Chapter 1 General introduction

1. Background of the present study

Dairy farming on grassland has expanded in Hokkaido, the northernmost island of Japan. The volume of raw milk produced by the Hokkaido dairy industry in 2010 was 3.9 million tons and made up 51% of the national output (Hokkaido District Agriculture Office, Ministry of Agriculture, Forestry and Fisheries 2011), making dairy an important industry in Hokkaido. However, Hokkaido's feed self-sufficiency rate in total digestible nutrient (TDN) equivalent was 52.7% in 2009 (Hokkaido Government 2011). This dairy production system has therefore depended on imported feed, in particular concentrated feed with high nutritive value. This can result in the increase of nitrogen (N) from outside regions. A better production infrastructure for forage grasses is required for improvement of material recycling among soil - grass - cow. The Japanese government drew up the Basic Plan for Food, Agriculture and Rural Areas in March 2005. The plan proposed increasing the feed self-sufficiency rate and shifting to an environment-friendly agricultural system. The price of imported concentrated feed has gone up dramatically in response to changes in the international situation in recent years. Hence, there is a compelling need to reduce costs by increasing the feed self-sufficiency rate in livestock farming. One measure includes the breeding of forage grasses. In order to increase the feed self-sufficiency rate in TDN equivalent, breeding improvement of not only forage yield but also nutritive value is important. Furthermore, forage with high nutritive value can reduce the amount of concentrated feed required (Masuko 2004), suggesting that the application of grass cultivars with high nutritive value may promote economic and environmental sustainability. Approximately 80% of total grassland in Hokkaido is timothy grass (*Phleum pratense* L.) (Ueda 1990). Improvement of the nutritive value of timothy through breeding could therefore largely affect dairy farming in Hokkaido, and therefore it is one of the most important breeding goals.

2. Characteristics of timothy

Timothy (*Phleum pratense* L., Japanese name: ooawagaeri), which is also called Herd's grass, is the only species of *Phleum* of economic importance (Berg *et al.* 1996). It is one of the most important perennial cool-season forage grasses in cool temperate regions, for example, parts of Canada, the USA, northern Europe, and in Hokkaido. The name 'Timothy' probably comes from Timothy Hansen, who played an important role in promoting the use of this grass in Maryland, North Carolina and Massachusetts, USA (Powel and Hanson 1973). The cultivation of timothy was started firstly in North America. It has been spread, presumably by humans, into all temperate and subarctic parts of the world (Berg *et al.* 1996). The amount of timothy grass seed sold in eastern Canada is twice that of all other grasses (McElroy and Kunelius 1995). It was introduced to Japan from the USA in 1874 (Ueda 1990). Cultivation has spread to various parts of Hokkaido over the last more than 100 years.

Timothy is cross-pollinating due to its self-incompatibility and wind-pollinated flowers (Shimokoji 1998). It is a complicated species to breed with its hexaploid set of chromosomes (2n = 6x = 42). The genome construction of timothy remains controversial because there are three proposed explanations: (a) allohexaploid (Nordenskiold 1945); (b) autohexaploid (Wilton and Klebesabel 1973; Cai *et al.* 2003); (c) autoallohexaploid (Cai and Bullen 1991, 1994). The cytology of *Phleum* species is difficult to clarify because its chromosomes are small, have similar sizes and shapes, and good cytological preparations are difficult to obtain (Berg *et al.* 1996).

Timothy is very winter-hardy compared with other perennial cool-season forage grasses. It can survive harsh overwintering conditions and therefore is grown in cold winter regions. It is well adapted to cool moist climates, but is not suited to dry or hot conditions (Berg *et al.* 1996) because it is less resistant to drought. It is also a palatable and nutritious grass that is readily consumed by cattle, sheep and horses.

Maturity is one of the most important traits used to group timothy cultivars. Timothy is an obligatory long-day plant with no cold/short day requirement before photoperiodic induction (Berg *et al.* 1996). Timothy genotypes have great variation on maturity. The maturity in Hokkaido is classified into four groups: extremely early; early; medium; and late. The early-maturing genotypes mostly originate from Hokkaido local materials, while materials introduced from Europe consist mostly of the late-maturing group. The photoperiodic requirements for flower initiation and heading in timothy cultivars vary with their latitudinal origin (Junttila 1985; Hay and Pederson 1986). Genotypes developed at higher latitudes generally have longer photoperiod requirements than those developed at lower latitudes.

3. Utilization methods and cultivars of timothy in Hokkaido

Timothy is mainly used for conserved feed, either as hay or silage, and the latter is the primary application. Timothy meadows are harvested multiple times (mostly 2 times). The first crop accounts for about 60% of the total annual yield, and the second crop, which is harvested in summer or fall following the first one, constitutes approximately 40% of the total annual yield. Timothy is also included in pasture mixtures.

Before the start of timothy breeding, ‘Hokkaido local’, having early maturity, was used in most grassland. It is an ecotype and highly adapted to climatic and cultural conditions in Hokkaido after repeated natural selections on its self-propagated populations for many years from introduced materials in 1874 (Ueda 1990). As the harvest involves large areas, however, it takes many days. Thus, timothy silage or hay with low nutritive value through delayed harvest was a major problem for dairy farmers because the negative fiber traits increase according to the growth stage (Casler and Vogel 1999). In such a situation, the breeding programs in Hokkaido have been successful in widening the span of maturity in timothy cultivars. ‘Kunpu’ (Matsutani *et al*. 1981) with extremely early maturity, ‘Senpoku’ (Maki 1985), ‘Nosappu’ (Ueda *et al*. 1977b) and ‘Natsuchikara’ (Ashikaga *et al*. 2012) with early maturity, ‘Akkeshi’ (Furuya *et al*. 1992b) and ‘Kiritappu’ (Furuya *et al*. 1992a) with medium maturity, and ‘Hokusyu’ (Ueda *et al*. 1977a) and ‘Natsusakari’ (Yoshizawa *et al*. 2005) with late maturity were bred at the Hokkaido Kitami Agricultural Experiment Station (currently; Kitami Agricultural Experiment Station, Hokkaido Research Organization) to which the author belongs. Forage yield improvement has been considered important for stable production among the goals in timothy breeding. Also, resistance to the purple spot by *Cladosporium phlei* (Gregory) de Vries, lodging resistance and competitive ability after the first cut have been particularly important breeding goals. Recent cultivars were mainly improved for these traits, and were improved by about 20% compared to ‘Hokkaido local’ before the start of breeding in forage yield (Tamaki 2005). However, the nutritive value in commercial cultivars has not been improved so far.

4. Nutritive value traits

Digestibility is a measure of energy availability to the ruminant. Improvement of dry matter (DM) digestibility may lead to improved animal performance by improving the rate of passage from rumen and intake. Casler and Vogel (1999) pointed out that the most important single event in the history of forage grass breeding was the publication of the *in vitro* DM digestibility (IVDMD) analysis of Tilley and Terry (1963). This analysis had nearly all the necessary characteristics for reasonable selection criterion: rapid, repeatable, amenable to a relatively small sample size, heritable and directly correlated with animal performance (Casler 2001).

The detergent analysis method (Van Soest 1963) has been widely applied to the nutritive evaluation of forages. Cell wall constituents are fractionated into neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by this method. NDF is the structural residue. It contains hemi-cellulose (HEM), cellulose (CEL), and ADL. ADF, which is the total of CEL and ADL, is the less digestible fractions, and ADL is the final residue. NDF content, which is a measure of fibrous bulk, is associated with voluntary intake (Van Soest 1966). Voluntary intake is generally considered to be the single most important factor limiting animal performance on high-forage diets (Fahey and Hussein 1999). Up to 70% of the variation in animal production can be attributed to variation in intake, while only 20% can be attributed to variation in digestibility (Crampton *et al*. 1960). Voluntary intake can be increased by reducing the bulk volume of the feed, which increases intake before satiation, or by increasing fiber clearance from the rumen, which reduces the time required to stimulate appetite (Casler 2002).

The enzymatic analysis method (Abe *et al*. 1979) has been most widely employed for evaluating fiber digestibility in Japan. The organic cell wall (OCW), high-digestible fiber (Oa) and low-digestible fiber (Ob) contents are fractions based on the digestibility of fiber determined according to the method. The OCW content, which is almost equal to the NDF content, reflects the fraction of HEM, CEL, lignin and insoluble protein (Abe 2007). The Oa content reflects the fraction of non-lignified and low-crystallized portion in OCW content, and the Ob content reflects the fraction of lignified and high-crystallized portion in OCW content (Abe 2007). Physical distention of the rumen is the major factor limiting voluntary intake of high producing ruminants on high-forage diets (Mertens 1994). The Ob content influences DM intake more strongly than NDF content (Deguchi *et al*. 1996) as well as *in vitro* indigestible NDF content under 24 h incubation (Deguchi *et. al*. 2010). The Ob/OCW ratio, which is an index of fiber digestibility, correlates closely with *in vivo* TDN content (Deguchi K. *et al*., unpublished data).

Water-soluble carbohydrate (WSC), which is a total of the component of non-structural carbohydrates, is a particularly useful criterion for feeding ruminants. The WSC content affects the milk yield of dairy cattle grazing on pasture (Miller *et al*. 2001). WSC is also utilized as a source of energy, and is related to the regrowth after cutting and persistence use of grazing pasture (Fulkereson amd Donaghy 2001), summer drought (Volaire *et al*. 1998) and snow mold resistance (Sanada *et al*. 2010) in forage grasses. Moreover, WSC is the main source of fermentation substrates during ensiling. Cultivars with high WSC content can lead to high quality silage (Wilkins and Humphreys 2003).

Crude protein (CP) of forage is an important indicator because protein is necessary for animal production (Morimoto 1984). In order to attain high levels of milk production, high lactating cows are typically fed high CP diets.

5. Previous studies on genetic improvement of the nutritive value traits in forage grasses

5-1. Importance of the improvement of the nutritive value traits in timothy

Improvement of the forage nutritive value through breeding should lead to enhanced livestock productivity since forage with improved nutritive value can benefit a number of aspects related to animal production. A 1% decrease in the Ob content of hay DM increases the DM intake of dairy cattle by 0.17 kg (Abe 2007). Genetic improvement in IVDMD generally results in improved animal daily gains of beef cattle and the relationship is broadly positive, with a 3.2% increase in daily gains for each 1% increase in IVDMD (Casler and Vogel 1999). Milk yield from dairy cattle offered high WSC cultivar was about 20% higher compared with that offered standard cultivar in grazing use (Miller *et al*. 2001). Genetic gains in forage nutritive value, if documented by increased animal performance, can improve overall profit margins associated with a new cultivar, compared with older cultivars, by literally millions of dollars (Casler and Vogel 1999). Improvement of the nutritive value of timothy as feed for livestock is therefore essential and has high priority in timothy breeding.

5-2. Previous researches on breeding for the nutritive value traits of grasses except for timothy

Contemporary literature includes references to the selection of genotypes for superior quality of forage and the concept of superior strain selection dating back at least 300 years (Casler and Vogel 1999). The selection criteria by early selectionists consisted of plant traits such as reduced disease symptoms and senescence.

Increasing DM digestibility was ranked as the most important goal for grasses (Smith *et al*. 1997). Although studies on IVDMD have been conducted on many forage grasses (Cooper *et al*. 1962; Ross *et al*. 1970; Saiga 1981b; Carpenter and Casler 1990; Hopkins *et al*. 1993; Yahagi *et al*. 2001), few commercial cultivars with high IVDMD have been developed so far. IVDMD can be increased either by improving the digestibility of the fiber or by increasing the ratio of the cell contents to fiber (Wilkins and Humphreys 2003). Also, materials with high cell contents would have high IVDMD since the cell contents, which include CP and WSC contents, are all highly digestible. These may confuse the direction of the improvement for IVDMD. The IVDMD procedure may also not be practical for all breeding programs because a reliable source of rumen fluid may not always be available (Casler 2001).

Genetic progress toward reduced NDF content has been reported for smooth bromegrass (*Bromus inermis* Leyss.) (Casler 1999) and reed canarygrass (*Phalaris arundinacea* L.) (Surprenant *et al*. 1988). The live weight gain of beef cattle was 15% higher when fed silage or dried grass of the

Italian ryegrass (*Lolium multiflorum* Lam.) cultivar 'Sabalan' than cultivar 'Tetila' although there was a significant difference between these cultivars in fiber contents but little difference in IVDMD (Wilkinson *et al*. 1982).

Increasing the WSC content of grasses ranked highly in the Delphi survey technique (Smith *et al*. 1997). Genetic studies for increasing WSC content have been conducted in perennial ryegrass (*Lolium perenne* L.) (Humphreys 1989a, b; Humphreys 1995; Smith *et al*. 2004) and cocksfoot (*Dactylis glomerata* L.) (Sanada *et al*. 2004; Sanada *et al*. 2007). The content of WSC in perennial ryegrass is not correlated with yield, but is positively correlated with IVDMD (Humphreys 1989b). Selection in the UK has been conducted to develop high WSC perennial ryegrass cultivars, which appear to be more palatable to sheep than the cultivars with standard WSC contents (Jones and Roberts 1991). Perennial ryegrass cultivars with high WSC content are already in wide use in Europe and New Zealand.

Many efforts to genetically improve CP content have been made along with some methods to improve digestibility and fiber contents (Casler 2001). However, N fertilization can negate the progress achieved through breeding for increased CP content in grasses (Arcioni *et al*. 1983). Some of the selection experiments showed that genetic increases in CP content were achieved at the expense of forage yield (Clements 1969; Arcioni *et al*. 1983). CP content is of questionable relevance to the nutrition of ruminants and generally not considered to be a useful selection criterion for improving the nutritive value of forages (Smith *et al*. 1997).

Breeding the nutritive traits of forage grasses has made much progress as a result of near-infrared reflectance spectroscopy (NIRS) developed in the 1970s. The application of NIRS makes breeding programs more efficient compared with laboratory analysis in three ways: a) reduction of cost; b) swiftness; c) simultaneous evaluation of multiple traits. NIRS has enhanced the capability of breeders to improve the forage nutritive value by allowing efficient screening of large populations. Selection for the nutritive traits has been conducted using NIRS in many forage grasses.

Meanwhile, difficulties are often associated with the nutritive breeding of forage grasses. According to Casler (2001), long-term natural selection for adaptive traits, such as lodging or disease resistance, may favor plants with higher and/or stronger cell walls. Hence, the nutritive value traits may be negatively affected by natural selection. Undesirable correlations between forage yield and nutritive value traits have been reported. In particular, undesirable genetic correlation (r_G) between NDF content and forage yield may be a biological necessity (Casler and Diaby 2008) because NDF content comprises the majority of plant cell walls (Casler and Hatfield 2006). Furthermore, many nutritive traits are typically intercorrelated, and nearly all traits are regulated by numerous enzymes that are involved in their biosynthesis or metabolism (Casler 2001), demonstrating the need for detailed genetic analysis of the nutritive value traits.

5-3. Previous researches on timothy breeding for the nutritive value traits

Studies on timothy nutritive breeding overseas have received the greatest attention with respect to digestibility and fiber content or ratio. Cultivar differences for IVDMD were reported (Koch 1976; Mason and Flipot 1988; Collins and Casler 1990). Berg and Hill (1983) reported the narrow-sense heritability (h_N^2) of IVDMD to be 0.38 using plants selected at random from 'Climax', with significant variance for general combining ability but not specific combining ability. They also showed significant year × general combining ability variances in IVDMD. One cycle of divergent selection for IVDMD produced populations that differed significantly for IVDMD and ADF content under space planted conditions (Surprenant *et al*. 1990a). Medium to high h_N^2 values for IVDMD, NDF and ADF contents were found (Surprenant *et al*. 1990b). Belanger *et al*. (2004) confirmed the presence of genetic variability for leaf and stem nutritive value traits (*in vitro* true digestibility, *in vitro* cell wall digestibility and stem NDF content) in timothy both under limiting and non-limiting N conditions. As for an approach to increase the digestibility of the cell wall, ADL/(HEM+CEL) and ADL/CEL ratios seem to be promising selection criteria to increase digestibility while maintaining or increasing yield (Claessens *et al*. 2004). The ADL/CEL ratio selection resulted in stable responses across years for *in vitro* true digestibility and *in vitro* NDF digestibility (Claessens *et al*. 2005).

There are few studies on breeding of WSC in timothy with the exception of the present study although WSC content is an important criterion in other cool-season grasses (Tamaki *et al*. 2010). A large variation was found in WSC content among timothy cultivars (Jonaviciene *et al*. 2008). As for CP content, Berg and Hill (1983) reported the h_N^2 to be 0.30 in the material from a single cultivar, 'Climax', and found general combining ability but not specific combining ability to be significant. Surprenant *et al*. (1990b) reported the h_N^2 of CP content to be over 0.80. One cycle of divergent selection in a wide genetic background produced populations that differed significantly for CP content using space planted conditions but not under sward conditions (Surprenant *et al*. 1990a).

As an initial attempt at nutritive timothy breeding in Hokkaido, Furuya (1987) attempted the selection for IVDMD. He indicated that the h_N^2 by the parent-offspring regression method was high in IVDMD; and that genetic variability in IVDMD was large. Unfortunately the development of improved cultivar on IVDMD could not be achieved due to other poor abilities, e.g., yield, of the breeding strains.

6. Objectives and composition in this thesis

This study was performed to develop effective breeding methods for improving the nutritive value traits in timothy. Estimation of the genetic parameters (e.g., genetic variation, heritability and genotype × environment interaction (G × E)) of the traits is essential for developing effective genetic improvement. The study focused on the genetic amelioration of the traits in harvesting timothy. This dissertation consists of 8 Chapters. Chapters 2 to 6 focus on the nutritive value traits of the first crop, while Chapter 7 focuses on the nutritive value traits of the second crop. In more detail, Chapter 2 examines the broad-sense heritability (h_B^2) and h_N^2 of the nutritive value traits in the first crop to identify suitable selection criterion for improving the nutritive value. Phenotypic (r_P), r_G and environmental (r_E) correlations among the traits and yield are also analyzed if simultaneous improvement among the traits and improvement of both yield and the traits are possible. The studies following Chapter 2 use the three selection criteria, presented in Chapter 2, for improving the nutritive value. Chapter 3 investigates the magnitude of genotype × year interaction (G × Y) and genotype × location interaction (G × L) on the nutritive traits to evaluate the stability of the traits in different environments. Chapter 4 investigates the magnitude of genotype × maturity stage interaction (G × M), genotype × harvest time on sunny day interaction (G × TS) and genotype × harvest time on cloudy day interaction (G × TC) on the nutritive traits to estimate how the relative ranking of genotypes based on the nutritive traits varies with different maturity stages and harvest time within a day. Chapter 5 investigates the relationships between the nutritive value and agronomic or morphological traits if simultaneous improvement among the traits is possible or whether indirect selection for the nutritive traits through agronomic and morphological traits is applicable. Chapter 6 examines the extent of genotype × N fertilization interaction (G × N) on the nutritive traits to evaluate the stability of the traits in different N application levels. Regarding the second crop, Chapter 7 investigates the magnitude of genotype × crop interaction (G × C) between the first and second crops, and the extent of G × Y and h_N^2 of the second crop to estimate the relationship between the two crops and the genetic parameters of the second crop. And, on the basis of these results, Chapter 8 presents a model of an effective breeding scheme on the nutritive traits in timothy. Potential methods of simultaneous improvement of the nutritive traits and yield are also discussed. Furthermore, the benefits of the superior cultivars with high nutritive value are discussed based on previous studies on the nutritive value and some assumptions.

Chapter 2 Heritability of the nutritive value traits in the first crop of timothy

Introduction

Intensive breeding studies on nutritive value traits have been conducted in many forage grasses except for timothy. Ross *et al*. (1970), Saiga (1981a) and Yahagi *et al*. (2001) indicated that the high h_N^2 in IVDMD was observed in smooth bromegrass, cocksfoot and tall fescue (*Festuca arundinacea* Schreb.), respectively. Surprenant *et al*. (1988) and Casler (1999) reported a large genetic variation and high realized heritability in NDF content in reed canarygrass and smooth bromegrass, respectively. Humphreys (1995) and Sanada *et al*. (2004) also indicated high h_B^2 in WSC content in perennial ryegrass and cocksfoot, respectively. These suggest the possibility of improving these nutritive traits in terms of breeding. On the other hand, Humphreys (1989a) suggested that the inheritance of WSC content of perennial ryegrass also appeared to be complex with a large non-additive component. Tan *et al*. (1978) and Shenk and Westerhauss (1982) showed low h_N^2 and medium h_B^2 of CP content in smooth bromegrass and cocksfoot, respectively. Cultivars with higher nutritive value show less yield productivity in general because of negative relationships between forage yield and nutritive traits (Furuya 1987; Casler and Vogel 1999; Belanger *et al*. 2001). Therefore, it is necessary to consider the trade-off relations between these traits.

Breeding studies on the nutritive value in timothy have mainly been conducted on digestibility. In IVDMD Furuya (1987) reported high h_N^2 and Surprenant *et al*. (1990a) indicated the selection effect in individual plant selection. These studies suggest a high possibility of genetic improvement of timothy digestibility. Surprenant *et al*. (1990a) and Claessens *et al*. (2005a) proposed the selection criteria of digestible DM yield and ADL/CEL ratio, which can improve digestibility without decreasing yield, respectively.

Few studies on nutritive traits except for the traits related to digestibility have been reported. Moreover, there is no report on breeding using fractions by enzymatic analysis method, which has been most widely employed in evaluating fiber digestibility in Japan. It is necessary for the breeders to estimate the following two points for effective improvement when a new trait is applied as a breeding goal: (a) the h_B^2 and h_N^2 under the condition that the parents and their progeny are grown in the same environment; (b) the effect of G × E (Tamaki 2005).

The objective of this study was to investigate the h_B^2 and h_N^2 of the nutritive traits in the first crop of timothy. This study also investigated the r_P, r_G and r_E coefficients among the nutritive traits or between the nutritive traits and yield to estimate the possibilities of simultaneous improvements among these traits.

Materials and methods

Fifteen early-maturing parental clones and their polycross half-sib progeny, which had not been selected for the nutritive value traits, were used in this experiment. These materials had also been selected for various agronomic traits such as yield, lodging resistance and competitive ability after the first cut. The range of heading date of the parental clones in the 2003 evaluation was three days. The parents were planted on 28 August 2001 at the Hokkaido Kitami Agricultural Experiment Station in Kunneppu (43°47’N, 143°42’E; currently the Kitami Agricultural Experiment Station, Hokkaido Research Organization), Hokkaido, Japan, at a spacing of 0.6 × 0.6 m in a randomized complete block design with four replications. The progeny were sown on 29 June 2001 in two rows 0.9 m long, spaced at 0.6 m intervals, with 0.6 m between each plot, in a randomized complete block design with four replications in the same field as the parents. The parents received 6.0 g N, 6.9 g P_2O_5 plus 6.0 g K_2O m^{-2} in April after snowmelt in 2004. The progeny also received 7.5 g N, 15.0 g P_2O_5 plus 7.5 g K_2O m^{-2} at the same time as the parents. They were harvested at 10 cm stubble height on 25 June 2004 when they reached full heading stage. Forage samples were collected at harvest.

The harvested samples were dried in an oven at 70 °C for 48 h, then milled and passed through a 0.75-mm screen. Seven nutritive value traits (Ob, Oa, OCW, WSC and CP contents and Ob/OCW and ADL/CEL ratios) were used as selection criteria in this study. The nutritive traits were analyzed using a NIRS (Foss NIRSystems Model 6500, Laurel, MD, USA). The equations used for prediction for Ob, OCW and CP contents were described by Deguchi (2003). The R^2 values of prediction for ADF (n = 44), ADL (n = 46) and WSC (n = 20) contents were 0.97, 0.58

and 0.92, respectively, with standard errors of prediction of 1.18, 0.43 and 0.93%. Biases of prediction were 1.14, –1.00 and 0.32, respectively (Deguchi K *et al*., unpublished data). These equations used for prediction were developed by using the partial least-squares regression. Oa content was estimated by subtracting Ob content from OCW content. CEL content was estimated by subtracting ADL content from ADF content. The predicted values of Ob and OCW contents were used to estimate the Ob/OCW ratio. The predicted values of ADL and CEL contents were used to estimate the ADL/CEL ratio. The samples for developing the calibration equations were analyzed by the enzymatic analysis method for Ob and OCW contents (Abe *et al*. 1979), by the anthron method for WSC content (Yemm and Willis 1954), by the detergent analysis method for ADL and ADF contents (Van Soest 1963) and by multiplying Kjeldahl N by 6.25 for CP content.

The h_B^2 of the parents and their progeny were estimated on a phenotypic mean basis averaged replications from the variance components in analysis of variance (ANOVA) as

$$h_B^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2 / r),$$

where r is the number of replications and σ_G^2 and σ_E^2 are the genotypic and error variance components, respectively. The h_N^2 was estimated by doubling the regression coefficient of the progeny means on the parental means. The r_G and r_E coefficients among the nutritive traits or between the nutritive traits and DM weight in the parental clones were calculated as

$$r_G = COV_{GAB} / \sqrt{(\sigma_{GA}^2 \times \sigma_{GB}^2)},$$

$$r_E = COV_{EAB} / \sqrt{(\sigma_{EA}^2 \times \sigma_{EB}^2)},$$

where COV_{GAB} is the genotypic covariance component between the two traits A and B, and σ_{GA}^2 and σ_{GB}^2 are the genotypic variance components for the traits A and B, respectively; COV_{EAB} is the error covariance component between the two traits A and B, and σ_{EA}^2 and σ_{EB}^2 are the error variance components for the traits A and B, respectively.

Results

Estimation of h_N^2

The means of the traits in the parental clones were almost the same as those of their half-sib progeny, but for all traits, the standard deviations (SD) and ranges were greater in the parents than in their progeny (Table 2-1). The estimates of h_B^2 ranged from 66.3 to 89.9% in the parents and 5.2 to 65.5% in their progeny. The estimates of h_N^2 ranged from 37.5 to 90.1%. Correlation coefficients between the parents and their progeny in the traits ranged from 0.39 to 0.81 ($P < 0.001$).

Relationships among the nutritive traits in the parental clones

Medium to strong positive r_G were shown between Ob content and Ob/OCW ratio, between ADL/CEL and Ob/OCW ratios, between ADL/CEL ratio and WSC content, and between Ob and OCW contents (Table 2-2). Medium to strong negative r_G were also detected between OCW content and ADL/CEL ratio, between OCW and WSC contents, between OCW and CP contents, between Oa content and Ob/OCW ratio, between Oa content and ADL/CEL ratio, between Oa and CP contents, and between Ob and WSC contents. The r_G among the seven nutritive traits were generally near the r_P. Most of r_G between the traits were weak or medium to strong desirable for the direction of the improvement of their traits. By contrast, undesirable r_G for simultaneous improvement between their traits were detected between ADL/CEL ratio and WSC content, between ADL/CEL ratio and OCW content, and between CP and Oa contents. The r_E varied with combination of the traits.

Relationships between the nutritive traits and DM weight in the parental clones

The DM weight in the parents showed large SD and ranges (Table 2-1). In contrast, the DM weight in the progeny was negative h_B^2. This indicates that this study could not evaluate the genetic variation of the DM weight in the progeny. Hence, the data in the parents were used for the relationships between the nutritive traits and DM weight. Ob, Oa, WSC contents and Ob/OCW and ADL/CEL ratios showed weak r_G with DM weight (Table 2-2). OCW and CP contents showed undesirable r_G with DM weight for simultaneous improvement between their traits. The r_E between the nutritive traits and DM weight were weak in general.

Table 2-1 Means, standard deviations (SD), ranges, broad-sense heritabilities (h_B^2) of 15 timothy parental clones and their half-sib progeny, and narrow-sense heritabilities (h_N^2) and correlation coefficients between them of the nutritive value traits and DM weight of the first crop in 2004

	Ob (%DM)	Oa (%DM)	OCW (%DM)	Ob/OCW (%)	ADL/CEL (%)	WSC (%DM)	CP (%DM)	DM weight (g)
Parent								
Mean	61.6	9.9	71.5	86.2	9.8	9.5	6.1	260.9
SD	2.27	1.58	2.27	2.11	0.74	1.58	0.71	32.83
Max	65.5	12.5	74.8	90.2	11.5	13.4	7.8	318.1
Min	57.2	6.7	67.6	82.6	8.7	7.7	5.2	199.7
h_B^2	80.5	77.4	84.7	76.6	66.3	89.9	80.3	78.5
Half-sib progeny								
Mean	62.9	9.8	72.7	86.5	10.1	9.9	5.3	415.0
SD	1.36	0.97	0.96	1.34	0.60	0.62	0.34	13.55
Max	64.8	12.6	74.8	87.8	11.3	11.1	5.9	447.6
Min	59.5	8.8	71.4	82.6	9.0	8.8	4.8	393.9
h_B^2	65.5	36.9	5.2	49.7	54.7	52.7	48.5	0.0[a]
h_N^2	73.2	86.4	48.4	90.1	87.5	63.8	37.5	–[b]
r_{PO}	0.61*	0.70**	0.57*	0.71**	0.54*	0.81***	0.39	0.27

*, **, *** indicate significance at the 0.05, 0.01, 0.001 levels, respectively.

[a] Negative value in the estimate of h_B^2 shows as zero.

[b] Data not shown due to different planting pattern between the parents and their progeny.

ADL, acid detergent lignin; CEL, cellulose; CP, crude protein; DM, dry matter; Oa, high-digestible fiber; Ob, low-digestible fiber; OCW, organic cell wall; r_{PO}, correlation between the parents and their half-sib progeny; WSC, water-soluble carbohydrate

Discussion

The estimates of h_B^2 in the parents were high in all traits (Table 2-1), indicating that these traits have a high proportion of genetic variation. This also suggests that the test accuracy in the present study was high due to the low proportion of environmental variation in these traits. Moreover, large SD and ranges in the parents indicate that the variations of these materials, which had been under high selection pressure for important agronomic traits, were large in all the traits. The estimates of h_B^2 in the progeny showed low values compared to those in the parents. This may coincide with the genetic variation among polycross half-sib progeny being a half of that among parental clones (Tamaki 2004).

The h_N^2 values estimated by the parent-offspring regression method were high in Ob, Oa and WSC contents and Ob/OCW and ADL/CEL ratios (Table 2-1), indicating the preponderance of an additive gene effect. Therefore, these traits are likely to be improved by individual selection. Many studies for the heritability of forage nutritive value traits have been reported on IVDMD using the realized heritability method. Saiga (1981a) showed that the realized heritability was 42 - 60% in cocksfoot. Casler and Vogel (1999) reported that about 20 - 30% realized heritability was commonly detected in perennial forage grasses. The conflict in the heritability values between the present study and these previous studies in terms of the realized heritability method seems to derive from the methods of measuring the heritability. Although the latter studies measured the heritability in different years, the former study measured the heritability in the same environment. Hence, it is considered that genetic covariance between the parents and their progeny in the former was difficult to diminish by environmental effect compared to the latter. Furuya (1987) reported that the h_N^2

Table 2-2 Phenotypic (r_P), genetic (r_G) and environmental (r_E) correlation coefficients among the nutritive value traits and DM weight of 15 timothy parental clones of the first crop in 2004

		Ob (%DM)	Oa (%DM)	OCW (%DM)	Ob/OCW (%)	ADL/CEL (%)	WSC (%DM)	CP (%DM)
DM weight	r_P	0.34	0.24	0.51	–0.15	0.13	0.10	–0.64**
(g)	r_G	0.41	0.26	0.57	–0.16	0.24	0.19	–0.84
	r_E	0.09	0.16	0.23	–0.12	–0.14	–0.39	0.12
CP	r_P	–0.30	–0.31	–0.51	0.21	–0.23	–0.22	–
(%DM)	r_G	–0.16	–0.49	–0.49	0.41	–0.07	–0.15	–
	r_E	–0.84	0.39	–0.61	–0.51	–0.71	–0.62	–
WSC	r_P	–0.61*	–0.07	–0.66**	–0.06	0.58*	–	–
(%DM)	r_G	–0.79	0.04	–0.74	–0.21	0.56	–	–
	r_E	0.43	–0.73	–0.12	0.73	0.80	–	–
ADL/CEL	r_P	0.09	–0.70**	–0.39	0.66**	–	–	–
(%)	r_G	–0.12	–0.67	–0.56	0.59	–	–	–
	r_E	0.70	–0.77	0.14	0.82	–	–	–
Ob/OCW	r_P	0.51*	–0.98***	–0.17	–	–	–	–
(%)	r_G	0.48	–0.98	–0.18	–	–	–	–
	r_E	0.65	–0.99	–0.10	–	–	–	–
OCW	r_P	0.76**	0.35	–	–	–	–	–
(%DM)	r_G	0.77	0.37	–	–	–	–	–
	r_E	0.69	0.27	–	–	–	–	–
Oa	r_P	–0.35	–	–	–	–	–	–
(%DM)	r_G	–0.30	–	–	–	–	–	–
	r_E	–0.51	–	–	–	–	–	–

*, **, *** indicate significance at the 0.05, 0.01, 0.001 levels, respectively.

ADL, acid detergent lignin; CEL, cellulose; CP, crude protein; DM, dry matter; Oa, high-digestible fiber; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

estimated by the parent-offspring regression method in the same environment was 89.1% in IVDMD of timothy. The estimates of h_N^2 in the Ob/OCW and ADL/CEL ratios on fiber digestibility were also 90.1 and 87.5% in this study, respectively. The h_N^2 of the five traits except for OCW and CP contents in the same environment should therefore be high. The estimate of h_N^2 of CP content was low, suggesting that the application of individual selection would include a risk of failure. Although successful selection for CP content was reported in timothy (Surprenant *et al*. 1990a), N fertilizer application may negate the beneficial breeding effect on CP content (Casler and Vogel 1999). Further studies are needed to determine an effective selection method of CP content.

Most of the r_G among the seven nutritive traits in the parents were weak or medium to strong desirable for the direction of the improvement among the traits (Table 2-2), indicating the possibility of simultaneous improvement among most of the traits. However, undesirable r_G for simultaneous improvement among the traits was detected between the ADL/CEL ratio and WSC content and between the ADL/CEL ratio and OCW content. This demonstrates the validity of applying the Ob/OCW ratio as a selection criterion for improvements among fiber digestibility, WSC and OCW contents. The present study suggests no definitive reasons for different r_G in Ob/OCW and ADL/CEL ratios, but the author believes it to be causally related to that although the stem portion has a considerable impact on the ADL/CEL ratio and WSC content (Smith 1973; Claessens *et al*. 2005b), Ob/OCW ratio may have weak influence of the stem portion due to including the influence of the leaf portion because the Ob/OCW ratio is the ratio of a bigger fiber fraction than the ADL/CEL ratio. Undesirable r_G was also detected between Oa and CP contents, suggesting that simultaneous improvement between the two traits would be difficult. This relationship may be a common trend between CP content and fiber traits as negative r_G between Ob and CP contents or between OCW and CP contents.

OCW and CP contents showed medium to strong undesirable r_G with DM weight in the parents (Table 2-2). The two traits also showed weak r_E with DM weight, suggesting that simultaneous improvements between the two relationships would be difficult. On the other hand, the five traits except for OCW and CP contents were weak r_G with DM weight although the previous studies showed undesirable correlations between the nutritive traits and yield (Furuya 1987; Casler and Vogel 1999). This seems to be because this study used materials, which (a) belonged to the same maturity, and (b) had been selected for agronomic traits including yield. It is necessary to consider the nutritive traits and maturity separately to evaluate the nutritive traits in each maturity class because the nutritive value and yield of forage generally show contradictory changes according to the growth stage (Casler and Vogel 1999). Selection combining nutritive traits with yield can improve nutritive traits without sacrificing yield (Surprenant *et al*. 1990a; Casler 1999; Claessens *et al*. 2004). It is therefore considered that the above two points should be taken into account for simultaneous improvement between the nutritive traits and yield. Moreover, the materials designed for the two points showed great variation in the nutritive traits (Table 2-1), suggesting that there is little possibility that the variation of the nutritive traits becomes narrow by consideration of the two points.

In conclusion, the three traits of Ob and WSC contents and Ob/OCW ratio in the first crop not only have potential for improvement by means of individual selection, but there are also prospects for simultaneous improvement among the three traits and with yield productivity.

Chapter 3 Effects of year and location on the nutritive value in the first crop of timothy

Introduction

The differential response of genotypes to various environments is an important consideration in plant breeding. The study in Chapter 2 showed that the h_N^2 is high in the three traits (Ob and WSC contents and Ob/OCW ratio) of the nutritive value of the first crop of timothy. It is therefore necessary to study how the relative genotype ranking of these traits changes in different environments for effective improvement of the nutritive value.

The G × E in forage quality traits has been studied in several grass species. Casler (2001) concluded that the G × E in IVDMD was unimportant in smooth bromegrass. Similarly, genetic gains in IVDMD and WSC content of perennial ryegrass were generally consistent across multiple sites and years (Humphreys 1989a; Wilkins 1997; Smith *et al.* 2004). Correlation of WSC content of cocksfoot across two years was significant (Sanada *et al.* 2004). The G × E in IVDMD of cocksfoot (Shenk and Westerhaus 1982) and that in WSC content of tall fescue (Burner *et al.* 1983) were non-significant or very small.

The nature of G × E in the nutritive value traits in timothy still remains unclear. There were no significant genotype × low solar radiation (Furuya *et al.* 1994) and genotype × groundwater level interactions (Furuya and Tsutsui 1996) in IVDMD. Selection for ADL/CEL ratio resulted in stable responses across years in *in vitro* true digestibility (Claessens *et al.* 2005a). On the other hand, IVDMD in rumen fluid showed a strong genotype × temperature interaction (Ames *et al.* 1993). Significant G × Y and G × L were found in IVDMD (McElroy and Christie 1986a), NDF, ADF and CP contents (Surprenant *et al.* 1993). Furthermore, no studies have examined the G × E in the three nutritive traits of Ob and WSC contents and Ob/OCW ratio which were revealed the effectiveness in Chapter 2.

The author therefore investigated the three nutritive value traits of the first crop of timothy genotypes in different years in Kunneppu, a dry area where timothy is bred, Hokkaido, Japan. The author also measured the traits of the genotypes in Nakashibetsu, a cool wet area where timothy is commonly grown, Hokkaido, Japan, as well as in Kunneppu in the same year. The objectives of the study were to investigate the magnitude of G × Y and G × L for the three nutritive value traits of the first crop of timothy.

Materials and methods

The experiment for the G × Y estimation used the same 15 early-maturing clones as in Chapter 2. The transplantation time and planting pattern of the materials were the same as in Chapter 2. Of them, two replications were used in this study. The plants received 6.0 g N, 6.9 g P_2O_5 plus 6.0 g K_2O m^{-2} in April after snowmelt in 2004 and 2005, and sampled at 10 cm stubble height with hand sickles on 25 June 2004 and 1 July 2005 when all the plants were at the full heading stage. Weather conditions in Kunneppu in 2004 and 2005 are shown in Table 3-1.

In the experiment for the G × L estimation, the author planted the same clones as in the G × Y estimation on 2 August 2006 in Kunneppu (the same site as above) at a spacing of 0.6 × 0.9 m in a randomized complete block design with two replications, and on 11 May 2006 at the Hokkaido Konsen Agricultural Experiment Station in Nakashibetsu (43°34’N, 144°58’E; currently the Konsen Agricultural Experiment Station, Hokkaido Research Organization) at a spacing of 0.6 × 0.6 m in a randomized complete block design with two replications. The plants were fertilized in April and sampled at the heading stage in 2007 (20 June in Kunneppu and 21 June in Nakashibetsu) in the same way as in the G × Y experiment. Four of the 15 clones were removed because of the errors in transplanting. Some abiotic conditions of the two experimental sites are shown in Table 3-2.

The harvested samples were dried in an oven at 70 °C for 48 h, and were milled and passed through a 0.75-mm screen. Ob/OCW ratio, Ob and WSC contents were analyzed by a NIRS (FOSS NIRSystems Model 6500, USA). For developing the calibration equations, 120 samples that included 17 samples used in the present study were selected by removing redundant samples on the basis of Mahalanobis distance in the first to third principal components by the principal component analysis from a total of 1486 samples of the first crop harvested from 2005 to 2007, and then divided them into 100 samples for calibration and 20 samples for prediction. The analysis me-thods of samples for developing the calibration equations

Table 3-1 Weather conditions from late April to June in Kunneppu in 2004 and 2005 (G × Y evaluation)

		April	May			June		
Attribute	Year	L	E	M	L	E	M	L
Mean air temperature	2004	4.0	6.8	14.5	13.4	15.0	16.6	19.1
(°C)	2005	6.0	4.5	7.3	11.4	14.6	16.9	18.6
Accumulated daylight	2004	42.1	61.3	49.7	48.2	51.2	47.0	12.8
hours (h)	2005	44.4	23.9	63.7	51.7	43.7	61.6	55.9
Accumulated rainfall	2004	15.0	12.0	11.0	49.0	19.5	13.0	37.0
(mm)	2005	36.5	26.0	5.0	11.5	7.0	33.0	8.5

E, early; G × Y, genotype × year interaction; L, late; M, middle

Table 3-2 Abiotic conditions of Kunneppu and Nakashibetsu (G × L evaluation)

	Location	
Attribute	Kunneppu	Nakashibetsu
Geographic coordinates	43°47’N, 143°42’E	43°34’N, 144°58’E
Elevation (m)	196	50
Soil type	Andosol	Andosol
Mean air temperature (°C)[a]	11.8	10.8
Accumulated daylight hours (h) [a]	341.0	367.0
Accumulated rainfall (mm) [a]	195.0	249.0

[a] Values from late April to June in 2007.

G × L, genotype × location interaction

in Ob, OCW and WSC contents and the method for estimation of Ob/OCW ratio were the same as those described in Chapter 2. The equations used for prediction were developed by using the partial least-squares regression. The R^2 values of prediction for Ob, OCW and WSC contents were 0.94, 0.90 and 0.92, respectively, with standard errors of prediction of 1.14, 1.18 and 0.93%. Biases of prediction were –0.53, 0.23 and 0.32, respectively.

Data from the two experiments were separately analyzed by ANOVA to evaluate the effects of genotype, year and their interaction (G × Y experiment) or the effects of genotype, location and their interaction (G × L experiment) on the traits. Genotype, year and location effects were estimated by a random effects model. Variance components were estimated from the linear function of the mean square. The h_B^2 in each experiment was estimated from variance components in ANOVA as in Chapter 2.

Results

Evaluation of G × Y

Although the effects of both genotype and year were significant for all the traits, those of G × Y were always non-significant showing smaller mean square values than the error (Table 3-3). Among the three sources, year produced the largest mean square in each trait. Variance components of G × Y were the smallest and near zero for all the traits. Means of Ob/OCW ratio and Ob content in Kunneppu were almost same across the two years, but those of WSC content differed greatly (Table 3-4). The SD and ranges of the traits showed similar tendencies. Estimates of h_B^2 ranged from 58.7 to 92.2%. The relationships of the traits between the two years showed correlation coefficients of ≥ 0.70 (P < 0.01 or P < 0.001, Figure 3-1).

Evaluation of G × L

The effects of both genotype and location were significant for all the traits, but those of G × L were always non-significant with smaller mean square values than the error (Table 3-5). Mean squares of location were by far the largest for all the traits. Likewise, variance components of location were the largest for all the traits. Variance components of G × L were the smallest and near zero for all the traits. Means of Ob/OCW ratio and Ob content were lower and that of WSC content was higher in Kunneppu than in Nakashibetsu (Table 3-6). The SD and ranges of the traits showed opposite tendencies. Estimates of h_B^2 ranged from 50.3 to 86.8%. The relationships of the traits between the two locations showed correlation coefficients of ≥ 0.62 ($P < 0.05$ or $P < 0.01$, Figure 3-2).

Table 3-3 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 15 timothy clones in Kunneppu in 2004 and 2005 (G × Y evaluation)

		Ob (%DM)		Ob/OCW (%)		WSC (% DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	14	11.67**	2.42	3.71**	0.77	21.35***	5.16
Year	1	51.14***	1.64	23.25***	0.75	617.03***	20.54
G × Y	14	1.20	0.11	0.65	–0.01	0.73	–0.09
Error	30	1.78	1.78	0.67	0.67	0.91	0.91

** and *** indicate significance at the 0.01 and 0.001 levels, respectively.

DM, dry matter; G × Y, genotype × year interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 3-4 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 15 timothy clones in Kunneppu in 2004 and 2005 (G × Y evaluation)

	Ob (%DM)		Ob/OCW (%)		WSC (% DM)	
	2004	2005	2004	2005	2004	2005
Mean	61.9	60.0	86.0	87.3	9.6	16.1
SD	1.61	2.06	1.05	1.04	2.20	2.49
Range	58.2–64.7	56.5–62.9	84.6–88.4	85.5–89.3	6.5–14.0	12.4–20.5
h_B^2 (%)	66.9	77.9	84.3	58.7	92.2	91.1

DM, dry matter; G × Y, genotype × year interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 3-5 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 11 timothy clones in Kunneppu and Nakashibetsu in 2007 (G × L evaluation)

		Ob (%DM)		Ob/OCW (%)		WSC (% DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	10	10.87*	2.05	6.00**	1.29	8.54**	1.84
Location	1	335.34***	15.12	198.26***	8.97	184.50***	8.33
G × L	10	2.67	–0.30	0.83	–0.09	1.19	–0.19
Error	22	3.28	3.28	1.00	1.00	1.58	1.58

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels, respectively.

DM, dry matter; G × L, genotype × location interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

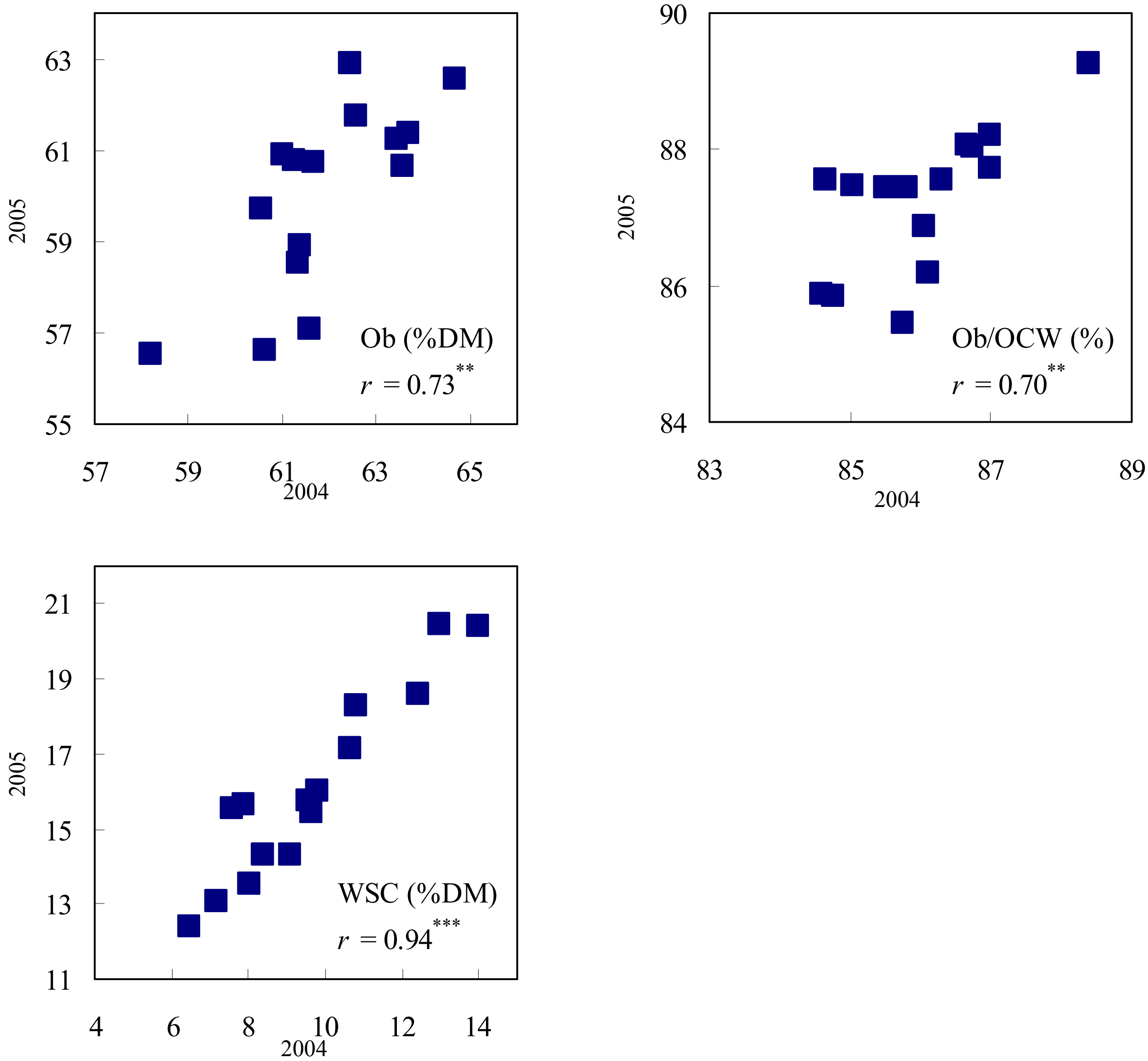

Figure 3-1 Correlations of the nutritive value traits of the first crop of 15 timothy clones between 2004 and 2005 in Kunneppu (G × Y evaluation). ** and *** indicate significance at the 0.01 and 0.001 levels, respectively. DM, dry matter; G × Y, genotype × year interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 3-6 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 11 timothy clones in Kunneppu and Nakashibetsu in 2007 (G × L evaluation)

	Ob (%DM)		Ob/OCW (%)		WSC (% DM)	
	Kunneppu	Nakashibetsu	Kunneppu	Nakashibetsu	Kunneppu	Nakashibetsu
Mean	47.6	53.1	76.3	80.5	10.0	5.9
SD	2.03	1.63	1.55	1.00	1.38	1.72
Range	44.7–50.4	49.7–56.2	74.1–78.4	78.5–81.8	8.2–12.4	3.6–10.0
h_B^2 (%)	74.9	65.0	86.8	50.3	51.8	72.8

DM, dry matter; G × L, genotype × location interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate.

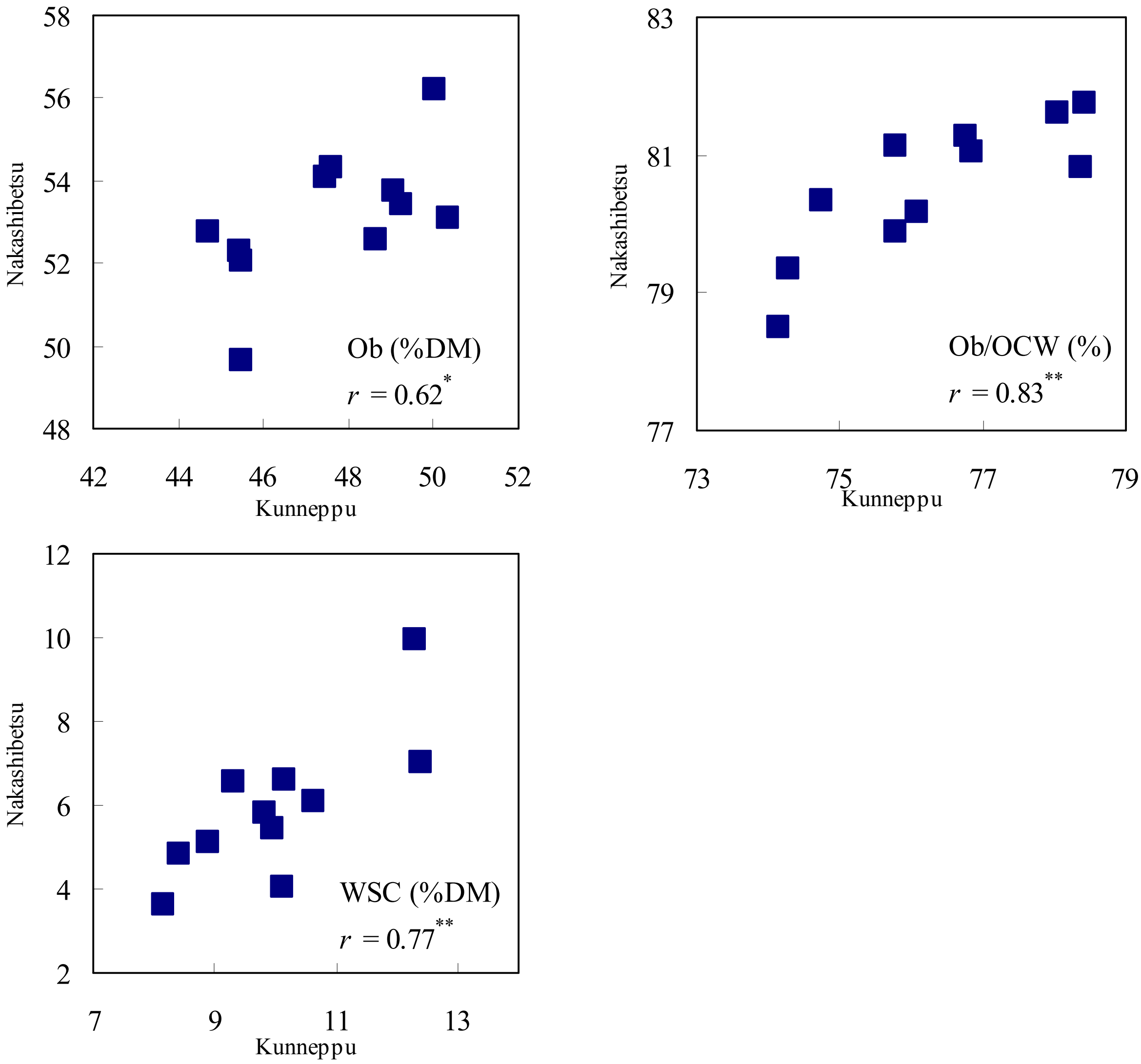

Figure 3-2 Correlations of the nutritive value traits of the first crop of 11 timothy clones between Kunneppu and Nakashibetsu in 2007 (G × L evaluation). * and ** indicate significance at the 0.05 and 0.01 levels, respectively. DM, dry matter; G × L, genotype × location interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Discussion

Timothy is well adapted to cool, moist climates but is not suited to dry or warm conditions (Berg *et al.* 1996). The weather conditions varied between the years in the G × Y experiment (higher temperature and rainfall from mid May to early June in 2004; Table 3-1) and between the locations in the G × L experiment (lower rainfall from late April to June in Kunneppu; Table 3-2). As a result, the means of the three nutritive value traits usually varied between the years and the locations (Tables 3-4 and 3-6). However, the G × E had no significant effect on any of the traits (Tables 3-3 and 3-5) and the correlation coefficients of all the traits were significant across the years (Figure 3-1) and the locations (Figure 3-2), indicating the unimportance of the G × E effect.

These results are similar to those for IVDMD of smooth bromegrass (Carpenter and Casler 1990), IVDMD and WSC content of perennial ryegrass (Humphreys 1989a; Smith *et al.* 2004), WSC content of cocksfoot (Sanada *et al.* 2004) and IVDMD of timothy (Furuya *et al.* 1994; Furuya and Tsutsui 1996), supporting the stability of relative genotype ranking for the nutritive traits in different environments. Although some previous studies reported significant G × E effects on IVDMD (Ames *et al.* 1993; McElroy and Christie 1986a), NDF, ADF and CP contents (Surprenant *et al.* 1993) in timothy, the mean square values of the G × E were smaller than those of genotype or environment, suggesting the G × E to be a minor factor responsible for the total variability (Burner *et al.* 1983). Among the traits, WSC content showed the greatest variation in the mean values between the years and the locations (Tables 3-4 and 3-6). This is considered to be in large part due to the difference in the rainfall amount between the years and the locations, as seen in that cultivars with high-DM content showed a higher WSC content in cocksfoot (Sanada *et al.* 2004). Furthermore, the weather at the harvest differed between the locations; sunny in Kunneppu but rainy in Nakashibetsu. Despite such a difference in weather, the relative genotype ranking of WSC contents remained stable between the locations. This suggests that high-WSC cultivars bred in a dry area can produce good silage even if they are grown in a rainy area because cultivars with high-WSC content enable high-quality silage to be made (Wilkins and Humphreys 2003).

The present results provide information relevant to designing an effective breeding method. Berdahl *et al.* (1994) suggested that selection for IVDMD in a single environment employing recurrent phenotypic selection or other selection methods that utilize additive genetic variance should be effective in intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkw. and D.R. Dewey) because IVDMD has high general combining ability and low G × E effects. With regard to the number of replications in the selection, England (1977) suggested that replicated selection is even less efficient than unreplicated selection when the selection traits have high heritability or receive low G × E effect if increased replication in the field reduces the intensity of selection on account of reduction in the number of materials. These indicate that unreplicated phenotypic selection for the traits of timothy in a single year or location may be effective since the traits have high h_N^2 (Chapter 2) and low G × E effects in the present study. In addition, it is important to accumulate superior alleles within each breeding population by some form of recurrent selection for the improvement of the traits influenced mainly by additive gene effects (Chapter 2). In perennial ryegrass, four generations of combined phenotypic and half-sib family selection within a breeding population over 12 years has been effective in simultaneously improving the DM yield by 10 to 14% and WSC content by 4 to 6% compared with the base population (Wilkins and Humphreys 2003). Therefore, the use of similar assiduous recurrent selection methods based on additive genetic variation should be useful in simultaneously increasing both the nutritive value and forage yield of timothy. Also, it would be important to avoid excessive inbreeding in recurrent selection because recurrent selection necessarily involves a restriction of population size (Casler 2001).

Based on the highly non-significant effects of G × Y and G × L on the three nutritive traits and the significant correlations of the traits between the 2 years and between the two locations, the author concludes that the relative ranking of genotypes in different years and at different locations should be consistent for the traits and that selection for the traits in a single environment are likely to be useful in effective improvement of the nutritive value of timothy.

Chapter 4 Effects of harvest time across maturity stages and within a day on the nutritive value in the first crop of timothy

Introduction

The nutritive value of forage generally changes according to the growth stage. Numerous changes occur as forage plants mature, most notably that the positive measures of forage quality decline, while negative fiber measures increase (Casler and Vogel 1999). In timothy, WSC content increases with the advancement of growth stage after heading (Souma *et al*. 2006). Moreover, the WSC content of timothy is affected by environmental conditions such as solar radiation, and even varies at different sampling times within the day (Souma *et al*. 2006; Masuko *et al*. 2008). These facts need to be considered in a forage-breeding program where simultaneous harvesting of test genotypes at the desirable stage (early heading stage for timothy; Winch *et al.* 1970) for evaluation and selection is difficult or impossible due to a large number of plants and weather limitations. For effective improvement of the nutritive value, it is therefore necessary to determine how the relative ranking of genotypes based on the three nutritive traits (Ob and WSC contents and Ob/OCW ratio) varies with different maturity stages and harvest times within a day. In addition, the study of harvest times within a day requires the examination in different solar radiation cases such as sunny and cloudy weather (Souma *et al.* 2006).

However, no studies have examined G × M or genotype × within-day harvest time interaction in the three nutritive traits of timothy, although McElroy and Christie (1986b) have reported significant G × M in IVDMD from very early heading to full or post anthesis. This prompted us to investigate the three nutritive value traits of the first crop of timothy genotypes at two maturity stages. Measures were also performed on the traits of the genotypes at two harvest times within a day in sunny and cloudy weather conditions. The objectives of the study were to investigate the magnitude of G × M, G × TS and G × TC for the nutritive value of the first crop of timothy.

Materials and methods

The experiment for the G × M estimation used the same 15 early-maturing clones as in Chapter 2. The transplantation time and planting pattern of the materials were the same as in Chapter 2. The plants received 6.0 g N, 6.9 g P_2O_5 plus 6.0 g K_2O m^{-2} in April after snowmelt in 2005, and were sampled at 10 cm stubble height with hand sickles at 13.00–14.00 hours on 17 June 2005 when all the plants reached the early heading stage and on 1 July 2005 when all the plants reached the full heading stage (two replications for each cutting date).

In the experiments for the G × TS and TC estimation, we planted 13 early-maturing clones in 2001 in Kunneppu (the same site as above), Hokkaido, Japan, with a spacing of 0.6 × 0.6 m in a randomized complete block design with four replications. The same selection process was applied as the 15 clones in Chapter 2 and the range of early heading date of the clones in the 2003 evaluation was six days. The plants were fertilized in April and sampled on 23 (sunny day; G × TS experiment) and 27 June 2005 (cloudy day; G × TC experiment) when all the plants reached the heading stage (Table 4-1), in the same way as in the G × M experiment. In each experiment, the author harvested the same plant in two identical parts at 09.00–10.00 hours (morning harvest) and at 16.00–17.00 hours (evening harvest) with two replications.

The harvested samples were dried in an oven at 70 °C for 48 h, then milled and passed through a 0.75-mm screen. The Ob, OCW and WSC contents were analyzed using a NIRS (Foss NIRSystems Model 6500, Laurel, MD, USA). The equations used for prediction were developed using the partial least-squares regression and are described in Chapter 3. The analysis methods of samples for developing the calibration equations in Ob, OCW and WSC contents and the method for estimation of Ob/OCW ratio were the same as those described in Chapter 2.

Data from the three experiments were separately analyzed by ANOVA to evaluate the effects of genotype, maturity stage and their interaction (G × M experiment) or the effects of genotype, within-day time and their interaction (G × TS and TC experiments) on the traits. Genotype, maturity stage, harvest time on sunny day and harvest time on cloudy day effects were estimated by a random effects model. Variance components were estimated from the linear function of the mean square. The h_B^2 was estimated on a phenotypic mean basis averaged replications from the variance components in ANOVA as

Table 4-1 Weather conditions on the sunny (23 June) and cloudy (27 June) days in 2005 (G × TS and G × TC evaluation)

	Air temperature (°C)			Daylight hour
Day	Mean	Max	Min	(h)
Sunny day	24.4	32.8	17.0	6.6
Cloudy day	17.3	20.9	15.3	4.2

G × TC, genotype × harvest time on cloudy day interaction; G × TS, genotype × harvest time on sunny day interaction

in Chapter 2.

Results

Evaluation of G × M

Means of DM weight, plant height and degree of heading at the full heading stage were greater than those at the early heading stage (Table 4-2). Although the effects of both genotype and maturity stage were highly significant for all the traits, those of G × M were always non-significant (Table 4-3). Among the three sources, the maturity stage produced the largest mean square in each trait. Variance components of G × M were the smallest and near zero for all the traits. Means of Ob and WSC contents and Ob/OCW ratio were smaller at the early heading stage than at the full heading stage (Table 4-4). The SD and ranges of the traits showed opposite tendencies. Estimates of h_B^2 ranged from 56.6 to 91.1%. The relationships of the traits between the two stages showed a correlation coefficient of ≥ 0.76 (P < 0.001, Figure 4-1).

Table 4-2 Means of some agronomic traits of the first crop of 15 timothy clones at the early and full heading stage (G × M evaluation)

Stage	DM weight (g)	Plant height (cm)	Degree of heading (1– 9; 9 = full)
Early heading	135.9	82.8	3.8
Full heading	256.3	131.6	9.0

DM, dry matter; G × M, genotype × maturity stage interaction

Table 4-3 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 15 timothy clones at the early and full heading stage (G × M evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (% DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	14	15.80***	3.41	5.95***	1.30	23.69***	5.44
Maturity stage	1	1132.92***	37.69	686.48***	22.86	102.60***	3.36
G × M	14	2.14	0.02	0.74	–0.36	1.93	0.08
Error	30	2.10	2.10	1.46	1.46	1.77	1.77

*** indicates significance at the 0.001 level.

DM, dry matter; G × M, genotype × maturity stage interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 4-4 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 15 timothy clones at the early and full heading stage (G × M evaluation)

	Ob (% DM)		Ob/OCW (%)		WSC (%DM)	
	Early	Full	Early	Full	Early	Full
Mean	51.3	60.0	80.5	87.3	13.4	16.1
SD	2.17	2.06	1.51	1.04	2.57	2.49
Range	46.4–55.0	56.5–62.9	77.5–83.1	85.5–89.3	10.1–18.5	12.4–20.5
h_B^2 (%)	73.6	77.9	56.6	58.7	80.5	91.1

DM, dry matter; G × M, genotype × maturity stage interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

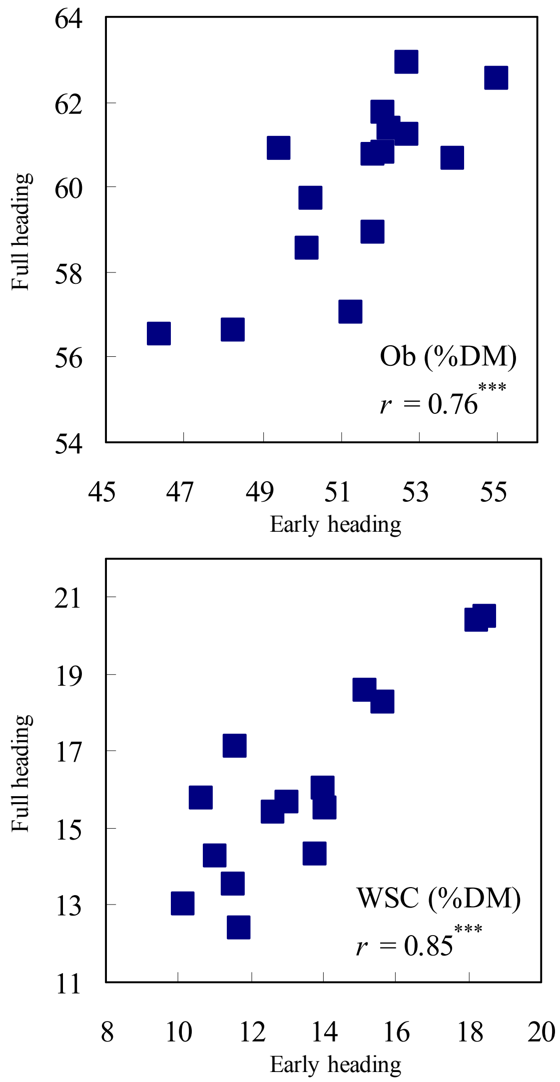

Figure 4-1 Correlations of the nutritive value traits of the first crop of 15 timothy clones between the early and full heading stages (G × M evaluation). *** indicates significance at the 0.001 level. DM, dry matter; G × M, genotype × maturity stage interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Evaluation of G × TS and G × TC

The effects of genotype on all the traits were highly significant, but the harvest time on sunny day only influenced WSC content (Table 4-5). The interactive effects of G × TS on the traits were not significant with smaller mean square values than the error. Variance components for genotype were the largest and those of G × TS were the smallest for all the traits. Means of Ob content and Ob/OCW ratio were almost the same between the two harvest times and the mean of WSC content was greater in the evening than in the morning (Table 4-6). The SD and ranges of Ob content and Ob/OCW ratio also showed the same patterns, but WSC content showed opposite tendencies. Estimates of h_B^2 ranged from 55.3 to 91.6%. The relationships of the traits between the two harvest times showed a correlation coefficient of ≥ 0.84 ($P < 0.001$, Figure 4-2).

On the cloudy day, the effects of genotype and harvest time were significant for all the traits (Table 4-7). Those of G × TC were always non-significant with smaller mean square values than the error. Variance components of G × TC were the smallest for all the traits. Means of Ob content and Ob/OCW ratio were almost the same between the two harvest times and the mean of WSC content was higher in the evening than in the morning (Table 4-8). The SD and ranges of Ob content and Ob/OCW ratio were greater in the morning than in the evening, but those of WSC content were smaller in the morning than in the evening. Estimates of h_B^2 ranged from 71.2 to 91.8%. The relationships of the traits between the two times showed a correlation coefficient of ≥ 0.92 ($P < 0.001$, Figure 4-2).

Table 4-5 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 13 timothy clones in the morning and evening on the sunny day (G × TS evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (%DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	12	10.69***	2.44	11.07***	2.71	11.82***	2.89
Harvest time on sunny day	1	2.28	0.05	0.02	–0.01	30.68***	1.17
G × TS	12	0.91	–0.70	0.24	–0.60	0.24	–0.38
Error	26	2.32	2.32	1.44	1.44	1.00	1.00

*** indicates significance at the 0.001 level.

DM, dry matter; G × TS, genotype × harvest time on sunny day interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 4-6 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 13 timothy clones in the morning and evening on the sunny day (G × TS evaluation)

	Ob (% DM)		Ob/OCW (%)		WSC (% DM)	
	Morning	Evening	Morning	Evening	Morning	Evening
Mean	59.6	59.2	84.3	84.3	9.7	11.2
SD	1.74	1.67	1.67	1.69	1.84	1.62
Range	56.7–62.0	56.9–61.7	81.9–86.9	81.5–86.7	7.4–13.1	9.3–14.1
h_B^2 (%)	78.3	55.3	73.5	90.0	91.6	76.9

DM, dry matter; G × TS, genotype × harvest time on sunny day interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 4-7 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 13 timothy clones in the morning and evening on the cloudy day (G × TC evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (%DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	12	11.01***	2.59	9.30***	2.22	7.22***	1.73
Harvest time on cloudy day	1	9.49**	0.34	3.20*	0.11	47.77***	1.82
G × TC	12	0.64	–0.40	0.43	–0.03	0.32	–0.19
Error	26	1.44	1.44	0.49	0.49	0.70	0.70

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels, respectively.

DM, dry matter; G × TC, genotype × harvest time on cloudy day interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 4-8 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 13 timothy clones in the morning and evening on the cloudy day (G × TC evaluation)

	Ob (% DM)		Ob/OCW (%)		WSC (%DM)	
	Morning	Evening	Morning	Evening	Morning	Evening
Mean	61.6	60.7	87.0	86.5	11.3	13.2
SD	1.92	1.46	1.68	1.43	1.22	1.51
Range	58.4–65.0	58.4–62.6	84.1–89.3	84.0–88.2	10.1–14.4	11.5–16.9
h_B^2 (%)	75.4	71.2	87.3	91.8	72.6	84.7

DM, dry matter; G × TC, genotype × harvest time on cloudy day interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Discussion

The agronomic (Table 4-2) and nutritive value traits (Table 4-4) of the timothy genotypes varied between the early and full heading stages, agreeing with the observation by Belanger *et al.* (2001). However, the G × M had no significant effect on the nutritive value traits (Table 4-3) as illustrated by the significant positive correlations between the maturity stages in all the traits (Figure 4-1). This means that timothy genotypes with high nutritive value should maintain this characteristic during the early to full heading stage and therefore selection for the nutritive traits even at delayed harvest, such as the full heading stage, should improve the nutritive value at the early heading stage as well. Although McElroy and Christie (1986b) reported a significant G × M effect on IVDMD in timothy, the maturity stages covered by their study, i.e. from the very early heading to full or post anthesis, were wider than those in the author's study. This may account for the difference in response related to the G × M effect.

WSC content varied with times of a day (morning and evening; Tables 4-6 and 4-8), agreeing with previous reports (Souma *et al.* 2006; Masuko *et al.* 2008). Nevertheless, G × TS and TC effects had no significance for Ob and WSC contents and Ob/OCW ratio (Tables 4-5 and 4-7), showing significant positive morning-evening correlations in all the traits (Figure 4-2). Hence, highly nutritious genotypes should show consistent performance from morning to evening. WSC contents on the cloudy day were higher than those on the sunny day (Tables 4-6 and 4-8), despite that low solar radiation generally reduces WSC content of a plant. This may be attributed in large part to the fact that the cloudy-day harvest was conducted later than the sunny-day harvest (27 and 23 June 2005, respectively).

The experiment for the G × M estimation used clones with similar maturity. If the test materials are highly variable in maturity, it is difficult to evaluate the nutritive

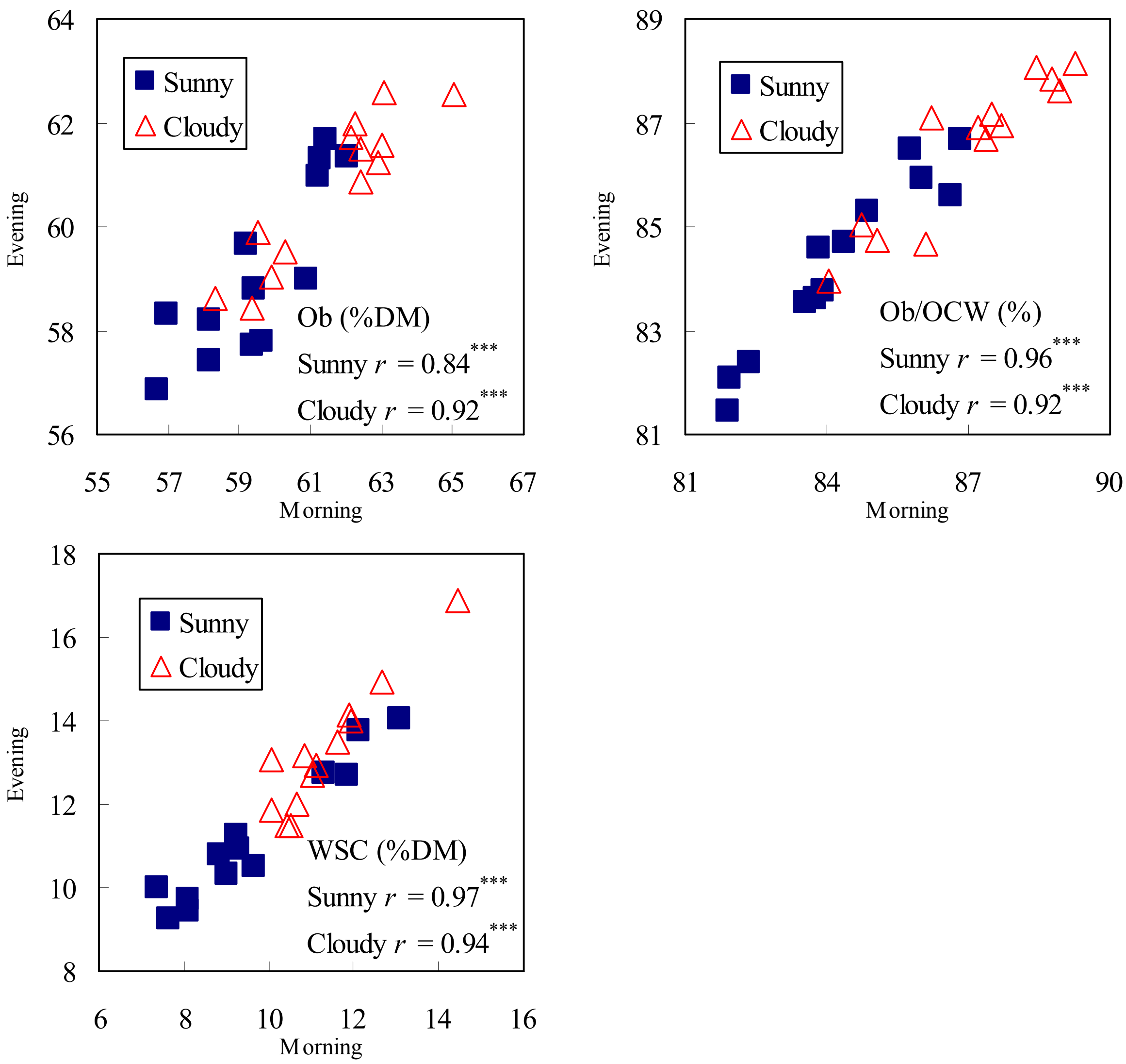

Figure 4-2 Correlations of the nutritive value traits of the first crop of 13 timothy clones between morning and evening on the sunny and cloudy days (G × TS and G × TC evaluation, respectively). *** indicates significance at the 0.001 level. DM, dry matter; G × TC, genotype × harvest time on cloudy day interaction; G × TS, genotype × harvest time on sunny day interaction; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

value without depending on maturity. In such a case, selection only within maturity classes or adjustment of the nutritive data to a constant maturity stage should be effective (Casler and Vogel 1999). The experiments for the G × TS and TC estimation harvested all the plants at about the same time (09.00–10.00 hours and at 16.00–17.00 hours, respectively). In the author's another experiment utilizing the same clones as the G × M experiment in 2004 in Kunneppu, the correlation coefficient between WSC content and harvest time within one hour was non-significant (r = 0.12, P > 0.05, n = 60, unpublished data). This indicates that the harvesting of the test materials within the one-hour time had little effect on the relative ranking of the timothy genotypes. If harvesting takes longer, additional attention may be required for data analysis and interpretation, since WSC content varies during the day as shown previously (Souma *et al.* 2006; Masuko *et al.* 2008) and currently (Tables 4-6 and 4-8).

The present results also provide useful information on designing management systems of timothy cultivation and utilization. It appears that silage of delayed-harvested timothy with higher-WSC content may provide better fermentation quality than harvest at the early heading stage because cultivars with high WSC content are usually considered as high-quality silage (Wilkins and Humphreys 2003). However, delayed-harvest results in increased negative fiber measures of forage (Casler and Vogel 1999), decreased IVDMD of timothy (Belanger *et al.* 2001), reduced voluntary intake of timothy silage and lower live weight gain in lambs (Bernes *et al.* 2008). It is therefore necessary to improve the nutritive value through breeding with consideration given to high digestibility during a delayed harvest and high WSC content even if harvested early. Furthermore, the results suggest that delaying harvest before the end of the day may be desirable to produce better silage because WSC content was higher in the evening than in the morning in both sunny and cloudy conditions (Tables 4-6 and 4-8). Fisher *et al.* (1999) also suggested that mowing hay late in the day increased forage preference in tall fescue. This harvest system, however, requires care if wilting for the production of silage is required. Masuko *et al.* (2008) reported that WSC content prior to ensiling in overnight-wilted grass following harvest in the evening was slightly lower than that in daytime-wilted grass following harvest in the morning. Hence, wilting in the daytime following harvest, not in the morning but in the afternoon, may assist the production of high-quality silage. For more effective management, there may be potential, for breeding purposes, for the selection of genotypes with higher WSC content after nighttime wilting.

On the basis of the no significant effects of G × M, G × TS and TC on any tested traits and the significant correlations of the three nutritive traits between the two maturity stages and between the two times under the two weather conditions, the author concludes that the relative ranking of genotypes in different maturity stages from the early to full heading stage and at different times within a day should be consistent for the nutritive value traits, and that selections for these traits at any stage of heading and any time of a day would permit effective improvement of the nutritive value of timothy only if the plants are harvested at about the same time (within one hour).

Chapter 5 Relationship between the nutritive value and agronomic or morphological traits in the first crop of timothy

Introduction

Negative effects have been observed between lowered lignin contents and agronomic traits such as lodging and survival in some perennial species (Pederson *et al*. 2005). Survivability for two years significantly decreased in the low lignin population versus the high (Buxton and Casler 1993). Winter survival of the cocksfoot and switchgrass (*Panicum virgatum* L.) populations negatively correlated to the cycles of selection for increased IVDMD when evaluated as space transplanted populations (Casler *et al.* 2002). Successful divergent selection for WSC in perennial ryegrass for three generations resulted in higher susceptibility to crown rust infection (caused by *Puccinia coronata* Corda) for families with high WSC (Breese and Davies 1970). In contrast, divergent selection for stalk-crushing strength in two maize (*Zea mays* L.) populations resulted in no changes to the lignin or fiber contents of the stalks (Undersander *et al*. 1977). Similarly, correlation between Ob content and lodging in Italian ryegrass was weak (Fukazawa and Yahagi 2008). The evaluation of relationships between environmental stress tolerance, such as winter survival and lodging resistance, and nutritive traits is therefore an important consideration in increased nutritive selection strategies. Undesirable relationships with some agronomic traits require special attention for developing timothy utility cultivars with high nutritive value.

In individual selection of initial screening in breeding, selection for desirable genotypes from a large number of plants is needed. However, evaluation of the nutritive traits of all individuals is time-consuming and costly to harvest the plants and prepare grinding samples. Time and labor for breeding could be saved in initial screenings if breeders had better information on agronomic or morphological traits with forage nutritive value (Lentz and Buxton 1991). In cocksfoot, selections for wide blades and late maturity were effective methods for initial screening in IVDMD selection (Lentz and Buxton 1991). The results of multiple regression analysis suggested that the thickness of stems and resistance to brown stripe (caused by *Cercosporidium graminis* (Fuckel) Deighton), which could be evaluated easily, were important indices for high WSC selection in cocksfoot (Sanada *et al*. 2004). In timothy, weak correlations between IVDMD and agronomic or morphological traits had the consequence that initial screenings for IVDMD based on agronomic or morphological traits would be difficult (Furuya 1987).

However, no studies have examined the relationships between the three nutritive traits (Ob and WSC contents and Ob/OCW ratio) and agronomic or morphological traits except for the report on DM yield in Chapter 2. The purposes of the present research were: to investigate the relationship between the nutritive value and agronomic traits; and to explore agronomic or morphological traits that offer indirect measures on the nutritive traits in timothy.

Materials and methods

Thirty medium-maturing clones, which had not been selected for the nutritive value traits, were used in this experiment. They were planted on 2 August 2006 at the Hokkaido Kitami Agricultural Experiment Station in Kunneppu (43°47’N, 143°42’E; currently the Kitami Agricultural Experiment Station, Hokkaido Research Organization), Hokkaido, Japan, at a spacing of 0.6 × 0.9 m in a randomized complete block design with two replications. In each of the following two years, they received 6.0 g N, 6.9 g P_2O_5 plus 6.0 g K_2O m^{-2} after snowmelt in April. They were sampled at 10 cm stubble height with hand sickles on 28 June 2007, and on 4 July 2008. The plants of the first crop reached the heading stage. The DM content and plant height were measured at harvest. The agronomic and morphological traits were scored on a scale of 1-9 as follows: winter survival (1, very poor; 9, very good), spring vigor (1, very poor; 9, very vigorous), degree of lodging (1, non or slight; 9, severe), density of stems (1, very few; 9, very many), degree of purple spot caused by *Cladosporium phlei* (Gregory) de Vries (1, healthy; 9, severe), vigor of the first crop (1, very poor; 9, very vigorous), degree of heading (1, none; 9, very many), plant type (1, erect; 9, prostrate), thickness of stems (1, thin; 9, thick), flexibility of stems (1, soft; 9, hard), leaf color (1, light; 9, dark) and leafiness (1, very few; 9, very many). The date of heading was scored as days after 1

June.

The harvested samples were dried in an oven at 70 °C for 48 h, then milled and passed through a 0.75-mm screen. The Ob, OCW and WSC contents were analyzed using a NIRS (Foss NIRSystems Model 6500, Laurel, MD, USA). The equations used for prediction were developed using the partial least-squares regression and are described in Chapter 3. The analysis methods of samples for developing the calibration equations in Ob, OCW and WSC contents and the method for estimation of Ob/OCW ratio were the same as those described in Chapter 2.

The h_B^2 was estimated on a phenotypic mean basis of averaged replications from the variance components in ANOVA as in Chapter 2. The r_G and r_E coefficients between the three nutritive traits and agronomic or morphological traits were calculated as in Chapter 2. Stepwise multiple regression analyses with Ob or WSC content or Ob/OCW ratio as the dependent variables were carried out using agronomic and morphological traits as independent variables. The winter survival and spring vigor measured in spring were removed from stepwise multiple regression analyses because the two traits were not measured at harvesting time in the first crop. Means for the two years were used as data for each clone in all measured traits.

Results

Significant correlations were found between Ob content and winter survival, between Ob content and density of stems, and between Ob content and leaf color (Table 5-1). Significant correlations were also detected between WSC content and winter survival, and between WSC and DM contents. The r_G between their traits were medium. The r_G between the other traits were weak. Estimates of h_B^2 of all measured traits ranged from 64.2 to 94.2% (Table 5-2). As a result of stepwise multiple regression analysis for Ob content, density of stems, degree of purple spot, DM content, degree of heading and flexibility of stems were selected as independent variables, and their contribution ratio was 48% (Table 5-3). From stepwise multiple regression analysis of Ob/OCW ratio, date of heading, DM content, thickness of stems and density of stems were selected as independent variables, and their contribution ratio was 30% (Table 5-4). DM content, leafiness, plant height, leaf color, degree of heading, and degree of purple spot were selected as independent variables from stepwise multiple regression analysis for WSC content and their contribution ratio was 49% (Table 5-5).

Table 5-1 Phenotypic (r_P), genetic (r_G) and environmental (r_E) correlation coefficients between the nutritive value and agronomic or morphological traits in spring and the first crop of 30 timothy clones based on averages for 2007 and 2008

		Winter survival	Spring vigor	Date of heading	Degree of lodging	Density of stems	Degree of purple spot	Vigor of the first crop	Plant height (cm)	Degree of heading	Plant type	Thickness of stems	Flexibility of stems	Leaf color	Leafiness	DM content (%)
Ob	r_P	0.38*	0.30	–0.19	0.19	0.43*	0.14	0.26	0.19	0.34	0.18	0.02	–0.15	–0.41*	0.31	–0.02
(%DM)	r_G	0.45	0.35	–0.03	0.22	0.50	0.17	0.14	0.08	0.29	0.18	0.02	–0.11	–0.47	0.42	–0.05
	r_E	0.18	0.15	–0.51	0.13	0.25	0.06	0.53	0.65	0.45	0.19	0.02	–0.38	–0.20	–0.09	0.10
Ob/OC-	r_P	0.20	0.00	–0.21	0.12	0.21	0.10	0.02	–0.06	0.14	0.11	–0.32	–0.20	–0.31	0.18	0.24
W (%)	r_G	0.21	–0.02	–0.20	0.12	0.23	0.09	–0.05	–0.12	0.11	0.07	–0.35	–0.19	–0.31	0.19	0.25
	r_E	0.16	0.11	–0.36	0.17	0.15	0.19	0.38	0.51	0.34	0.37	–0.20	–0.26	–0.28	0.04	0.09
WSC	r_P	–0.37*	–0.28	0.05	–0.03	–0.28	–0.09	–0.03	–0.05	0.04	–0.04	–0.32	–0.04	–0.03	–0.28	0.48*
(%DM)	r_G	–0.45	–0.32	0.10	–0.04	–0.30	–0.14	–0.04	–0.05	0.09	–0.04	–0.44	–0.07	0.02	–0.31	0.50
	r_E	–0.03	–0.03	–0.09	–0.01	–0.20	0.16	–0.01	–0.06	–0.12	–0.08	0.10	0.12	–0.53	–0.10	0.42

* indicates significance at the 0.05 level.

DM, dry matter; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 5-2 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value, agronomic and morphological traits in spring and the first crop of 30 timothy clones based on averages for 2007 and 2008

	Ob (%DM)	Ob/OCW (%)	WSC (%DM)	Winter survival	Spring vigor	Date of heading	Degree of lodging	Density of stems	Degree of purple spot
Mean	55.1	81.8	9.4	5.3	6.1	25.0	1.6	6.5	3.2
SD	1.62	1.80	1.43	0.84	0.77	1.11	0.83	0.59	0.95
Range	51.7–57.7	77.7–85.2	6.5–12.8	3.3–7.5	4.3–7.3	23.0–26.5	1.0–3.8	5.3–7.5	1.8–6.0
h_B^2	69.1	91.6	83.2	77.3	84.4	64.2	73.8	76.3	89.0
	Vigor of the first crop	**Plant height (cm)**	**Degree of heading**	**Plant type**	**Thickness of stems**	**Flexibility of stems**	**Leaf color**	**Leafiness**	**DM content (%)**
Mean	6.3	117.5	7.2	4.7	6.0	6.4	6.2	5.4	22.7
SD	0.76	11.53	0.74	1.03	0.87	1.03	0.97	0.83	1.04
Range	4.8–7.5	89.3–137.0	5.3–8.0	3.0–6.8	4.0–8.0	4.0–7.8	3.8–8.3	3.8–7.5	20.5–24.9
h_B^2	71.7	87.7	70.8	85.4	73.9	92.1	94.2	87.0	88.9

DM, dry matter; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 5-3 Result of multiple regression analysis with Ob content as dependent variable using agronomic and morphological traits as independent variables in the first crop of 30 timothy clones based on averages for 2007 and 2008

Characteristics selected as independent variables	Partial regression coefficient	Standard regression coefficient	F-value
Density of stems	2.28	0.84	14.23
Degree of purple spot	0.82	0.48	8.50
DM content (%)	0.57	0.37	4.39
Degree of heading	0.77	0.35	4.03
Flexibility of stems	0.62	0.39	3.90
$R = 0.69$, $R^2 = 0.48$, $F = 4.42$, $P < 0.01$			

DM, dry matter; Ob; low-digestible fiber

Table 5-4 Result of multiple regression analysis with Ob/OCW ratio as dependent variable using agronomic and morphological traits as independent variables in the first crop of 30 timothy clones based on averages for 2007 and 2008

Characteristics selected as independent variables	Partial regression coefficient	Standard regression coefficient	*F*-value
Date of heading	–0.51	–0.31	3.22
DM content (%)	0.56	0.33	3.02
Thickness of stems	–0.60	–0.29	2.53
Density of stems	0.91	0.30	2.45
$R = 0.55$, $R^2 = 0.30$, $F = 2.66$, $P = 0.056$			

DM, dry matter; Ob, low-digestible fiber; OCW, organic cell wall

Table 5-5 Result of multiple regression analysis with WSC content as dependent variable using agronomic and morphological traits as independent variables in the first crop of 30 timothy clones based on averages for 2007 and 2008

Characteristics selected as independent variables	Partial regression coefficient	Standard regression coefficient	*F*-value
DM content (%)	0.81	0.59	14.11
Leafiness	–0.86	–0.50	7.18
Plant height (cm)	–0.05	–0.40	3.90
Leaf color	–0.50	–0.34	3.35
Degree of heading	0.66	0.35	3.18
Degree of purple spot	–0.40	–0.27	2.61
$R = 0.70$, $R^2 = 0.49$, $F = 3.72$, $P < 0.01$			

DM, dry matter; WSC; water-soluble carbohydrate

Discussion

Undesirable r_G between Ob content and winter survival, and between WSC content and winter survival were detected (Table 5-1). This indicates that attention should be paid to selection for these traits although the strengths of r_G were medium. Because winter survival and lignin synthesis are both affected by an array of genes, it is likely that both pleiotropy and linkage are involved in the observed negative relationships between winter survival and lignin content (Casler *et al*. 2002). Casler *et al*. (2002), however, suggested that selection for winter survival within high digestibility or low lignin germplasm can be successful based on the results of experiments on switchgrass. Selections and crosses among desirable genotypes for these traits would be necessary for nutritive breeding also in timothy. The r_G between the three nutritive traits and degree of lodging were weak, suggesting that increased nutritive value through selections of the traits should not cause higher lodging potential. The

r_G between the three nutritive traits and yield related traits (vigor of the first crop and plant height) were weak, agreeing with the observation in Chapter 2. Although this study was conducted using unselected materials for the nutritive traits, materials selected for the nutritive traits with little regard for agronomic traits may result in undesirable r_G between these traits. Recurrent selection strategies for the nutritive traits should include the evaluation of these agronomic traits for plants to ensure that environmental stress tolerances are retained throughout the breeding history. Performing the second screening for the nutritive traits after the first screening for important agronomic traits is likely to be desirable for effective selection when large numbers of individuals are evaluated.

Significant multiple regression equations were found for Ob and WSC contents with about 50 % contribution ratios (Tables 5-3 and 5-5), but use of these equations may be difficult for reliable selection because the nutritive traits estimated from these agronomic and morphological traits contain errors and their contribution ratios were less than half. However, the results presented here provide information on timothy breeding plans. Although the r_G between Ob content and degree of purple spot and between WSC content and degree of purple spot were weak (Table 5-1), desirable relationships for the direction of improvement were detected in multiple regression equations (Tables 5-3 and 5-5). Correlation between the degree of purple spot under inoculation test in a greenhouse and IVDMD was strong desirable for the direction of improving the two traits (Furuya 1987). Since plant disease that decreases the forage nutritive value affects not only the preference of livestock but also yield, disease resistance is one of the most important objectives in grass breeding (Sanada *et al*. 2004). The present results from both analyses reveal that DM content is intimately related to WSC content. WSC in the vacuole of the plant cell is a larger component as the water content of the plant decreases (Sugawara 1983). Selection for DM content may be available for initial screening in timothy WSC content. Evaluating large numbers of materials for DM content would be, however, difficult because it requires a huge amount of labor and destructive sampling. Meanwhile, these multiple regression equations clearly demonstrate the different effects on maturity related traits (date of heading, degree of heading and DM content) among the traits. The results indicate that later-maturing genotypes for Ob content and Ob/OCW ratio, and earlier-maturing genotypes for WSC content can have better quality, suggesting that selection for all the targeted traits should pay special attention to maturity.

These results suggest that selections and crosses among desirable genotypes for Ob and WSC contents and winter survival would be needed for the simultaneous improvement among these traits; and that the application of indirect selection for the three nutritive traits through the agronomic and morphological traits may be difficult for reliable selection.

Chapter 6 Evaluating the genotype × nitrogen fertilization interaction on the nutritive value of the first crop in timothy clones

Introduction

N plays a significant role in forage crop production, since N fertilizer accelerates the growth and yield of forage grasses in general. It can also affect forage nutritive value, but there are conflicting reports on the N effect on timothy quality. Tremblay *et al*. (2005) and Okamoto *et al*. (2011) reported decreased WSC content and silage quality at higher N rates. In contrast, Pelletier *et al*. (2009) reported that increasing N fertilization rates did not decrease timothy carbohydrate content. Conflicts also exist as for the extent of the change of timothy genotype ranking under varying N application levels. Furuya and Tsutsui (1995) obtained a significant G × N in IVDMD of stem and foliage for cultivars with different maturity levels, while Belanger *et al*. (2004) reported both significant and nonsignificant G × N effects on NDF content. The magnitude of G × N in timothy quality is critical to both improvement and use of this grass. If the effect of G × N is not negligible, the improvement of timothy nutritive traits requires the evaluation of materials under multiple N application levels. Farmers have difficulty in using cultivars which have stable performance under varying situations of N application levels. No studies, however, have investigated the G × N effect on the three nutritive traits (Ob and WSC contents and Ob/OCW ratio) in timothy, highlighting the necessity of studying how the relative genotype ranking of the traits changes with N rates.

The objective of this study was thus to investigate the magnitude of G × N effect on the three nutritive value traits in the first crop of timothy. The author also analyzed the WSC content of mono- and disaccharides (fructose, glucose and sucrose) and fructan (degree of polymerization (DP) ≥ 3) separately with the aim of examining in detail how the sugar components respond to varying N application levels.

Materials and methods

Sixteen medium-maturing clones, which had been selected for various agronomic traits, in particular, lodging resistance and competitive ability after the first cut, were used in the experiment. They were planted on 9 May 2008 at the Hokkaido Kitami Agricultural Experiment Station in Kunneppu (43°47’N, 143°42’E; currently the Kitami Agricultural Experiment Station, Hokkaido Research Organization), Hokkaido, Japan, at a spacing of 0.6 × 0.6 m in a randomized complete block design with two replications per N treatment. Three rates of N fertilizer, 3, 7 and 11 g N m^{-2} (LN, SN and HN, respectively), were applied in April after snowmelt in 2009, with a common dose of 8 g P_2O_5 and 7 g K_2O m^{-2}. The N level of the SN treatment and the common P and K levels followed the standard levels adopted in spaced plant tests in the author’s breeding team. The N rates for LN and HN were set so that they almost cover the N application levels in timothy cultivation in Hokkaido with equal deviations (± 4 g N m^{-2}) from SN.

Plants at the heading stage were sampled at a 10 cm stubble height with hand sickles on 30 June 2009. The samples were dried in an oven at 70 °C for 48 h, then milled and passed through a 0.75-mm screen. The contents of Ob, OCW, WSC, mono- and disaccharides and fructan were analyzed using a NIRS (Foss NIRSystems Model 6500, Laurel, MD, USA). The equations used for prediction of Ob and OCW contents were developed using the partial least-squares regression and are described in Chapter 3. The analysis methods of samples for developing the calibration equations in Ob and OCW contents and the method for estimation of Ob/OCW ratio were the same as those described in Chapter 2. The same samples as the above two traits were used for developing the calibration equations for WSC, mono- and disaccharide and fructan contents as measured using high performance liquid chromatography (HPLC). The WSC content of the samples was separately analyzed for mono- and disaccharides and fructan, and was expressed as the total content of the two. Total WSC of the samples were extracted from a 0.25-g ground sample by boiling de-ionized water containing 1 mg ml^{-1} of propylene glycol as the internal standard for 1 h. The extract was passed through a 0.45-μm pore filter and was analyzed using HPLC. Mono- and disaccharides and fructan in the extract were separated using gel permeation HPLC columns (Shodex KS-802 and KS-803 combined, Showa Denko, Tokyo, Japan) with a flow rate of 0.8 ml min^{-1} of HPLC grade water at 50 °C, and were detected using a refractive index detector (L-2490, Hitachi, Tokyo, Japan). The equations used for prediction of their contents were developed using the partial least-squares regression. The R^2 values of prediction for total WSC, mono- and disaccharide and fructan contents were 0.96, 0.94 and 0.91, respectively, with standard errors of prediction of 0.63, 0.52 and 0.43%. Biases of prediction were 0.07, –0.11 and –0.11, respectively.

Data from the experiment were analyzed by ANOVA to evaluate the effects of genotype, N fertilization and their interaction (G × N experiment). Genotype and N fertilization effects were estimated using a random effects model and a fixed effects model, respectively. Variance components were estimated from the linear function of the mean square. The h_B^2 was estimated on a phenotypic mean basis averaged replications from the variance components in ANOVA as in Chapter 2.

Results

The interactive effects of G × N were non-significant with smaller mean square values than the errors for all of the traits (Table 6-1). By contrast, the effects of genotype were significant for all of the traits. The effects of N fertilization were significant for Ob and fructan contents and Ob/OCW ratio. Variance components of G × N were the smallest for all of the traits. Variance components of N fertilization in WSC, mono- and disaccharide and fructan contents were near zero. The relationships among N fertilization rates for the five traits showed significant correlations except for mono- and disaccharide content between SN and HN (Table 6-2). Correlations between LN and HN showed weaker trends than those between SN and LN or between SN and HN in Ob and fructan contents and Ob/OCW ratio. The means of Ob content and Ob/OCW ratio decreased with increasing N application levels (Table 6-3). Increasing N application levels, however, did not lead to a large decrease in the means of WSC, mono- and disaccharide or fructan content. The SD and ranges of all of the traits were generally not affected by N fertilization rates. Estimates of h_B^2 ranged from 50.0 to 74.0% for Ob content, 76.9 to 83.4% for Ob/OCW ratio, 84.2 to 86.6% for WSC content, 42.5 to 65.7% for mono- and disaccharide content and 67.2 to 91.8% for fructan content.

Table 6-1 Mean squares and variance components estimated from analysis of variance for the nutritive value traits of the first crop of 16 timothy clones at three N fertilization levels (G × N evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (% DM)		Mono- and disaccharides (%DM)		Fructan (%DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	15	25.21***	3.64	17.62***	2.73	7.91***	1.20	1.97***	0.25	1.94***	0.29
N fertilization	2	88.34***	2.65	61.60***	1.89	1.48	0.02	0.28	–0.01	0.83*	0.02
G × N	30	3.39	–0.37	1.22	–0.07	0.73	–0.07	0.47	–0.04	0.18	–0.03
Error	48	4.12	4.12	1.35	1.35	0.88	0.88	0.56	0.56	0.23	0.23

* and *** indicate significance at the 0.05 and 0.001 levels, respectively.

DM, dry matter; G × N, genotype × nitrogen fertilization interaction; N, nitrogen; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Discussion

The non-significant G × N effects (Table 6-1) and significant correlations among the N rates (Table 6-2) in four of the five traits suggest the stability of the relative ranking of genotypes in the traits across different N fertilizer application levels. The timothy cultivars with improved nutritive traits should therefore provide a stable performance in various N fertilizer application levels. Meanwhile, the correlations between LN and HN were not as strong as those between SN and LN or between SN and HN in three of the five traits (Table 6-2). This suggests that timothy cultivar selection is most effective at the standard N rate, because the selection results at this level are likely to apply to a range of N levels (from LN to HN) used commonly for cultivation of the grass in Hokkaido.

Content of mono- and disaccharides provides an informative index of silage fermentation, since they are degraded more rapidly than fructan during the primary stage of ensilaging (Merry *et al.* 1995). This study, however, showed a non-significant correlation between SN and HN for mono- and disaccharide content (Table 6-2), indicating its instability as a selection index. Fructan is a polymer of fructose and is stored as an energy reserve in many cool-season grasses (Pollock and Cairns 1991), but the SD and ranges of fructan content were small (Table 6-3). It demonstrates that its application as a selection index would be difficult for evaluating the genetic variation. Hence, selection for WSC content, which is the sum of all these, should provide stable improvement of the

Table 6-2 Correlation coefficients among three N fertilization levels of the nutritive value traits of the first crop of 16 timothy clones (G × N evaluation)

	Ob (%DM)		Ob/OCW (%)		WSC (% DM)		Mono- and disaccharides (%DM)		Fructan (%DM)	
	SN	HN	SN	HN	SN	HN	SN	HN	SN	HN
LN	0.66**	0.58*	0.81***	0.77***	0.86***	0.77***	0.69**	0.61*	0.84***	0.80***
SN	–	0.84***		0.89***	–	0.74***	–	0.22	–	0.93***

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels, respectively.

LN, SN and HN correspond to nitrogen fertilization of 3, 7 and 11 g m^{-2}, respectively.

DM, dry matter; G × N, genotype × nitrogen fertilization interaction; N, nitrogen; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 6-3 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first crop of 16 timothy clones at three N fertilization levels (G × N evaluation)

	Ob (%DM)			Ob/OCW (%)			WSC (% DM)			Mono- and disaccharides (%DM)			Fructan (%DM)		
	LN	SN	HN	LN	SN	HN	LN	SN	HN	LN	SN	HN	LN	SN	HN
Mean	56.7	55.3	53.4	84.7	82.9	81.9	7.1	6.7	6.9	6.6	6.6	6.8	0.5	0.3	0.2
SD	2.43	2.46	2.01	1.91	1.75	1.82	1.49	1.02	1.20	0.80	0.59	0.68	0.81	0.48	0.51
Max	59.4	59.5	56.7	88.7	87.1	87.7	10.9	9.1	9.9	7.8	7.8	8.0	2.7	1.7	2.0
Min	50.1	49.8	48.9	81.8	80.8	80.0	4.7	4.9	5.2	5.3	5.6	5.7	0.0	0.0	0.0
h_B^2 (%)	74.0	73.6	50.0	83.4	81.4	76.9	84.7	86.6	84.2	42.5	44.8	65.7	67.2	75.1	91.8

LN, SN and HN correspond to nitrogen fertilization of 3, 7 and 11 g m^{-2}, respectively.

DM, dry matter; G × N, genotype × nitrogen fertilization interaction; N, nitrogen; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate fermentation quality of silage at different N fertilizer application levels.

Increasing N fertilization rates did not largely decrease the WSC, mono- and disaccharide or fructan content of timothy genotypes (Table 6-3), which agreed with the report by Pelletier *et al*. (2009) but disagreed with the reports by Tremblay *et al*. (2005) and Okamoto *et al*. (2011). This conflict seems to come from the N rates used in the studies. The N rates used by Tremblay *et al*. (2005) and Okamoto *et al*. (2011) covered higher ranges (from no application to 18 g N m^{-2} and from 10.7 to 16 g N m^{-2}, respectively) than those used in the present study and in Pelletier *et al*. (2009) (3–11 g N m^{-2} in both studies). The effect of N fertilization on the three traits of non-structural carbohydrate may thus be minor if the N application levels are not extremely different from the standard.

The present study also provides information on phenotypic selection for the nutritive traits in timothy. Both Ob content and Ob/OCW ratio decreased with increasing N fertilization (Table 6-3), agreeing with Furuya and Tsutsui (1995) who reported increased IVDMD of foliage with increasing N levels. It implies that attention should be paid to differences in soil fertility in field tests designed to select materials according to their nutritive traits. Non-uniformity in soil fertility levels may lead to inaccurate selection for these traits. In such cases, replicated selection would be necessary to obtain reliable improvement in these traits. England (1977), however, suggested that replicated selection is less efficient than unreplicated selection if increased replication in the field reduces the intensity of selection on account of a reduction in the number of testable materials. Alternatively, employing grid selection to reduce environmental variation and its negative effects on the progress of selection (Gardner 1961; Bos 1983) should also be effective for evaluating the traits in individual plant tests. Burton (1982) reported that the forage yield of Pensacola bahiagrass (*Paspalum notatum* var. *saurae* Parodi) was successfully improved by recurrent restricted phenotypic selection using grid selection. Casler (1992) also indicated the usefulness of grid selection for NDF content in smooth bromegrass. The application of grid selection may increase the efficiency of unreplicated phenotypic selection for the nutritive traits also in timothy if the application of replicated selection is restricted in terms of labor or area in field tests.

In conclusion, the relative ranking of timothy genotypes at different N application levels is almost consistent for the desirable traits of Ob and WSC contents and Ob/OCW ratio; and selections for these traits at the standard N level are potentially useful for effective improvement of the nutritive value of timothy.

Chapter 7 Relationship between the first and second crops and estimation of genetic parameters of the second crop on the nutritive value of timothy

Introduction

The harvesting of a second crop is important for the effective utilization of timothy meadows, since it accounts for about 40% of total annual yield. However, it has generally received poorer grades for reasons of lower preference and lower milk productivity (Suzuki 2009), and also has lower digestibility than the first crop because it is summer aftermath (Ishiguri 1991). The improvement of the nutritive value of the second crop is therefore an important goal in timothy breeding. Timothy breeders should bear in mind for selection of the second crop that timothy genotypes show large variations in yield and regrowth of the second crop. Some strains have internodes that are as markedly elongated as in the first crop, while others do not, even though their growth and maturity are similar to those in the first crop. This variation is assumed to be due to the degree of winter preparation in addition to reproductive ability, since timothy is an obligatory long-day plant with no cold/short-day requirement (Berg *et al*. 1996). This may complicate investigation of the relationship between the first and second crops on the nutritive value of timothy.

Studies of the three nutritive traits (Ob and WSC contents and Ob/OCW ratio) in Chapters 2 to 6 focused on first-crop timothy. The study in Chapter 2 indicated the high h_N^2 using the parent-offspring regression method in the three traits of the first crop. The study in Chapter 3 also suggested that the relative ranking of genotypes for these traits should be consistent in different years and at different locations (Kunneppu and Nakashibetsu). However, no similar studies have been made for the second crop. It would be highly efficient if selection of the traits of the first crop could improve the traits of the second one. In addition, estimation of the genetic parameters (e.g., heritability and genotype × environment interaction) of the traits of the second crop is needed for effective improvement.

The objectives of this study were to investigate the magnitude of G × C between the first and second crops, and the extent of G × Y and h_N^2 of the second crop for effective improvement of the nutritive value traits in the second crop of timothy. The relationships between the first and second crops were in some cases analyzed through grouping the materials in terms of the ratio of internode elongation stems (IES) of the second crop, since forage quality is affected by the presence of reproductive stems (Saiga 1981b).

Materials and methods

All experiments were carried out at the Hokkaido Kitami Agricultural Experiment Station in Kunneppu (43˚47’N, 143˚42’E; currently the Kitami Agricultural Experiment Station, Hokkaido Research Organization), Hokkaido, Japan.

Evaluations of G × C and G × Y

Twenty-six medium-maturing clones of 30 clones evaluated in Chapter 5 were used in the experiments for the G × C and G × Y estimations. Four of 30 clones used in Chapter 5 were not evaluated the nutritive traits of the second crop due to laborsaving. The transplantation time and planting pattern of the materials were the same as in Chapter 5. In each of the following two years, they received 6.0 g N, 6.9 g P_2O_5 plus 6.0 g K_2O m^{-2} after snowmelt in April and 6.0 g N plus 6.0 g K_2O m^{-2} after harvesting the first crop. They were sampled at 10 cm stubble height with hand sickles on 28 June and 4 September 2007, and on 4 July and 11 September 2008. The plants of the first crop reached the heading stage. Weather conditions in Kunneppu in 2007 and 2008 are shown in Table 7-1. The plant vigor of the second crop was scored on a scale of 1-9 (1, very poor; 9, very vigorous). The IES ratio of the second crop was also scored on a scale of 1-9 (1, very low; 9, very high). In addition to the analysis of all 26 clones, the 12 clones whose average scores for the elapsed time of two years were 5 (meaning half of all stems were elongated) or more, and the 14 clones scoring less than 5 were separately analyzed as high and low IES ratio groups in the experiment for the G × C estimation.

The harvested samples were dried in an oven at 70 ˚C for 48 h, then milled and passed through a 0.75-mm screen. The Ob, OCW and WSC contents were analyzed using a NIRS (Foss NIRSystems Model 6500, Laurel, MD, USA). The equations used for prediction in the first crops are

Table 7-1 Weather conditions from late April to harvest date in September in Kunneppu in 2007 and 2008 (G × C and G × Y evaluations)

		April	May			June			July			August			September
Attribute	Year	L	E	M	L	E	M	L	E	M	L	E	M	L	Harvest[a]
Mean air temperature (°C)	2007	6.4	9.8	7.9	9.3	15.9	17.3	15.8	15.4	14.4	19.9	21.5	21.3	19.9	18.3
	2008	9.0	10.4	8.8	9.9	15.3	14.1	12.1	21.1	16.9	16.1	20.1	18.0	16.1	19.9
Accumulated daylight hours (h)	2007	69.6	47.3	23.7	48.3	76.5	54.1	21.5	58.5	61.0	70.8	23.3	51.9	70.8	26.0
	2008	42.0	39.3	55.9	31.3	60.2	35.8	46.7	63.6	2.8	13.0	55.6	40.0	13.0	76.6
Accumulated rainfall (mm)	2007	6.0	41.0	34.0	25.0	5.0	29.0	55.0	8.0	2.0	1.0	59.0	11.0	1.0	4.0
	2008	10.5	10.0	44.5	23.5	18.5	17.0	2.0	52.0	48.0	33.5	26.5	36.5	33.5	94.0

[a] 1st to 4th in 2007 and 1st to 11th in 2008.

E, early; G × C, genotype × crop interaction; G × Y, genotype × year interaction; L, late; M, middle

described in Chapter 3. For developing the calibration equations in the second crop, 128 samples that included the 42 samples used in the present study were selected by approach similar to Chapter 3 from a total of 985 samples of the second crop harvested from 2005 to 2008, and then divided them into 108 samples for calibration and 20 samples for prediction. The analysis methods of samples for developing the calibration equations in Ob, OCW and WSC contents and the method for estimation of Ob/OCW ratio were the same as those described in Chapter 2. The equations used for prediction were developed using the partial least-squares regression. The R^2 values of prediction for Ob, OCW and WSC contents were 0.94, 0.98 and 0.96, respectively, with standard errors of prediction of 1.24, 0.69 and 0.60%. Prediction biases were 0.38, 0.71 and -0.09, respectively.

Data from the two experiments were separately analyzed by ANOVA to evaluate the effects of genotype, crop and their interaction (G × C experiment) or the effects of genotype, year and their interaction (G × Y experiment) on the traits. Genotype, crop and year effects were estimated by a random effects model. Means for the two years were used as data for each clone in the G × C experiment. Variance components were estimated from the linear function of the mean square. The h_B^2 was estimated on a phenotypic mean basis averaged replications from the variance components in ANOVA as in Chapter 2.

Estimation of h_N^2

Seventeen medium-maturing parental clones and their polycross half-sib progeny were used in the experiments for h_N^2 estimation. The progeny were sown on 20 May 2009 in two rows 0.85 m long, spaced at 0.15 m intervals, with 0.6 m between each plot, in a randomized complete block design with four replications. The parents were planted on 28 May 2009 at a spacing of 0.6 × 0.75 m with two replications. The progeny received 9.0 g N, 18.0 g P_2O_5 plus 9.0 g K_2O m^{-2} in April after snowmelt in 2010, and 6.0 g N plus 6.0 g K_2O m^{-2} after harvesting the first crop. The amounts and times of fertilizer applied to the parents were the same as in the evaluations of G × C and G × Y. The progeny and parents were harvested at 10 cm stubble height on 29 June and 5 July 2010 in the first cut and on 30 August and 3 September 2010 in the second cut, respectively. Forage samples of the second crop were collected at harvest. The method of analysis of the three traits was the same as in the evaluations of G × C and G × Y. The h_B^2 of the parents and their progeny were estimated on a phenotypic mean basis averaged replications from the variance components in ANOVA as in Chapter 2. The h_N^2 was also estimated by the same method as in Chapter 2.

Results

Evaluation of G × C

The analysis of all 26 clones showed the effects of G × C to be significant in all of the traits (Table 7-2). Among the three sources, crop produced the largest mean square in each trait. Analysis with 12 clones of high IES ratio showed the effect of G × C to be non-significant only for WSC content. The variance component of G × C was also the smallest only for WSC content. Crop produced the largest mean square for all of the traits, and the effect of genotype was significant only for WSC content. The analysis of 14 clones with low IES ratio showed the effects of G × C to be significant for all of the traits. Crop produced the largest mean square in Ob content and Ob/OCW ratio. Genotype produced the largest mean square in WSC content. The correlation coefficients between the first and second crops of Ob and WSC contents and Ob/OCW ratio were 0.22, 0.64 ($P < 0.001$), and 0.42 ($P < 0.05$) in the analysis of all 26 clones; 0.38, 0.82 ($P < 0.01$), and 0.41 in the analysis of 12 clones with high IES ratio; and -0.05, 0.58 ($P < 0.05$), and 0.43 in the analysis of 14 clones with low IES ratio (Figure 7-1). Means averaged over the two years of Ob content were greater in the first than in the second crop in three analyses, and those for WSC content and Ob/OCW ratio were smaller in the first than in the second crop except for WSC content of the analysis of 14 clones with low IES ratio (Table 7-3). The SD and ranges were greater in the second than in the first crop except for Ob/OCW ratio in three analyses. Estimates of h_B^2 ranged from 62.2 to 91.5% in the analysis of all 26 clones, 80.4 to 95.6% in the analysis of 12 clones with high IES ratio, and 37.7 to 95.0% in the analysis of 14 clones with low IES ratio.

Table 7-2 Mean squares and variance components estimated from analysis of variance for the nutritive value traits between the first and second crops of timothy clones in three analyses based on averages for 2007 and 2008 (G × C evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (%DM)	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
All 26 clones							
Genotype	25	8.77	0.75	7.96*	1.16	10.85**	1.91
Crop	1	276.50***	5.21	153.24***	2.88	15.31*	0.23
G × C	25	5.76***	2.23	3.30***	1.40	3.21***	1.21
Error	52	1.30	1.30	0.50	0.50	0.79	0.79
12 clones with high IES ratio in the second crop							
Genotype	11	10.64	1.44	5.80	0.81	10.30**	2.11
Crop	1	70.91**	2.75	58.86***	2.35	27.45**	1.07
G × C	11	4.89***	2.14	2.57***	1.06	1.86	0.51
Error	24	0.62	0.62	0.44	0.44	0.84	0.84
14 clones with low IES ratio in the second crop							
Genotype	13	5.22	–0.13	10.27	1.54	11.26*	1.90
Crop	1	220.95***	7.69	95.40***	3.26	0.23	–0.12
G × C	13	5.75**	1.94	4.10***	1.77	3.65***	1.46
Error	28	1.88	1.88	0.56	0.56	0.74	0.74

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels, respectively.

DM, dry matter; G × C, genotype × crop interaction; IES, internode elongation stems; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Evaluation of G × Y

The effects of G × Y were highly significant for four traits except for WSC content, where the effect of genotype was significant and that of G × Y was not significant, with a smaller variance component than the error (Table 7-4). The correlation coefficients between the two years for Ob and WSC contents, Ob/OCW and IES ratios and plant vigor were 0.22, 0.79 ($P < 0.001$), 0.02, 0.59 ($P < 0.01$) and 0.54 ($P < 0.01$), respectively (Figure 7-2). The means of the four traits except for the Ob/OCW ratio were greater in 2007 than in 2008 (Table 7-5). The SD and ranges of the traits except for the Ob/OCW ratio also showed similar patterns. Estimates of h_B^2 ranged from 80.2 to 91.4% in 2007 and 85.3 to 91.7% in 2008.

Table 7-3 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value traits of the first and second crops of timothy clones in three analyses based on averages for 2007 and 2008 (G × C evaluation)

	First crop			Second crop		
	Ob (%DM)	Ob/OCW (%)	WSC (%DM)	Ob (%DM)	Ob/OCW (%)	WSC (%DM)
All 26 clones						
Mean	55.2	81.9	9.3	52.0	84.3	10.1
SD	1.57	1.82	1.28	2.19	1.52	2.32
Range	51.9–57.7	77.7–85.2	6.5–11.4	47.6–57.2	81.4–87.6	6.6–14.8
h_B^2 (%)	62.2	91.4	82.2	91.5	89.8	90.7
12 clones with high IES ratio in the second crop						
Mean	55.4	81.9	9.3	53.0	84.1	10.8
SD	1.69	1.65	1.19	2.21	1.20	2.16
Range	52.4–57.7	78.7–84.7	7.6–11.4	48.5–57.2	82.3–86.0	7.8–14.8
h_B^2 (%)	89.6	95.6	86.2	94.4	80.4	84.7
14 clones with low IES ratio in the second crop						
Mean	55.0	81.9	9.3	51.1	84.6	9.4
SD	1.50	2.01	1.40	1.80	1.77	2.34
Range	51.9–57.0	77.7–85.2	6.5–11.2	47.6–54.2	81.4–87.6	6.6–13.3
h_B^2 (%)	37.7	91.4	80.7	83.0	93.2	95.0

DM, dry matter; G × C, genotype × crop interaction; IES, internode elongation stems; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 7-4 Mean squares and variance components estimated from analysis of variance for the nutritive value and agronomic traits of the second crop of 26 timothy clones in 2007 and 2008 (G × Y evaluation)

		Ob (% DM)		Ob/OCW (%)		WSC (% DM)		IES ratio		Plant vigor	
Source	df	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component	Mean square	Variance component
Genotype	25	19.21	1.56	9.29	0.09	21.56^{***}	4.70	8.96^{***}	1.65	4.24^{*}	0.65
Year	1	31.62	0.36	65.07^{*}	1.08	14.63^{*}	0.23	48.47^{***}	0.89	5.54	0.07
G × Y	25	12.97^{***}	5.48	8.92^{***}	3.83	2.75	0.31	2.35^{***}	0.94	1.66^{***}	0.65
Error	52	2.00	2.00	1.26	1.26	2.13	2.13	0.47	0.47	0.37	0.37

* and *** indicate significance at the 0.05 and 0.001 levels, respectively.

DM, dry matter; G × Y, genotype × year interaction; IES, internode elongation stems; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

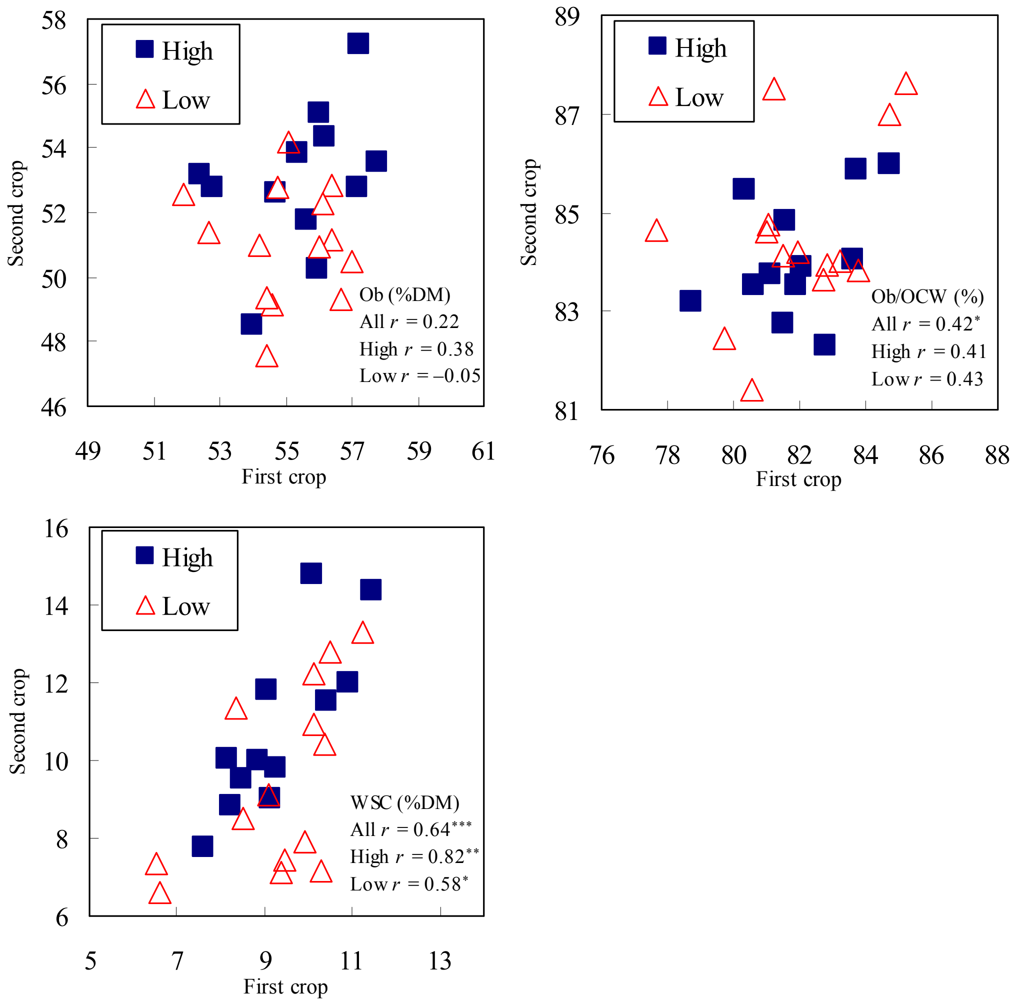

Figure 7-1 Correlations of the nutritive value traits between the first and second crops of timothy clones based on averages for 2007 and 2008 (G × C evaluation). *, ** and *** indicate significance at the 0.05, 0.01 and 0.001 levels, respectively. All, all 26 clones; DM, dry matter; G × C, genotype × crop interaction; High, 12 clones with a high ratio of internode elongation stems in the second crop; Low, 14 clones with a low ratio of internode elongation stems in the second crop; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Table 7-5 Means, standard deviations (SD), ranges and broad-sense heritabilities (h_B^2) of the nutritive value and agronomic traits of the second crop of 26 timothy clones in 2007 and 2008 (G × Y evaluation)

		Second crop				
Year		Ob (%DM)	Ob/OCW (%)	WSC(%DM)	IES ratio	Plant vigor
2007	Mean	52.5	83.6	10.4	5.1	5.7
	SD	3.42	2.14	2.70	1.74	1.53
	Range	47.3–60.5	78.6–87.6	6.5–16.0	1.5–7.5	3.0–8.0
	h_B^2 (%)	87.9	83.2	80.2	91.4	87.5
2008	Mean	51.4	85.1	9.7	3.8	5.3
	SD	2.10	2.13	2.20	1.63	0.78
	Range	47.7–55.5	81.3–90.1	6.4–14.6	1.0–8.0	4.0–7.0
	h_B^2 (%)	85.3	89.9	85.8	91.7	87.8

DM, dry matter; G × Y, genotype × year interaction; IES, internode elongation stems; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

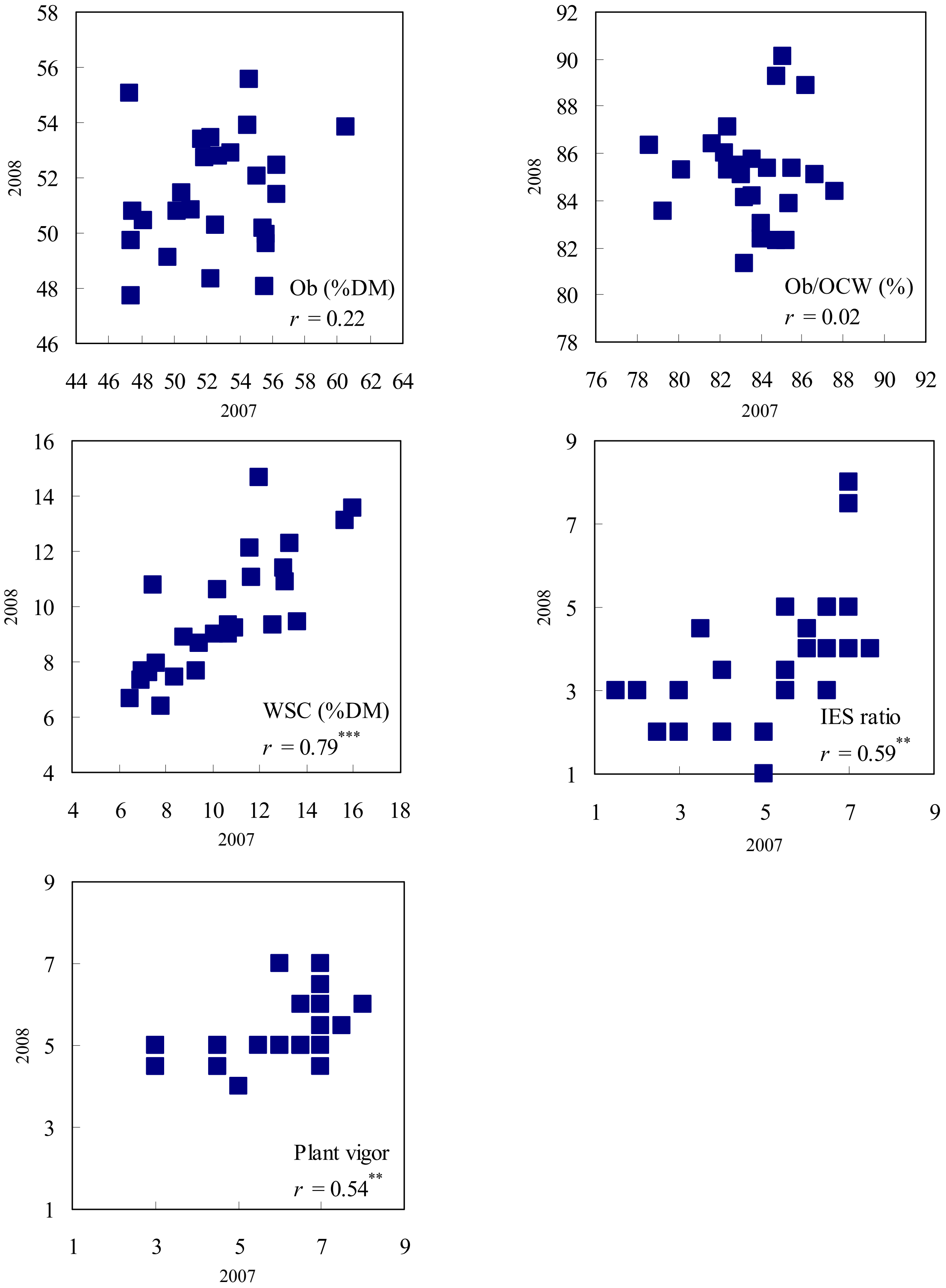

Figure 7-2 Correlations of the nutritive value and agronomic traits of the second crop of 26 timothy clones between 2007 and 2008 (G × Y evaluation). ** and *** indicate significance at the 0.01 and 0.001 levels, respectively. DM, dry matter; G × Y, genotype × year interaction; IES, internode elongation stems; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

Estimation of h_N^2

The means of the traits in the parental clones were smaller than in their half-sib progeny, but for all traits, the SD and ranges were greater in the parents than in their progeny (Table 7-6). The estimates of h_B^2 ranged from 72.2 to 78.8% in the parents and 61.2 to 79.3% in their progeny. The estimates of h_N^2 ranged from 40.6 to 71.1%. Correlation coefficients between the parents and their progeny in the traits were about 0.5 ($P < 0.05$).

Table 7-6 Means, standard deviations (SD), ranges, broad-sense heritabilities (h_B^2) of 17 timothy parental clones and their half-sib progeny, and narrow-sense heritabilities (h_N^2) and correlation coefficients between them of the nutritive value traits of the second crop in 2010

	Ob (%DM)	Ob/OCW (%)	WSC (%DM)
Parent			
Mean	55.9	86.6	10.8
SD	2.63	1.78	2.80
Range	51.7–61.0	83.0–89.5	6.5–16.6
h_B^2 (%)	73.5	72.2	78.8
Half-sib progeny			
Mean	56.4	87.7	11.2
SD	1.20	1.20	1.06
Range	54.2–58.6	86.2–89.9	9.1–13.1
h_B^2 (%)	61.2	62.0	79.3
h_N^2 (%)	50.0	71.1	40.6
r_{PO}	0.54*	0.49*	0.53*

* indicates significance at the 0.05 level.

DM, dry matter; Ob, low-digestible fiber; OCW, organic cell wall; r_{PO}, correlation between the parents and their half-sib progeny; WSC, water-soluble carbohydrate

Discussion

This study clearly demonstrated the different effects on G × C and G × Y among the traits. For WSC content in the 12 clones with a high IES ratio, G × C had no significant effect (Table 7-2) as illustrated by the significant positive correlation between the two crops (Figure 7-1). G × Y also had no significance (Table 7-4) and the correlation coefficient was also significantly positive between the two years (Figure 7-2). These results suggest that selection of timothy genotypes with high WSC content in the first crop should lead to indirect improvement of the second crop if genotypes have a high IES ratio in the second, and that the relative ranking of genotypes in different years should be consistent. In timothy breeding in Hokkaido, selection of genotypes with strong competitive ability after the first cut is regarded as the key to good recovery and maintenance of vegetation. This relationship in genotypes with many reproductive stems is thus useful for simultaneous improvement between them. This related characteristic found in the 12 clones with a high IES ratio was, however, not maintained when all 26 clones were analyzed. The reproductive stems of forage have a significant influence on WSC content (Smith 1973; Saiga 1981b). This suggests that if only the 12 clones with high IES ratios were evaluated, the relative genotype rankings in the two crops would show more similarities in WSC content if the two crops had similar morphological characteristics.

By contrast, regardless of degree of IES ratio, the Ob content and Ob/OCW ratio revealed that the G × C effects were significant (Table 7-2) and that the correlation coefficients of the traits were weak to medium between the two crops (Figure 7-1). The G × Y effects of the traits also had significance (Table 7-4) as shown by weak correlation coefficients of the traits between the two years (Figure 7-2). These observations suggest that selections through the first crop and in a single environment are not enough to improve the traits of the second crop. The present study suggested no definitive reasons, but the author believe it to be causally related to (a) the difference in weather conditions between 2007 and 2008 (higher rainfall from early July to harvest in September in 2008: Table 7-1); and (b) morphological differences between the two crops and the two years as significant effects of G × Y in the plant vigor and the IES ratio of the second crop (Table 7-4). The present results suggest that the two traits of the second crop are more sensitive to these environmental influences than WSC content. Further studies, including morphological and physiological analyses, will be needed to ascertain the different genetic parameters among the traits. Although forage quality is influenced by the presence of reproductive stems (Saiga 1981b), the variations and high h_B^2 were detected for the two traits in the group with a high IES ratio (Table 7-3 and Figure 7-1), suggesting the potential for improving these traits while maintaining competitive ability with reproductive stems. Meanwhile, the three nutritive traits of the first crop in the present study showed no significant influence on G × Y effects or significant positive correlations between the two years (data not shown), as did the study in Chapter 3. The two traits of the second crop may also have lower stability in different environments than the first crop.

The h_N^2 values estimated by the parent-offspring regression method were medium to high in all of the traits (Table 7-6), indicating the preponderance of an additive gene effect. The h_B^2 values of the half-sib progeny are also estimated as the h_N^2 assuming the genetic variance among half-sib families to be equivalent to one-fourth of the additive genetic variance, although the regression method provides a more satisfactory estimate of h_N^2 than does this variance component method (Nguyen and Sleper 1983). The estimates of h_N^2 in this case were also medium to high. These results suggest that the traits of the second crop can be improved by some form of recurrent selection method to accumulate desirable genes. Four generations of recurrent combined phenotypic and half-sib family selection proved successful in selection for WSC content and DM yield in perennial ryegrass (Wilkins and Humphreys 2003). The use of similar conscientious recurrent selection which utilizes additive genetic variances within and among families is likely to be effective for the improvement of the nutritive traits in timothy.

The present results give beneficial information on planning an effective breeding program. Selection for Ob content and Ob/OCW ratio with high G × Y effects in the second crop requires attention to the application of unreplicated individual selections if thousands of plants are tested, although the traits were chiefly influenced by the additive gene effect. Sanada *et al*. (2007) suggested that selection based on performance in progeny tests should be carried out in selection for forage quality traits in cocksfoot, since significant G × Y effects were detected in parental clones. The results for the two traits imply that genotypic selections in half-sib family or progeny tests may be more effective than phenotypic selection in individual plant tests, since these are more susceptible to the effects of genotype × environment interactions. Furthermore, the adoption of individual selection in the second crop requires a great deal of time and labor, since it requires the evaluation of hundreds of elite plants not only from the second crop but also from the first: these were selected by the author’s breeding team for key agronomic traits from thousands of individuals over three years. It also involves multi-year investigations of the elite plants in the second crop due to the high G × Y effects of the two traits. The author therefore conclude that the following breeding scheme, based on the above combined phenotypic and half-sib family selection, should be effective for the simultaneous improvement of the three traits if using a number of individuals. (a) individual selection should have applicability to selection for the first crop with high h_N^2 (Chapter 2), low genotype × year and location interaction effects (Chapter 3) for the three traits, and (b) the application of half-sib family selection, which is tested concurrently with individual selection, under multiple environments should be useful for the improvement of the traits of the second crop. This scheme would enable fewer years per selection cycle and higher accuracy of selection than when employing individual selection in both the first and second crops.

The conclusion of the present study is that, to acquire effective selection methods for these traits in the second crop, selections for WSC content through the first crop in genotypes with many reproductive stems in the second crop, and under multiple environments for Ob content and Ob/OCW ratio should be useful; and that recurrent selection based on additive genetic variance is likely to be effective for acquiring the three traits.

Chapter 8 General discussion

There is a growing need, due in part to the recent surge in the price of imported concentrated feed, for improvement to the nutritive yield of forage grass. Timothy is a primary perennial grass that assists self-reliance in feed production in Hokkaido, and it is therefore essential to prioritize the improvement by genetic means of its nutritive value as feed for livestock. To achieve this goal, it is necessary to establish an effective and reliable selection methodology. In this Chapter, the author discusses the following three points: (a) effective breeding methods using three desirable traits for selection, Ob and WSC contents and Ob/OCW ratio, based on the results given in Chapters 2 to 7; (b) potential breeding methods for simultaneous selection for both the nutritive traits and yield; and (c) the impact on livestock productivity and farm management of genetic improvement of timothy's nutritive value.

1. Effective and reliable selection methods for the nutritive traits in the timothy breeding program

Current studies on the nutritive traits have revealed the following: the three most important traits related to the nutritive value in the first crop not only have potential for improvement by means of individual selection, but there are also prospects for simultaneous improvement among the three traits and with yield productivity (Chapter 2). It has been shown that the relative ranking of genotypes for these traits should be consistent in different years and at different locations, and that selections for the traits in a single environment are likely to be feasible for effective improvement of the nutritive value (Chapter 3). It has been shown that the relative ranking of genotypes at different stages of maturity from the early to full heading stage and at different times within the day should be consistent for the three targeted traits, and that selections for these traits at any stage of heading and any time of day could permit effective improvement of the nutritive value only if the plants are harvested at about the same time (within one hour) (Chapter 4). Continuous selections and crosses among desirable genotypes for Ob and WSC contents and winter survival would be necessary for their simultaneous improvement, and that the application of indirect selection for the nutritive traits through agronomic and morphological traits may not lead to reliable selection (Chapter 5). The relative ranking of genotypes for different N application levels is virtually consistent for the three targeted traits, and selections for the traits at the standard N application level are of potential use for effective improvement of the nutritive value (Chapter 6). In the second crop, selections for WSC content through the first crop in genotypes with many IES in the second crop, and under multiple environments in Ob content and Ob/OCW ratio are potentially useful, suggesting that the evaluations in progeny tests are useful for effective improvement (Chapter 7).

The results of the present study indicate that the following breeding scheme model would be effective for developing high nutritive value cultivars (Figure 8-1). (a) Selection of several tens of promising half-sib families; (b) between- and within-family selection for the nutritive value (evaluations of the first crops in space planting and of the second crops in family selection by row seeding) and agronomic traits; (c) selection of 30 - 50 superior plants in promising families; (d) polycrossing among the elite 30 - 50 plants; (e) beginning the next cycle using the polycrossed progeny seeds from elite plants; (f) development of a new practicable cultivar with high nutritive value using desirable plants after one to several cycles of recurrent selection. In spaced-plant nurseries within family selection, selection from promising genotypes prescreened for important agronomic traits would be useful for developing practical timothy cultivars. Although individual selection for only one year in space planting would have applicability to selection for the nutritive traits of the first crop, replicated selection after individual selection or grid selection may useful to ensure accurate selection if non-uniformity in soil fertility levels is assumed in the test field. The results of the present study also provide helpful information on nutritive breeding, using the three targeted traits, in other perennial forage grasses.

The present status of nutritive breeding based on the findings from this study is as follows. The author's research showed that one generation of phenotypic selection for WSC content within a population, which had not been selected for the nutritive traits, has accomplished an improvement by 0.6 - 4.4% compared to the medium-maturing check cultivars 'Akkeshi' and 'Kiritappu' (Table 8-1).

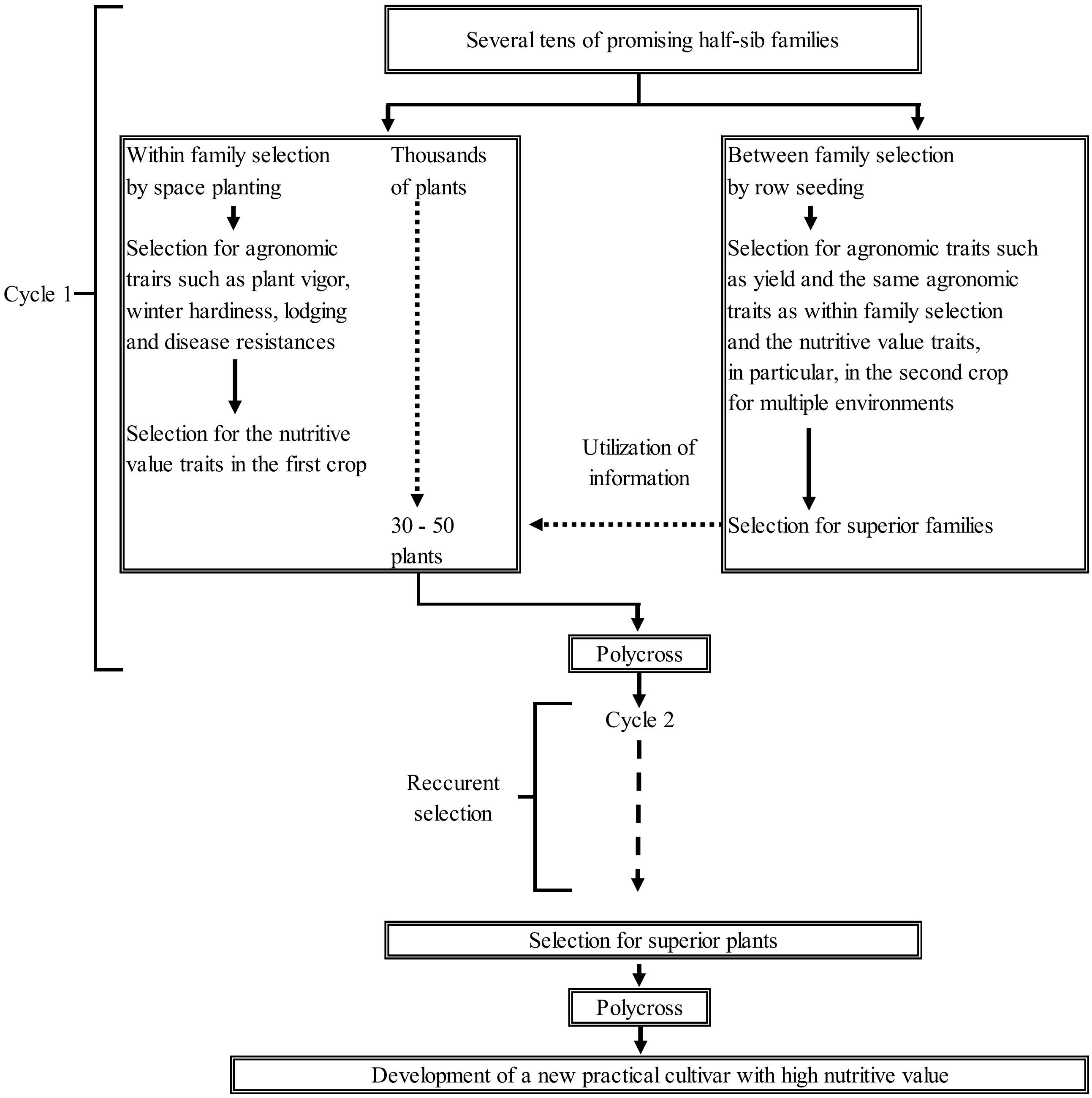

Figure 8-1 A model of breeding scheme to effectively improve the nutritive value traits for development of practical cultivars in timothy.

The V-score of this strain in the second crop, with no wilting and additive-free, showed a significant difference in fermentation quality relative to two check cultivars, substantiating the effectiveness of the improvement of individual plant selection for WSC content. However, there was little difference between these strain and cultivars in the V-score for the first crop, due to poor fermentation, probably resulting from lower WSC and DM contents in the first crop than in the second crop. New strains with higher WSC content may show significant differences from standard cultivars, even in such cases. One cycle of clonal selection focused on desirable nutritive traits for selection, especially Ob and WSC contents, also produced superior medium-maturing progeny which had mean values of 1.8 - 2.2% lower for Ob content, 0.5 - 0.9% lower for Ob/OCW ratio, and 1.0 - 1.4% higher for WSC content than ‘Kiritappu’ (Table 8-2). The application of recurrent selection based on additive genetic variance appears to be effective for acquiring a high nutritive value. The author has also conducted selections and crosses to obtain high nutritive value in other breeding populations with different maturities. It appears likely that employment of the above selection scheme using these populations could promote future timothy breeding for increased nutritive value.

Table 8-1 Early heading date in June and DM yield based on averages for 2009 and 2010 and WSC, DM contents and V-score in 2010 of ‘Kitakei 07304’ and check cultivars

	Averages for 2009 and 2010				2010					
	Early heading	DM yield (kg a^{-1})			WSC (%DM)		DM content (%)		V-score	
Cultivars	date	1st	2nd	Total	1st	2nd	1st	2nd	1st	2nd
Kitakei 07304	19.3	119.8	54.7	174.5	8.2	11.5	25.4	31.0	44.2	92.1
Akkeshi	18.9	117.7	43.2	160.9	7.6	9.1	24.3	28.0	47.5	82.8
Kiritappu	21.8	106.0	42.1	148.2	6.6	7.1	23.1	26.8	42.9	75.2
CV (%)	1.9	3.3	8.4	3.9	13.0	15.1	2.4	4.2	27.9	5.3
LSD (0.05)	0.57	5.79	6.05	9.68	NS	2.05	0.86	1.82	NS	6.77

CV, coefficient of variance; DM, dry matter; LSD, least-significant deference; NS, non-significant; WSC, water-soluble carbohydrate

Table 8-2 Early heading date in June, DM yield and the nutritive value traits averaged for 2010 and 2011 of the two groups mainly selected for agronomic (group A[a]) and the nutritive value (group B[b]) traits and check cultivar

	Early heading	DM yield (kg a^{-1})			Ob (%DM)		Ob/OCW (%)		WSC (%DM)	
Cultivars	date	1st	2nd	Total	1st	2nd	1st	2nd	1st	2nd
Mean in group A	21.5	85.6	39.7	125.3	60.0	56.7	83.8	88.0	9.9	12.6
Mean in group B	23.0	76.5	32.5	109.0	58.5	54.7	83.0	88.0	10.8	11.7
Kiritappu	22.0	81.3	32.4	113.7	60.7	56.5	83.9	88.5	9.8	10.3
CV (%)	2.9	6.2	7.6	5.8	1.8	1.9	0.8	1.4	6.0	6.6
LSD (0.05)	0.88	7.23	4.0	9.81	1.47	1.48	0.88	1.69	0.86	1.14

[a] n = 35. Only hopeful progeny (n = 17) were analyzed the nutritive value traits.

[b] n = 14.

CV, coefficient of variance; DM, dry matter; LSD, least-significant deference; NS, non-significant; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

2. Potential breeding methods for simultaneous improvement of both the nutritive value and yield in timothy

The trade-off relationships between the nutritive value traits and forage yield have been well documented (Furuya 1987; Casler and Vogel 1999; Belanger *et al*. 2001). Cultivars with improved yield may have a lower nutritive value; or, conversely, cultivars with improved nutritive value may have lower yields. Improvement of both the nutritive value traits and yield is therefore a necessary strategy in timothy breeding. Breaking this tradeoff is needed to be able to develop superior varieties. The following two strategies are discussed as potential means of realizing the simultaneous improvement of both the nutritive value and yield.

2-1. Selection combining the nutritive value traits with yield

Selection that combines the nutritive traits with yield is needed, on the grounds that simultaneous improvement is possible, as already mentioned in Chapter 2 and supported in reports by Surprenant *et al*. (1990a), Casler (1999) and Claessens *et al*. (2004). The author has attempted to select two groups as a means of development of the base population: group A, which focuses on agronomic traits, yield in particular, with medium to low selection intensity for the nutritive value traits; and group B, selecting for the nutritive value traits, with medium to low selection intensity for agronomic traits. The results of progeny testing of the two groups over three years showed a trend of improvement for each targeted trait (Table 8-2 and Figure 8-2). Moreover, some strains were noted to have the nutritive values and yields that were superior to 'Kiritappu', the check cultivar. This indicates the potential for improving the nutritive traits while maintaining or improving forage yield. It is therefore possible to break the tradeoff between the nutritive value traits and yield and to select for high-yielding genotypes with high nutritive value.

2-2. Developments and maintenance of two heterotic groups

The main limitation of recurrent selection is the numbers of families that can be evaluated at each generation (Wilkins and Humphreys 2003), although the application of recurrent selection is effective for the improvement of the nutritive traits, as pointed out above. Casler (1999) suggested that part of the reduction in DM yield of smooth bromegrass following recurrent selection for reduced NDF content was attributable to inbreeding. Recurrent selection for any nutritive traits will involve some inbreeding as a result of intercrossing a limited number of related individual plants. It is therefore important to avoid excessive inbreeding in recurrent selection.

The yield improvement in forage grasses has been much slower than that seen in maize. The yield advance in maize can be attributed in part to the harnessing of heterosis. Heterosis for forage yield is a common phenomenon in highly heterozygous, cross-pollinated forage crops, since it occurs at both the population-cross and clonal-cross levels (Brummer 1999). This indicates that in timothy, inbred lines are not a prerequisite for capturing heterosis. Further improvement of DM yield in timothy would need an examination of improvements not only within, but also between populations (Ashikaga *et al*. 2012). Casler (1999) proposed selections for low NDF content in multiple unrelated populations, followed by strain crossing to produce chance hybrids between the improved strains. In smooth bromegrass, 10 of 21 crosses showed a significant positive heterotic effect for forage yield, but no heterosis for NDF content (Casler *et al*. 2005). Hybridization of diverse populations has the potential to restore yield potential via heterosis, possibly retaining low NDF content, unless the heterotic response of NDF content is similar to that of forage yield (Casler 1999). In alfalfa (*Medicago saliva* L.), crosses that showed an average of 18% heterosis for forage yield (Riday and Brummer 2002) showed an average of only 3% heterosis for NDF content (Riday *et al*. 2002). Crosses of genotypes from diverse populations could be subject to fewer inbreeding depression effects than crosses within a single population, even if the crosses do not express heterosis effects. Tamaki *et al*. (2009) found considerable yield variation in topcrossed progeny using timothy cultivar 'Aurora', and they selected promising progeny with high combining ability toward the cultivar. Hence, heterotic groups could be identified by evaluating the performance of progeny resulting from crosses with other populations. Utilizing molecular markers may also assist the identification of heterotic groups, as reported in the discrimination of genetically diverse genotypes in perennial ryegrass

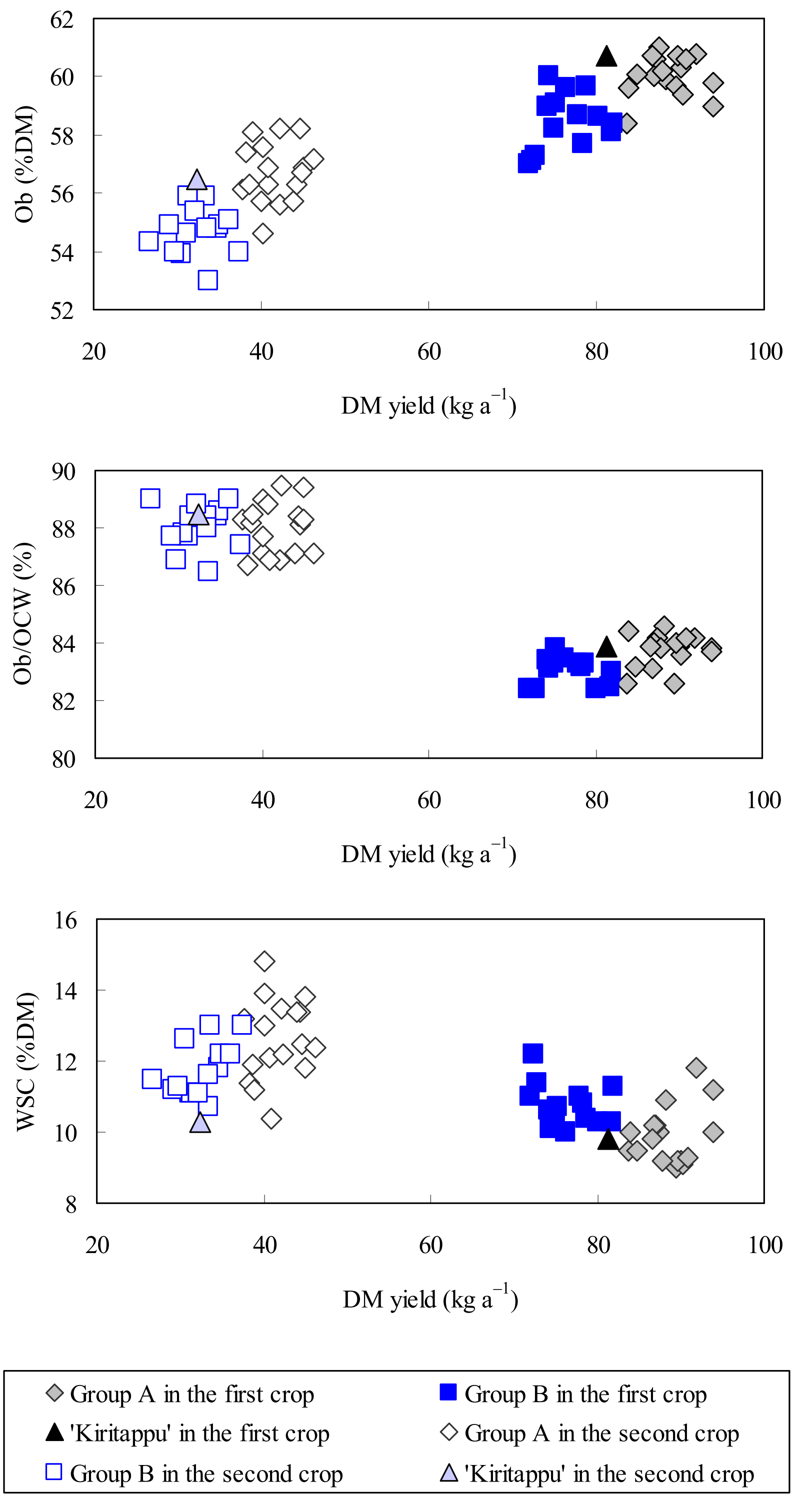

Figure 8-2 Relationships between the nutritive value traits and DM yield of the two groups mainly selected for agronomic (group A) and the nutritive value (group B) traits and check cultivar based on averages for 2010 and 2011. DM, dry matter; Ob, low-digestible fiber; OCW, organic cell wall; WSC, water-soluble carbohydrate

(Kölliker *et al*. 2005) and timothy (Tanaka *et al*. 2011). The application of a proposed breeding scheme using reciprocal recurrent selection between populations incorporating the above model (Figure 8-1) for the nutritive traits may lead to significant advances in timothy breeding (Figure 8-3).

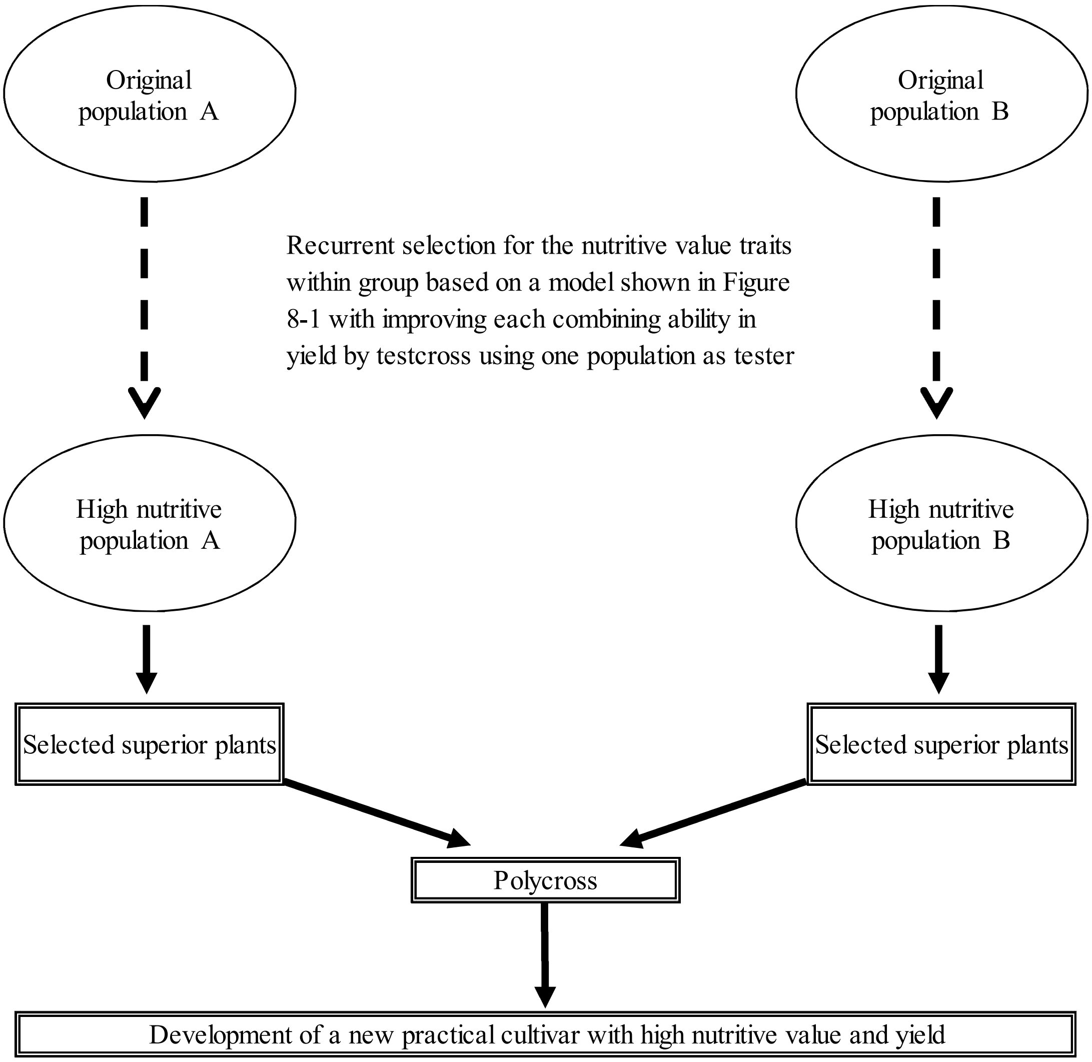

Figure 8-3 A schematic of breeding scheme using reciprocal recurrent selection to improve the nutritive value traits and yield for development of practical cultivars in timothy. Original populations A and B are unrelated to each other.

2-3. Conclusion

There are still no commercial timothy cultivars that show improvements in both forage yield and nutritive value. However, genotypes with high yield and nutritive traits have been identified (Tables 8-1 and 8-2 and Figure 8-2; Bregard *et al*. 2001; Claesses *et al*. 2005a, b). Belanger *et al*. (2001) suggested the potential for dissociating the nutritive value and yield by means of breeding and hence the improvement of the nutritive value of timothy while maintaining forage yield. This is in agreement with the conclusions drawn by Casler and Vogel (1999). A bermudagrass (*Cynodon* spp.) hybrid, ‘Tifton 85’, produced a higher yield and IVDMD than ‘Coastal’, which was released more than 40 years earlier than ‘Tifton 85’ (Hill *et al*. 1993). In perennial ryegrass, four generations of selection over 12 years has achieved progressive improvement (Wilkins and Humphreys 2003). Continuous and conscientious selection is therefore necessary to develop new cultivars with practical performance.

3. Impact on livestock productivity and farm management of genetic improvement on the nutritive value in timothy

Increasing digestibility improves ruminant output. A 1% increase in IVDMD brings benefits of more than 10% in live weight gain (Saiga 1981b), and a one-unit increase in IVDMD improves animal output by 5% (Humphreys *et al*. 2010). A one-unit increase in NDF digestibility *in vitro* or *in situ* is associated with a 0.17 kg increase in DM intake and a 0.25 kg increase in 4% fat-corrected milk (Oba and Allen 1999). A 1% decrease in the Ob content of hay DM increases DM intake by 0.17 kg (Abe 2007). Improvement of digestibility is therefore crucially important, even if a new cultivar is 1% above the check cultivar. Some desirable genotypes in the improved population for the nutritive traits showed a decrease of more than 2% in Ob content (Figure 8-2). Based on the following estimated regression equation of TDN intake on Ob content by Deguchi *et al*. (1996): TDN intake (g/metabolic body size/d) = $-1.29 \times \text{Ob} + 106.3$, and the relationship between 4% fat milk production and TDN requirement recommended in the Japanese Feeding Standard for Dairy Cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, Ministry of Agriculture, Forestry and Fisheries 2007), an improvement of 2% in Ob content can translate into a 1 kg increase in milk production by dairy cattle. Therefore, on the assumption that 64 dairy cattle per farming household, the mean for Hokkaido (Hokkaido District Agriculture Office, Ministry of Agriculture, Forestry and Fisheries 2011), are fed, and milk per 1 kg is priced at 75 yen, high nutritive cultivars with a 2% lower Ob content than the check cultivar would net about 1.8 million yen in the course of the year. Meanwhile, WSC content in timothy varies greatly due to the weather, climatic and harvest time conditions (Chapters 3 and 4). It is thus desirable to include higher WSC content in timothy plants, since high quality silage requires a WSC content of more than 6.5% with no wilting, or more than 5% with wilting, in additive-free grasses (Masuko 2004). Higher WSC content, however slight, can cut the risk of poor silage fermentation. The use of high quality silage can counteract nutrient loss in silage and increase the DM intake of dairy cattle (Masuko 2004). Feeding cattle with high nutritive silage with increased DM intake can also reduce the amount of concentrated feed needed and play an important role in maintaining the health of highly productive lactating dairy cows (Masuko 2001). The application of high WSC cultivars may assist the production of high quality silage, even if additive-free timothy fresh forage is used. Assuming that lactobacillus additive costs 500 yen per 1 t of fresh grass, and 1,320 tons of fresh timothy grasses are harvested annually from 40 ha of timothy meadows, the mean for farms in Hokkaido as estimated by the Hokkaido District Agriculture Office, Ministry of Agriculture, Forestry and Fisheries (2011), the use of high WSC cultivars without the use of additives may result in cost savings of about ¥700,000 per year to dairy farms that normally use lactobacillus additive. Although these preliminary calculations include several presumptions, they suggest that improvements to timothy's nutritive value would be of great significance.

The present study has not included a simulation of grazing use, but the application of cultivars with improved WSC content in grazing use would provide the following advantages. Dairy cows in late lactation fed with herbage from a high-WSC variety of perennial ryegrass excreted less N in their urine (Miller *et al*. 2001). The lower N output in the urine of cows on a high-WSC perennial ryegrass variety treatment is associated with a reduction in the proportion of dietary N being excreted in the urine of dairy cows in early lactation (Moorby *et al*. 2006). These results suggest a reduction in the potential environmental burden of dairy systems utilizing cultivars with elevated WSC content. To reduce environmental pollution, it would be practical for dairy farmers who use grazing pasture, and are not able to use corn silage, to utilize high WSC cultivars. Further breeding studies that simulate grazing use will be needed for future environment-friendly agricultural systems.

The decline of grassland vegetation quality is a major problem facing grass production in Hokkaido. The renewal rate of grassland in Hokkaido has remained stagnant in recent years. It was about 3% in 2008 (Sato 2011). Research into grassland vegetation in different regions of Hokkaido, e.g., Tokachi, Konsen and Abashiri, has revealed that weeds now account for about half of grassland vegetation (Sato 2011), suggesting that the nutritive yield of timothy meadows has declined due to weed infestation. The development of timothy cultivars with improved nutritive value may significantly enhance pasture renovation by forage producers, since high nutritive cultivars can benefit a range of aspects related to animal production. Furthermore, the use of forage with high nutritive value can allow farmers to reduce the amount of imported (and expensive) concentrated feed needed to maintain economic levels of production (Masuko 2004). Minimizing the input of imported concentrated feed is an important contributor to the economic and environmental sustainability of livestock systems. Use of timothy cultivars with high nutritive value would thus enable the reduction of imported concentrated feed, leading to improvements of material recycling among soil - grass - cow and the feed self-sufficiency rate in Hokkaido. The present study proposes an effective breeding method for the three nutritive traits. The application of this method can lead to the development of superior timothy cultivars with high nutritive value, and moreover the genetic improvement through this breeding method may contribute the shift to environment-friendly agricultural system. The future looks bright for progress in genetic improvements.

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Summary

Dairy farming on grassland has expanded in Hokkaido, the northernmost island of Japan. This dairy production system has, however, depended on imported concentrated feed with high nutritive value, leading to the increase of nitrogen (N) from outside regions. A better production infrastructure for forage grasses is required for improvement of material recycling among soil - grass - cow in Hokkaido. There is also a compelling need to reduce costs of imported concentrated feed in livestock farming from the recent surge in the price of the feed. One measure includes the breeding of forage grasses. The application of high nutritive grass cultivars may promote environmental preservation through the reduction of imported concentrated feed. Furthermore, the improvement of forage nutritive value through breeding leads to enhanced livestock productivity since forage with improved nutritive value can benefit a number of aspects related to animal production. Timothy (*Phleum pratense* L.) occupies approximately 80% of total grassland in Hokkaido. The improvement in timothy should therefore largely affect animal production in Hokkaido. This study was conducted to develop effective breeding methods for improving the nutritive value in timothy.

Heritability of the nutritive value traits in the first crop of timothy

It is necessary for the breeders to estimate the heritability of the traits for effective genetic improvement. The objective of this study was to estimate the broad (h_B^2) and narrow (h_N^2) -sense heritabilities of the nutritive value traits in the first crop of timothy. This study also investigated the phenotypic (r_P), genetic (r_G) and environmental (r_E) correlation coefficients among the nutritive traits or between the nutritive traits and yield to estimate the possibilities of simultaneous improvements among these traits. Fifteen early-maturing clones and their polycross progeny, both of which had been cultivated in the same environment in Kunneppu, Hokkaido, Japan, were used in 2004 by analyzing the nutritive value traits of the first crop. The estimates of h_N^2 were high in the three nutritive traits (low-digestible fiber (Ob) and water-soluble carbohydrate (WSC) contents and the ratio of Ob content to organic cell wall (OCW) content). The r_G among the traits in the parents were weak or medium to strong desirable for simultaneous improvement among the traits. The r_G between the nutritive traits and dry matter weight in the parents were weak using the materials which belonged to the same maturity and had been selected for agronomic traits including yield. These results suggest that the three traits in the first crop not only have potential for improvement by means of individual selection, but there are also prospects for simultaneous improvement among the three traits and with yield productivity.

Effects of year and location on the nutritive value in the first crop of timothy

Effective improvement of the nutritive value of the grass requires a study on how the relative genotype ranking of the nutritive value traits changes in different environments. The objective of the present study was to investigate the magnitude of genotype × year interaction (G × Y) and genotype × location interaction (G × L) in the nutritive value traits of the first crop of timothy. The author grew 15 early-maturing clones of timothy in Kunneppu, Hokkaido, Japan in 2004 and 2005 to estimate G × Y and 11 early-maturing clones in Kunneppu and Nakashibetsu, Hokkaido, Japan in 2007 to estimate G × L, and analyzed the nutritive value traits of the first crop. Ob and WSC contents and Ob/OCW ratio were significantly correlated between the two years and between the two locations, with non-significant effects of G × Y and G × L. The author concludes that the relative ranking of genotypes in different years and at different locations should be consistent for the traits; and that selection for the traits in a single environment are likely to be useful in effective improvement of the nutritive value of timothy.

Effects of harvest time across maturity stages and within a day on the nutritive value in the first crop of timothy

This study evaluated the magnitude of genotype × maturity stage interaction (G × M), genotype × harvest time on sunny day interaction (G × TS) and genotype × harvest time on cloudy day interaction (G × TC) for the nutritive value traits of the first crop of timothy. Fifteen early-maturing clones of timothy were used to evaluate G × M and 13 clones were used to evaluate G × TS and TC in Kunneppu, Hokkaido, Japan in 2005 by analyzing the

traits of the first crop. Contents of Ob and WSC and the Ob/OCW ratio measured at early heading significantly correlated with their measurements at full heading stage, with non-significant effects of G × M. These traits also showed a significant correlation between morning and evening in both sunny and cloudy weather conditions, with non-significant effects of G × TS and TC. The author concludes that the relative ranking of genotypes at different maturity stages from early to full heading stage and at different times within a day should be consistent for the required traits; and that selections for these traits at any stage of heading and any time of a day would permit effective improvement of the nutritive value of timothy only if the plants are harvested at about the same time (within one hour).

Relationship between the nutritive value and agronomic or morphological traits in the first crop of timothy

The objective of this study was to investigate the relationships between the nutritive value and agronomic traits; and to explore agronomic or morphological traits that offer indirect measures on the nutritive traits of the first crop in timothy. Thirty medium-maturing clones were used to evaluate the above relationships in Kunneppu, Hokkaido, Japan in 2007 and 2008 by analyzing the nutritive value traits of the first crop. Contents of Ob and WSC showed medium undesirable r_G with winter survival, indicating that attention should be paid to selections for these traits. On the other hand, most of the r_G between the three nutritive (the above two traits and Ob/OCW ratio) and agronomic traits, including degree of lodging and vigor of the first crop, were weak. Significant multiple regression equations were found for Ob and WSC content with about 50 % contribution ratios, and multiple regression equation was found for Ob/OCW ratio with about 30 % contribution ratio. However, use of these equations may be difficult for reliable selection because the nutritive traits estimated from agronomic and morphological traits contain errors and their contribution ratios were less than half. These results suggest that continuous selections and crosses among desirable genotypes for Ob and WSC contents and winter survival would be needed for the simultaneous improvement among these traits; and that the application of indirect selection for the three nutritive traits through the agronomic and morphological traits may be difficult for reliable selection.

Evaluating the genotype × nitrogen fertilization interaction on the nutritive value of the first crop in timothy clones

This study evaluated the effect of genotype × N fertilization interaction (G × N) on the nutritive value traits of the first crop of timothy. Five traits, the contents of Ob and WSC, mono- and disaccharides and fructan and the Ob/OCW ratio of the first crop were investigated as quality traits for 16 clones under three N rates (3, 7 and 11 g N m^{-2} as low, standard and high levels, respectively) in Kunneppu, Hokkaido, Japan in 2009. The G × N effects were non-significant in all the traits despite the significant main effects of genotype and N fertilization in all or most of the traits. Coinciding with this, there were significant correlations among the three N rates for Ob, WSC, fructan contents and Ob/OCW ratio. Correlations between the low and high N rates showed weaker trends than those between the standard and low N rates or between the standard and high N rates in three of the five traits. Fructan content showed small standard deviations and ranges, indicating the difficulty of using it as a selection index for evaluating the genetic variation. The results suggest that, for Ob and WSC contents and Ob/OCW ratio as the desirable traits for selection, the relative ranking of genotypes is almost consistent across different N application levels; and that selections for the traits at the standard N level are of potential use for effective improvement of the nutritive value of timothy.

Relationship between the first and second crops and estimation of genetic parameters of the second crop on the nutritive value of timothy

This study investigated the extent of genotype × crop interaction (G × C) between the first and second crops, and the extent of G × Y and h_N^2 of the second crop of the nutritive value of timothy. Twenty-six clones were used to evaluate these interactions in 2007 and 2008, and 17 clones and their half-sib progeny were used to evaluate the h_N^2 in 2010 in Kunneppu, Hokkaido, Japan by analyzing their nutritive value traits. The content of WSC was significantly correlated between the two crops in 12 clones with many internode elongation stems (IES) in the second crop and the two years, with non-significant effects of G × C in these clones and G × Y. However, Ob content and

Ob/OCW ratio showed weak to medium correlations between the two crops, regardless of IES ratio or the elapsed time of two years, with significant effects shown for the two interactions. The h_N^2 of the three traits was medium to high. These results suggest that, in the second crop, selections for WSC content through the first crop in genotypes with many IES in the second crop, and under multiple environments in other traits are potentially useful; and that recurrent selection which utilizes an additive genetic variance is likely to be effective for the three traits.

Development of effective breeding methods for the improvement of the nutritive value in timothy

The establishment of effective selection methodologies in timothy is necessary for the improvement of the nutritive value through breeding. Based on the results in Chapters 2 to 7, the author developed an effective breeding method on the three desirable traits for selection (Ob and WSC contents and Ob/OCW ratio). The author also discussed the potential breeding methods for simultaneous improvement of the nutritive traits and yield. High nutritive value genotypes with high yield productivity have been identified through selection combining the nutritive traits with yield, suggesting the potential for improving the nutritive value while improving yield. The impact on livestock productivity and farm management of genetic improvement of the nutritive value in timothy were also indicated. Although the preliminary calculations include several presumptions, they suggest that improvements to timothy's nutritive value would be of great significance. The application of this breeding method developed based on the results of the present study can lead to the development of superior timothy cultivars with high nutritive value, and moreover the genetic improvement through this method may contribute the shift to environment-friendly agricultural system. There is no sign that the genetic improvement will be not achieved aftertime.

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チモシー（*Phleum pratense* L.）の栄養価改良に向けた効果的な育種方法に関する研究

足　利　和　紀

要約

チモシー（*Phleum pratense* L.）は北海道内における草地面積の約 80%を占める代表的な牧草である。家畜へ高栄養価の牧草を給与させることは家畜の生産性を向上させるとともに輸入濃厚飼料の削減にもつながる。自給粗飼料多給型の環境保全型農業の推進のためには、チモシーの栄養価を改良することは重要な育種目標である。効率的に育種を実施するには、当該形質の遺伝率、遺伝子型と環境や管理条件との交互作用の程度、他の農業形質との関係等の情報を把握する必要があるが、チモシーでは消化性形質を除いて栄養価形質の選抜に関する報告が少なく、サイレージ発酵品質に影響する水溶性炭水化物（WSC）および日本で広く採用され、家畜の乾物摂取量を精度高く推定できる酵素分析分画による評価形質の選抜に関する報告はない。また、栄養価形質は、気象条件の異なる年次および場所間、異なる生育時期、刈取時刻および窒素施肥水準間で変動することが報告されている。他の飼料作物では、高栄養価を示す遺伝子型は環境ストレス耐性形質で劣るという負の相関関係がみられ同時改良する上での問題が指摘されており、チモシーでもその点を評価する必要がある。一方、農業形質から栄養価形質の間接評価が可能であれば、省労力なため数千点規模でのスクリーニングが可能となる。また、チモシー草地では年間に２回程度の収穫を行うが、１番草の選抜が２番草の改良につながれば、１回の検定で済むため効率的である。そこで、チモシーの栄養価形質を効果的に改良する育種方法を構築する目的で本研究を行った。最初に、利用で主体となる１番草について遺伝率、遺伝子型と年次、場所、生育時期、刈取時刻および窒素施肥水準との交互作用、他の農業形質との関係を調査した。次に、家畜の嗜好性が低いとされる２番草について遺伝子型と番草（１番草と２番草）および年次との交互作用、遺伝率を調査した。栄養価の評価形質として、高消化性繊維、低消化性繊維（Ob）、細胞壁物質（OCW）、繊維消化性として Ob/OCW と酸性デタージェントリグニン/セルロース、WSC および粗蛋白質を遺伝解析に用いた。

１番草における遺伝率を推定するため、早生の親栄養系とその後代系統 15 組を供試した。その結果、Ob および WSC 含量と Ob/OCW の３形質は狭義の遺伝率が高いことから個体選抜による改良が有効であること、形質相互間および乾物重との遺伝相関は効率的な同時改良が可能である相関かもしくは弱い相関で、それら形質間および収量性との同時改良が可能であることが明らかになった。以下の試験からは、この３形質について解析を行った。

１番草における遺伝子型と年次および場所との交互作用の程度を評価するため、早生の 15 栄養系と 11 栄養系を供試して、２年次および２場所（訓子府町と中標津町）においてそれぞれ検討した。分散分析および相関係数の検定の結果、３つの栄養価形質は、遺伝子型と年次および場所との交互作用は有意ではなく、年次および場所間において有意な相関が得られ、単一環境下での選抜が効果的であることが明らかになった。

１番草における遺伝子型と生育時期および刈取時刻との交互作用の程度を評価するため、早生の 15 栄養系と 13 栄養系を供試して、２つの生育時期（出穂始期と出穂揃期）および晴れと曇りの両日で２つの刈取時刻（朝と夕方）においてそれぞれ検討した。分散分析および相関係数の検定の結果、３形質は、遺伝子型と生育時期および刈取時刻との交互作用は有意ではなく、生育時期および刈取時刻間の相関係数は有意であった。このことから、それらの変動要因において相対的な遺伝子型の序列の変動程度が小さく、短時間内に収穫することで、どの出穂時期または刈取時刻でも効果的な選抜が可能であることが解明された。

中生の 30 栄養系を供試して、各農業形質との遺伝相関係数の検定を、また各農業形質を独立変数として重回帰分析を実施した。その結果、Ob と WSC 含量に

関して高栄養価を示す遺伝子型は越冬性にやや劣るという負の遺伝相関が認められ、実用的な品種を育成する上では各農業形質による１次選抜後、栄養価形質で２次選抜することが望ましいこと、また、重回帰分析では複数の農業形質を用いても約 50%以下の寄与率であるため、各農業形質からの間接選抜は難しいことが示された。

１番草における遺伝子型と窒素施肥水準との交互作用の程度を評価するため、中生の 16 栄養系を供試して、３つの窒素施肥水準（窒素３、７、11g/㎡）間で検討した。分散分析および相関係数の検定の結果、３つの栄養価形質において、遺伝子型と窒素施肥水準との交互作用は有意ではなく、水準間で有意な相関で、異なる窒素施肥水準における相対的な遺伝子型の序列は変動の程度が小さいことが明らかになった。

遺伝子型と番草および２番草における遺伝子型と年次との交互作用の程度を評価するため、中生の 26 栄養系を供試して、２つの番草および２年次においてそれぞれ検討した。また、２番草の遺伝率を推定するため、中生の親栄養系とその後代系統 17 組を供試して遺伝解析を実施した。分散分析および相関係数の検定の結果、WSC 含量は年次間での遺伝子型の序列の変動程度が小さく、節間伸長茎割合の高い材料では番草間での変動程度も小さかったが、WSC 含量を除く２形質は番草および年次間での変動程度が大きかった。遺伝解析の結果では、３形質とも狭義の遺伝率は中程度以上の値を示した。したがって、WSC 含量は節間伸長茎割合の高い材料の場合では１番草からの選抜が２番草の改良にもつながり、他２形質の選抜は複数環境下における後代検定での評価が望ましいことが解明された。

以上、３つの栄養価形質を指標にして選抜することが栄養価の改良には効率的であり、栄養価評価の前に各農業形質で選抜後、１番草では３形質による個体選抜を適用すること、２番草では複数環境下における後代検定での選抜を適用すること、相加的遺伝分散を活用する循環選抜が有効であることが本研究から明らかとなり、チモシーの栄養価改良の効果的な育種手法を構築することができた。現在、本手法を用いて育種を展開中であり、WSC 含量で１回の個体選抜を経た育成系統は、標準品種と比べ２番草の WSC 含量と発酵品質の指標である V スコアで有意に優れた。また、３形質で１回の栄養系選抜を経た後代集団は、平均において標準品種と比べ１、２番草の Ob 含量で 1.8～2.2%低く、Ob/OCW で 0.5～0.9%低く、WSC 含量で 1.0～1.4%高かった。これらも含めた様々な材料において、世代を繰り返す循環選抜により優良遺伝子を集積することで、更なる改良が見込まれている。今後、本研究で得られた効果的な育種手法により、栄養価に優れるチモシー優良品種が育成できる可能性が高まり、自給粗飼料を主体とした環境保全型農業の推進に貢献することが期待される。

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（チモシー（***Phleum pratense* L.**）の栄養価改良に向けた
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by
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