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2	Use of molecular marker diversity for forage yield increase in timothy
3	(Phleum pratense L.) polycross breeding
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1 Abstract

2 The aim of this study was to investigate genetic distances (GDs) based on molecular 3 markers in relation to forage yield improvement in timothy syn1 and polycross progenies. 4 In the first experiment, parental clones with high general combining ability (GCA) from 5 two contrasting syn1 progenies, 'Kitakei 98301' that showed promising high yields and 6 'Kitakei 98303' that exhibited low yields contrary to expectation, were analyzed. Average 7 GD among the parental clones of 'Kitakei 98301' was higher than that for 'Kitakei 8 98303'. These results indicate that differences in GD could be a major reason for 9 contrasting yield improvements. In the second experiment using 40 parental clones of a 10 polycross, GD values among the parental clones were partitioned into general genetic 11 distance (GGD) and specific genetic distance components. This study showed a 12 significant correlation between the GGD and GCA for yield and a significant residual mean square for the regression of yield with GGD. These observations reveal the 13 14 existence of considerable non-additive effects in GCA values and the possibility of partitioning GCA values into additive and non-additive effects. 15

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Keywords: forage yield — genetic distance — *Phleum pratense* L. — polycross —
simple sequence repeats marker

1 Introduction

2 Timothy is an important perennial forage grass in the Nordic countries, eastern Canada 3 and northern Japan, which have severe winters, as it has high nutritive quality and good 4 winter hardness. Cultivated forage-type timothy (Phleum pratense L.) is a hexaploid (2n 5 = 6x = 42) species with self-incompatibility (Tamaki et al. 2010). Its forage yield 6 improvement is regarded as one of the major breeding achievements in Japan. 7 Improvement of self-incompatible forage crops including timothy often relies on the 8 production of synthetic varieties. A variety developed by this method consists of an 9 advanced generation of a population initiated by inter-crossing a limited number of 10 parents selected on the basis of high general combining ability (GCA) using a polycross 11 breeding design. Theoretically, the variance among non-inbred half-sib families, 12 including polycross progeny lines, is a quarter of the additive genetic variance and is equivalent to the GCA (Nguyen and Sleper 1989) of the polycross parents. GCA is 13 defined as the average performance of a genotype in a series of crosses and is measured as 14 the deviation of its progeny from the mean of those crosses. GCA values are especially 15 16 useful in the prediction of synthetics (Posselt 2010). However, contrary to expectations 17 based on GCA values for forage yield from polycross progeny tests, there have been 18 several synthetic strains that fail to produce high yields in the Japanese timothy breeding 19 programs carried out at Hokkaido prefectural Kitami Agricultural Experiment Station (KAES) since the 1960s (Ueda 1990). There have been a number of reports that attempt 20 21 to explain these unsuccessful selections. One of the possibilities is the existence of 22 specific combining ability and/or inbreeding depression that can substantially influence 23 the success of improving perennial and self-incompatible forage crop species (Gau et al. 1989; Michaelson-Yeates et al. 1997; Riday and Brummer 2002; Tamaki et al. 2007). 24

Another convincing explanation is that the GCA value itself may include both additive
and non-additive effects (Hayward 1979) and/or be masked by genotype by environment
interactions such as for forage yield under spaced plants and swards conditions (Wilkins
and Humphreys 2003; Casler and Brummer 2008; Amini et al. 2011).

5 Today molecular markers are regarded as powerful tools for the analysis of genetic 6 diversity and as criteria for the selection of parents in developing a variety. If diversity at 7 marker loci reflects diversity at other linked loci, then selection for more heterozygosity 8 at marker loci selection should increase the probability of having favorable dominant 9 alleles at linked loci which affect forage yield and produce greater overall complementary 10 gene interaction. Some investigations in maize and oilseed rape have shown that genetic 11 diversity among parents measured with molecular markers significantly correlates with 12 hybrid performance and that molecular markers can be used as a tool to predict heterosis for yield (Smith et al. 1990; Riaz et al. 2001; Betrán et al. 2003; Reif et al. 2003). There 13 14 are existing studies on molecular marker diversity in relation to forage yield in crops such as alfalfa (Medicago sativa L.) (Kidwell et al. 1994, 1999; Tucak et al. 2011), white 15 16 clover (Trifolium repens L.) (Joyce et al. 1999), perennial ryegrass (Lolium prenne L.) 17 (Kölliker et al. 2005), tall fescue (Festuca arundinacea Schreb) (Amini et al. 2011) and 18 timothy (Tanaka et al. 2011). Some of these studies have revealed promising relationships 19 between forage yields and genetic diversity. The focus of most studies of relationships between molecular diversity and forage yield has been on selecting (and/or pre-selecting) 20 21 combinations of parents, and not on evaluating the proportion of additive and 22 non-additive effects in the observed yield performance of synthetic varieties. However, 23 Melchinger et al. (1990) partitioned genetic distance (GD) values into general genetic distance (GGD) and specific genetic distance components using a method analogous to 24

Griffing's Model I of Method 4 (Griffing 1956), where GGD was contrasted with GCA in a diallel cross design. This could be a tool to extract contributions of non-additive effects from GCA values obtained in polycross progeny tests, as a large positive GGD value is thought to indicate that a genotype possesses many alleles with low frequency (Melchinger et al. 1990). However, there are no published reports concerning relationships between the GGD and GCA based on polycross progeny tests in forage crops.

8 The objectives of this study were (i) to evaluate the effects of GD among parental 9 clones on forage yield of their first generation of synthetic (syn1) and polycross progenies, 10 and (ii) to investigate whether the GD values measured with molecular markers are useful 11 for improving forage yield in timothy polycross breeding, by dissecting the performance 12 of polycross progenies into additive and non-additive component effects.

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14 Materials and methods

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All field tests were carried out at KAES (43°47′N, 143°42′E; currently Kitami
Agricultural Experiment Station, Hokkaido Research Organization) on a wet andosol.

18

19 Experiment 1 (synthetic varieties) – Plant materials & field tests design

Eleven early-maturing timothy clones were included in this experiment. Five of the clones were the parents of 'Kitakei 98301' and the remaining six clones were the parents of 'Kitakei 98303'. Both of the synthetic strains were developed at KAES. The parental clones were selected up to 1998 based on high forage yields in polycross progeny tests (Tables 1 and 2). Seeds were planted in a randomized complete block design with four

replicates in May 1999. The seeding rate was 200 g m⁻². Plots were 2.5m by 1.2m with 1 2 two drilled rows. Seeding-year management consisted of two harvests without data collection to manage annual weeds and an application of 70 kg N ha⁻¹, 80 kg P₂O₅ ha⁻¹ 3 and 70 kg K_2O ha⁻¹. The experiment was managed with three harvests per year in 2000 4 and 2001. Plots were fertilized as follows: 75 kg N ha⁻¹, 150 kg P_2O_5 ha⁻¹ and 75 kg K_2O_5 5 ha^{-1} in early spring, 45 kg N ha^{-1} , 0 kg $P_2O_5 ha^{-1}$ and 45 kg $K_2O ha^{-1}$ immediately after 6 the first harvest and 30 kg N ha⁻¹, 0 kg P₂O₅ ha⁻¹ and 30 kg K₂O ha⁻¹ immediately after 7 8 the second harvest. Plots were clipped to a 10cm stubble height. Dry matter 9 determinations were made on random 300g to 500g forage samples and were used to 10 adjust plot yields to a dry matter basis. Dry matter yields (DMYs) for each plot were 11 summed over all six harvests. Total DMYs for the two years were subjected to analyses of 12 variance (ANOVA) according to a randomized complete block design.

13

14 Experiment 2 (polycross progenies) – Plant materials & field tests design

Forty-one timothy polycross progenies and their parental clones were used in this experiment. The 41 progenies, which were derived from a polycross corresponding to the third cycle of 'Maternal line selection combined with a progeny test' (Tamaki et al. 2010), were investigated in the following field tests. All parental clones apart from one clone ('27thPC-05') were analyzed using 28 simple sequence repeat (SSR) markers. The '27thPC-05' clone died during vegetative propagation of the germplasm collection in KAES.

Total DMY for the forty-one polycross progenies together with three check varieties including 'Nosappu' (Ueda et al. 1977) were evaluated over two years. The polycrosses with twelve replicates were produced in 1999 to 2000. The seeds were planted in a

1 randomized complete block design with four replicates in August 2000. Plots were 1.5m 2 by 0.6m with one drilled row. Seeding-year management consisted of an application of 40 kg N ha⁻¹, 80 kg P₂O₅ ha⁻¹ and 40 kg K₂O ha⁻¹ without harvests. The experiment was 3 4 managed for three harvests per year in 2001 through 2003. Fertilizer application and seeding rate were equivalent to Experiment 1. DMYs for each plot were summed over all 5 6 six harvests in 2002 and 2003 (three harvests per year for two years). Data of harvests 7 in 2001 were excluded because of some missing values. The total DMYs were 8 analysed using ANOVA according to a randomized complete block design.

9

10 Genotyping protocol & statistical analysis

Outlines of the protocol used were described in a previous study made at KAES (Tanaka et al. 2011). Briefly, 28 SSR primer pairs screened out of 55 SSR primer pairs were used. Among this set, at least three SSR loci were located on each linkage group of the diploid timothy map, and the mean distance between neighboring markers was approximately 13.9 cM. Details of the SSR loci and their map locations are given in the report of Cai et al. (2009).

General combining ability (GCA) values were calculated from percentages of 17 18 'Nosappu' for total DMYs in polycross progenies tests using the following equation: $GCA_i = [(p-1)/(p-2)] (X_i - \mu)$, where GCA_i is the GCA value of the parental clone *i*, X_i 19 is the value of the polycross progeny i, μ is the mean of all polycross progenies and p is 20 the parental number of the polycross, respectively. Genetic distance (GD) estimates were 21 22 calculated from SSR data for all possible pairs of parental clones using the following equation: $GD_{i,j} = 1 - [2N_{i,j} / (N_i + N_j)]$, where $GD_{i,j}$ is the GD estimate between clone *i* and 23 j, $N_{i,j}$ is the total number of bands common to clone i and j, and N_i is the total number of 24

1 bands present in *i*. This is equal to one minus the genetic similarity coefficient originally 2 devised by Dice (1945) and was first used for molecular diversity by Nei and Li (1979). 3 Principle coordinate analysis (PCOA) was carried out on the matrix of GD estimates by 4 using the function 'pcoa' in the R statistical package 'ape' (Paradis et al. 2004). General 5 genetic distance (GGD) estimates within parental clones of the polycross were calculated 6 from GD estimates using the following equation based on a method analogous to 7 Griffing's Model I of Method 4 (Griffing 1956; Melchinger et al. 1990): $GGD_i = [(p-1)]$ 8 (p-2)] (GD_i, -GD.), where GD_i is the average of GD estimates between clone *i* and 9 the other parental clones of the polycross, GD., is the average of GD estimates between 10 all possible pairs of parental clones of a polycross and p is the parental number of the 11 polycross, respectively. The data set including GGD and DMY of 40 polycross progenies 12 was analyzed using linear regression.

13

14 **Results**

15 **Experiment 1** (synthetic varieties)

16 Significant differences were detected among the DMY in 2000 and total DMY over 2 years (2000-2001) for the four entries (Table 3). 'Kitakei 98301' yielded 18%, 5% and 17 12% more than a check variety 'Nosappu' for DMY in 2000, in 2001 and in total 18 19 (2000-2001), respectively (Table 3). On the other hand, 'Kitakei 98303' was comparable to 'Nosappu' and lower than 'Kitakei 98301' for DMY (Table 3). The GD for all pairs 20 21 among the 11 parental clones ranged from 0.614 to 0.782 and the average GD for all pairs 22 was 0.701. The GD estimates among parental clones for each synthetic strain, ranged 23 from 0.677 to 0.782 with an average GD of 0.744 for 'Kitakei 98301' and from 0.614 to 0.752 with an average GD of 0.677 for 'Kitakei 98303'. Thus the average GD among 24

parental clones of 'Kitakei 98301' was clearly higher than that of 'Kitakei 98303'. The PCOA based on 28 SSR markers, where the first two principle coordinates termed PC1 and PC2 explained 16.1% and 14.9% of the variation respectively, could not separate the eleven parental clones into groups corresponding to the synthetic strains (Fig. 1). The distribution of the five parental clones of 'Kitakei 98301' (closed symbols in Fig. 1) tended to be more widely dispersed than that of 'Kitakei 98303' (open symbols in Fig. 1) in the PCOA scatter plot.

8

9 Experiment 2 (polycross progenies)

10 The 41 polycross progenies showed large variation for several traits, except for heading 11 date (data not shown), and ranged from 93 to 115% of 'Nosappu' for DMY (Table 4). The 12 ANOVA produced an F-value of 2.95 (p < 0.001) for the total DMY of 44 entries over two years. GCA values based on total DMY in the progeny test ranged from -10.0 to 11.4 13 (Table 4). The GD estimates of all possible pairs among the parental clones of the 14 polycross, not including '27thPC-05,' ranged from 0.588 to 0.908 (data not shown). The 15 16 GGD estimates calculated from the GD estimate matrix for all possible pairs of 40 entries ranged from -0.0244 to 0.0394 (Table 4). The relationship between genetic diversity and 17 18 performance in polycross progenies for forage yield was evaluated by calculating the 19 Pearson product-moment correlation coefficient between GCA values for total DMY and GGD estimates, not including '27thPC-05'. The correlation coefficient for this 20 relationship was 0.45 (p < 0.01) (Fig. 2). Analysis of variance of regression with GGD 21 22 (Table 5) showed that the mean square values for 'among polycross progenies' (F-value of 2.40), 'regression with GGD' (F-value of 9.67) and 'residual' of the regression 23 (F-value of 1.96) were significant for total DMYs of the 40 polycross progenies not 24

1 including '27thPC-05'.

2

3 **Discussion**

4 The synthetic strains 'Kitakei 98301' and 'Kitakei 98303', both derived from parental 5 clones with high GCA, exhibited contrasting forage yield levels (Table 3). Compared 6 with 'Kitakei 98303', which showed lower DMY contrary to expectations based on GCA 7 estimates, the parental clones of 'Kitakei 98301' had larger GD values among themselves 8 which spread wider in the PCOA scatter plot (Fig. 1). The results from SSR analyses 9 clearly show that genetic diversity among the parental clones of 'Kitakei 98301' was 10 higher than for 'Kitakei 98303'. Differences in molecular diversity among parental clones 11 could be a major reason for contrasting yield performance between the two synthetic 12 strains because of non-additive gene effects and/or inbreeding depression. These 13 observations agree with a previous study where high molecular marker diversity led to a 14 significantly increased DMY in synthetic varieties of perennial ryegrass (Kölliker et al. 15 2005). The results support a hypothesis that the selection of polycross parents with high 16 molecular diversity is a possible way to exploit heterosis and avoid inbreeding depression. 17 However, there is no conclusive proof that populations derived from parents with high 18 molecular marker diversity invariably result in high performance for forage yield. There 19 is a report of unsuccessful selection experiments using molecular marker diversity for forage yield in alfalfa (Kidwell et al. 1999). Kidwell et al. (1999) pointed out the 20 21 importance of additive gene effects as well as dominance effects and complementary 22 gene interactions for improving forage yield. Thus both additive and non-additive effects 23 must be considered when investigating the relationship between molecular marker diversity and forage yield in the performance of polycross progenies. Experimental 24

1 polycross parent selections based on GD together with an evaluation of additive and 2 non-additive genetic effects are necessary to elucidate contrasting synthetic performance. 3 Although estimates of GCA are generally useful in the prediction of synthetic performance (Posselt 2010), there are unsuccessful selection experiments based on GCA 4 5 values such as the timothy synthetic strain 'Kitakei 98303' in this study (Tables 2 and 3) 6 and the tall fescue syn1 progenies (HGCA) described by Amini et al. (2011). It is true that 7 a polycross can provide satisfactory discrimination between the breeding values of 8 genotypes when both additive and dominance effects are present with equal gene 9 frequencies. However, as Hayward (1979) points out, unequal gene frequencies are likely 10 to arise as a consequence of selection and here the discrimination among genotypes 11 would be more difficult. The present study showed that differences in GGD among the 12 parental clones of polycrosses correlated with their respective GCA values for DMY based on polycross progeny tests (Fig. 2) and that the mean of square for regression with 13 14 GGD was significant for DMY (Table 5). These results indicate that the GCA values included a genetic effect associated with genetic diversity in addition to additive effects, 15 16 as suggested by Hayward (1979). This situation might have arisen from the low levels of 17 additive genetic variation remaining after three cycles of selection. 'Maternal line 18 selection combined with a progeny test' allows breeders to conduct individual selection 19 and a polycross progeny test simultaneously. Although genetic variation among 20 individuals within timothy populations and their interrelationships have usually been 21 assessed using morphological and agronomic traits, these are not sufficient to provide 22 good estimates of genetic relatedness among timothy populations. Molecular markers 23 provide useful tools to assess the genetic relatedness of breeding material when selecting within genetically broad-based populations. In addition, it is interesting that 24

both the mean square for the residual of regression and that for regression with GGD were significant (Table 5). It shows that the former residual mean square includes considerable genetic effects unrelated to genetic diversity, i.e. additive effects. Therefore, these approaches will result in a rigorous evaluation for yield performances in polycross progeny tests dividing the GCA values into additive and non-additive effects based on molecular marker diversity, and lead to more efficient improvement of forage yield in timothy polycross breeding.

8 In conclusion, GD values based on molecular marker diversity were related to the 9 forage yield of progenies. These investigations support the hypotheses that selection of 10 polycross parents for high molecular diversity is a possible way to exploit heterosis or to 11 avoid inbreeding depression and that molecular diversity can be useful for understanding 12 the contribution of both additive and non-additive effects to GCA values in polycross progeny tests. Our results confirmed the usefulness of molecular markers in the selection 13 of parents for forage yield in polycross breeding with selection strategies that capitalize 14 15 on both additive and non-additive genetic variation.

16

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23 References

24 Amini, F., A. Mirlohi, M.M. Majidi, S. ShojaieFar, and R. Kölliker, 2011: Improved

1	polycross breeding of tall fescue through marker-based parental selection. Plant Breed.
2	130, 701–707.
3	Betrán, F.J., J.M. Ribaut, D. Beck, and D. Gonzalez de León, 2003: Genetic diversity,
4	specific combining ability, and heterosis in tropical maize under stress and nonstress
5	environments. Crop Sci. 43, 797–806.
6	Cai, HW., M. Inoue, N. Yuyama, and M. Hirata, 2009: Genome mapping in cool-season
7	forage grasses. In: T. Yamada, and G. Spangenberg (eds), Molecular breeding of forage
8	and turf, 173–183. Springer, New York.
9	Casler, M.D., and E.C. Brummer, 2008: Theoretical expected genetic gains for
10	among-and-within-family selection methods in perennial forage crops. Crop Sci. 48,
11	890–902.
12	Dice, L.R., 1945: Measures of the amount of ecological association between species.
13	Ecology 26, 297–302.
14	Gau, M., A. Sawai, S. Ueda, and M. Matsuura, 1989: Significance of specific combining
15	ability for yield in alfalfa (Medicago sativa L.) breeding. J. Japan Grassl. Sci. 34,
16	292–299.
17	Griffing, B., 1956: Concept of general and specific combining ability in relation to diallel
18	crossing systems. Aust. J. Biol. Sci. 9, 463–493.
19	Hayward, M.D., 1979: The application of the diallel cross to out breeding crop species.
20	Euphytica 28, 729–737.
21	Joyce, T.A., M.T. Abberton, T.P.T. Michaelson-Yeates, and J.W. Forster, 1999:
22	Relationships between genetic distance measured by RAPD-PCR and heterosis in

- inbred lines of white clover (*Trifolium repens* L.). Euphytica **107**, 159–165.
- 24 Kidwell, K.K., D.R. Woodfield, E.T. Bingham, and T.C. Osborn, 1994: Molecular marker

1	diversity and yield of isogenic 2x and 4x single-crosses of alfalfa. Crop Sci. 34,
2	748–788.
3	Kidwell, K.K., L.M. Hartweck, B.S. Yandell, P.M. Crump, J.E. Brummer, J. Moutray, and
4	T.C. Osborn, 1999: Forage yields of alfalfa populations derived from parents selected
5	on the basis of molecular marker diversity. Crop Sci. 39, 223–227.
6	Kölliker, R., B. Boller, and F. Widmer, 2005: Marker assisted polycross breeding to
7	increase diversity and yield in perennial ryegrass (Lolium perenne L.). Euphytica 146,
8	55–65.
9	Melchinger, A.E., M. Lee, K.R. Lamkey, and W.L. Woodman, 1990: Genetic diversity for
10	restriction fragment length polymorphisms: relation to estimated genetic effects in
11	maize inbreds. Crop Sci. 30 , 1033–1040.
12	Michaelson-Yeates, T.P.T., A. Marshall, M.T. Abberton, and I. Rhodes, 1997:
13	Self-compatibility and heterosis in white clover (Trifolium repens L.). Euphytica 94,
14	341–348.
15	Nei, M., and W.H. Li, 1979: Mathematical model for studying genetic variation in terms
16	of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76, 5269-5273.
17	Nguyen, H.T., and D.A. Sleper, 1989: Theory and application of half-sib matings in
18	forage grass breeding. Theor. Appl. Genet. 64, 187–196.
19	Paradis, E., J. Claude, and K. Strimmer, 2004: APE: Analyses of phylogenetics and
20	evolution in R language. Bioinformatics 20, 289–290.
21	Posselt, U.K., 2010: Breeding methods in cross-pollinated species. In: B. Boller, U.K.
22	Posselt, and F. Veronesi (eds), Fodder crops and amenity grasses, Handbook of plant
23	breeding 5, 39–87. Springer, New York.
24	Reif, J.C., A.E. Melchinger, X.C. Xia, M.L. Warburton, D.A. Hoisington, S.K. Vasal, G.

1	Srinivasan, M. Bohn, and M. Frisch, 2003: Genetic distance based on simple sequence
2	repeats and heterosis in tropical maize populations. Crop Sci. 43, 1275–1282.
3	Riaz, A., G. Li, Z. Quresh, M.S. Swati, and C.F. Quiros. 2001: Genetic diversity of oilseed
4	Brassica napus inbred lines based on sequence-related amplified polymorphism and its
5	relation to hybrid performance. Plant Breed. 120, 411–415.
6	Riday, H., and E.C. Brummer, 2002: Forage yield heterosis in Alfalfa. Crop Sci. 42,
7	716–723.
8	Smith, O.S., J.S.C. Smith, S.L. Bowen, R.A. Tenborg, and S.J. Wall, 1990: Similarities
9	among a group of elite maize inbreds as measured by pedigree, F ₁ grain yield, grain
10	yield, heterosis, and RFLPs. Theor. Appl. Genet. 80, 833-840.
11	Tamaki, H., A. Yoshizawa, H. Fujii, and K. Sato, 2007: Modified synthetic varieties; a
12	breeding method for forage crops to exploit specific combining ability. Plant Breed.
13	126, 95–100.
14	Tamaki, H., J. Baert, and P. Marum, 2010: Timothy. In: B. Boller, U.K. Posselt, and F.
15	Veronesi (eds), Fodder crops and amenity grasses, Handbook of plant breeding 5,
16	324–338. Springer, New York.
17	Tanaka, T., H. Tamaki, HW. Cai, K. Ashikaga, H. Fujii, and T. Yamada, 2011: DNA
18	profiling of seed parents and a topcross tester and its application for yield
19	improvement in timothy (Phleum pratense L.). Crop Sci. 51, 612-620.
20	Tucak, M., S. Popvic, S. Grljusic, T. Cupic, and S. Bolaric, 2011: Implementation of
21	molecular markers diversity in parental selection of alfalfa (Medicago sativa L.).
22	Biotechnol. and Biotechnol. Eq. 25, 2310–2314.
23	Ueda, S., T. Masutani, S. Higuchi, M. Furuya, and S. Tsutsui, 1977: New timothy variety
24	'Nosappu' (in Japanese with English summary). Bull. Hokkaido Pref. Agric. Exp. Stn.

- 1 **38,** 34–46.
- 2 Ueda, S, 1990: Timothy breeding in Japan. Jpn. Agric. Res. Q. 24, 195–201.
- 3 Wilkins, P.W., and M.O. Humphreys, 2003: Progress in breeding perennial forage grasses
- 4 for temperate agriculture. J. Agric. Sci. **140**, 129–150.

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Table 1: General combining ability (GCA¹) for forage yields of five parental clones of a synthetic variety, 'Kitakei 98301', based on total dry matter yields (DMY) in a polycross progeny test over three years (1991-1993)

Parental clones names	DMY in a polycross progeny test		
	(Mg ha ⁻¹)	(% of 'Nosappu')	(GCA)
17thPC-06	18.74	101	4.4
17thPC-20	19.14	104	6.6
17thPC-24	19.02	103	5.9
17thPC-32	18.69	101	4.1
17thPC-35	19.30	104	7.5
Mean of selections ²	18.98	103	5.7
Mean of entries ³	17.98	97	0.0
Nossapu ⁴	18.48	100	

¹GCA calculated by the following equation: $GCA_i = [(p-1)/(p-2)] (X_i - \mu)$, where *p* is number of parents in a polycross, X_i is DMY (percentage of 'Nosappu') of a polycross progeny *i*, and μ is means of DMY (percentage of 'Nosappu') of all polycross progenies, respectively.

²Mean of polycross progenies derived from selected five parental clones.

³Mean of all thirty-five polycross progenies evaluated in progenies test.

⁴A check variety.

Table 2: General combining ability (GCA¹) for forage yields of six parental clones of a synthetic variety, 'Kitakei 98303', based on total dry matter yields (DMY) in a polycross progeny test over two years (1996-1997)

	(Mg ha ⁻¹)	(% of 'Nosappu')	(GCA)	
23thPC-01	18.27	104	5.9	
23thPC-15	18.30	104	6.1	
23thPC-27	17.56	99	1.8	
23thPC-30	17.71	100	2.7	
23thPC-31	17.58	100	1.9	
23thPC-34	18.34	104	6.4	
Mean of selections ²	17.96	102	4.2	
Mean of entries ³	17.24	98	0.0	
Nossapu ⁴	17.66	100		

Parental clone names DMY in a polycross progeny test

¹GCA calculated by the following equation: $GCAi = [(p-1) / (p-2)](Xi - \mu)$, where *p* is number of parents in a polycross, X*i* is DMY (percentage of 'Nosappu') of a polycross progeny *i*, and μ is means of DMY (percentage of 'Nosappu') of all polycross progenies, respectively.

²Mean of polycross progenies derived from selected six parental clones.

³Mean of all thirty-four polycross progenies evaluated in a progenies test.

⁴A check variety.

Entry	$DMY (Mg ha^{-1})$				
	2000	2001	Total (2000-2001)		
Kitakei 98301	6.73	4.66	11.39		
Kitakei 98303	5.77	4.30	10.07		
Check varieties					
Nossapu	5.69	4.46	10.15		
Aurora	5.69	4.29	9.98		
$LSD_{0.05}^{1}$	0.59	NS ²	1.06		

Table 3: Dry matter yields (DMY) of synthetic strains in a yield trial over two years(2000-2001)

¹Least significant difference at the 0.05 probability level.

²NS, not significant.

Parental clone names	Total	GGD		
	(Mg ha ⁻¹)	(% of 'Nosappu')	(GCA)	-
27thPC-01	24.76	101	-2.2	0.0209
27thPC-02	25.76	105	1.8	0.0113
27thPC-03	23.93	98	-5.5	-0.0215
27thPC-04	25.78	105	1.9	-0.0047
27thPC-05	26.35	108	4.1	_3
27thPC-06	25.37	104	0.2	-0.0061
27thPC-07	26.57	109	5.0	0.0205
27thPC-08	25.39	104	0.3	0.0329
27thPC-09	24.61	101	-2.8	0.0072
27thPC-10	26.39	108	4.3	-0.0051
27thPC-11	25.40	104	0.3	-0.0031
27thPC-12	25.24	103	-0.3	-0.0105
27thPC-13	25.96	106	2.6	-0.0057
27thPC-14	25.73	105	1.7	-0.0047
27thPC-15	25.66	105	1.4	-0.0145
27thPC-16	25.62	105	1.2	-0.0047
27thPC-17	25.60	105	1.1	0.0141
27thPC-18	25.20	103	-0.5	-0.0061
27thPC-19	23.83	97	-5.9	-0.0074
27thPC-20	24.44	100	-3.5	0.0032
27thPC-21	24.41	100	-3.6	-0.0116
27thPC-22	26.41	108	4.4	0.0394
27thPC-23	28.17	115	11.4	0.0107
27thPC-24	24.88	102	-1.7	-0.0227
27thPC-25	25.93	106	2.5	-0.0131
27thPC-26	24.34	99	-3.9	-0.0115
27thPC-27	23.35	95	-7.8	-0.0244
27thPC-28	26.53	108	4.9	-0.0040
27thPC-29	25.32	103	0.0	-0.0011
27thPC-30	24.01	98	-5.2	-0.0074
27thPC-31	24.84	102	-1.9	-0.0201
27thPC-32	24.81	101	-2.0	0.0001
27thPC-33	26.02	106	2.8	0.0150
27thPC-34	24.80	101	-2.0	0.0218
27thPC-35	23.84	97	-5.9	-0.0042
27thPC-36	25.91	106	2.4	0.0112
27thPC-37	24.24	99	-4.3	-0.0084
27thPC-38	22.81	93	-10.0	-0.0014
27thPC-39	27.95	114	10.5	0.0147
27thPC-40	24.41	100	-3.6	-0.0111
27thPC-41	27.28	111	7.8	0.0121
Mean ⁴	25.31	103	0.0	
Check varieties				
'Nossapu'	24.47	100		
'Aurora'	21.36	87		
'Hokusei'	23.92	98		
$LSD_{0.05}^{5}$	2.08	8		

Table 4: General combining ability (GCA^1) for total dry matter yields (DMY) over two years (2002-2003) in a polycross progeny test and general genetic distances (GGD^2) within the parental clones of the polycross

¹GCA calculated by the following equation: $GCAi = [(p-1) / (p-2)] (Xi - \mu)$, where *p* is number of parents in a polycross, X*i* is DMY (percentage of 'Nosappu') of a polycross progeny *i*, and μ is means of DMY (percentage of 'Nosappu') of all polycross progenies, respectively. ²GGD calculated from genetic distances based on molecular marker diversity by methodology analogous to

²GGD calculated from genetic distances based on molecular marker diversity by methodology analogous to Griffing's Model I of Method 4 (Griffing 1956; Melchinger et al. 1990).

³The clone was excluded from the SSR analysis due to its non-existence.

⁴Mean of all forty-one polycross progenies.

⁵Least significant difference at the 0.05 probability level.

Table 5: Analysis variance of regression with general genetic distances (GGD) based on molecular marker diversity for total dry matter yields (DMY) of 40 timothy polycross progenies over two years (2002-2003)

Source of variance	df	Mean of squares	<i>F</i> -value	<i>P</i> -value
Plaak	2	72 169	22.01	<0.001
DIOCK	5	/2.108	52.91	<0.001
Among polycross progenies	39	5.259	2.40	< 0.001
Regression with GGD ¹	1	41.595	9.67	0.004
Residual	38	4.303	1.96	0.003
Error	117	2.193		
Total	159	4.266		

¹A regression with GGD based on molecular marker diversity among parental

clones for DMY of the polycross progenies.

Figure legends

Fig. 1: Scatter plot of the first two principle coordinate scales for eleven parental clones of the strains 'Kitakei 98301' and 'Kitakei 98303' which were developed by a synthetic variety method. PC1 and PC2 are the first two principle coordinates, respectively. The closed symbols show five parental clones of 'Kitakei 98301' and the open symbols show six parental clones of 'Kitakei 98303'.

Fig. 2: Relationship between general combining ability (GCA) for total dry matter yields (DMY) of polycross progenies and general genetic distances (GGD) of each parental clone within polycross. *r* denotes Pearson's correlation coefficient. ** indicates significance *r*-value (p < 0.01). GCA values calculated by the following equation: GCA*i* = [(p-1) / (p-2)] (X*i* – μ), where *p* is number of parents in a polycross, X*i* is DMY (percentage of 'Nosappu') of a polycross progeny *i*, and μ is means of DMY (percentage of 'Nosappu') of all polycross progenies, respectively. GGD values calculated from genetic distances based on molecular marker diversity by methodology analogous to Griffing's Model I of Method 4 (Griffing 1956; Melchinger et al. 1990).