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Evaluation of nitrogen use efficiency in the Maksimir 3 Synthetic maize population

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Abstract

Nitrogen use efficiency (NUE) has become an important trait for sustainable agriculture. Traits present in maize (*Zea mays* L) landraces that contribute to adaptation in nitrogen-limited environments are not easily implemented directly into modern maize breeding programs. Thus, the landraces might be improved by recurrent selection and afterwards used as source of certain traits for elite breeding material. The Maksimir 3 Synthetic (M3S) maize population was created from landraces traditionally grown under low levels of soil fertility. The synthetic was subjected to recurrent selection for yield during three cycles and for improved NUE in the fourth cycle. In order to determine the effect of four cycles of recurrent selection, performance of populations per se (S0), population testcrosses, and populations per se selfed (S1) were evaluated in field trials under high and low nitrogen input conditions at four locations during 2010. The S0 populations had significantly higher grain yield (+20.6%), ear diameter (+2.6%), 1000 kernel weight (+3.3%) and ear leaf chlorophyll content (+31.2%) at the high nitrogen fertilization rate as compared to the low nitrogen fertilization rate. The S1 populations and testcross populations responded similarly to nitrogen fertilization. Genotype x nitrogen interaction for yield was not significant, but indication of specific adaptation to the nitrogen deficient environments was found. After the fourth cycle of recurrent selection, a significant increase in grain yield was found at both levels of nitrogen fertilization.

Keywords: maize, nitrogen use efficiency, recurrent selection

Introduction

The increase of nitrogen (N) fertilizer use in modern cereal production systems has been accompanied by steady increases in average crop yields (Presterl et al, 2002b; Gallais and Coque, 2005). On the other hand, negative impacts of nitrogen on the atmosphere, groundwater and other components of ecosystems have also been reported (Socolow, 1999). According to Raun and Johnson (1999), cereal production receives approximately 60% of the total worldwide N fertilizer, and the world's cereal grain N fertilizer use efficiency was estimated to be only 33%. In Europe, the European Community has developed a specific directive (91/676/EEC) to reduce nitrate pollution of groundwater by restricting the use of nitrate fertilizers. Farmers should therefore try to optimize their use of nitrate fertilizers in order to respect the nitrate directive, but also try to maintain sufficient production profit margins. An important aspect of this goal can be met by using varieties which efficiently accumulate and utilize nitrogen from applied fertilizers, i.e. by use of the varieties that have superior NUE (Gallais and Coque, 2005). For grain maize, NUE has been defined by Moll et al (1982) as grain yield per unit of nitrogen available in the soil (including fertilizers). According to these authors, grain NUE can be broken down as the product of the nitrogen uptake efficiency and nitrogen utilization efficiency. Presterl et al (2002b) defined NUE as ability of a genotype to realize superior grain yield at low soil nitrogen condition in comparison with other genotypes. Genetic variability for NUE or its components is an important prerequisite for development of nitrogen use efficient cultivars (Coque and Gallais, 2007). Evaluation of genetic variability for nitrogen use efficiency and its components in European maize was performed for maize landraces (Alonso Ferro et al, 2007), varieties (Coque and Gallais, 2007), breeding populations (Gallais et al, 2008), hybrids (Presterl et al, 2002b) and inbred lines and testcrosses (Presterl et al, 2002a; Presterl et al, 2003). It is generally considered that NUE of modern maize cultivars is lower because they were developed under conditions of high nitrogen availability in order to eliminate nitrogen as a variable (Kamprath et al, 1982). The consequences of lower NUE of modern cultivars are proportionally lower yields when lower N

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fertilization rates are used (Gallais and Coque, 2005). According to Lafitte et al (1997), it can be argued that modern improved maize cultivars developed after many generations of selection under adequate N levels in breeding nurseries may have lost certain adaptive traits for performance on soils with limited fertility which were present in the landraces from which they were derived. Landraces may provide basic material to breeding programs for selecting traits with adaptive value for nitrogen deficient environments because they were traditionally managed at restricted levels of soil fertility (Alonso Ferro et al, 2007). Presterl et al (2003) reported that lines derived from landraces showed the highest genetic variability at low levels of N input. Although landraces may exhibit specific adaptation to nitrogen-limited environments, they do not outperform modern cultivars when grown under limited nitrogen conditions (Lafitte et al, 1997). Several problems with direct use of landraces and other exotic maize materials in modern breeding programs relate to their poor agronomic characteristics and photoperiodism.

According to Gallais and Coque (2005), landraces can be used in recurrent selection programs to develop synthetics from which new lines can be derived in order to introduce new traits into elite breeding material.

Recurrent selection methods were developed to gradually improve the mean trait performance of genetically broad base populations (Hallauer, 1992). In the literature, one can find numerous examples of successful improvement of important agronomic quantitative traits by recurrent selection in maize. At the current time, there are few published results describing implementation of recurrent selection to improve NUE in maize (Kamprath et al, 1982; Gallais et al, 2008).

The objective of this study was to determine the effect of three cycles of recurrent selection for yield and an additional cycle of selection for improved yield and NUE in the Maksimir 3 Synthetic (M3S) maize population under low and high N input conditions.

Materials and Methods

Development of the Maksimir 3 Synthetic and selection trials

The Maksimir 3 Synthetic (M3S) population was created at the Faculty of Agriculture, University of Zagreb, by intermating 12 maize inbred lines. Their origin traces back to several open-pollinated varieties and landraces from different regions of the former Yugoslavia. M3S parental inbred lines showed equally good combining ability and similar number of isozyme/allozyme loci with different alleles compared to Iowa Stiff Stalk Synthetic, BSSS (B73) and Lancaster Sure Crop, LSC (Mo17) testers (Pejic, 1992), as well as similar mean genetic distance to both BSSS (B73, B83, A632) and LSC (C103, H109, Oh43) lines as determined by SSR markers (Sarcevic et al, 2008). After creation, M3S was subjected to four cycles of recurrent selection (Table 1). In the first cycle, C1, the emphasis of selection was on disease resistance among S1 progenies [artificially inoculated with *Helminthosporium turcicum* Pass and *Colletotrichum graminicola* (Ces) GW Wils fungi] and grain yield among S2 progenies. In the second and third cycles (C2 and C3), selection for higher grain yield was conducted among S1 and FS progenies, respectively. Details of the experimental procedures used in population syntheses and the first three cycles of selection were described previously (Sarcevic et al, 2004; Sabljo et al, 2008).

The fourth cycle of recurrent selection (C4N0 and C4N150) in the M3S population began in 2006. The primary selection criteria for the fourth cycle of selection was improved NUE. It was expected that the M3S population has valuable alleles which could contribute to improvement of NUE and disease resistance since the 12 parental inbreds were derived from populations cultivated under low N input conditions and no fungicide use.

The starting materials were obtained by selfing approximately 300 plants with good agronomic characters out of a block of approximately 1,000 plants of the third cycle of selection. A total of 196 ears were saved for testing on the basis of the visual assessment of their health and sufficient kernel number.

In 2007, the 196 S1 progenies were planted in two selection field trials at two locations in Northern Croatia (Maksimir and Rugvica) and one in the Eastern part of the country (Kutjevo) and fertilized with 0 kg N ha^{-1} (N0) in one and 150 kg N ha^{-1} (N150) in the other trial. To have better control of a 0 kg N ha^{-1} fertilization rate, trials were established in fields where the previous crop was grown under reduced N fertilization. At the Maksimir and Rugvica locations, the previous crop was winter wheat, which received 80 kg N ha^{-1}. After harvest, no N was added for plant residue decomposition. At the Kutjevo location, the trial was planted after tobacco which received only 40 kg N ha^{-1}, because higher values of nitrogen decrease cured tobacco leaf quality (Budimir et al, 2007). Soil analysis revealed relatively low levels of soil organic matter (1.62%, 1.79%, and 2.09%) and relatively low levels of total soil nitrogen (0.08%, 0.09%, and 0.011%) in Kutjevo, Maksimir and Rugvica, respectively. In the absence of supplemental fertilizer, these low residual levels of soil organic matter and soil nitrogen were considered to contribute to a low N fertility environment. The experimental design for each environment was a 14 x14 row-column design with two fertilized (150 kg N ha^{-1}) and two non fertilized replicates (0 kg N ha^{-1}) at each location. At one location, artificial infection with inoculum of corn anthracnose was performed when plants had six to eight leaves. Thirty progenies were selected (15.3% selection intensity) based upon the yield performance across locations within each N-fertilization treatment.

Table 1- Selection protocols for the four cycles of selection conducted in the M3S maize population

Cycle of selection	Type of progeny Evaluated	 Intermated	No. of progeny Evaluated	 Intermated	Primary trait under selection	Selection intensity, %
C1	S1		750		Disease resistance	
	S2	S2	225	40	Grain yield	5
C2	S1	S1	225	30	Grain yield	13
C3	FS	FS	100	15	Grain yield	15
C4N0*	S1	S1	196	30	Grain yield	15
C4N150**	S1	S1	196	30	Grain yield	15

*selection performed with 0 kg N ha^{-1} fertilization; **selection performed with 150 kg N ha^{-1} fertilization

Consideration was also given to anthracnose stalk rot when making selection decisions.

In 2008, remnant seed of 30 S1 progenies, selected at the N0 and at the N150 fertilization rates, was planted in isolated nurseries separately. At each nursery, 30 selected progenies were randomized and planted in 30 rows spaced 0.7 m apart with 0.2 m distance within the plants in the row. Intercrossing was performed via the wind facilitated pollination. All ears from each nursery were harvested, and equal quantities of seed from each ear were bulked to obtain seed of the C4N0 and C4N150 populations, respectively.

The same year, 500 plants of C0, C1, C2, and C3 population were grown at separate locations. Seed of the S0 populations was produced by chain sibbing approximately 150 plants of each population. Also, approximately 150 plants of each population were crossed to the hybrid tester A619 x A632 for production of testcross population seed. In addition, approximately 150 plants of each population were self-pollinated to obtain S1 population seed. In 2009, the same matings conducted in 2008 were also performed in the C4N0 and C4N150 populations to produce seed of their S0 populations, S1 populations, and testcross populations.

Evaluation trials for estimating response to selection

In 2010, two field trials were conducted at each of two locations in Northern (Maksimir, Rugvica), and two in Eastern Croatia (Kutjevo, Osijek). The first trial was a four replicate 4 x 4 row-column design in which C0, C1, C2, C3, C4N0 and C4N150 populations per se (S0) and their testcross progenies were grown at two nitrogen fertilization rates, 0 and 150 kg N ha^{-1}. It also included four checks, three commercial hybrids, and the experimental three-way hybrid A619 x A632 x Bc14.

The second trial was a four replicate RCBD, comprised of the six populations per se selfed (S1) also grown at the two fertilization rates. In both experiments, randomizations were initially carried out ignoring nitrogen rates. Afterwards, nitrogen (150 kg N ha^{-1}) was applied to two of four replicates.

Control of a 0 kg N ha^{-1} fertilization rate was performed in the same manner as for the selection trials in 2007, and no additional soil analyses were performed. In Osijek, the previous crop was winter wheat, fertilized with 80 hg of N ha^{-1}, with no additional N added for plant residue ecomposition after harvest in 2009. This was also the case for the other locations.

Experimental plots for each trial were 2.8 m wide and 4.0 m long, and were represented by four rows spaced 0.7 m apart with 0.2 m distance between the plants within rows. Each experimental plot was overplanted and later thinned to a plant density of approximately 71,500 plants ha^{-1}. Plant and ear height were recorded on 20 competitive plants per plot, and measured as the distance from the soil surface to the node of the flag leaf and to the highest ear bearing node, respectively. Ten competitive plants per plot were used for measuring ear leaf chlorophyll content at silking. Readings were taken midway between the stalk and leaf tip, and midway between the midrib and leaf margin, using CCM-200 Portable Chlorophylll Content Meter (Opti-Sciences Inc). Anthesis-silking interval was determined at one location (Maksimir) only. All the plants of the middle two rows of each plot were harvested. After drying to uniform grain moisture content, all harvested ears per plot were used for measurements of ear diameter (mm), ear length (cm) and kernel row number. After shelling, grain yield per plot was determined and expressed in t ha^{-1}. At one location (Maksimir), 1,000 kernel weight (g) was determined.

Statistical analysis

Data from the individual locations were first analyzed separately using the appropriate model for a row-column design. Least square estimates of the genotypic performance, along with their standard errors, were then used as input for the second stage of analysis. Estimates of the six S0 populations were considered separately from the six testcross populations and experimental three-way crosses. At this stage, weighted two-stage analysis according to Piepho (1999) was performed using a model comprised of fixed effects for genotype, fertilization, location, and genotype x fertilization. All other interactions were considered as random effects. The data for the three commercial hybrids were not included in the

Table 2 - Analysis of variance across locations for nine traits in S0 populations, S1 populations and testcross populations of the M3S maize population

	Pr > F								
Source of variation	Grain yield, tha^{-1}	Ear length, cm	Ear diameter, mm	Kernel row number	1000 kernel weight, g	Plant height, cm	Ear height, cm	LCC	ASI, days
				S0 populations					
F[1]	0.0481	0.0604	0.0233	0.0585	0.0161	0.1301	0.1120	0.0139	0.0425
L[2]	0.0483	0.2972	0.1724	0.0279		0.0948	0.0410	0.0568	
C[3]	0.0117	0.0373	0.0130	0.0006	0.0002	0.2010	0.1460	0.1955	0.0045
F x C	0.4250	0.1434	0.4958	0.8793	0.0218	0.3513	0.0214	0.3533	0.2372
				S1 populations					
F	0.2936	0.3697	0.5991	0.5357	0.0425	0.4583	0.6480	0.6837	0.1930
L	0.0281	0.1376	0.1525	0.1241		0.0844	0.0667	0.3240	
C	0.0289	0.1126	0.1375	0.0691	0.2551	0.7824	0.5806	0.4072	0.0093
F x C	0.3804	0.7716	0.7111	0.4439	0.1517	0.1610	0.0400	0.8293	0.0975
				Testcross populations					
F	0.0478	0.0260	0.0131	0.0177	0.0014	0.2661	0.1542	0.0275	0.8386
L	0.1463	0.2793	0.6654	0.0631		0.1769	0.6460	0.0770	
C	0.6358	0.0306	0.0016	0.0000	0.0175	0.2504	0.0008	0.0297	0.2368
F x C	0.8580	0.3876	0.1100	0.0983	0.0871	0.7368	0.1936	0.3142	0.4936

[1]fertilization rate; [2]location; [3]cycle of selection

second part of the analysis.

A similar analysis was applied for the second trial, with the different first stage related to the experimental design, RCBD. After the second stage, multiple comparisons between genotypic means were carried out, applying the Bonferroni adjustment

The exceptions from this analysis were for anthesis-silking interval and 1,000 kernel weight, where data were collected only at one location. These traits were analyzed applying only the first stage part of the analysis for the RCBD trials.

All described analyses were performed using the SAS/STAT software (SAS Institute Inc, 2009).

Results

For the S0 populations, the combined analysis of variance across locations (Table 2) revealed significant differences between the 0 and 150 kg N ha^{-1} fertilization rates for grain yield, ear diameter, 1,000 kernel weight, leaf chlorophyll content (LCC) and anthesis silking interval (ASI). Significant location effects were observed for grain yield, kernel row number, and leaf chlorophyll content. Selection cycles differed significantly for grain yield, ear length and diameter, kernel row number, 1,000 kernel weight and ASI. The interaction between fertilization and cycle of selection was significant for 1,000 kernel weight and ear height.

For the S1 populations, fertilization rate was significant for 1,000 kernel weight. The location effect was significant for grain yield. Significant differences between cycles were observed for grain yield and ASI. Interaction fertilization x cycle of selection was significant only for ear height.

For the testcross populations, fertilization rate was significant for grain yield, ear length, ear diameter, kernel row number, 1,000 kernel weight and leaf chlorophyll content. Cycle of selection was significant for ear length, ear diameter, kernel row number, ear height, 1,000 kernel weight and ASI.

For S0 population performance, the average yield was 20.60% higher at the fertilization rate of 150 kg N ha^{-1} relative to 0 kg N ha^{-1}. A general increasing trend of grain yield improvement over cycles of selection was observed at both nitrogen fertilization levels, although C1 exhibited slightly decreased grain yields as compared to C0 (Table 3).

Generally, higher relative yield improvements were observed for the later cycles (compared to the C0) at 0 than at 150 kg N ha^{-1}. The C4N0 showed the highest improvement at the low N level, and C4N150 at the high N fertilization rate.

In the S1 populations, a significant yield improvement at 0 kg N ha^{-1} was observed until the C2 where it remained at a relatively similar level in further selection cycles. At 150 kg N ha^{-1}, a tendency for yield increase in later cycles of selection was found, but the differences among the cycles of selection were not statistically significant.

In the testcross populations, a significant difference in yield between the two fertilization rates resulted in 24.3% lower yield at 0 kg N ha^{-1}. At both levels of N fertilization, there were no significant differences in grain yield among the cycles of selection including the control three-way hybrid.

In S0 populations, an increasing trend over cycles of selection was observed for ear length, ear diameter, and 1,000 kernel weight at 150 kg N ha^{-1} with a more positive response for C4N150 then for C4N0 (Table 4). At 0 kg N ha^{-1}, 1,000 kernel weight showed a similar trend of increase, but only until the third cycle of selection.

For the S1 populations, ear length at 150 kg N ha^{-1} did not change over the cycles of selection, while at 0 kg N ha^{-1} a significant increase of ear length was observed until the C3.

The 1,000 kernel weight showed an increasing trend over cycles of selection only at 150 kg N ha^{-1} with the highest value observed in the C4N0.

Table 3 - Grain yield in t ha^{-1} and as percentage of yield improvement relative to C0 (in parentheses) of the S0 populations, S1 population and testcross populations of the M3S maize population.

	Populations																	
Cycle of selection	S0 populations						S1 populations						Testcross populations					
	Fertilization																	
	N0[1]			N150[2]			N0			N150			N0			N150		
C0	3.89	ab[3]		5.27	ab		2.54	a		3.35	a		4.55	a		5.95	a	
C1	3.65	a	(-6.6)	4.60	a	(-14.6)	3.16	ab	(19.6)	3.35	a	(0.0)	4.60	a	(1.1)	5.75	a	(-3.5)
C2	4.04	ab	(3.7)	5.42	ab	(2.8)	3.76	b	(32.4)	3.95	a	(15.2)	4.19	a	(-8.6)	5.99	a	(0.7)
C3	4.75	b	(18.1)	5.52	b	(4.5)	3.83	b	(33.7)	3.49	a	(4.0)	4.46	a	(-2.0)	6.39	a	(6.9)
C4N0	4.98	b	(21.9)	5.59	b	(5.7)	3.55	b	(28.5)	4.20	a	(20.2)	4.83	a	(5.8)	6.83	a	(12.9)
C4N150	4.56	ab	(14.7)	6.20	b	(15.0)	3.39	b	(25.1)	4.01	a	(16.5)	5.11	a	(11.0)	6.23	a	(4.5)
Control													4.99	a	(8.8)	6.04	a	(1.5)
Mean		4.31			5.43			3.37			3.73			4.67			6.17	
Significance				*					n.s.						*			

[1] 0 kg N ha^{-1}; [2] 150 kg N ha^{-1}; [3] means within each fertilization rate followed by the same; letter are not statistically different at the 0.05 level of significance; * significant at $p<0.05$;

In the testcross populations, ear length and ear diameter at both nitrogen fertilization levels showed increasing trends with the cycle of selection. For these traits, later cycles were comparable in performance to the experimental three-way hybrid grown as a control (Table 4). At 0 kg N ha^{-1} the C2 population had significantly higher 1000 kernel weight than the C0, C1, and C3 populations, while at 150 kg N ha^{-1}, the C4N150 population had significantly higher 1000 kernel weight than the C0, C1, and C2 populations. At both nitrogen fertilization levels, 1000 kernel weight values for the best populations and two populations of the fourth selection cycle were either comparable or slightly higher than the 1,000 kernel weight for the control.

Kernel row number was the only trait that, in all three types of populations, exhibited a decreasing trend over cycles of selection at both nitrogen fertilization levels.

For the S0 populations, plant and ear height at 150 kg N ha^{-1} showed an increasing trend until the C3. In the fourth cycle, a significant decrease for both traits was observed in C4N0 (Table 5).

For the two other population types (S1 and testcross), none of the two traits changed significantly over cycles of selection at lower or higher N fertilization rates.

Ear leaf chlorophyll (LCC) content was significantly lower at 0 kg N ha^{-1} than at 150 kg N ha^{-1} for S0 populations and testcross populations. In all three types of populations, improved cycles of selection were not significantly different from C0 at both nitrogen fertilization levels, although in S0 populations, at 0 kg N ha^{-1}, the lowest value of LCC was recorded for C4N0.

For S0 populations, mean anthesis-silking interval (ASI) was longer at the 0 kg N ha^{-1} than at 150 kg N ha^{-1}. However, the two fourth cycle populations, C4N0 and C4N150, had similar magnitudes of ASI at both nitrogen fertilization levels, and at 0 kg N ha^{-1}, these populations had significantly shorter ASI than C0.

In the S1 population, only C4N150 had significantly shorter ASI at 0 kg N ha^{-1} compared to the other cycles of selection, while in the testcross populations ASI among the cycles of selection stayed at the same level at both nitrogen fertilization levels.

Discussion

Grain yield of the S0 populations and their testcrosses were significantly lower at 0 than at 150 kg N ha^{-1}, but an increasing trend over cycles of selection was observed at both N fertilization levels. For the S1 populations, N stress did not result in significant yield differences between the two nitrogen rates, which confirms the statement of Gallais and Coque (2005) and results of Balko and Russell (1980) that N stress only slightly affects yields of inbreds. In the present study, yield improvement for the S1 populations was observed only at 0 kg N ha^{-1} which might indicate that M3S maize population has potential for selection under low N conditions. Generally, relative yield improvement in the S0 populations was more evident in the later cycles of selection at 0 than at 150 kg N ha^{-1}, with C4N0 exhibiting the greatest improvement at the low N rate and C4N150 at high N rate. Yield results for S0 population performance in this study might indicate a tendency for specific adaptation of C4N0 to low and C4N150 to high N input conditions. Gallais et al (2008) showed that for an environment where N-stress was as expected, the genetic advance between C1 and C2 populations selected under high N-input conditions was higher under high N-input test environments. Coque and Gallais (2005) showed that selection at low N-input for response at high N-input was less efficient than direct selection at high N-input, whereas selection at high N-input for response at low N-input was close to direct selection at low N-input, mainly due to nearly similar heritabilities at low and at high N-inputs. Greater efficiency of direct selection at

Table 4 - Ear length (cm), ear diameter (mm), kernel row number and 1,000 kernel weight (g) for S0 populations, S1 populations and testcross populations of the M3S.

Cycle of selection	Ear length				Ear diameter				Kernel row number				1,000 kernel weight			
	N0[1]		N150[2]		N0		N150		N0		N150		N0		N150	
S0 populations																
C0	16.80	b[3]	16.45	a	41.04	a	41.24	a	14.43	b	14.60	a	260.67	a	276.00	ab
C1	15.44	a	16.54	a	40.62	a	41.13	a	14.61	b	15.18	c	276.00	a	261.67	a
C2	15.68	ab	17.67	ab	41.29	a	42.52	b	14.56	b	14.90	bc	277.17	a	280.67	ab
C3	16.88	ab	18.01	b	41.37	a	42.94	b	13.74	a	14.16	a	295.33	b	293.00	bc
C4N0	16.38	ab	17.09	ab	41.25	a	42.71	b	13.97	a	14.16	a	284.00	ab	309.67	cd
C4N150	16.44	ab	17.97	b	41.79	a	43.45	b	14.07	ab	14.32	a	289.67	ab	319.67	d
Mean	16.27		17.29		41.23		42.33		14.23		14.55		280.47		290.11	
Significance		n.s.				*				n.s.				*		
S1populations																
C0	14.77	a	15.47	a	39.29	a	39.29	a	14.42	b	14.21	ab	269.00	a	268.33	a
C1	14.62	a	15.33	a	38.52	a	39.33	a	13.80	ab	14.49	b	270.67	a	268.50	a
C2	15.05	ab	15.82	a	39.80	a	40.20	a	14.01	ab	14.45	b	260.67	a	278.67	ab
C3	15.81	b	15.92	a	39.84	a	40.05	a	13.87	ab	13.68	ab	281.17	a	280.67	ab
N0	14.95	ab	15.48	a	38.75	a	39.44	a	13.78	ab	13.62	a	261.33	a	295.83	b
N150	15.27	ab	15.41	a	39.84	a	39.33	a	13.43	a	13.55	a	278.33	a	286.00	ab
Mean	15.08		15.57		39.34		39.61		13.89		14.00		270.19		279.67	
Significance		n.s.				n.s.				n.s.				*		
Testcross populations																
C0	16.67	ab	17.35	a	41.65	ab	43.39	a	14.79	cd	15.12	c	279.33	a	284.50	a
C1	16.87	ab	18.12	ab	41.04	a	43.75	ab	14.42	bc	14.84	bc	281.00	a	287.67	a
C2	16.77	ab	18.26	b	42.54	b	44.82	b	14.59	bc	15.88	e	296.83	b	291.33	a
C3	16.53	a	18.61	b	41.29	a	44.18	ab	13.57	a	14.54	ab	277.33	a	302.67	ab
N0	17.60	bc	18.47	b	42.44	bc	44.43	b	14.12	ab	14.74	abc	289.67	ab	305.00	ab
N150	17.58	bc	18.52	b	42.60	bc	44.21	ab	14.18	b	14.56	abc	290.17	ab	311.67	b
Control	19.97	c	18.56	b	43.24	c	44.20	ab	15.00	d	15.20	cd	292.17	ab	297.67	ab
Mean	15.08		15.57		39.34		39.61		13.89		14.00		270.19		279.67	
Significance		*				*				*				**		

[1]0 kg N ha^{-1}; [2]150 kg N ha^{-1}; [3]means within each furtilization rate followed by the same letter are not statistically different at the 0.05 level of significance; *, ** significant at $p< 0.05$ and $p<0.01$, respectively; n.s. - not significant

low-N input was observed by Lafitte and Edmeades (1995) and Bänziger et al (1997). Muruli and Paulsen (1981) found, under conditions of low N supply, that a synthetic selected at low N-input outyielded the one selected at high N-input by approximately 35%. When selection was conducted under high N-input conditions, a yield advantage of 25% in favor of the synthetic selected at high N-input was observed. Brun and Dudley (1989) demonstrated that selection in high N environments resulted in higher predicted yield gains over two nitrogen levels than selection in lower N environments. A similar conclusion was reported by Lafitte and Edmeades (1995) who observed greater environmental errors and lower heritability under low N-input conditions. According to Gallais and Coque (2005), specific genes for adaptation to low N stress probably exist and could be revealed only in such environments.

In the present study, a significant increase in ASI was observed under low nitrogen fertilization rate for S0 populations. This result is in agreement with the findings of Gallais and Coque (2005). At low nitrogen fertilization, both C4N0 and C4N150 showed significant decreases in ASI as compared to earlier cycles of selection, with a greater decrease observed for C4N0. According to Gallais and Coque (2005), growing of maize plants in presence of some stress, such as drought or nitrogen deficiency, results in increased ASI. In 2007, when selection for improved NUE in M3S was conducted, drought (DHMZ, 2007) during the anthesis period might have favored the selection of genotypes with shorter ASI at both nitrogen levels. The selection pressure for shorter ASI was probably enhanced in the low N environment leading to a further decrease in ASI in the C4N0 population as compared to C4N150 population. In the study of Bänziger et al (2002), ASI was reduced in drought-tolerant selection cycles in all experiments in comparison to original cycles, but it was more strongly reduced in selection to mid-season drought stress in two experiments with the lowest grain yield (i.e. where N stress was most severe). Gallais and Coque (2005) and Bänziger et al (1999) suggest that selection for drought tolerance likely increases tolerance to low N conditions, showing that both criteria must be considered simultaneously.

The decrease in yield observed between the C0 and C1 cycles at both nitrogen fertilization levels could be explained by the two-stage inbred selection that was used between the two cycles in M3S. In the first stage, plants resistant to corn anthracnose were selected and, in the second stage, yield selection and

Table 5 - Plant height (cm), ear height (cm), Leaf chlorophyll content and anthesis-silking interval for S0 populations, S1 populations and testcross populations of the M3S.

Cycle of selection	Plant height				Ear height				LCC				ASI			
									S0 populations							
	N0[1]		N150[2]		N0		N150		N0		N150		N0		N150	
C0	156.22	a[3]	174.78	a	72.10	ab	81.49	a	49.03	ab	67.62	a	5.50	c	2.50	ab
C1	158.12	a	170.28	ab	74.42	ab	77.80	a	48.31	ab	66.31	a	5.00	bc	4.00	b
C2	157.52	a	177.56	ab	66.15	a	83.61	ab	54.69	b	75.82	a	5.50	c	4.00	b
C3	157.05	a	182.59	b	71.56	ab	92.17	b	50.30	ab	69.71	a	5.00	bc	4.50	b
C4N0	160.79	a	171.73	a	71.75	ab	81.84	a	43.97	a	73.85	a	1.50	a	1.50	a
C4N150	163.54	a	185.46	b	75.51	b	90.92	b	48.81	ab	75.54	a	3.00	ab	3.50	ab
Mean	158.87		177.07		71.92		84.64		49.18		71.48		4.25		3.33	
Significance		n.s.				n.s.				**				*		
									S1populations							
C0	141.86	a	133.58	a	60.83	b	60.38	ab	64.43	a	65.29	a	4.50	b	4.50	ab
C1	149.44	a	132.72	a	54.55	a	65.75	b	55.84	a	59.37	a	4.00	b	3.50	ab
C2	143.51	a	138.79	a	60.50	ab	63.60	ab	60.84	a	70.42	a	4.00	b	6.00	b
C3	142.69	a	136.09	a	58.45	b	60.11	a	62.00	a	66.60	a	4.50	b	5.00	b
C4N0	147.39	a	137.10	a	60.44	b	63.64	ab	56.49	a	60.82	a	4.00	b	3.00	a
C4N150	137.43	a	140.73	a	61.77	b	59.44	a	59.75	a	63.75	a	2.00	a	3.50	ab
Mean	143.72		136.50		59.42		62.15		59.89		64.37		3.83		4.25	
Significance		n.s.				n.s.				n.s.				n.s.		
									Testcross populations							
C0	171.03	ab	186.91	a	74.57	b	82.42	a	49.02	ab	72.00	b	2.50	ab	3.00	ab
C1	170.11	ab	192.52	a	70.04	ab	82.83	a	43.40	ab	67.05	ab	2.50	ab	4.00	b
C2	171.23	ab	188.09	a	69.80	ab	86.43	a	48.95	ab	68.44	b	3.50	ab	3.50	ab
C3	168.39	a	187.85	a	66.37	a	87.91	a	43.16	a	69.47	b	4.00	b	3.50	ab
N0	176.51	ab	194.22	a	75.44	ab	88.02	a	51.35	ab	68.52	b	3.50	ab	2.50	ab
N150	175.91	ab	193.21	a	72.03	ab	89.01	a	51.99	b	75.53	b	3.50	ab	2.50	ab
Control	178.10	b	191.82	a	83.93	c	97.24	b	45.98	ab	58.76	a	2.00	a	2.00	a
Mean	173.04		190.66		73.17		87.69		47.69		68.54		3.07		3.00	
Significance		n.s.				n.s.				*				n.s.		

[1] 0 kg N ha^{-1}; [2] 150 kg N ha^{-1}; [3] means within each furtilization rate followed by the same letter are not statistically different at the 0.05 level of significance; *, ** significant at $p < 0.05$ and $p < 0.01$, respectively; n.s. - not significant

intercrossing was performed. Similar yield decreases when selection was primarily for pest resistance were reported by Devey and Russell (1983) and Klenke et al (1986).

The tester used as the seed parent for testcross production was single-cross hybrid A632 x A619, whose inbred lines originate from the BSSS and Lancaster heterotic groups, respectively. From the testcross yields, it could be concluded that M3S has good general combining ability with both heterotic groups, which was one of the goals during its creation (Pejic 1992; Sarcevic et al, 2004). After four cycles of recurrent selection, the general combining ability of the improved population was preserved, a result similar to those reported in the studies of Genter (1973), Walejko and Russell (1977), Horner et al (1989), and Doerksen et al (2003).

The increasing trend for ear length and diameter observed in the present study is in agreement with the results of Sarcevic et al (2004), who found an increase of ear length after the first two cycles of recurrent selection in the M3S population. For the S1 families, increased ear length with the cycle of selection was the only trait that might explain the trend of yield improvement of S1 families at 0 kg N ha^{-1}. The 1,000 kernel weight in the present study was significantly increased over cycles of selection at 0 kg N ha^{-1} for S0 population performance and for testcross performance, and at 150 kg N ha^{-1} for all three groups of genetic materials. Additionally, significant differences in 1,000 kernel weight between the two fertilization rates were observed in all three groups. Similar results for lower 1000 kernel weight at lower than high N fertilization were reported by Alonso Ferro et al (2007) and Bänziger et al (2002).

Increases in plant and ear height were observed in the evaluation of S0 population performance at 150 kg N ha^{-1}. Sarcevic et al (2004) found plant and ear height increases in M3S after the second cycle of recurrent selection. Vasal et al (1995a, b; 2001) reported delayed maturity and increased height due to selfed progeny recurrent selection for grain yield. In the present study, significantly delayed maturity was not observed (data not shown). Hallauer and Miranda (1988) reported that maize grain yield, plant height, and maturity traits were generally positively correlated with each other.

In this study, the trend of yield improvement by recurrent selection in the M3S population was confirmed after four cycles of recurrent selection. Traits which might be related to improvement of yield are yield components, ear length, ear diameter, 1,000 kernel weight, and also increased plant and ear height.

LCC was significantly lower in the present study at 0 kg N ha^{-1} than at 150 kg N ha^{-1} in S0 populations and testcross evaluations, but among the cycles of selection, no significant differences for LCC were observed. Bänziger and Edmeades (1997) observed low but positive genetic correlations between grain yield and leaf chlorophyll content in 15 of 19 maize experiments grown under low levels of N. Zaidi et al (2003) found medium to strong positive phenotypic correlations between grain yield and leaf chlorophyll content, and according to Presterl et al (2003) hybrids from selection at low N-input showed a higher leaf chlorophyll content than hybrids from selection at high N-input. However, Bukan et al (2010) recorded a decrease in leaf chlorophyll content from C0 to C4 in M3S population, accompanied by a significant trend of yield improvement in relation to cycle of selection when populations were grown at relatively low levels of N fertilization.

Results of this study show that, at 0 kg N ha^{-1}, yield response of improved cycles of selection was generally higher than at 150 kg N ha^{-1}, indicating that the M3S population has potential for improvement of NUE. The cycle population developed under low nitrogen conditions (C4N0) might be better adapted to low nitrogen growing conditions, while the cycle population developed under high nitrogen conditions (C4N150) might be better adapted to high nitrogen growing conditions. This indicates a greater efficiency of direct selection at both levels of nitrogen fertilization. Further selection should be performed in contrasting nitrogen environments.

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