Table 1. Yield reduction in genotypes under low-N and ESM conditions.

Genotype	% yield reduction	Response	% yield reduction	Response	Genotype	% yield reduction	Response	% yield reduction	Response
	Low-N	Low-N	ESM	ESM		Low-N	Low-N	ESM	ESM
POB. 33 C_3-12-2-1-1-2-2 (L_1)	39.58	S	59.02	S	L_5T_1	2.55	T	32.71	S
POB. 33 C_3-12-2-1-2-2-5 (L_2)	9.31	T	20.35	T	L_5T_2	3.02	T	30.09	S
POB. 33 C_3-142-1-6-1-1-4 (L_3)	12.16	T	58.29	S	L_5T_3	7.86	T	30.05	S
POB. 45 C_8-86-1-3-7-6-4 (L_4)	13.74	T	22.48	T	L_5T_4	32.39	S	70.63	S
POB. 45 C_8-45-2-6-1-2-7 (L_5)	23.16	T	42.22	S	L_6T_1	15.87	T	27.84	S
POB. 45 C_8-269-2-4-6-3-3 (L_6)	11.64	T	23.08	T	L_6T_2	9.69	T	25.92	S
POB. 45 C_8-86-1-1-7-5-1 (L_7)	20.89	T	44.41	S	L_6T_3	3.8	T	22.16	T
CLG 1708-1-1-9 (L_8)	40.27	S	37.19	S	L_6T_4	6.62	T	3.32	T
POB. 45 C_8-45-2-6-1-1-1 (L_9)	68.57	S	85.88	S	L_7T_1	83.25	S	50.34	S
POB. 45 C_8-86-1-3-4-5-2 (L_{10})	27.18	S	30.88	S	L_7T_2	3.23	T	19.40	T
POB. 45 C_8-86-1-3-2-2-5 (L_{11})	56.36	S	65.98	S	L_7T_3	19.97	T	44.57	S
POB. 45 C_8-269-2-4-6-6-1 (L_{12})	33.11	S	58.63	S	L_7T_4	18.34	T	29.49	S
POB. 445 ⊗ 58-6-3-B-B-B (T_1)	16.26	T	16.22	T	L_8T_1	0.37	T	33.03	S
POB. 446-74-2-B-B-B (T_2)	9.68	T	19.18	T	L_8T_2	17.21	T	19.11	T
CML-421(T_3)	4.3	T	3.87	T	L_8T_3	26.69	S	53.25	S
CML-423(T_4)	7.26	T	80.46	S	L_8T_4	6.05	T	35.52	S
L_1T_1	8.82	T	28.25	S	L_9T_1	27.57	S	27.71	S
L_1T_2	13	T	37.12	S	L_9T_2	4	T	22.88	T
L_1T_3	17.41	T	45.22	S	L_9T_3	8.1	T	43.40	S
L_1T_4	0.70	T	26.52	S	L_9T_4	8.89	T	38.26	S
L_2T_1	6.03	T	45.52	S	$L_{10}T_1$	4.01	T	19.17	T
L_2T_2	23.96	T	55.3	S	$L_{10}T_2$	5.58	T	51.02	S
L_2T_3	8.72	T	52.67	S	$L_{10}T_3$	5.42	T	41.35	S
L_2T_4	5.1	T	39.17	S	$L_{10}T_4$	18.08	T	31.13	S
L_3T_1	7.34	T	41.53	S	$L_{11}T_1$	22.04	T	26.50	S
L_3T_2	6.13	T	20.30	T	$L_{11}T_2$	18.68	T	49.89	S
L_3T_3	20.02	T	55.40	S	$L_{11}T_3$	9.64	T	41.94	S
L_3T_4	5.41	T	45.44	S	$L_{11}T_4$	16.52	T	33.02	S
L_4T_1	12.90	T	17.70	T	$L_{12}T_1$	6.12	T	39.27	S
L_4T_2	1.0	T	50.33	S	$L_{12}T_2$	28.53	S	84.73	S
L_4T_3	20.72	T	48.79	S	$L_{12}T_3$	17.79	T	45.41	S
L_4T_4	4.39	T	42.12	S	$L_{12}T_4$	11.18	T	21.18	T

Note: S = susceptible (greater than 25% yield reduction), T = tolerant (less than 25% yield reduction).

Estimation of yield losses in ESM conditions. The percent yield reduction among the crosses varied from 3.32 percent in L_6T_4 to 84.73 percent in $L_{12}T_2$. Crosses with moderate reductions in yield were L_8T_2 (19.11 percent), $L_{10}T_1$ (19.17 percent) and L_7T_2 (19.40 percent). Among the lines, the lowest reduction in yield was found in L_2 (20.35 percent) and the highest reduction in yield was found in L_9 (85.88 per cent). Among the testers, T_3 showed the least reduction in yield (3.87 percent) and T_4 showed maximum yield reduction (80.46 percent). Excess soil moisture conditions reduced the yield of nine lines, 1 tester and 39 hybrids by more than 25%, whereas the remaining test materials showed less than 25% yield reduction (Table 1).

Kernel carotenoids in 37 maize lines

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Vitamin A deficiency is a global problem. Among the three major cereals, only maize grain contains coloured carotenoid compounds that can be converted into vitamin A in humans and other animals. Maize exhibits considerable natural variability for kernel carotenoids, with some lines accumulating as much as 66 μg/g of dry weight (Brunson and Quackenbush, Crop Sci. 2:344-347, 1962; Buckner et al., Plant Cell 2:867-876, 1990; Harjes et al., *Science* 319:330-333, 2008). The present investigation was undertaken to characterize a set of potential inbred lines and populations for carotenoid content for further analysis and use in development of hybrid with enhanced level of carotenoids.

Thirty inbred lines and 7 improved populations of maize were characterized for kernel carotenoid content using the extraction protocol developed by Torbert Rocheford's Lab (http://www. cropsci.uiuc.edu/faculty/rocheford/quick_carotenoid_analysis_protocol.pdf) and optical density measurement. The total carotenoid content was found to vary from a minimum of 3.54 μg/g dry weight to a maximum of 29.27 μg/g dry weight (Table).

Table. Carotenoid content of different maize lines.

S. No.	Pedigree	Carotenoids (μg/g)	S. No.	Pedigree	Carotenoids (μg/g)
1.	Hyd07R-104-6	18.29	20.	Hyd07R-456-2	20.41
2.	Hyd07R-300-6	12.75	21.	Hyd07R-419-2	24.52
3.	Hyd07R-325-3	17.72	22.	Hyd07R-421-2	17.26
4.	Hyd07R-301-3	22.35	23.	Hyd07R-451-1	27.21
5.	Hyd07R-456-1	18.15	24.	Hyd07R-419-1	27.84
6.	Hyd07R-301-2	23.35	25.	Hyd07R-438-4	19.21
7.	Hyd07R-441-1	21.41	26.	Hyd07R-445-4	29.21
8.	Hyd07R-302-1	21.55	27.	Hyd07R-418-2	22.92
9.	Hyd07R-325-6	23.07	28.	Hyd07R-418-4	22.21
10.	Hyd07R-437-2	19.26	29.	Hyd07R-443-4	27.87
11.	Hyd07R-325-2	22.58	30.	D-131	22.78
12.	Hyd07R-408-2	29.27	31.	D-765	16.55
13.	Hyd07R-438-1	22.29	32.	Kanchan	12.41
14.	Hyd07R-302-5	26.24	33.	Tarun	12.24
15.	Hyd07R-300-4	27.47	34.	Surya	21.24
16.	Hyd07R-407-5	29.10	35.	Amar	24.89
17.	Hyd07R-445-5	26.10	36.	Pragati	14.15
18.	Hyd07R-437-5	25.72	37.	CM-300	3.54
19.	Hyd07R-444-3	26.98			
C.D. (5%)		2.454			2.454

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Clustering methods for determining heterotic patterns using molecular markers

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In hybrid maize breeding programs, efficiency of procedures to identify inbreds used to develop outstanding single crosses strongly affects the success of the program (Hallauer and Miranda, 1988). The best hybrid combinations can be identified using information from diallel (which are prohibitive with large numbers of inbreds) or topcrosses to testers (Terron et al., Agron. Meso. 8:26-34, 1997). When a large number of germplasm exists but no established heterotic groups are available, genetically similar germplasms can be identified with molecular markers. On the basis of this information, field trials can be planned more efficiently (Reif et al., Crop Sci. 43:1275-1282, 2003).

Several studies have been published in the last few years using molecular markers to study genetic divergence with variable results (Dias et al., 2004 Genet. Mol. Res. 3:356-368). According to Reif et al. (Crop Sci. 41:1-7, 2005), the choice of a coefficient for studying divergence depends on the marker system properties involved and on the study objectives, among other conditions. According to these authors, several studies ignore these conditions, especially those related to the coefficient properties, which are connected to the study objective, which are very important for decision making considering the proper coefficient to be used. These studies usually employ the same similarity coefficients for dominant markers, such as RAPDs, and codominant and multiallele markers, such as simple sequence repeats (SSR), even though some of these coefficients are specific for dichotomic variables. Most similarity coefficients are based on comparisons between the occurrence of common and different bands (indicated by ones and zeros in common in a data matrix), while genetic dissimilarity coefficients, such as Roger's modified distance and Nei's distance, make use of information on allele frequency obtained by molecular markers, especially microsatellites (Balestre et al., Genet. Mol. Res. 7:695-705, 2008; Reif et al., 2005).

Pritchard et al. (Genetics 155:945-959, 2000) introduced the software, Structure, which has been used with relative success in maize (Camus-Kulandaivelu et al., Crop Sci 47:887-890, 2007). Given a value for the number of populations (*K*), Structure uses a Bayesian framework to assign lines from the entire sample to clusters in such a way that Hardy-Weinberg disequilibrium and linkage disequilibrium (LD) are maximally explained (Pritchard, et al., 2000). The purpose of this study was to evaluate the reliability of clustering methods based on molecular marker information to replace and/or complement topcross trials in assigning lines to heterotic groups of temperate germplasm.

For the analysis, we used the results of the molecular characterization of 21 microsatellite loci evenly distributed in the genome of 26 inbred lines. All lines except one (B73) were developed by INTA (Instituto Nacional of Tecnologia Agropecuaria) from different sources (mainly landraces) and belong to the Argentine Orange Flint heterotic group. Results were partially published in Morales Yokobori et al. (MNL 79:36-37, 2005). The entire set of 26 lines was previously grouped into four heterotic groups by topcross (Table 1) (Eyherabide et al., Plant Breeding: The Arnel R. Hallauer International Symposium, Blackwell Publishing, pp. 352–379, 2006).

Table 1. Clustering of lines established by topcross (Eyhérabide et al., 2006; Nestares et al., 1999).

Heterotic Group	Inbreds
I	B73, lp17, lp32, lp521, lp122
II	lp123, lp153, lp22, lp44, lp662, lp70, P1338
III	lp13, lp146, lp147, lp19, lp199, ZN6
IV	lp38, lp62, lp103, lp109, lp110, lp138, lp152, lp140

Cluster analysis was performed using the Unweighted Pair Group Method using Arithmetic averages (UPGMA) and on the basis of Modified Roger's distance (MRD). According to Melchinger (The Genetics and Exploitation of Heterosis in Crops, pp. 99–118, 1999*)*, heterosis is a function of the dominance effect of the QTL and of MRD between parents. Reif et al. (2005) states that MRD is especially suitable in studies based on (i) the prediction of heterosis with genetic dissimilarities or (ii) the establishment of heterotic groups.

Both distance and clustering were performed using InfoStat/P, v1.1 (Grupo InfoStat, FCA, Córdoba Argentina). Four groups were determined by visual inspection of dendrograms (Table 2). Lines were also subdivided into 4 genetic clusters using **Structure** (Pritchard et al., 2000). We set the parameter K = 4, the number of heterotic populations previously established by topcross. Burn-in time and replication number were both set to 500,000. Results can be seen in Table 3.

Table 2. Clustering of lines based on UPGMA (Unweighted Pair Group Method with Arithmetic average) and Roger's Modified Distance.

Cluster	Inbreds
1	lp38, lp44
2	lp152, p1338, ZN6, lp199, lp521,lp117
3	lp138, lp22, lp32,lp62,lp110,lp19
4	lp103, lp122, lp123, lp109, lp13, lp662, lp153, lp70, B73, lp140, lp146, lp147

Table 3. Clustering of lines according to Structure software (Pritchard et al., 2000).

Cluster	Inbreds
A	lp103, lp122 , lp123, lp22,lp32 , lp38, lp44
B	B73, lp110, lp138, lp140, lp19, lp62, lp662
C	lp117, lp152, lp199, lp521,p1338, ZN6
D	lp109, lp13, lp146, lp147, lp153, lp70

A script in R language (http://www.r-project.org/) was made in order to determine the best level of agreement between clustering based on molecular data (this work) and clustering based on topcross (Eyhérabide et al., 2006). This allows identification of the best match between molecular and topcross groups. Concordance was measured by Cohen's Kappa coefficient (psy package of R Project). Cohen's kappa measures the agreement between two raters who each classify *N* items into *C* mutually exclusive categories. K< 0 indicates no agreement whereas 1 indicates a perfect match. Kappa values ranged from 0.16 to 0.24 (Table 4), which indicates a fair agreement.

To the present, distance-based methods are most frequently applied (Reif et al., 2005); however, we found that STRUCTURE grouping shows better agreement with topcross data than distance-based methods (Table 4). This could be attributed to: a) the