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Agronomic evaluation of inbred lines derived from tissue cultures of maize*

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Summary. Tissue culture-induced variation has been proposed as a novel source of variation for crop improvement. In maize (*Zea mays* L.), chromosome aberrations and qualitative genetic variants have been induced during in vitro culture. The proportion of regenerated plants carrying such variants has been shown to increase with culture age. The objective of this research was to evaluate the relationship between culture age and somaclonal variation for several agronomic traits. Six sib-pollinated ears of S_0 (F_2) plants in four OH43 *ms*/A188 populations each provided control seed and embryos for culture initiation. S_2 lines derived from control seed and from plants regenerated 4 and 8 months after culture initiation were grouped according to their source ear and grown in 6 separate trials. A total of 305 tissue culture-derived and 48 control lines were evaluated as lines per se and in a testcross at each of three locations. Tissue culture-derived lines and their testcrosses generally had lower grain yield and moisture. Since grain yield and moisture were not positively correlated in any trial, the highest yielding lines could be selected without increasing grain moisture. Grain yield and plant height tended to decrease with culture age. Although tissue culture-derived lines were, on average, inferior, the highest yielding line per se in three of six trials and the top-ranked line in five of six trials for yield and moisture were derived from tissue culture. The results indicate that tissue culture may generate variation for agronomic traits. Some of the variation, particularly the trend towards earlier maturity, could be useful. However, this method may require screening large populations because of the tendency to generate a large proportion of inferior lines.

Key words: *Zea mays* L. – Somaclonal variation – Agronomic traits – Tissue culture age – Earliness

Introduction

The propensity of plant tissue culture for generating genetic variation has been well documented (Larkin and Scowcroft 1981; Orton 1983). Larkin and Scowcroft (1981) suggested that this source of variation could be a useful adjunct to plant breeding programs. While culture-induced variation for agronomic traits has been reported in several crop species (Larkin and Scowcroft 1981; Orton 1983), its utility has not been firmly established.

Information regarding somaclonal variation for agronomic traits in maize (*Zea mays* L.) has been limited. Beckert et al. (1983) evaluated progeny of regenerated plants of several inbreds in a single year location and reported significant variation for several agronomic traits. However, they emphasized that the magnitude of the variation was small. Earle and Gracen (1985) also reported variation for several traits among progeny of regenerated plants of a single inbred.

In maize, tissue culture-induced variation has been more thoroughly described in terms of qualitative and cytological variation. Progeny of regenerated maize plants often segregate for variants inherited as single gene recessives (Edallo et al. 1981; McCoy and Phillips

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1982; Benzion 1984; Armstrong and Phillips 1988; Lee and Phillips 1987a), with estimates ranging from 0.3 (McCoy and Phillips 1982) to 1.3 (Lee and Phillips 1988) mutations per regenerated plant. Cytological examinations of regenerated plants have detected changes in chromosome number and structure with the latter predominating (Benzion 1984; Armstrong 1986; Rhodes et al. 1986; Lee and Phillips 1987). The detected proportion of cytologically abnormal plants has ranged from 2/110 (mitotic analyses of Edallo et al. 1981) to 119/257 (meiotic analyses of Rhodes et al. 1986). Variation in the mitochondrial genome in T cytoplasm has also been described (Pring et al. 1981).

The potential influence of several biological (e.g., genotype, explant tissue) and environmental factors on culture-induced variation has been noted, but general principles have not emerged (Orton 1983). With regard to maize, the data for qualitative and cytological variation indicate that culture age may be an important consideration. Lee and Phillips (1984) reported that the number of variants per regenerated plant and the proportion of regenerated plants with progeny segregating for variants were higher for plants regenerated from older cultures. Armstrong (1986) also observed an increase in the proportion of regenerated plants with segregating progeny as culture age increased. A similar tendency was noted by Benzion (1984), who suggested that the apparent age effect could be due to mutations accumulating within cultured cells and not an increased mutation rate in older cultures. There is also some evidence that mutations accumulate in callus cells in rice (Fukui 1983). Results bearing on the relationship between culture age and the recovery of cytologically abnormal plants from maize tissue cultures have been contradictory. The percentage of cytologically abnormal regenerated plants was observed to increase with culture age in several studies (Benzion 1984; Armstrong 1986; Lee and Phillips 1987). In contrast, McCoy and Phillips (1982) and Rhodes et al. (1986) did not observe a relationship between culture age and the percentage of cytologically abnormal regenerated plants.

An association between culture age and variation for agronomic traits has not been observed directly, but there has been some circumstantial evidence. Second-cycle doubled haploid lines have exhibited additional yield declines in comparison to first-cycle doubled haploids in *Nicotiana tabacum* L. (Brown et al. 1983) and *Nicotiana sylvestris* L. (De Paepe et al. 1981). Wenzel et al. (1979) observed increased phenotypic variation among protoplast-derived potato plants from older cultures.

In this report we describe the agronomic performance of S_2 lines derived from maize plants regenerated 4 and 8 months after culture initiation. The objective of this study was to evaluate the relationship between culture age and tissue culture-induced variation for several agronomic traits.

Materials and methods

Male-sterile plants in inbred Oh43 isolines segregating for recessive nuclear male-sterility genes (ms7, ms8, ms10, ms13) were pollinated with inbred A188. The male-sterility genes were included for an objective of another study. The F_1 plants were self-pollinated to produce F_2 family rows.

Immature embryos (1.5–3.0 mm long) from ears of sib-pollinated male-sterile F_2 plants were the explants for each male-sterility strain. One-half of an ear provided embryos for culture initiation and the other half was left on the plant to produce control seed. Organogenic callus cultures were initiated and maintained according to the procedures of Green and Phillips (1975) using media supplemented with 0.75 mg^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Subcultures were transferred to fresh media every 3–4 weeks. Pedigree records were kept that maintained the identity of regenerated plants, cultures and their respective source embryos.

Four months after culture initiation, plants were regenerated from 18 cultures representing 18 embryos from 6 ears. One plant was regenerated from each culture at this time. Eight months after culture initiation, plants were regenerated from 12 cultures, including 4 cultures that also produced plants at 4 months. The number of plants produced per culture during the second cycle of plant regeneration ranged from 1–10.

Regenerated plants (R0) were screened for pollen sterility using a pocket microscope (30×, Nikon) and chromosome aberrations were detected through meiotic analysis of microsporocytes. Plants with normal levels of pollen sterility (0–10%) were selfpollinated to produce the R1 generation. Within each R1 progeny, at least five plants were self-pollinated to produce the R2 generation. R1 and R2 progeny were screened under field conditions for segregating qualitative variants. Five R2 progeny rows per regenerated plant were selected for further evaluation on the basis of adequate seed production and absence of segregating qualitative variants. In addition, eight independently-derived S_2 lines were produced from control seed by single seed descent from each of the six source ears. For each source ear, lines derived from regenerated plants and control seed were at the same level of inbreeding. All lines were, in effect, the equivalent of an F_4 generation, and therefore highly homozygous ($F=0.875$). For the purpose of this report, the symbols R0, R1 and R2 have been replaced by S_0, S_1 and S_2, respectively.

Each S_2 line was increased by making five sib-pollinations within lines using a plant once as either male or female. Lines were also crossed as males to a related-line single cross tester, A641×CM105.

This study was conducted at three locations in Minnesota during the 1985 growing season: Rosemount, Waseca and Lamberton. Management practices consistent with high yield maize production for the region were followed at each location.

Lines derived from control seed and regenerated plants from each of the six ears were evaluated in six separate trials due to anticipated differences in maturity and vigor. The same procedure was followed for testcross evaluation. Each trial consisted of eight control lines plus lines derived from plants regenerated 4 and 8 months after culture initiation, to give

Table 1. No. of maize lines from each tissue culture age group in individual trials

Trial	No. of control lines	No. of lines derived from regenerated plants	
		4 months	8 months
1	8	10	60
2	8	10	35
3	8	25	50
4	8	10	35
5	8	20	5
6	8	15	30
Totals	48	90	215

three culture age groups in each trial (Table 1) all tracing back to the same ear. Each regenerated plant was represented by five S_2 lines.

Materials in each trial were planted in a randomized complete block design with two replications per location. One-row plots (line per se evaluation) and two-row plots (testcross evaluation) were machine-planted with 76 cm between rows and were hand-thinned to approximately 32 plants per row (57,400 plants ha^{-1}). Lines per se were hand-harvested and testcrosses were machine-harvested.

For line evaluation, the following traits were measured on a plot basis at all locations: grain yield adjusted to a perfect stand and 15.5% moisture, plant height (distance from soil level to flag leaf collar of five plants per plot), combined root and stalk lodging (percent plants leaning more than 30° from vertical and percent plants with stalk broken below uppermost ear), dates of 50% flowering (pollen shed and silk emergence) and grain moisture at harvest. The weight of a 200-kernel sample of grain per plot was measured at Lamberton and Waseca only. For testcross evaluation, grain yield, moisture and lodging (all as previously defined) were recorded at each location.

Combined location analyses of variance were computed for each trial to evaluate differences due to culture age. For all analyses, S_2 lines and culture age were considered fixed effects and environmental factors were assumed to be random. When culture age was found to be significant according to appropriate *F*-tests, Fisher's L.S.D. (Steel and Torrie 1980) was calculated.

Results and discussion

There was significant cytological and qualitative variation among regenerated plants and their progeny (Lee and Phillips 1987, 1987a). In addition, many inbred lines derived from regenerated plants were characterized by poor seed set, independent of cytological and qualitative variation. Thus, prescreening for cytological and qualitative variants and for adequate seed production reduced the number of tissue culture-derived lines in the trials from 1,240 to 305 (five lines for each of 61 regenerated plants). In spite of this prescreening, substantial variation for agronomic traits was observed.

There was significant variation among culture age groups for yield in five of the six trials with S_2 lines (analyses of variance not shown). Significant variation also existed among culture age groups for grain moisture and kernel weight (four of six trials), days to pollen shed and silk emergence (all trials) and plant height (all trials). Significant culture age × location effects were not large and were primarily due to changes in magnitude of the differences between culture age groups rather than changes in rank.

Mean grain yields of regenerated groups were generally lower than their respective controls. Culture age group means for S_2 lines combined over locations are presented in Table 2. In three trials, 4-month groups were significantly lower yielding than control groups by 13%–35%. Eight-month groups had significantly lower yields than controls in five trials by 18%–44%. Eight-month groups were also significantly lower yielding than 4-month groups in five trials by 5%–35%. The pattern of yield reductions observed in this study indicate there was a tendency for yield to decrease with culture age. Similar reductions in grain yield were reported by Baenziger et al. (1983) for anther-derived doubled haploids in wheat.

Mean plant height also declined with culture age. Four-month groups were significantly shorter than controls in five trials by 4–24 cm. Eight-month groups were shorter than controls in all trials by 8–30 cm. In four trials, 4-month group means exceeded 8-month group means by 5–13 cm. The variation did not appear to be qualitative in nature. Short stature variants have also been reported in rice (Oono 1981; Schaeffer 1982) and tobacco (Burk and Matzinger 1976).

The tendency for grain yield and plant height to decline with increasing culture age implies that variation may be induced throughout the period of in vitro culture. In maize, results from several studies (Benzion 1984; Lee and Phillips 1988; Armstrong 1986) have shown that the proportion of regenerated plants carrying qualitatively-inherited variants tends to increase with culture age. The nature of this culture age effect has not been firmly established. Mutations may be continuously induced throughout the duration of in vitro culture or, alternatively, most mutations may occur early in the culture period in cells that subsequently give rise to a large proportion of the callus, and thus a large proportion of regenerated plants. Evidence for continuous induction of mutations was reported by Fukui (1983) in rice callus cultures. Evidence for the alternative hypothesis was reported by Benzion (1984). Through extensive pedigree analysis, Benzion documented that some phenotypically similar variants were clonally related in the callus at an early stage of in vitro culture. Thus, variants regenerated after several months in culture may be traced to a single mutational event that occurred early in the culture phase. It appears that mutations affecting grain yield and plant

Table 2. Tissue culture age group means from S_2 maize lines for several agronomic traits

Trial	Culture age	No. of lines	Grain yield (Mg ha^{-1})	Grain moisture (%)	Pollen shed (days)	Silking (days)	Plant height (cm)	Lodging (%)	Kernel weight (g)
1	0	8	5.65	36.7	77.4	78.8	151	15.3	40.64
	4	10	4.58	33.0	75.7	77.5	144	28.5	38.93
	8	60	5.00	33.3	76.5	77.5	142	21.3	40.11
LSD 0.05			1.00	1.6	1.1	0.5	3	4.7	2.55
2	0	8	4.63	41.4	80.9	82.3	151	25.8	39.34
	4	10	3.74	38.7	78.7	80.8	127	16.4	40.14
	8	35	2.85	39.2	80.3	82.3	121	17.9	38.26
LSD 0.05			0.46	0.6	0.6	0.9	3	12.5	0.72
3	0	8	4.59	35.3	78.3	80.4	160	9.9	39.27
	4	25	4.60	36.3	77.8	79.9	153	13.5	38.76
	8	50	3.11	36.4	79.2	81.4	148	6.8	39.48
LSD 0.05			0.44	1.4	1.3	1.2	5	2.4	0.73
4	0	8	4.51	37.2	79.9	82.3	167	9.9	36.96
	4	10	2.94	35.8	80.2	82.5	155	19.5	35.34
	8	35	2.53	37.9	81.8	84.1	159	22.6	33.38
LSD 0.05			0.41	3.0	1.3	0.5	2	14.5	0.72
5	0	8	5.03	32.9	76.9	78.4	134	31.0	35.60
	4	20	4.90	29.4	75.2	77.2	130	24.8	37.14
	8	5	3.23	30.3	75.1	78.1	117	13.3	33.15
LSD 0.05			0.78	0.7	1.4	1.6	7	22.5	1.29
6	0	8	5.06	34.2	79.3	80.7	140	13.2	37.78
	4	15	4.41	30.9	75.1	76.9	126	16.1	37.11
	8	30	4.17	31.1	75.2	76.5	128	15.1	38.01
LSD 0.05			0.17	0.8	0.8	0.4	8	4.9	0.78

height occurred within the initial 4 months of in vitro culture and that subsequent mutations were induced during the following 4 months.

Culture age means for remaining traits did not follow the trends observed for grain yield and plant height. Mean grain moisture for 4- and 8-month groups were similar in most trials and both groups were generally lower than the controls by 2.1%–3.7%. The trend towards lower grain moisture was reflected by the generally lower mean number of days to pollen shed and silk emergence for regenerated groups. The earliest flowering group in each trial was derived from tissue culture. In addition, grain moisture was positively correlated with number of days to pollen shed and silk emergence (not shown). Regenerated groups also had the lowest mean kernel weights in all trials, although mean kernel weights did not change markedly with culture age. Significant differences among culture age group means for lodging were detected in only two trials. In each case, the 4-month group showed the greatest lodging. Although means for these traits did not exhibit a clear trend with culture age, results for grain moisture, flowering dates and kernel weight indicate that mutations affecting these traits may have occurred within 4 months of culture initiation.

The tendency of tissue culture-derived lines to flower and mature earlier may relate to genomic alterations known to occur in maize callus cultures. Meiotic analysis of regenerated maize plants has identified chromosome breakage and rearrangement as the predominant source of cytological variation (Rhodes et al. 1986). More detailed analysis has indicated that most of the breakage events could be initiated by late replication of knob heterochromatin (Benzion et al. 1986; Lee and Phillips 1987). Such a mechanism could also produce changes in the knob constitution of regenerated plants (Lee and Phillips 1987). The changes in knob constitution could range complete elimination of knobs, which has been observed (Benzion 1984), to subtle changes in knob size.

In maize, several studies have noted a relationship between knob constitution, DNA C-value and geographic distribution of germplasm. Knob number has been shown to be negatively correlated with latitude and altitude (Brown 1949; Bennett 1976; Bennett 1987). DNA C-values have also been shown to be negatively correlated with latitude (Rayburn et al. 1985). These relationships may reflect a response by the maize genome to selection for earlier flowering and maturity in higher latitudes (Bennett 1985). One result

of such selection could be a reduction in the amount of heterochromatin, since DNA C-value has been shown to be positively correlated with C-band heterochromatin and knob number (Rayburn et al. 1985). Chughtai and Steffensen (1987) have reported that each additional homozygous knob combination delays plant development and flowering by 3–4 days. Earliness among tissue culture-derived lines may result from the reduction of knob heterochromatin through chromosome breakage events.

In a related study, the frequency of regenerated plants containing a chromosome aberration increased from 0%–48% from 4–8 months in culture (Lee and Phillips 1987). The same cultures were used to produce plants for cytological and agronomic evaluation. Meiotic analysis revealed that most aberrations were due to chromosome breakage and rearrangement of arms containing large blocks of heterochromatin (knobs or nucleolus organizer region). The location of breakpoints suggested that late replication of heterochromatin during the mitotic cell cycles of callus cells could generate gross chromosome aberrations (translocations, deficiencies and duplications) as well as more subtle variations (change in sequence copy number and heterochromatin content). Regenerated plants used for agronomic studies were cytologically normal as determined by meiotic analysis or pollen abortion frequencies. These techniques would not detect many genomic changes known to occur in tissue cultures such as changes in C-banding patterns (Ashmore and Gould 1981; Lapitan et al. 1984) or copy number changes of highly repetitive sequences (Cullis and Cleary 1985). The maize genome has been shown to be capable of such modulation (Bennett 1985; Rivin et al. 1986) but this has not been investigated in tissue culture-derived material.

The production of earlier lines from tissue culture has also been observed in sorghum (R. Smith, personal communication). However, derivation of earlier lines may be restricted to certain genetic backgrounds. In our study, both parents Oh43 and A188 contained a relatively high number of knobs (five and four, respectively) for United States inbred lines. Therefore, this genetic background may have a greater propensity for heterochromatin-related changes to occur in tissue culture. This may also account for some of the observed genotypic differences for the occurrence of mutations during tissue culture (Williams and Widholm 1986).

Frequency distributions of S_2 line grain yields are presented in Table 3. Each culture age group appeared to have a different distribution pattern. Of the 8-month lines, 55%–100% were below their trial mean. In most trials, this group also had the greatest range of grain yields. Four-month lines were more evenly distributed. In contrast, 50%–88% of the control lines in each trial yielded above the mean. The proportion of lines three standard errors above the trial mean decreased with culture age.

Despite the generally poor yields of lines derived from tissue culture, some individual lines yielded well. S_2 lines within each trial were ranked according to mean yield over locations and by a rank summation index using grain yield and moisture (not shown). The highest yielding line in three trials and the highest yielding line over all trials were lines derived from tissue culture. At least four of the ten highest-yielding lines in each trial were derived from tisue culture. Based on the rank summation index, the top line in five trials and at least seven of the top ten in each trial were derived from tissue culture. This was roughly equivalent to the proportion of S_2 lines derived from tissue culture in each trial (76%–90%). These results indicate that agronomically acceptable lines may be derived from tissue culture. However, use of this method may require screening large populations because of the tendency to generate a large proportion of inferior lines, especially from older cultures.

Several cultures produced plants during both cycles of plant regeneration. Comparisons among these clonally-related lines permits a more direct assessment of the extent of somaclonal variation and the effect of increased culture age (Table 4). For this purpose, inbred lines were grouped according to their source regenerated plant and the mean performance for each group of five lines was used to estimate the genetic potential of each regenerated plant. There were significant differences among clonally-related, regenerated plant means within culture age groups and between culture age groups. For example, culture 4–41 produced three regenerated plants 4 months after culture initiation and one regenerated plant 8 months after culture initiation. Within the 4-month group, regenerated plant means were significantly different for grain yield and plant height. Although the differences were small (3% and 5% for plant height and grain yield, respectively), the results indicate that variation for these traits was induced within the initial 4 months in culture. The differences between the 4- and 8-month means were substantially larger. For culture 4-41, the plant from the second period of plant regeneration produced inbred lines that averaged 33%–37% lower grain yield and a 9%–11% height reduction. Similar observations were noted for clonally-related lines from cultures 1-42 and 6-42. The only exception to the trend of decreased grain yield and plant height was plant 13 b from culture 6-42. Mean performance of inbred lines derived from that plant was not significantly different from lines obtained from plant 1, regenerated 4 months after culture initiation. The remaining plants regenerated 8 months after culture initiation produced inbred lines with much

Table 3. Frequency distributions of S_2 line grain yields

Trial	Mean ± SE[a]	Culture age (months)	Range	Class center (Mg ha^{-1})											
				2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25	7.75	8.25
1	5.01 ± 0.11	0	4.59–6.70					1	2	3	1	1			
		4	3.58–6.13			3	1	3	2		1				
		8	2.81–7.76	1		5	9	21	14	2	4			1	3
				1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75
2	3.29–0.20	0	2.29–6.91				1	1		2	1		1		2
		4	1.10–6.03	1		2	1			3	2			1	
		8	1.35–5.76	2	7	9	3	5	4		3	1	1		
				0.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25
3	3.71 ± 0.14	0	2.88–5.30					1			2	2	3		
		4	2.60–6.32					1	4	3	5	1	3	5	3
		8	0.78–4.67	2	1	6	7	5	8	6	11	4			
				0.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25
4	2.91 ± 0.14	0	2.90–5.19					1	1		1	2	2	1	
		4	2.23–4.30				3	3	2	1	1				
		8	0.77–4.38	1	2	5	10	8	6	2	1				
				2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75			
5	4.68 ± 0.15	0	3.29–6.60		1		1	2	1	2		1			
		4	4.24–5.81				4	8	5	3					
		8	2.62–3.67	2	2	1									
				1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25
6	4.37 ± 0.15	0	3.50–7.51					1	2	2	1	1			1
		4	3.54–5.24					5	3	3	4				
		8	2.43–8.00		1	2	4	9	4	4	3		2		1

[a] Overall trial mean and SE

Table 4. Mean performance of S_2 lines derived from clonally-related regenerated plants for agronomic traits

Trial	Culture	Regenerated plant	Culture age (months)	Grain yield	Flowering date		Grain moisture	Plant height
					Pollen	Silk		
5	4-41	1	4	5.11	76	78	29.1	131
	4-41	2	4	4.92	75	77	30.1	128
	4-41	3	4	4.85	75	77	28.5	132
	4-41	4	8	3.23	75	78	30.3	117
LSD (0.05) among regenerated plant means				0.15	0.3	0.3	1.7	2
6	1-42	1	4	4.64	76	77	31.5	123
	1-42	2	8	3.31	76	78	32.6	121
LSD (0.05) among regenerated plant means				0.27	0.3	0.3	1.4	2
6	6-42	1	4	4.70	75	76	33.2	131
	6-42	2a	8	4.00	75	77	29.6	123
	6-42	9	8	3.50	74	76	28.9	115
	6-42	13a	8	3.67	78	77	32.8	135
	6-42	13b	8	4.89	75	76	30.7	131
LSD (0.05) among regenerated plant means				0.27	0.3	0.3	1.4	2

lower performance. Apparently, the genetic content of plant 13b was quite different from clonally-related plants, even very closely related plants such as 13a. Plants 13a and 13b were twin plants from the same piece of callus, yet the average performance of their inbred lines differed for all traits. This example illustrates the extensive genetic variation that can exist between clonally-related plants and their sexual progenies.

There was significant variation among culture age groups for testcross grain yield in three trials (analyses of variance not shown). There was also significant

variation among culture age groups for grain moisture (five of six trials) and lodging (four of six trials) of testcrosses.

Regenerated groups tended to have lower mean testcross grain yields than control groups (Table 5). Grain yield of 4-month groups were significantly lower than controls by 6%–7% in two trials. Eight-month groups had significantly lower yields than controls by 5%–6% in three trials. In general, grain yields of the 4- and 8-month groups were not significantly different from each other. In contrast to group means of S_2 lines for grain yield, there were fewer and smaller differences among culture age groups for testcrosses. This could be due to masking effects of dominant favorable alleles in the tester.

Mean grain moisture of 4-month groups were significantly lower than controls in four trials by 1.1%–1.3%. Eight-month groups had significantly lower grain moisture than controls in three trials by 0.8%–1.1%. In general, regenerated groups were not significantly different for grain moisture.

Culture age group means for grain yield and moisture followed similar trends in testcross and S_2 line evaluations: regenerated groups tended to have lower means for both traits. Phenotypic correlations between S_2 and testcross performance for yield, moisture and lodging were calculated using genotype means over locations (not shown). Despite the similar trends for culture age group means, grain yield of S_2 lines was not significantly correlated with testcross yield in any trial. S_2-testcross correlations for grain moisture and lodging were positive and significant ($P < 0.01$) in most trials, but the magnitude of the correlation coefficients was generally low (< 0.70).

Grain yield was negatively correlated with moisture in all trials, but the magnitude of the correlation coefficients was not large, ranging from –0.07 to –0.57. Grain yield was also negatively correlated with days to silk emergence in four trials (–0.35 to –0.75). This relationship may have resulted from the relatively cool growing conditions and shorter season, which would prevent later-flowering lines from completing a normal grain-filling period.

Mean grain yield and moisture for the highest and lowest yielding 10% of the lines in each trial are presented in Table 6. In each trial, the mean grain yield of the high group was 2–4 times greater than the low group without adverse changes in grain moisture. The weak relationship between grain yield and moisture indicates that higher yielding lines could be selected without increasing grain moisture.

The results indicate that tissue culture may generate variation for agronomic traits. In addition, the numerous differences between culture age groups in most trials support the hypothesis that variation was gen-

Table 5. Tissue culture age group means from testcrosses for several agronomic traits

Trial	Culture age	No. of lines	Grain yield (Mg ha^{-1})	Grain moisture (%)	Lodging (%)
1	0	8	9.07	20.5	22.0
	4	10	8.54	19.3	28.8
	8	60	8.66	20.1	23.2
LSD 0.05			0.31	0.5	3.5
2	0	8	7.95	23.7	19.0
	4	10	7.94	22.4	22.3
	8	35	7.51	22.7	18.6
LSD 0.05			0.25	0.4	8.8
3	0	8	8.02	20.5	16.4
	4	25	8.15	21.0	20.3
	8	50	8.05	20.3	15.5
LSD 0.05			0.68	0.3	10.5
4	0	8	7.63	23.8	16.3
	4	10	7.75	23.4	21.9
	8	35	7.55	23.1	20.4
LSD 0.05			0.28	1.4	3.5
5	0	8	8.26	20.8	16.8
	4	20	8.22	19.6	15.8
	8	5	8.11	20.0	11.0
LSD 0.05			0.75	0.7	4.0
6	0	8	8.47	21.5	17.0
	4	15	7.83	20.4	22.5
	8	30	7.93	20.4	19.5
LSD 0.05			0.26	0.4	3.5

Table 6. Mean grain yield and moisture of the highest and lowest yielding 10% of the S_2 maize lines in each trial

Trial	Yield group	No. of lines per culture age group			Grain yield	Grain moisture
		0	4	8	(Mg ha^{-1})	(%)
1	High	2	0	6	7.04	33.1
	Low	0	2	6	3.86	33.1
	SE[a]				0.12	0.2
2	High	3	1	2	5.98	34.9
	Low	0	1	5	1.43	42.9
	SE[a]				0.11	0.2
3	High	0	9	0	5.80	34.7
	Low	0	0	9	1.49	41.8
	SE[a]				0.10	0.1
4	High	6	0	0	4.96	36.2
	Low	0	0	6	1.60	39.3
	SE[a]				0.12	0.2
5	High	3	1	0	5.95	34.8
	Low	1	0	3	3.03	30.4
	SE[a]				0.14	0.2
6	High	3	0	3	6.53	33.6
	Low	0	0	6	2.82	32.6
	SE[a]				0.12	0.2

[a] SE of a 10% group mean

erated throughout the period of in vitro culture. From the perspective of maize improvement, this source of variation did not appear promising for increasing grain yield, but the trend towards earlier maturity could be beneficial.

There were several limitations to the study that preclude conclusive statements about the observed variation. Since the starting material was a segregating population, a better estimate of the variation in the original material would have been obtained through a larger sample for control lines. In most trials, there was also a relatively small sample of 4-month lines. Since the materials in each trial were derived from a single cross (Oh43 *ms* isolines × A188) the results may only apply to this genetic background.

The consistent performance of regenerated groups relative to their controls in most trials indicates that a substantial proportion of the variation was generated during tissue culture. Additional research is needed to establish the extent of the phenomenon. The use of several elite inbreds as starting materials would reduce the sampling problem, making it easier to assess the value of the variation, and would provide an opportunity to detect differences among genotypes for genetic stability in culture. Tissue culture would be especially useful if a unique array of mutational events occurred as compared with other mutagenic methods. The trend toward ealier maturing maize lines while maintaining at least as high yields as the donor materials indicates that tissue culture may offer a useful tool in plant breeding.

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