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A Study on 'Genomewide Selection' for Maize (*Zea mays* L.) Breeding in Japanese Public Sectors: Heritability of Maturity- and Yield-Related Traits in the Training Populations

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Abstract

The authors have considered introducing a molecular breeding technique called 'genomewide selection (GwS)' to effectively improve the yield of maize for whole-crop silage use. The GwS for this purpose requires training populations (TPs) where individual plants having been developed from three-way crosses are examined in their phenotypes as well as in molecular-marker genotypes. The purpose of this study was to evaluate the (broad-sense) heritability of maturity- and yield-related traits in such TPs, because a former simulation study by the authors showed that the heritability should exceed 25% for the success of the GwS. Five preliminary TPs were grown to estimate the heritability of silking date, ear dry weight and culm length from 2011 to 2013 on two planting density levels, the higher of which was for productivity testing and the lower for accurate individual phenotyping. The results indicated that heritability exceeded 25% in all cases, and that the higher planting density did not result in lower heritability. Accordingly, it was concluded that the maize GwS to improve yield is sufficiently feasible in terms of maintaining high heritability in the TPs, and that planting density for TPs should be that for productivity testing because it is known to have remarkable interaction on yield with genotypes.

Discipline: Plant breeding
Additional key words: best linear unbiased prediction (BLUP), culm length, inbred line, planting density, silking date

Introduction

There are concerns in Japan over the difficulties in ensuring long-term food supply, which has prompted the Japanese government to promote a policy to boost food self-sufficiency. A primary measure for this goal is to raise the feed self-sufficiency rate from 26 (in 2011) to 38% (MAFF 2011). The Japanese public sectors, to which the authors belong, are now expected to support this policy by breeding high-yield maize (*Zea mays* L.) varieties for whole-crop silage use highly adapted to Japanese climates.

As the cost of molecular genotyping has rapidly declined in recent years (e.g. Yan et al. 2010), it has become feasible for maize breeding teams in the Japanese public sectors to adopt certain molecular breeding techniques, where markers should be arranged over the whole genome (Tamaki et al. 2012). 'Genomewide selection (GwS)' is one such technique, the details and concept of which are explained in Meuwissen et al. (2001) and Bernardo & Yu (2007). The focus of GwS is on accumulating favorable genes in many minor quantitative trait loci (QTLs) whereby the yield is deemed controlled (Yu & Buckler 2006), and can be started from a biparental population, i.e. with molecular-marker information on a small number of inbred lines (hereinafter referred to as inbreds). Accordingly, the authors considered introducing GwS to effectively improve yield because maize breeding teams of the Japanese public sectors have accumulated less molecular-marker information on their materials.

The purpose of this study was to evaluate heritability (in the broad sense, hereafter the same) of maturity- and yield-related traits (on a whole-crop basis) in training populations (TPs) for GwS. For decades, the maize breeding teams of the Japanese public sectors have adopted programs

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where hybrid variety candidates have been developed from 'D×F' (abbreviations for dent and flint genetic groups, respectively) combinations. Based on these breeding programs, it was assumed in the first computer simulation study by the authors (Tamaki et al. 2012) (1) that the breeding target was to develop a new dent inbred D_N, with high combining ability toward (i.e. eliciting a high yield in the hybrid) a specific flint tester inbred F_T, from a crossing of two existing inbreds D_1 and D_2, (2) that a TP was developed from a three-way cross '$(D_1 \times D_2) \times F_T$' to survey which of D_1 or D_2 would have higher combining ability toward F_T on each genome region represented by each molecular marker, and (3) that eight individual plants of '$(D_1 \times D_2)$' in F_2 generation are selected from 1000; based solely on the genotypic information obtained in (2). The simulations have shown that GwS can be a powerful tool to effectively improve yield provided the following two conditions are fulfilled: i.e. (1) if molecular markers can be arranged over the whole genome at intervals of 20cM or shorter, and (2) if heritability in the TP is 25% or higher. The subsequent study (Tamaki et al. 2014) has investigated in the feasibility of the first condition by surveying single nucleotide polymorphisms (SNPs) in the inbreds of the Japanese public sectors. The results have been very positive; thousands of polymorphisms have been found over the whole genome. Accordingly, in this study, the focus of the authors' interest has shifted to the second condition, namely heritability in TPs. In maize breeding teams of the Japanese public sectors, heritability evaluation has only been performed from the analyses of variance (ANOVAs) for productivity testing where varieties and/or strains are arranged in a randomized complete block design, meaning that it has never been estimated on the individual phenotyping basis as required in GwS. Investigation and discussion will also be made in the adequate planting density of TPs for this purpose, and optimal linear unbiased prediction (BLUP) equation to adjust the yield fluctuation among rows in a TP.

Materials and methods

1. Plant materials and investigated traits

Table 1 shows the details of the preliminary training populations (PTPs, where the molecular genotyping of each individual plant remains pending). Entries in a PTP can be classified into two groups in terms of development; three-way and single crosses. The latter is mainly for heritability calculation (or, more strictly, to evaluate the size of non-genetic variance); details of which will be described below. All the inbreds were developed in the Japanese public sectors; National Agriculture and Food Research Organization (NARO) Hokkaido Agricultural Research Center (NARO/HARC), Nagano Animal Industry Experiment Station (NAIES), NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC) and NARO Institute of Livestock and Grassland Science (NARO- ILGS) developed inbreds with names starting with "Ho", "Ki", "Mi" and "Na", respectively. The three PTPs containing "D", "11DL", "12DL" and "12DH", are for the assumed target to develop a new dent inbred, having high combining ability toward a flint inbred 'Na50', from two existing inbreds 'Mi29' and 'Na71'. These three inbreds are parents of registered commercial hybrid varieties (Ikegaya et al. 1999, Miki et al. 2011). The other two PTPs containing "F", "12FL" and "13FH" are for the assumed target to develop a new inbred, having high combining ability toward a dent inbred 'Mi88', from biparental crossings between a flint inbred 'Na101' and one of flint or semi-dent inbreds, 'Ho95', 'Ki75', 'Na50', 'Mi106' or 'Mi111', because the hybrid from 'Mi88' and 'Na101' has a very high yield level, despite the difficulty in handling 'Na101'. (Semi-dent genetic group, to which 'Mi106' belongs, differs from either dent or flint, and has been found in previous studies, including Tamaki et al. (2014), to be as genetically distant to the dent as the flint. Accordingly, it was expected in the beginning of this study that 'Mi106' would have the potential to have equivalent combining ability toward dent 'Mi88' to the other four flint inbreds).

All PTPs were grown in a field of NARO-ILGS at Nasushiobara (36°55'N, 139°56'E), to which 500 kg a^{-1} of manure was applied in early spring as well as chemical fertilizer containing 1.0 kg a^{-1} N + 1.0 kg a^{-1} P_2O_5 + 1.0 kg a^{-1} K_2O before the seeding. Seeds of all PTPs were sowed in the second half of May, following practical maize cultivation around NARO-ILGS. Each individual plant was harvested 43-45, 39-40 and 39-41 days after silking in 2011, 2012 and 2013, respectively, following the usual harvesting rules for productivity testing in the maize breeding program of NARO-ILGS.

As shown in Table 1, PTPs were grown at two planting density levels, whose inter-row×intra-row spacing were 75×30 and 75×20 cm, corresponding to 444 and 667 plants a^{-1}, respectively. In the maize breeding team of NARO-ILGS, where GwS operations are considered, the higher density is for productivity testing in line with the practical maize cultivation around NARO-ILGS, while the lower one is for individual selection (mainly for developing inbreds), where individual plants should be accurately phenotyped. Each PTP has a name ending in "H" and "L" to indicate higher and lower planting density, respectively.

The top of Table 3 shows the traits phenotyped in this study. Silking date and ear dry matter ratio are considered important indices for maturity, while culm length and basal stalk (60cm-long) dry weight (only in "13FH") are candidate indices for whole stover yield, which is important for whole-crop silage use. Details of these two traits will be described below.

Table 1. Details of preliminary training populations (PTPs) tested in this study

Year	Name	Entries					Planting density (plants a^{-1})	Date of	
		Three-way cross			Single cross (D×F)[1]			Seeding	Harvest (days after silking)
		Combination	Type[1]	Number	Combination	Number			
2011	11DL	(Mi29×Na71)×Na50	(D×D)×F	200	Mi29×Na50 Na71×Na50	32 each	444	May-18	43-45
2012	12DL	〃	〃	200	〃	32 each	444	May-25	39-40
	12DH	〃	〃	120	〃	20 each	667	〃	〃
	12FL	Mi88×(Ho95×Na101) Mi88×(Ki75×Na101) Mi88×(Na101×Na50) Mi88×(Na101×Mi111) Mi88×(Na101×Mi106[2])	D×(F×F)	30 each	Mi88×Ho95 Mi88×Ki75 Mi88×Na50 Mi88×Na101 Mi88×Mi111 Mi88×Mi106[2]	15 each	444	〃	〃
2013	13FH	Mi88×(Ki75×Na101) Mi88×(Na101×Na50)	〃	202 207	Mi88×Ki75 Mi88×Na50 Mi88×Na101	27 each	667	May-17	39-41

[1] D and F are abbreviations for dent and flint genetic groups, respectively.

[2] ‘Mi106’ is defined as a flint genetic group member in this study, though it belongs to the semi-dent group, different from either dent or flint.

2. Heritability estimation

The heritability (in the broad sense) in a PTP, H_B^2, where phenotyped were N_3 entry(ies) developed from three-way cross(es) and N_S single cross entries, was estimated in this study from the following equation:

$$H_B^2 = 1 - \frac{\dfrac{\sum_{L=1}^{N_S} v_S(L)}{N_S}}{\dfrac{\sum_{K=1}^{N_3} v_3(K)}{N_3}} \qquad \dots(1)$$

where $v_S(L)$ and $v_3(K)$ are unbiased variances of the L-th ($1 < L \leq N_S$) single cross entry and of the K-th ($1 \leq K \leq N_3$) entry developed from three-way cross, respectively. Equation (1) is based on the assumption of negligible genetic variance within a single cross entry.

3. Assumption of non-genetic yield fluctuation among rows in a field test

In the former simulation study (Tamaki et al. 2012), the authors assumed that rows in a TP (i.e. a test field) would have fluctuating environmental values on yield, which could be adjusted with the following best linear unbiased prediction (BLUP) equation

$$y = X\beta + Zw + e \qquad \dots(2)$$

where y is an $N_I \times 1$ vector for the phenotypic data of the N_I individual plants, X is an $N_I \times N_R$ design matrix to express which individual plant is located on which row in the TP, β is an $N_R \times 1$ unknown vector for environmental values of the N_R rows, Z is an $N_I \times N_M$ design matrix to express which individual plant has a molecular marker derived from which parent on which locus, w is an $N_M \times 1$ unknown vector for breeding values of the N_M markers, and e is an $N_I \times 1$ vector for residual effects.

For the purpose of preliminary verification of these assumptions, the authors adopted the following equation modified from equation (2) in this study.

$$y = X\beta + Z'w' + e \qquad \dots(3)$$

where Z' is an $N_I \times (N_3 + N_S)$ design matrix to express which individual plant belongs to which entry in the PTP, and w' is an $(N_3 + N_S) \times 1$ unknown vector for (average) values of $(N_3 + N_S)$ entries (efforts have been made to establish each PTP for well-balanced material arrangement so that this BLUP matrix equation can be solved smoothly). From equation (3), we can obtain a vector for the best linear unbiased estimation (BLUE) values, or the estimated yield potential of each row, $\hat{\beta}$. In case $\hat{\beta}$ would effectively reflect the non-genetic yield fluctuation among the rows, this adjustment would remarkably raise the heritability. This adjustment was examined in terms of ear dry weight and basal stalk dry weight, but not culm length because its correlation to whole stover weight has been found to be relatively low (as described below).

4. Evaluation of candidate indices for whole stover yield

As mentioned above, two traits were adopted in this study as candidate indices to predict whole stover yield, culm length and basal stalk dry weight. Here, they have been compared in terms of the accuracy of prediction, because neither has ever been evaluated as indices for this purpose. In 40 individual plants of "13FH", the whole stover of which was kept intact on harvest, the sampled contents included not only basal stalks but also other stover parts to compute their whole stover weight. The samples were then weighed after drying with a hot-air dryer (70 degrees centigrade) for more than 10 days.

Results and discussion

Table 2 shows the calculated heritability of ear and basal stalk dry weight with and without adjustment by the BLUP equation (3). The adjustment saw the heritability of the ear dry weight in "11DL" soar from 19.0 to 31.4%, but decline somewhat in other cases where heritability was calculated as exceeding 40% without the adjustment. Based on these results, the authors decided to adopt this adjustment only for the ear dry weight of "11DL". Though more examples are required for convincing discussion, the authors currently estimate that the phenotypic fluctuation among the rows mainly reflects genetic and non-genetic factors in the cases of high (>40%) and low (<20%) heritability (without adjustment), respectively, and that the BLUP adjustment can only work well in the latter.

As shown in Table 3, the heritability ranged from 31.4 to 88.8%, i.e. exceeded the threshold for the success of GwS which emerged in the former simulation study (Tamaki et al. 2012), 25%, in all cases. It is interesting that higher planting density did not result in lower heritability, contrary to the assumptions the authors made beforehand that the higher density would trigger more severe intervention among individual plants, which would then lead to larger non-genetic variance and lower heritability. It has been concluded from these results that the planting density for TPs should be as high as that for productivity testing because it is well known to have remarkable interaction on yield with materials (genotypes) (e.g. Koinuma et al. 2004), and that the maize GwS to effectively improve yield remains sufficiently feasible in terms of maintaining high heritability in the TPs. Also considering numerous molecular-marker polymorphisms over the whole genome of the inbreds (Tamaki et al. 2014) as well as the recent rapid decline in the cost of molecular genotyping, maize GwS can be a powerful tool in the near future to accelerate yield breeding; not only in the Japanese public sector but also other breeding organizations having accumulated little information on molecular marker polymorphisms of their parental inbreds.

Fig. 1, showing the correlations of whole stover weight with its two candidate indices i.e. basal stalk dry weight (Fig. 1a) and culm length (Fig. 1b), clearly indicates that the former exceeds the latter. Though basal stalk dry weight has never been considered in maize breeding as an index for whole stover yield, these results suggest that it can be a powerful tool in the future, not only for GwS but also for productivity testing.

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Table 2. Calculated heritability (%) with and without BLUP[1)] adjustment, and the ratio of population variance among the ridges

Trait	Ear dry weight					BSDW[2)]
PTP[3)]	11DL	12DL	12DH	12FL	13FH	13FH
Calculated heritability						
without BLUP	19.0	42.2	53.5	48.1	55.4	49.2
with BLUP	31.4	39.4	47.3	45.2	50.0	47.4
Adoption of the adjustment	✓	–	–	–	–	–

1) Best linear unbiased prediction. See equation (3) in the text.
2) Basal stalk dry weight
3) Preliminary training population

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Table 3. Field tests results and calculated heritability

PTP1) and entry / trait (unit)			Silking date (days after June 30)		Ear dry matter ratio (%)		Ear dry weight (g individual^{-1})		Culm length (cm)		Basal stalk dry weight (g individual^{-1})	
			Avg.2)	USD3)	Avg.2)	USD3)	Avg.2)	USD3)	Avg.2)	USD3)	Avg.2)	USD3)
11DL	Three-way	(Mi29×Na71)×Na50	34.3	2.208	52.1	3.425	*214*	*43.24*	303	19.84		
	Single cross	Mi29×Na50	36.1	1.370	53.0	2.039	*222*	*36.02*	302	12.85		
		Na71×Na50	33.9	2.014	52.1	2.875	*210*	*35.62*	302	13.23		
	Heritability (%)		39.2		47.0		*31.4*		56.8			
12DL	Three-way	(Mi29×Na71)×Na50	34.9	1.606	50.0	3.659	224	32.48	315	16.93		
	Single cross	Mi29×Na50	36.7	1.274	49.3	1.235	226	24.30	316	8.20		
		Na71×Na50	34.4	1.305	48.8	1.211	203	25.08	312	10.80		
	Heritability (%)		35.5		88.8		42.2		67.9			
12DH	Three-Way	(Mi29×Na71)×Na50	35.1	1.821	47.9	2.973	171	41.99	319	16.00		
	Single cross	Mi29×Na50	37.4	0.966	48.5	1.355	176	25.84	318	5.97		
		Na71×Na50	35.3	1.421	47.9	2.587	136	31.20	309	7.50		
	Heritability (%)		55.5		51.8		53.5		82.1			
12FL	Three-way	Mi88×(Ho95×Na101)	32.6	2.230	52.5	2.029	172	18.45	303	21.40		
		Mi88×(Ki75×Na101)	33.1	1.262	53.2	2.022	186	21.62	317	18.48		
		Mi88×(Na101×Na50)	35.6	1.502	51.9	1.927	191	30.99	319	15.32		
		Mi88×(Na101×Mi111)	36.2	1.204	52.4	3.806	187	25.61	318	13.37		
		Mi88×(Na101×Mi106)	35.5	1.351	52.5	2.153	170	26.03	305	14.85		
	Single cross	Mi88×Ho95	30.2	1.401	51.7	1.399	145	19.18	280	9.90		
		Mi88×Ki75	31.8	1.229	51.9	3.320	158	29.38	301	13.35		
		Mi88×Na50	35.6	0.996	52.6	1.137	204	10.50	308	9.68		
		Mi88×Na101	34.6	1.016	52.9	1.089	216	13.04	320	12.03		
		Mi88×Mi111	34.6	1.158	51.6	1.691	162	13.50	309	13.08		
		Mi88×Mi106	35.2	1.168	53.0	1.877	156	15.41	286	10.22		
	Heritability (%)		43.5		41.4		48.1		54.1			
13FH	Three-way	Mi88×(Ki75×Na101)	23.5	2.103	51.0	1.894	145	31.32	317	18.65	30.7	7.440
		Mi88×(Na101×Na50)	26.1	2.360	50.9	1.760	171	31.56	323	19.11	32.4	6.778
	Single cross	Mi88×Ki75	21.3	1.288	50.7	1.716	105	21.09	309	16.34	24.3	4.983
		Mi88×Na50	25.9	0.933	50.4	0.989	176	17.64	322	13.31	29.3	3.145
		Mi88×Na101	25.0	1.214	51.2	0.863	193	23.81	329	11.94	36.5	6.520
	Heritability (%)		73.3		53.5		55.4		45.1		49.2	

Italic letters indicate the results with the best linear unbiased prediction (BLUP) adjustment shown in the text.

1) Preliminary training population

2) Average

3) Unbiased standard deviation

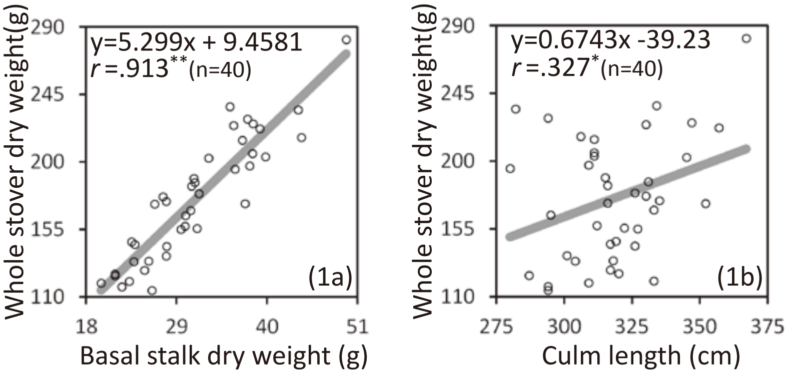

Fig. 1. The relationships of whole stover dry weight in the preliminary training population "13FH" with basal stalk (60cm-long) dry weight (1a) and with culm length (1b)

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