Determination of the Best Heat Stress Tolerance Indices in Maize (*Zea mays* **L.) Inbred Lines and Hybrids under Khuzestan Province Conditions**

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ABSTRACT

Maize improvement for high temperature tolerance requires the reliable assessment of parental inbred lines and their combinations. Fifteen maize inbred lines were evaluated during 2007 and 2008 in Shushtar city (Khuzestan Province). The inbred lines were planted at two dates: 6 July, to coincide heat stress with pollination time; and 27 July, as normal planting to avoid high temperature during pollination and grain filling period. In addition, 28 hybrids from a combination of eight selected lines, were evaluated under the same conditions in 2008. Five stress tolerance indices, including mean productivity (MP), stress tolerance (TOL), stress susceptibility (SSI), stress tolerance index (STI) and geometric mean productivity (GMP) were used in this study. Data analysis revealed that the SSI, STI and GMP indices were the more accurate criteria for selection of heat tolerant and high yielding genotypes. The positive and significant correlation of GMP and grain yield under both conditions revealed that this index is more applicable and efficient for selection of parental inbred lines in producing hybrids to be tolerant to high temperatures and high yielding under both conditions. Based on two years' data and using the STI, GMP and MP indices, K166B, K166A and K18×K166B proved to be the most heat tolerant lines and hybrid. Biplot analysis allowed us to distinguish groups of tolerant and sensitive inbred lines and hybrids. Based on the results of this study, the hybrid K18×K166B can be recommended for the Khuzestan region.

Keywords: Biplot, Correlation, Heat stress, Maize, Tolerance index.

INTRODUCTION

 Stress can reduce maize grain yield and quality and any further rise in temperature reduces the pollen viability and silk receptivity, resulting in poor seed set and reduced grain yield (Johnson, 2000; Aldrich *et al.*, 1986; Samuel *et al.*, 1986). In the southern part of Iran, especially in Khuzestan, high temperature stress is one of the most important abiotic stresses in the maize growing area. Increasing heat tolerance of hybrids is consequently a challenge for maize breeders. For this, it is necessary for promising inbred lines as well as their combinations to be tested under both normal and heat stress conditions.

Different indices have been employed for screening stress tolerant genotypes. These indices are based either on stress resistance or susceptibility of genotypes (Fernandez, 1992). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the non-stress (Yp) and stress (Ys) environments and mean productivity (MP) as the average yield of *Yp* and *Ys*.

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Fischer and Maurer (1978) proposed a genotype stress susceptibility index (SSI) as a ratio of genotypic performance under stress and non-stress conditions. Fernandez (1992) introduced a stress tolerance index (STI) which can be used to identify genotypes that yield well under both stress and non-stress conditions. Geometric mean productivity (GMP) is another index which is often used by breeders interested in relative performance (Ramirez and Kelly, 1998). Rosielle and Hamblin (1981) reported a positive correlation between *MP* and *Ys*, therefore selection based on *MP* will improve average yield under both stress and non-stress environments. Other studies also showed a high and positive correlation between *MP* and yield under stress conditions (Sanjari, 1998; Ghajar Sepanlo *et al.*, 2000).

A low *TOL* index indicates higher tolerance to stress. Selection based on this criterion favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions (Fernandez, 1992). This criterion does not permit us to separate genotypes yielding well under stressed conditions from genotypes yielding well under both stress and unstressed conditions. Stress indices based on loss of yield under stress conditions in comparison to normal conditions have been used for screening stress tolerant genotypes. Mitra (2001), Fernandez (1992) and Kristin *et al.* (1997) used genotypes' *GMP* under both conditions for the determination of susceptibility to avoid the effects of stress variation in different years. Clarke *et al.* (1992) used *SSI* for evaluation of drought tolerance in wheat genotypes and found a year-by-year variation in *SSI* for genotypes and their ranking pattern. Ramirez and Kelly (1998) reported that selection based on a combination of *GMP* and *SSI* indices may provide a more desirable criterion for improving drought resistance in common bean.

In wheat, Bansal and Sinha (1991) proposed to use SSI and grain yield as stability parameters to identify drought resistant genotypes. Moghaddam and Hadizadeh (2000) reported that *STI* is more applicable for selection of maize genotypes tolerant to stress than *SSI. STI* and *GMP* tend to select hybrids with high yield under stress and non-stress conditions, while *SSI* identifies genotypes yielding well under stress conditions (Khalili *et al.*, 2004; Souri *et al.*, 2005; Karami *et al.*, 2006). The present study was conducted to examine the accuracy of different stress tolerance indices in identifying maize inbred lines and hybrids for heat stress tolerance.

MATERIALS AND METHODS

The study was conducted at Shushtar city located in Khuzestan Province, Iran (32°2 N and 48°50 ′ E, 150m asl) during two years 2007 and 2008. The soil type at this location is clay loam, pH= 7.6 with *EC*= 0.5 mmhos cm^{-1} .

Fifteen maize inbred lines were evaluated using a randomized complete block design with three replications, under two planting dates: 6 July, to coincide with heat stress and pollination time; and 27 July (the normal planting date) to avoid a high temperature during pollination and grain filling period. Twenty-eight hybrids obtained from combinations of eight selected inbred lines with different reactions to heat stress were evaluated under the same conditions in 2008. Each plot contained three rows 75 cm apart and 9 m in length and consisted 45 hills; each of two seeds were sown, one of whose seedlings were removed at the six leaves stage. The experiment was irrigated every five days, fertilizers were applied prior to sowing at a rate of 120 kg N ha⁻¹ and 140 kg P ha $^{-1}$, and additional side dressing of 120 kg N ha $^{-1}$ was applied at the six leaves stage of maize plants. Minimum and maximum air temperatures at pollination time were 29°C and 45° C in 2007 and 31° C and 45° C in 2008 under heat stress conditions (planting date 6 July) and 24° C and 38° C in 2007 and

Months	Temperature $({}^{\circ}C)$						
		Minimum	Maximum				
	2007	2008	2007	2008			
July	30°C	$31 \, \text{oC}$	46°C	46 °C			
August	32. ^o C	$32 \, ^{\circ}C$	47°C	$46 \degree C$			
September	29°C	$31 \, \text{oC}$	45°C	45°C			
October	24°C	23°C	38 °C	38 °C			
November	19 oC	17°C	32°C	27 oC			

Table 1. Average minimum and maximum temperature of research farm in heat stress and non-stress conditions in 2007 and 2008.

23°C and 38°C in 2008 under normal conditions (planting date 27 July) (Table 1).

Stress tolerance indices were calculated by the following formula:

TOL= Y_p - Y_s (Rosielle and Hamblin, 1981),

 $MP = \frac{Yp + Ys}{2}$ *(Rosielle and Hamblin,*

1981),
$$
GMP = \sqrt{Yp.Ys}
$$
 (Fernander, 1992),

Ys

$$
SSI = \frac{1 - \frac{TS}{Yp}}{SI}
$$
 (Fischer and Maurer, 1978),

in which
$$
SI = 1 - \frac{\overline{Ys}}{\overline{Yp}} \text{ and } STI = \frac{Ys.Yp}{(\overline{Yp})^2}
$$

(Fernandez,1992) with *Ys* and *YP* being the yields of genotypes evaluated under stress and non-stress conditions and *Ys* and *Yp* the mean yield over all genotypes evaluated under stress and non-stress conditions.

Analysis of variance was performed for each individual experiment and year, using the SPSS computer program as well as mean comparison and correlation coefficients. The biplot display was used, which provides a useful tool for data analysis. To display the genotypes in biplot, a principal component analysis was performed.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences between inbred lines and between hybrids (Table 2). Among inbred lines, K166B and K166A produced high grain yield under both stress and nonstress conditions in the two years. K3651/2 had the highest yield under non-stressed, but low yield under stressed conditions. K47/2- 2-13-3-1-1-1; K19 lines had relatively high yield in non-stressed conditions, but

Table 2. Analysis of variance of stress tolerance indices and yield in heat stress and non-stress conditions in maize inbred lines in 2007-2008.

	Source of	Degree	Mean of squares						
variance		of	Y_P^a	Y_{S} b	MP ^c	GMP ^d	STI ^e	SSI	TOL ^g
		freedom							
2007	Block	2	1020982ns	63452ns	145555ns	120ns	0.23 ns	0.04 _{ns}	1075678ns
	Line	14	1560356**	666166**	965432**	8994**	$0.65**$	$0.35**$	1877321**
	Error	28	353517	86319	100421	95625	0.18	0.008	586526
2008	Block	2	970002ns	44086ns	150215 _{ns}	97ns	0.12ns	0.002ns	1431137*
	Line	14	1589764**	710195**	864802**	908376**	$0.52**$	$0.28**$	1144279**
	Error	28	355490	87176	122076	95625	0.1	0.004	396527
2008 ^h	Block	2	2317944*	535426*	216145ns	171559ns	$0.2**$	0.04 _{ns}	4792526**
	Hybrid	27	1316319**	$1092651**$	912147**	1169363**	$0.34**$	$0.38**$	1154495*
	Error	54	584733	145969	174202	137082	0.04	0.16	740475

* and **, Significant at 5% and 1% levels, respectively. ns= Non significant.

^{*a*} Yield in non-stress conditions, ^{*b*} Yield in stress conditions, ^{*c*} Mean Productivity, ^{*d*} Geometric Mean Productivity, ^{*e*} Stress Tolerance Index, ^{*f*} Stress Susceptibility Index, ^{*g*} Tolerance Index.

h Analysis of variance of stress tolerance indices and yield in heat stress and non-stress conditions in maize hybrids in 2008.

relatively low yield under stressed conditions. In contrast, K18 and K19/1 lines had a high yield under stressed and intermediate yield under non-stressed conditions (Table 3). Among the hybrids, K18×K166B had the highest yield under both conditions. K166A×K3640/5, K166A×K47/2-2-1-21-2-1-1-1 and K166A×K19 had the highest yield under non-stressed and intermediate yield under stressed conditions. K18×K47/2-2-1-21-2-1- 1-1 showed the smallest yield difference between stressed and non-stressed conditions (Table 4).

Based on the *MP* index, the K166B, K3651/2 and K166A lines and K18×K166B and K18×K47/2-2-1-21-2-1-1-1 hybrids were identified as tolerant (Tables 3 and 4). Therefore, according to these results, selection based on *MP* will improve mean yield under both conditions, but does not allow to discriminate lines of groups A (high yield under both conditions) and B (high yield under non-stress and low yield under stress conditions). The same results were reported by Moghaddam and Hadizadeh (2002) and Khalili *et al.* (2004).

TOL index allowed us to select MO17, K166A and K3640/5 lines and K18×K19 hybrid as tolerant genotypes (Tables 3 and 4). All of these genotypes, except K166A, were low yielding under both conditions. This is due to low yield differences between the two conditions, that decreased the value of the *TOL* index. Therefore, low *TOL* does not mean high yielding, and genotype yield should be taken in consideration in addition to this criterion. Similar results were reported by Ahmadzadeh (1997) for maize hybrids. Limitations of using the *TOL* index have also been discussed in relation to wheat (Clark *et al.,* 1992) and common bean (Ramirez and Kelly, 1998). Although low *TOL* has been used for selecting genotypes with tolerance to stress, the likelihood of selecting low yielding genotypes can be anticipated (Ramirez and Kelly, 1998).

According to *SSI*, the K166A and then, K166B and K18 inbred lines and K18×K47/2-2-1-21-2-1-1-1 hybrid were revealed as tolerant to heat stress. K166A yielded relatively highly in both conditions, while K18 and K18× K47/2-2-1-21-2-1-1-1 had an intermediate yield under non-stressed and a relatively high yield under stressed conditions. K166B yielded well under both conditions (Tables 3 and 4). Therefore, this index discriminated group A genotypes from others. This finding is consistent with that reported by Moghaddam and Hadizadeh (2000) in maize.

 Based on *STI,* the lines K166A and K166B and the hybrid K18×K166B showed the highest tolerance to heat stress. K18×K166B produced the highest yield in both conditions (Tables 3 and 4). This index also separate group A from other groups. This is in consistent with those reported by Ahmadzadeh (1997), Moghaddam and Hadizadeh (2000) and Khaili *et al.* (2004) in maize.

The study of *GMP* showed more comprehensive results. Based on this index, the K166B, K166A and K18 lines and K18×K3640/5 and K18×K47/2-2-1-21-2-1- 1-1 hybrids were revealed as tolerant, and had high yield under both conditions (Tables 3 and 4). The ability to separate group A genotypes from others using the *GMP* index is consistent with the results reported by Ahmadzadeh (1997) and Khalili *et al.* (2004) in maize, Kristin *et al.* (1997) and Fernandez (1992) in common bean, Souri *et al.* (2005) in pea, Karami *et al.* (2005) in barley and Rezaeizad (2007) in sunflower. This makes *GMP* the most accurate criterion in selecting genotypes with tolerance to heat stress and high yield under both stressed and non-stressed conditions.

To determine the most desirable stress tolerant criterion, the correlation coefficient between *Yp, Ys* and quantitative indices of stress tolerance were calculated (Table 5). There were significant correlations between *Yp*, and (MP, GMP and TOL); and between *Ys*, and (MP, STI, GMP and SSI); *GMP* and *MP* consequently appeared as better predictors of *Yp* and *Ys* than *TOL*, *SSI* and *STI.* The relationships observed between both *Yp* and *Ys*, and *MP* are consistent with

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Table 4. Mean comparison related to maize hybrids' yield in non-stress conditions (Yp), yield in stress conditions (Ys), Mean Productivity (MP), Geometric Mean Productivity (CMP), Tolerance Index (TOL), Stress Susceptibili

		$Yp^{\overline{a}}$	\overline{Ys}^b	TOL \overline{c}	STI^d	SSI ^e	MP ^f
	Y_{S}	$0.53*$					
	TOL	$0.76**$	-0.14 ns				
Line 2007	STI	0.34 ns	$0.95**$	-0.33 ns			
year	SSI	-0.35 ns	$-0.95**$	0.31 ns	$-0.99**$		
	MP	$0.93**$	$0.81**$	0.46 ns	$0.66**$	$-0.67**$	
	GMP g	$0.74**$	$0.96**$	0.13 ns	$0.87**$	$-0.87**$	$0.94**$
	Y_{S}	$0.52*$					
	TOL	$0.75**$	-0.17 ns				
Line 2008	STI	$0.66**$	$0.93**$	0.02ns			
year	SSI	-0.38 ns	$-0.96**$	0.31 ns	$-0.82**$		
	MP	$0.93**$	$0.80**$	0.44 ns	$0.88**$	$-0.69**$	
	GMP	$0.75**$	$0.95**$	0.13ns	$0.95**$	$-0.88**$	$0.94**$
	Ys.	$0.52**$					
Hybrid	TOL	$0.57**$	$-0.40*$				
	STI	$0.66**$	$0.95**$	-0.21 ns			
	SSI	-0.07 ns	$-0.81**$	$0.69**$	$-0.62**$		
	MP	$0.88**$	$0.86**$	0.12ns	$0.92**$	$-0.49**$	
	GMP	$0.71**$	$0.96**$	-0.15 ns	$0.96**$	$0.65**$	$0.95**$

Table 5. Phenotypic correlation coefficients between maize inbred lines yield (2007 and 2008) and hybrids in stress and non-stress conditions and heat stress tolerance indices.

* and **, Significant at 5% and 1% levels, respectively.

ns= Nonsignificant.

^{*a*} Yield in non-stress conditions, ^{*b*} Yield in stress conditions, ^{*c*} Tolerance Index, ^{*d*} Stress Tolerance Index, ^{*e*} Stress Susceptibility Index, ^{*f*} Mean Productivity, ^{*g*} Geometric Mean Productivity.

those reported by Fernandez (1992) in mungbean and Farshadfar and Sutka (2002) in maize. In the present study, the correlation coefficients between *SSI* and *Ys* were $r = -0.95$ and -0.96 in the two years, respectively, for indeed lines and $r = -0.81$ for hybrids. Thus, selection for *SSI* should give decreased yield under heat stress conditions. Therefore selection for stress tolerance should give a positive yield response in a hot environment. The correlation coefficients between *STI* and *Yp* were $r = 0.95$, $r = 0.93$ for indeed lines in

2007 and 2008, and *r*= 0.95 for hybrids in 2008. The correlation coefficients between *STI* and *Ys* were *r*= 0.34, *r*= 0.66 and *r*= 0.66, respectively. Thus, selection for *STI* should give positive responses under nonstressed conditions. These results are similar to those reported by Ahmadzadeh (1997), Moghaddam and Hadizadeh (2000) and Khalili *et al.* (2004). A high correlation coefficient between *Ys* and *STI* and a negative correlation coefficient between *Ys* and *SSI* indicated that selection for tolerance based on *STI* and *SSI* would be worthwhile

Table 6. Eigen values, cumulative proportion and component of first and second tolerance indices and yield in stress and non-stress conditions in maize inbred lines in two years and maize hybrids.

Treatment	Component	Eigen values	Cumulative proportion	Yр	Ys	MP	GMP	TOL	SSI	STI
Line		4.909	%70.12	0.384	0.978	-0.297	0.703	-0.992	0.992	0.900
Year 1	2	2.025	%99.05	0.923	0.165	0.950	0.709	0.302	-0.046	0.427
Line		5.062	%72.32	0.507	0.994	0.796	0.949	-0.188	-0.967	0.937
Year 2	∍	1.795	%97.96	0.861	0.014	0.605	0.311	0.980	0.138	0.217
Hybrid		4.790	%68.43	0.854	0.886	0.997	0.969	0.067	-0.521	0.938
	∍	1.982	%96.74	0.510	-0.461	0.055	-0.212	0.985	0.784	-0.250

only when the target environment is heat stressed. Fernandez (1992) proposed *STI* as

an index which discriminates genotypes with high yield and stress tolerance potentials. In this study, we found positive and high correlation between grain yield under heat stress and *STI*. The correlation coefficients between *GMP* and yield in stress and nonstress environments were highly positive and significant, especially under stressed conditions (Table 5). Hence, selection for high *GMP* should give positive responses in both environments. The correlation coefficients between *MP* and, *Yp* and *Ys* were high and positive (Table 5). Therefore, selection for *MP* should give positive responses in both environments. Similar results were reported by Ahmadzadeh (1997) in maize and Ghajar Sepanlo *et al.* (2000) and Sanjari (1998) in wheat. Selection based on a combination of indices may provide a more useful criterion for improving stress tolerance of maize.

Principal component analysis (PCA) of inbred lines revealed that the first PCA explained 70.12% and 72.32% of the

variation with *Yp, Ys, MP, GMP, SSI, TOL* and *STI* in 2007 and 2008, respectively. In the case of hybrids, the first PCA explained 68.43% of the variation with the same attributes (Table 6). Thus, the first axis (PCA1) can be identified as yield potential and heat tolerance. Considering the high and positive value of this PCA on biplot, selected genotypes will be high yielding under stress and non-stress environments. The second PCA explained 28.93%, 25.64% and 28.31% of the variation with different attributes in 2007 and 2008 in inbred lines and in 2008 in hybrids, respectively (Table 6). Therefore the second component (PCA2) can be named as a stress susceptible component with low yield in a stressful environment. Thus selection of genotypes that have high PCA1 and low PCA2 are suitable for both stress and non-stress environments. Therefore, K166B, K166A, K18 and K19/1 inbred lines and K18×K166B, K18×K47/2-2-1-21-2-1-1-1, K166B×K3640/5, K166B×K47/2-2-1-21-2-

1-1-1 and K166B×K19 hybrids are superior for both environments with high PCA1 and low PCA2.

Kaya *et al.* (2002) revealed that genotypes with larger PCA1 and lower PCA2 scores gave high yields (stable genotypes), and genotypes with lower PCA1 and larger PCA2 scores had low yields (unstable genotypes). The use of biplot display in selecting drought tolerant genotypes has already been used by Ahmadzadeh (1997) in maize, Fernandez (1992) in common bean, Souri *et al.* (2005) in pea and Karami *et al.* (2006) in barley. The correlation coefficient among any two indices is given approximately by the cosinus of the angle between their vectors. Hence, $r = \cos 180^\circ = -$ 1, $\cos 0^\circ = 1$, and $\cos 90^\circ = 0$ (Yan and Rajcan, 2002). Thus, a strong positive association between *GMP*, *MP* and *STI* with *Yp* and *Ys* was revealed by the acute angles between the corresponding vectors. A negative association between *SSI* and *Ys* was reflected by the larger obtuse angles between their vectors in a biplot display (Figure 1). The results obtained from the biplot graph, confirmed the correlation analysis. Results of this study are in good agreement with Golabadi *et al*. (2006) in durum for drought tolerance.

CONCLUSIONS

According to the results of the two years, the use of the *SSI, STI* and *GMP* indices should help to improve heat tolerance in inbred lines. *GMP* that showed high positive correlations with grain yield in both stressed and non-stressed environments should be more efficient in inbred line selection. In the case of hybrids, *MP, GMP* and *STI* are all applicable. In general, selection of inbred maize lines and hybrids based on *GMP* might allow us to improve heat tolerance and potential yield under both environments. Based on biplot display, the lines K166B, K166A, K18 and K19/1 appeared as having high yield potential and low stress susceptibility. Based on biplot analysis, the

Figure 1. The biplot display of yield in seven heat tolerance indices based on the first and second main components [A] maize inbred lines in first year; [B] maize inbred lines in second year; [C] maize hybrids.

hybrids K18×K166B, K18×K47/2-2-1-21-2- 2-1-1-1, K166B×K3640/5, K166B×K47/2- 2-1-21-2-1-1-1 and K166B×K19 exhibited high yield potential and low stress susceptibility. The K18×K166B hybrid showed high yield under both conditions.

Parents of this hybrid are high yielding inbred lines in both environments. Based on the results of this study, the hybrid K18×K166B can be recommended for the Khuzestan region. Therefore, regarding the frequency of heat tolerant combinations, we can conclude that K166B should be a source of heat tolerance in crosses for hybrid production.

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تعيين بهترين شاخص (هاي) تحمل به تنش گرما در لاينها و هيبريدهاي ذرت در شرايط خوزستان

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چكيده

اصلاح ذرت براي تحمل به دماي بالا نيازمند بررسي دقيق لاينهاي والديني و تركيبات آنها است. پانزده لاين خالص ذرت در سال0هاى ۱۳۸۶ و ۱۳۸۷ در شهرستان شوشتر (استان خوزستان) ارزيابي شدند. اينبرد لاینِها در دو تاریخ ۱۵ تیرماه به منظور همزمانی تنش گرما با زمان گرده افشانی و ۵ مردادماه به عنوان زمان كشت معمول جهت اجتناب از دماي بالا در زمان گرده افشاني و دوره پرشدن دانه كشت گرديدند. علاوه بر اين، 28 هيبريد حاصل از تركيب هشت لاين برگزيده در شرايط مشابه در سال 1387 مورد ارزيابي قرار گرفتند. پنج شاخص تحمل به تنش شامل ميانگين بهرهوري (MP)، تحمل به تنش (TOL)، حساسيت به تنش (SSI(، شاخص تحمل به تنش (STI(و ميانگين هندسي بهره وري (GMP(در اين مطالعه مورد استفاده قرار گرفتند. تجزيه دادهها نشان داد كه شاخصهاي SSI، STI و GMP معيار دقيقتري براي گزينش ژنوتيپهاي با تحمل گرما و عملكرد بالا در هر دو شرايط تنش و بدون تنش هستند. همبستگي مثبت و معني دار GMP و عملكرد دانه در هر دو شرايط نشان داد كه اين شاخص براي گزينش لاينهاي والديني به منظور توليد هيبريدهاي متحمل به دماي بالا و عملكرد بالا تحت هر دو شرايط كارايي بيشتري دارد. براساس دادههاي دو سال و با استفاده از شاخصهاي STI، GMP و MP، لاينهاي B166K و K166A و هيبريد K18×K18 متحمل $ترين لاينها و هيبريد به تنش گرما شناخته شدند. تجزيه باي$ پلات، گروههاي متحمل و حساس لاينها و هيبريدها را به تنش گرما تشخيص داد. بر اساس نتايج اين مطالعه هيبريد K18×K166Bرا ميتوان براى منطقه خوزستان توصيه نمود.