 a	9 a
Preceding crop	Sweet potato	Forage
Mulching	Done	Done
Ridging	3 times on Apr., May, and Jul.	3 times on May, Jun., and Jul.
Fertilizer /10a	Basal:Chicken manure 300kg Fused phosphate with calcium silicate 100kg N,P,K = 9.6 ,16.0, 8.0 kg Supplemental :N,K = 9.0, 9.0 kg	Basal:Cattle manure 1.5ton Chicken manure 150kg Fused phosphate with calcium silicate 200kg N,P,K = 9.6 ,16.0, 8.0 kg Supplemental :N,K = 9.0, 9.0 kg
Watering	Rainfed	Rainfed

Note: Based on interviews with the growers.

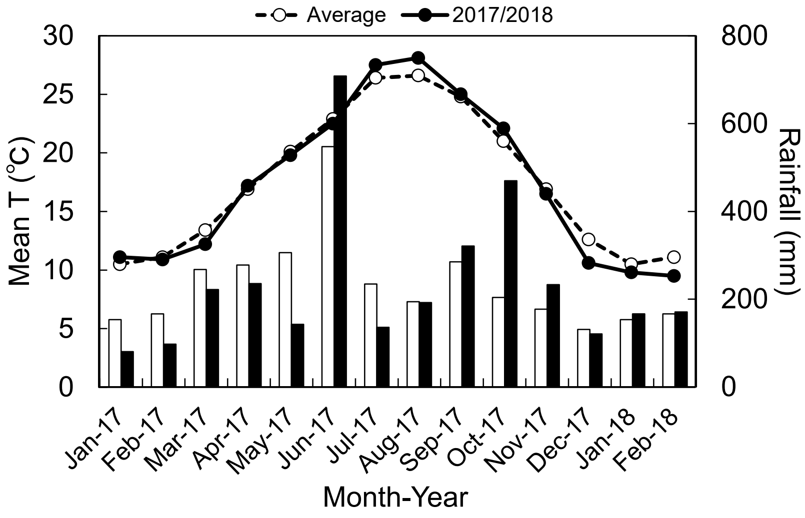


Fig. 3.4.3. Climate data of 2017/2018 season at the experimental site.

Note: Data from Kaminaka meteorological station (Japan Meteorological Agency 2018). Average values are calculated using data from 1980 to 2010.

3.4.3 Results and Discussion

Weather conditions based on climate data from the Kaminaka meteorological station nearest to the studied field during the growing season are presented in Fig. 3.4.3. Compared with the 30-year average, mean temperatures were high in July–August and low in December–February. Rainfall was lower during the early growth stage and increased during the rainy season in June and because of several typhoons. In 2017, the region was attacked by four typhoons. The typhoon in August caused stalks to break and led to lodging during the grand growth stage. The three other typhoons in September and October caused leaves to break and also led to lodging. Severe damage caused by the typhoons resulted in a reduction in *NMS* and low sugar content (approx. 11%). Average cane yield and sugar content in Nakatane-cho were 5.8 t $10a^{-1}$ and 11.1%, respectively, in 2017–2018 (from sugar mill data).

Fig. 3.4.4 Outline of the mixture field on 27 July, 2017.
Note: Leaf sheath color was purple in Ni22 and green in NiTn18 (above).
The length of each section of the red-and-white scale bar is 20 cm (below).

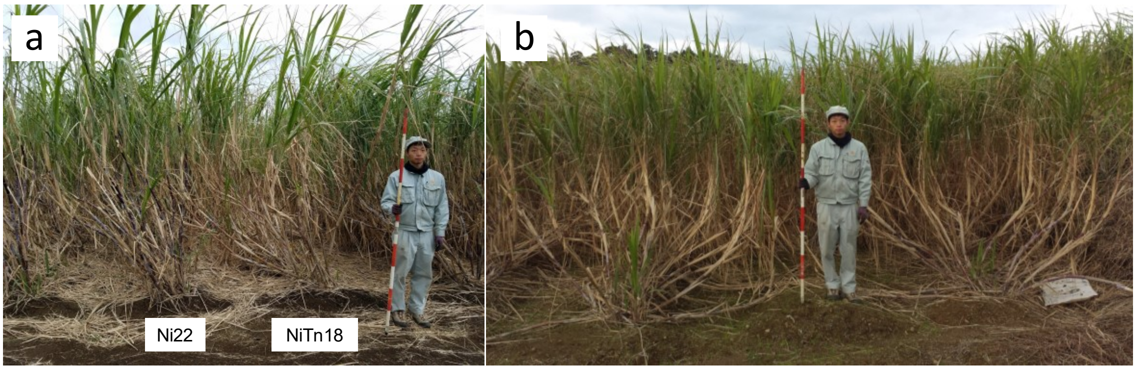


Fig. 3.4.5 Sugarcane stands of mixture (a) and mono-cropped NiTn18 (b) on December 5, 2017.
Note: The length of each section of the red-and-white scale bar is 20 cm.

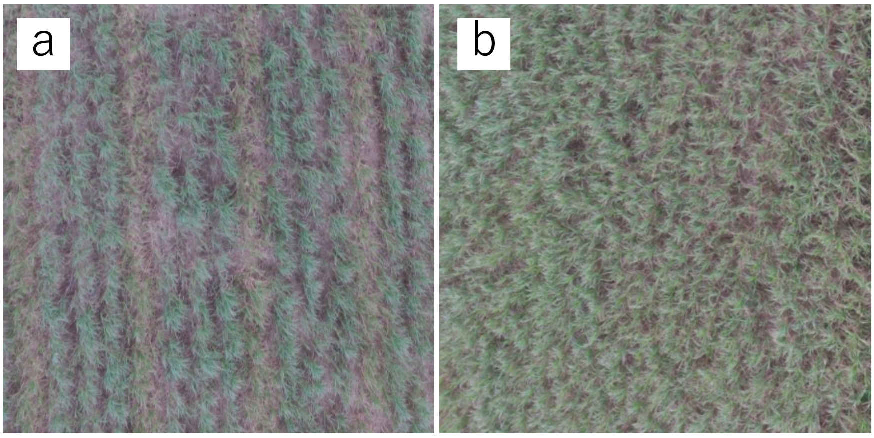


Fig. 3.4.6 Sugarcane canopy of mixture (a) and mono-cropped NiTn18 (b) viewed from above on December 5, 2017.

Note: Photograph was taken by DRONE (Phantom3, DJI) at 20-30 m height.

SSL was higher in lodging-sensitive NiTn18 than in Ni22 (Table 3.4.2). Mixed NiTn18 tended to have lower *SSL* and lower incidence of broken stalks than mono-planted NiTn18 (Table 3.4.2, Figs. 3.4.5 and 3.4.6). *SSL* and incidence of broken stalks were higher in *Ni22 next* than in *Ni22 center*. These results indicate that the varietal composition in mixed planting may be important in strengthening canopy lodging tolerance (Takao and Miyasaka 1981). The harvesting speed was faster for Ni22 than for NiTn18 because the operator may have found it difficult to control the direction of movement and cut the cane if the sugarcane was severely and disorderly lodged. *SSL* correlates positively with yield loss for crops mechanically harvested (Shinzato 2015). The cane that could not be harvested mechanically (yield loss) was picked up manually by other workers after the harvester had passed. A reduction in lodging through mixed planting could potentially reduce yield loss and the manual labor needed to pick it up; however, these parameters were not recorded in this study. Sugarcane lodging depends on two factors: direct external force exerted by wind or rain, and collision between plants either because of wind or the weight of the plant itself. In paddy rice, a lodging-tolerant variety provided support and reduced lodging of a sensitive variety under mixed planting conditions (Takaya and Miyasaka 1981). In addition, high-tillering sugarcane varieties have the ability to act as windbreaks (Terauchi et al. 2000). In our study, the higher-tillering and relatively higher lodging-tolerant Ni22 may have played the role of support and windbreak for the mixed NiTn18. In other words, pressure on mixed NiTn18 was reduced because the high stalk density of Ni22 provided increased windbreak ability and the lodging from next centres were decreased. Stalk weight and sucrose content in juice were not significantly different between varieties (Table 3.4.2). The difference in *NMS* was likely the main factor impacting cane and sugar yields. Values for *NMS* were highest in *Ni22 center* and lowest SSL, followed by *Ni22 next* and mixed NiTn18. Finally, *NMS* values and cane yield in the *Mix* field tended to be higher than in the *Mono* field.

Based on an interview with the grower who has planted various mixtures, growers in Tanegashima are often forced to plant several varieties because seed cane shortage means they are unable to source enough of one specific variety to plant all their fields. In addition, growers intentionally plant several varieties because they well understand that using only one variety carries a degree of risk in terms of yield reduction through injury of continuous cultivation and the damage caused by natural disasters (Takaragawa et al. 2018d). In such cases, the grower does not plant each variety separately as a monoculture, not mix varieties randomly, but mixes varieties systematically in each field as an effective option.

Mixed planting of two varieties with different lodging resistance presented one option to reduce lodging damage, with the potential to maintain *NMS* levels and improve yield, even after multiple severe typhoons. In addition, mixed planting improved harvesting speed and efficiency. Finally, although the varietal mixture was evaluated as an innovative growing method in Tanegashima, additional field trials under controlled conditions, such as with the same growers and under the same cultivation methods, are required.

Table 3.4.2 Sugarcane yield and its components in experimental fields.

Cropping type		Severity score of lodging	Incidence of broken stalks (%)	Speed to harvest (m s^{-1})	Stalk length (cm)	Stalk weight (g)	Number of millable stalks (10a^{-1})	Cane yield (t 10a^{-1})	Sucrose in juice (%)	Sugar yield (t 10a^{-1})
Mix	NiTn18	3.0	3.9	0.218	225	908	9583	8.7	11.6	0.76
	Ni22 next	2.5	5.1	0.226	211	850	10052	8.6	12.1	0.82
	Ni22 center	2.0	3.3	0.224	214	892	11250	10.0	12.1	0.93
	Total	2.5	4.2	0.219	215	875	10234	9.0	12.0	0.83
Mono	NiTn18	3.9	7.4	0.196	213	818	8403	6.9	12.5	0.65

Note: n = 12 for mono-cropped NiTn18, n = 4 for mixed varieties. Total for mix means weighted mean of four rows (NiTn18: Ni22 next: Ni22 center = 1:2:1) for each parameter.

Chapter 4: General discussion

4.1. Effective management of varietal diversity

The present study attempted to gain a better understanding of the current status of use of varietal diversity, prior to attempting to develop an effective method to exploit it. Richness diversity from the viewpoint of plant ecology (Chapter 2.1), functional diversity with respect to plant type (Chapter 2.2), the current status of variety use (Chapter 2.3), and long-term performance of varieties after release (Chapter 2.4) were examined to help identify the optimum method to manage and maintain varietal diversity. From these results, Japanese varietal diversity in sugarcane was defined and its current and potential issues were identified. The summary of findings was shown below:

1. Current status of Japanese varietal diversity is dependent on administrative divisions (Kagoshima and Okinawa prefectures), which have different policies for registration and use of variety. Kagoshima has put more effort into achieving evenness than in maximizing the number of varieties while Okinawa has put greater emphasis on number of varieties than on evenness of varietal dominance. There is a limit to how we can increase varietal diversity by increasing the number of varieties. Therefore, to establish a system to manage evenness (area composition of each variety) with administrative efforts such as limitation of the number of varieties and improvement of grower awareness of optimal variety use is also required to maintain or increase the diversity at a regional level.
2. As an example of the important functional traits associated with sugarcane productivity, a quantitative evaluation method for plant type (index), which had previously been evaluated only qualitatively, was developed by analyzing leaf features to suggest the varietal diversity of plant type. Further phenotyping functional diversities should be carried out to contribute to better decision making in terms of selection of varieties.
3. The current issue of variety use was revealed to exhibit a trend toward dependence on a few specific varieties in a few regions, the difficult selection from many varieties, and chronicity of inactive varietal mixture in growers' field. The follow-up and after-care support, such as administrative support to establish the management system and the creation of a guidebook for extension work about variety use, were suggested as requirements to increase maintain and exploit the diversity.
4. Monitoring long-term performance of variety after release suggested that the ability of the variety itself seemed not to change though some sign of apparent varietal yield decline due to accumulation of pathogens could be possible.
5. Comparing with monoculture of one old standard variety which had maintained high productivity with no trend of deterioration, growing diversified varieties has improved regional productivity, especially with respect to ratoon cropping.

From these findings, four future directions for the management of varietal diversity were suggested (Fig. 4.1). The first direction should be to put to greater efforts toward increasing public awareness of effective variety use. One method to achieve this is to stop leaving the selection of variety to growers and to control varietal composition with a strict policy determining registration and propagation of varieties. A second method would be to create a guidebook for growers, extension workers, and sugar mill workers to suggest how to use various varieties. The development of guidelines for the identification of varieties would also be helpful for this.

The second direction is to develop a nursery system to raise and sell seedlings by private and local companies, which would also be useful in controlling variety use. At present, the branches of the *National Center for Seeds and Seedlings* propagate seedlings in Okinawa and Kagoshima and distribute to municipalities, whereupon seedling are then maintained in the fields by sugar mill workers and finally distributed to growers (Maezono 1999; Suzuki 2012;). This center was founded to propagate pathogen-free seedlings, but only for recommended varieties, especially dominant varieties. However, propagation and seedling production by private and local companies should be accepted and supported aggressively by government to meet the diversified demands in many regions and to prevent the unauthorized importation and propagation of varieties (including unrecommended ones) by growers. This would help result in the suitable management of variety use. Micropropagation using meristem exists at Tokunoshima in Kagoshima, as only one case, which has been run by private sugar mill company (Matsumoto 2000). Once in Ishigaki, seedling from sprouted lateral bud has been studied as promising system to provide and transplant seedling, which bright the light for future nursery system at private company scale (Iritakenishi et al. 1996; Iritakenishi 1999). However, these techniques and production system have not been adopted in other regions because of the need for significant initial and management costs. In addition to the production of pathogen-free cuttings, a mass-production system of one-bud seedling setts raised in cell trays is a commonly used cost-saving method (Shinzato et al. 2010; Shinzato 2015). while a true seed production and sowing system has also been suggested for sugarcane (Sugimoto 1994), which needs further study to assess the hybridity of seeds and the stability of varietal performance carefully. Note that a private company should preliminarily contract with the person who has the Plant Breeder's Rights of the variety in question to propagate and sell seedlings of the variety (Murabayashi et al. 2013). In particular, cooperation between seedling production and consignment work for planting could result in major labor savings for growers and would be useful to manage variety information of field and grower. In Brazil, the

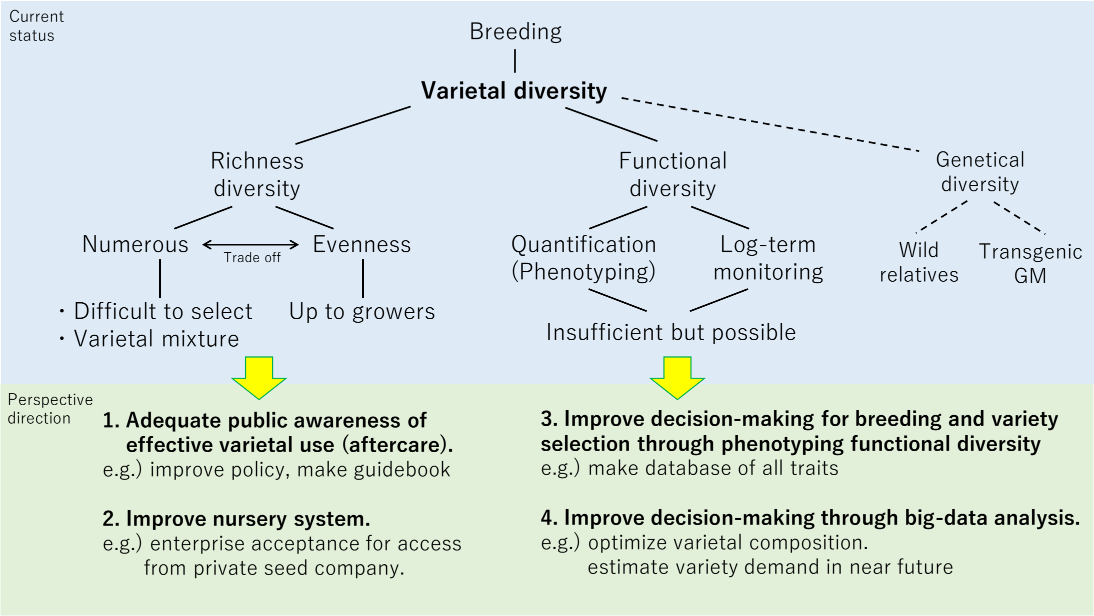


Fig. 4.1 Current status and future perspective direction for use of varietal diversity.

optimization of sugarcane seedling production was achieved by utilizing a transplanting system similar to that used for rice transplanting (Ueno 2014). In Minami Daito, the agricultural production corporation associated with the sugar mill company is managing variety information in each field by expanding the consignment business for planting. Recently, the system for planting billets harvested by harvester has been studied and both domestic and overseas examples, not only in sugarcane but also in other crops, may be useful. Unfortunately, in Japan, information on variety and field area was highly dependent on interviews with growers. The modification and digitization of the area record using GPS and GIS techniques as in Daito Islands and the further development of applications to identify varieties could enable us to manage variety information in each field (Takaragawa et al. 2018a).

The third direction is to improve decision making for breeding and variety selection through phenotyping functional diversity. Distinctness is necessary to register varieties, which means that all varieties could be distinguished using one or more different traits (Plant Variety Protection and Seed Act 3-1-1, Murabayashi et al. 2013). Unfortunately, in Japan, there is no database to cover all characteristics of all sugarcane varieties. Creating an appfor decision making for variety selection through such a database could be a useful tool with which to effectively use varieties.

The fourth direction is to improve decision making for breeders and growers to select varieties through big-data analysis. There are many big-data sources in Japanese sugarcane industry: data of breeding trials, factory data, manual interview with growers, and so on. Big-data analysis using these data sources could be useful in optimizing varietal composition and to estimate variety demand in the near future.

4.2. Evaluation of varietal mixtures as an option to achieve effective use of varietal diversity

The present study suggested systematic mixed-planting of different combinations of varieties (i.e. varietal mixtures) as a novel planting method and attempted to identify the ideal combination of varieties for such a mixture. Differences in plant type identified in Chapter 3.1 (‘Ni29’ and ‘Ni12’, ‘NiF8’ and ‘Ni17’), tillering and ratooning abilities in Chapter 3.2 (‘NiF8’ and ‘Ni22’), root elongation ability (deep vs. shallow) in Chapter 3.3 (‘NiF8’ and ‘Ni22’), and lodging resistance in Chapter 3.4 (‘NiTn18’ and ‘Ni22’) were examined to reveal the effects of varietal mixture on biomass production in sugarcane. The summary of findings was shown as below:

1. Varietal mixture with different plant types improved canopy light use because the horizontal-leafed variety may have captured radiation transmitted through erect-leafed variety, resulting in reduction of light loss during early growth while the erect-leafed variety improved the light conditions at lower canopy layers. Such habitat segregation in varietal mixtures may have increased the number of millable stalks at harvest under field condition due to improving its radiation-use efficiency.
2. Effects of a mixture of stalk-weight type variety, which is vigorous in new plantings, and stalk-number type variety, which is vigorous in ratoon cropping, were highly dependent on the pattern of mixture and the growing season. Number of millable stalks were increased in mixed-planting by row in new plantings while increased in mixed-plantingby plant in ratoon cropping. From these results, stalk-number type variety may have a high compensatory ability, whose vigor may induce improved performance of varietal mixtures over monocultures.
3. Mixed-planting of deep-rooting and shallow-rooting varieties induced habitat segregation within

the rooting zone. Roots under mixed varieties grew well in the middle soil layer (20–50 cm depth) and were thoroughly distributed in each soil layer. Such root densification did not increase shoot biomass in the present study; however, it has the potential to improving underground resource-use efficiency, especially under some abiotic stress conditions.

4. Under mixed-planting, a lodging-resistant variety exhibited the role of supporter and windbreak to mitigate the damage incurred by a lodging-sensitive variety as a result of frequent typhoons. Such a mixture may have a potential to improve the number of millable stalks and harvest efficiency.

From these findings, it was suggested that mixture-induced plasticity would bring out the abilities of habitat segregation and compensation in varieties. Although mixture index sometimes surpassed 1.0, mixtures rarely exhibited growth and yield values greater than those of monocultures of both component varieties. This observation suggests that many potential issues with respect to varietal combinations remain before growers can adopt this growing method for attaining increased numbers of millable stalks and cane yield.

Barot et al. (2017) suggested that, because there is still no generalist sugarcane variety that possesses all the desirable traits, selecting or breeding varieties for mixture-planting could be a necessity. So far, a variety which shows increased vigor even when grown in mixed-planting communities and one which shows compensatory ability are considered to be the ideal combination for mixed-planting (Kodama and Nagatomi 1969; Barot et al. 2017). In the Nansei Islands, crop growth conditions including climate and soil are so adverse and diverse that breeding has paid much attention to the geo-ecological adaptability of varieties (Takaragawa et al. 2018e). Japanese varieties have vigor with respect to elongation, tillering, and ratooning abilities, as well as tolerances to biotic and abiotic stresses. Therefore, there is a possibility that the ideal varieties for mixtures could exist among the varieties already available. When targeted traits such as plant type, tillering ability, ratooning ability, root elongation ability, and lodging tolerance were considered when selecting varieties for mixtures, varieties were classified into a 32- variety code, and 16 combinations of varieties, each of which had traits opposite to each other were predicted (Fig. 4.2, Table 4.1 and 4.2). At present, 11 of these combinations are available using recommended varieties. As it would be good to mix lodging tolerant varieties, an additional eight combinations are predicted and 15 combinations of recommended varieties are available. As it would be also useful to mix stalk (tiller)-number type varieties, an additional eight combinations are predicted and four combinations of recommended varieties are available. The reason for the poor options of certain combinations may be that there are few varieties with horizontal leaves. Both attempts to mixed-plant many promising combinations in many regions and to maintain the functional diversity among recommended varieties, as well as rejected varieties and breeding lines, as the first steps to successful mixed-planting, will be important to get the maximum benefit from varietal mixtures. In addition, to facilitate the successful operation in the field, component varieties would need to show little diversity with respect to traits such as similar fertilization application time (shoot elongation ability) and harvest time (maturity) when selecting combinations for mixtures.

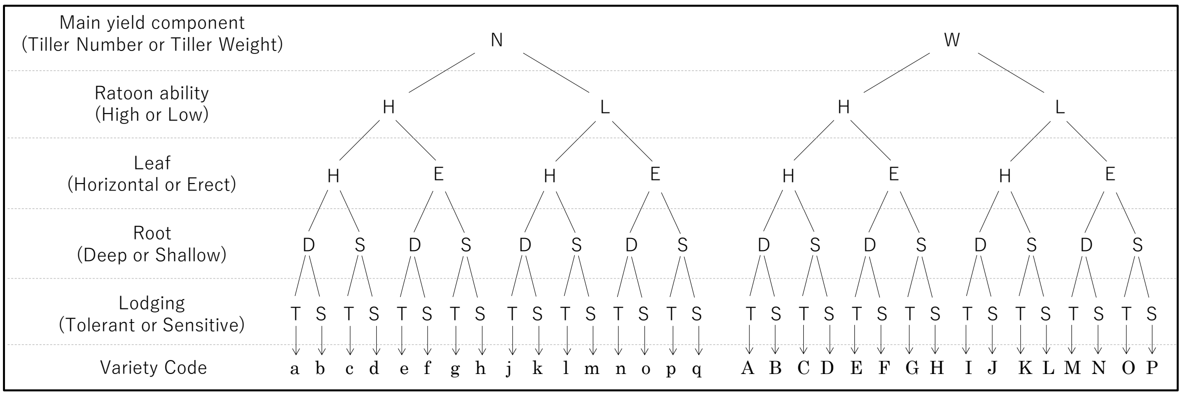


Fig. 4.2 Classification codes for mixture using five main functional traits.

Table 4.1. Main functional traits and class code of Japanese recommended varieties.

Variety	Clone name	Main yield component	Ratoon ability	Plant type	Rooting ability	Lodging resistance	Class code
F161	—	Weight	mid	erect	shallow	high	G, O
NiF8	KF81-11	Weight	high	erect	shallow	high	E
Ni9	RK80-1010	Number	high	mid	deep	high	G
Ni15	RK90-0039	Weight	low	erect	mid	high	a, e
Ni17	RK91-1004	Weight	high	horizontal	shallow	high	O
Miyako1	—	Weight	high	erect	deep	high	C
NiTn18	KF92-93	Number	high	erect	—	low	f, h
NiTn20	KF92T-519	Number	high	mid	deep	low	f
Ni21	RK94-4035	Weight	mid	erect	deep	low	F, N
Ni22	KY96-189	Number	high	mid	deep	high	a, e
Ni23	KY96T-537	Number	high	mid	deep	high	a, e
NiN24	KN91-49	Weight	high	erect	shallow	high	G
NiH25	RH86-410	Weight	low	horizontal	deep	low	J
Ni26	RK95-1	Number	low	erect	shallow	mid	o, p
Ni27	KR96-93	Weight	high	erect	deep	high	E
Ni28	RK96-6049	Number	high	mid	mid	high	a, c, e, g
Ni29	RK97-7020	Number	mid	erect	mid	high	e, g, m, o
NiN30	KN00-114	Number	high	erect	—	high	e, g
Ni31	KY99-176	Number	low	erect	—	high	m, o
NiTn32	KTn03-54	Weight	low	erect	—	high	M, O
Ni33	RK97-14	Weight	low	mid	—	low	J, L, N, P

Note: Information of traits were derived from Alic (2013) and Okinawa prefectural government, development of agriculture, forestry and fisheries (2015). Rooting ability was estimated using information of drought tolerance (tolerant: deep root, sensitive: shallow root). Class code of variety was determined according to Fig. 4.2.

Table 4.2. Estimated combination of Japanese recommended varieties for mixture.

	Combination of variety code	a-P	b-O	c-N	d-M	e-L	f-K	g-J	h-I	i-H	j-G	k-F	l-E	m-D	n-C	o-B	p-A	Total
Combination of varieties with all traits oppositte	Small	Ni9,Ni22,Ni23,Ni28	-	Ni28	-	Ni9,Ni22,Ni23,Ni28,Ni29,NiN30	NiTn18,NiTn20	Ni27,Ni28,Ni29	NiTn18	-	-	-	-	Ni29,Ni31	-	Ni26,Ni29,Ni31	Ni26	
	Capital	Ni33	F161,Ni15,NiTn32	Ni21,Ni33	NiTn32	Ni33	-	NiH25,Ni33	-	-	F161,NiF8,NiN24	Ni21	Miyako1,Ni27	-	Ni17	-	-	
	No. of combinations	4	0	2	0	6	0	6	0	0	0	0	0	0	0	0	0	11
Combination of lodging tolerant varieties	Combination of variety code	a-O	c-M	e-K	g-I	i-G	k-E	m-C	o-A									Total
	Small	Ni9,Ni22,Ni23,Ni28	Ni28	Ni9,Ni22,Ni23,Ni28,Ni29,NiN3	Ni27,Ni28,Ni29	-	-	Ni29,Ni31	Ni26,Ni29,Ni31									
	Capital	F161,Ni15,NiTn32	NiTn32	-	-	F161,NiF8,NiN24	Miyako1,Ni27	Ni17	-									
	No. of combinations	12	1	0	0	0	0	2	0									15
Combination of tiller number type varieties	Combination of variety code	a-p	b-o	c-n	d-m	e-l	f-k	g-j	h-i									Total
	Small	Ni9,Ni22,Ni23,Ni28	-	Ni28	-	Ni9,Ni22,Ni23,Ni28,Ni29,NiN30	NiTn18,NiTn20	Ni27,Ni28,Ni29	NiTn18									
	Capital	Ni26	Ni26,Ni29,Ni31	-	Ni29,Ni31	-	-	-	-									
	No. of combinations	4	0	0	0	0	0	0	0									4
Combination of tiller weight type varieties	Combination of variety code	A-P	B-O	C-N	D-M	E-L	F-K	G-J	H-I									Total
	Small	-	-	Ni17	-	Miyako1,Ni27	Ni21	F161,NiF8,NiN24	-									
	Capital	Ni33	F161,Ni15,NiTn32	Ni21,Ni33	NiTn32	Ni33	-	NiH25,Ni33	-									
	No. of combinations	0	0	2	0	2	0	6										10

Note: Varietal class code written as small and capital alphabets is according to Fig.4.2.

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Summary

Technological innovations leading to labor-saving, ecofriendly, and high and stable yield are needed to achieve sustainable sugarcane production. Although breeding new varieties is one of the most promising options, effective utilization of existing varieties had only been discussed prior to the current study. The objective of the present study was not only to discuss but also to suggest how varietal diversity could be exploited.

Firstly, an attempt was made to grasp the current status of the use of varietal diversity before developing strategies for the optimal methods to manage and maintain varietal diversity. From the results, Japanese sugarcane varietal diversity was defined and its current and potential issues were identified. Current status of Japanese varietal diversity was dependent on administrative divisions (Kagoshima and Okinawa prefectures), which have different policies for registration and use of varieties. Kagoshima has paid more attention on evenness, with a limited number of varieties being cultivated on similar cultivation areas than increasing the number of varieties while Okinawa has emphasized the number of varieties. The current issue of variety use was revealed to be the trend of dependence on a small number of varieties in some regions, difficulty to select effectively from the many varieties bred, and chronicity (i.e. normalization) of inactive varietal mixture in growers' fields. The follow-up and after-care services, such as administrative support to establish the management system and the creation of a variety use guidebook for extension work, were suggested to be required to increase and maintain the diversity.

As an example of important functional traits for sugarcane productivity, a quantitative evaluation method for plant type (index), which has previously been evaluated only qualitatively, was developed by analyzing leaf features, suggesting varietal diversity of plant type. Further phenotyping of functional diversities should be done and would contribute to better decision making for the selection of varieties. Monitoring long-term performance of varieties after release suggested that performance of the variety itself seemed not to change though apparent varietal yield decline due to accumulation of pathogen and climate disaster could be possible. Comparing performance with that of a monoculture of one old variety that has maintained high productivity over the years with no trend of deterioration, growing diversified varieties has improved regional productivity, especially in ratoon cropping.

Secondly, the present study suggested systematic mixed-planting of different varieties (i.e. a varietal mixture) as a novel growing method and attempted to identify the ideal combination of varieties for use in a mixture. Varietal mixture with different plant types improved canopy light use because horizontal-leafed variety may have captured radiation transmitted through erect-leafed variety, resulting in reduction of light yield loss during early growth, while erect-leafed variety improved the light conditions at the lower canopy layer. Such habitat segregation may have increased the number of millable stalks at harvest under field conditions due to its improved radiation-use efficiency. The effects of mixtures of a stalk-weight type variety, vigorous in new plantings, and a stalk-number type variety, vigorous in ratoon cropping, were highly dependent on the pattern of the mixture and the growing season. Number of millable stalks were increased in mixed-planting by row in new planting while increased in mixed-planting by plant in ratoon cropping. From these results, stalk-number type variety may have a high compensatory ability, and its vigorousness may induce greater performance from a varietal mixture. Mixed-planting of deep-rooting and shallow-rooting varieties induced habitat segregation for the rooting zone. Under mixed varieties, roots grew better in the middle soil layer (20–

50 cm depth) than under the corresponding monocultures and were thoroughly distributed in each soil layer. Such root densification did not increase shoot biomass in the present study, although it had the potential for improving underground resource-use efficiency, especially under abiotic stress conditions. Under mixed-planting, a lodging-resistant variety played the role of supporter and windbreak to mitigate the damage suffered by a lodging-sensitive variety as a result of frequent typhoons. Such a mixture may have the potential to increase the number of millable stalks and harvest efficiency.

From these findings, it was suggested that mixture-induced plasticity would induce habitat segregation and compensation in varieties. Although mixture index sometimes surpassed 1.0, mixtures rarely exhibited growth and yield greater than that of both corresponding monocultures of the variety mixture components. This fact suggests that many potential issues such as varietal combination remain before growers can adopt this growing method for attaining increased numbers of millable stalks and cane yield. The attempts to create a database of varietal characteristics, to mix-plant many promising combinations in different regions, and to reconsider many previously rejected varieties and breeding lines to maintain functional diversity among the recommended varieties are the first basic steps to success in mixture-planting.

要　旨

持続的なサトウキビ生産には省力化、環境配慮、安定多収につながる技術革新が必要である。新品種育成はその手段として有用であるが、普及後の既存品種の活用は十分に議論されぬまま現在に至っている。本研究は、既存品種群の多様性に着目し、その有効活用方法を提案することを目的として行われた。

まず、品種多様性の有効活用方法の掲示に先立ち、既存品種群の品種多様性およびその利用の現状の把握に努めた。その中で、日本の品種多様性を定義し、利活用に関する課題も抽出した。日本の品種多様性は、行政的なポリシーの違いもあり、2 つの行政区分（鹿児島県と沖縄県）で実態が異なっていた。すなわち、多様性増幅に関し、鹿児島県では品種数よりも均等度を重んじていること、沖縄県では均等度よりも品種数を重んじていることが明らかとなった。品種利用の実態調査から、地域により数品種への偏重傾向があること、品種選択が難しいこと、消極的理由による混植が慢性化していることなど複数品種利用の課題が明らかとなった。品種多様性の増幅および維持管理に関し、品種登録や増殖に関する政策的な取り決めの強化、品種利用に関する明確な指針を示した指導書の作成、植え付けの受託作業と連携した民間レベルでの種苗生産施設の積極的な受け入れといった方向性を提案した。

機能的多様性についてはこれまで定性的に評価されてきたものが多く、サトウキビの草型を定量的に評価する草型指数を葉身形質の解析から考案し、草型の品種多様性を示すことによりフェノタイピングの方向性を示した。品種普及後の生産能力を長期的に観察したところ、病害の蓄積や度重なる気象災害などによる、一時的あるいは見かけ上の生産能力の低下は否定できないが、品種そのものの生産能力は衰えていないことが示唆された。生産能力の低下が見られず長期的に活躍する品種の単植栽培と比較し、多様化された品種群の栽培は株出し栽培を中心に地域全体の生産性を向上させていることが明らかとなった。

また、本論では、既存品種の有効活用方法として異品種の混植を挙げ、各形質に注目して品種を選抜し、最適品種組み合わせを明らかにしようと試みた。水平葉型品種と直立葉型品種の混植により、初期成育時は水平葉型品種が畝間をよく被覆し光のロスを軽減し、生育後期は直立葉型品種により群落下層への光透過が良好となった。このような光利用効率の向上により、圃場試験では有効茎数が増加する可能性も示唆された。新植に強い茎重型品種と株出しに強い茎数型品種の混植の影響は混植様式や栽培年度によって異なり、新植では畝毎の混植により有効茎数が増加する可能性が示唆され、株出しでは株毎の混植により有効茎数が増加する可能性が示唆された。茎数型品種は補償能力が高いと考えられ、その生育が旺盛な年度で特に混植の好影響が見いだされると考えられた。深根性品種と浅根性品種の混植により、根の重心がそれら品種の単植の中間程度に移り、土壌領域を万遍なく占有し、地下部構造が緻密化した。このような地下部構造の可塑性は水利用効率など資源利用効率の向上に資するものであると考えられた。倒伏に強い品種と弱い品種を混植することにより、倒伏に強い品種が支柱的、防風的役割を果たし、倒伏に弱い品種の被害が軽減された。その結果、茎数確保や収穫効率が向上する可能性が示唆された。

以上のように、形質の異なる異品種を混植することにより可塑性が発揮され、棲み分けや補償作用が生じた。混植指数が 1 を超え単植する場合と比べて混植で高い値が示されたものの、両単植の示す値を凌駕するような生育、収量を示すことは珍しく、茎数確保や収量増加に資する技術としては依然として課題が多い。最も大きな課題である混植に適した品種の選択に関し、各品種形質をデータベース化するとともに、有望な組み合わせを各地で栽培試験すること、これまで単植用に淘汰されてきた品種・系統を混植用に再考することが混植品種選択の第 1 歩となるだろう。

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