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Anthesis to Silking Interval Usefulness in Developing Drought Tolerant Maize

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Abstract - Maize, the most important stable crop in Kenya is affected by drought stress mostly at flowering stages causing delay in silk emergence. The Anthesis to Silking Interval (ASI) is highly correlated to grain yields under drought stress as shown by experiments conducted at CIMMYT, Mexico. This study aimed at combining both earliness and short ASI in one maize genotype. Early-maturing, open pollinated varieties (OPVs) from Kenya Agricultural Research Institute (KARI), with a long ASI and late-maturing, inbred lines from CIMMYT with a short ASI were evaluated in a randomized complete block design trial replicated three times. F2 progenies arising from crosses between these two types of germplasm were generated and tested under drought stress conditions. There were significant differences among the OPVs and inbred lines, in all traits including ASI. ASI was significantly correlated to kernel weight (r=0.76), days to anthesis (r=-91), days to silking (r=-0.81) and to leaf senescence (r=0.86). F2 population of KVD2 x CML442 produced 74-80% of the early flowering genotypes of between 49 and 57 days and had an ASI segregation range of -2 to 10 days. The results showed that ASI and earliness can be combined and selected for in the same maize genetic background.

Keywords - ASI, Days to Anthesis, Days to Silking, Drought Tolerance, Inbred Lines, Open Pollinated Varieties (OPVs)

1. Introduction

Drought has had adverse effects on yields in most of the farming regions around the world [1]. According to [2], maize hybrids worldwide will only make an impact and result into increased production if a reasonable level of drought tolerance is incorporated in the germplasm. However, variable field conditions and weather factors in addition to the lack of an integrated measure make it difficult to characterize drought stress and tolerance.

Secondary traits can improve the precision with which drought tolerant genotypes are identified, as opposed to measuring grain yield only [3]. Flowering in maize is a crucial trait in breeding for drought tolerance. When maize flowers under drought, there is the delay in silking and the period between male and female flowering increases giving rise to anthesis silking interval (ASI). Studies done by [3] showed that high grain yield under a range of stress intensities was associated with a short ASI and earlier flowering dates, increased plant and ear height, increased number of ear per plant and delayed leaf senescence [4].

Drought stress affects maize productivity most, when it occurs during the two weeks of the flowering period [5]. It is during flowering and pollination, when there is a high demand for water to ensure grain filling [6,7].

In segregating genotypes under drought, lengthening of ASI period is an indicator of poor tolerance to drought stress. ASI is highly correlated with grain yield and has a high heritability under drought stress [7]. Richards [8] reported that drought susceptible genotypes allocate less assimilate toward ear growth when the ears are quite small and this is well indicated by a delay in silking. According to [7] recurrent selection for low ASI, small tassels and delayed senescence resulted in increased partitioning to early ear growth and high grain yields.

Drought affects maize production in most parts of Kenya considering that over 80% of the land is arid and semi-arid. Edmeades and Bolanos [9,10] consistently showed that a short ASI is a strong measure of drought tolerance in maize genotypes. Genotypes that gave high grain yields under severe drought stress levels had a short ASI and were late maturing whereas those that matured early had a long ASI and lower grain yields. Indeed even after eight cycles of recurrent selection, grain yield under severe drought stress levels was highly positively correlated with a short ASI. Subsequent studies at Centro Internacional de Mejoramiento de Maiz y Trigo, (CIMMYT) genetically mapped the Quantitative Trait Loci (QTL) for ASI and grain yield and confirmed that indeed, a short ASI is a real measure of drought tolerance in maize [11,12]. Nevertheless, up to now maize breeders in CIMMYT have been selecting only for short ASI in their elite materials whereas in Kenya, breeders have developed maize varieties such as Katumani and Makueni composites, Dryland composites and Pwani maize using early maturity as the only selection criterion. The objective of this study was an attempt to combine early maturity and a short ASI in the same genotype in order to enhance the drought tolerance of maize genotypes and subsequently increase grain yields of this important staple crop in the country.

2. Material and Methods

2.1. Field evaluation of parental lines

Eleven genotypes which included four CIMMYT inbred lines and KARI composites and synthetics were evaluated under drought stress environment. These genotypes were CML 440, CML 442, CML 444, CML 445, KDV1, KDV2, KDV3, KDV4, KDV5, KCB, and ZEWA, respectively.

The materials were sown at KARI, Kiboko sub-station in Eastern Province, Kenya. Kiboko is at Longitude of 37.75oE, Latitude 2.15oS and has an elevation of 974 m above the sea level. The soils at Kiboko Research Farm are ferric fluvisols, which are mainly sandy clay type with a top soil pH of 7.9. The annual rainfall is 530 mm, while the maximum and minimum temperatures are 35.1oC and 14.3oC respectively. The genotypes were evaluated in a randomized complete block design of three replications. Each plot consisted of four rows of maize spaced 75cm apart with an intra-row spacing of 25cm.

The experiment was conducted in a relatively rain free environment and irrigation was applied as recommended by [13,14]. According to the methods used by [15] and [14] one can be able to induce drought stress and select for drought tolerance by applying over-head sprinkler irrigation and withholding water, 14 days before 50% male flowering without necessarily measuring the exact amount of water applied or the evapo-transpiration rates experienced. Therefore in this trial, the overhead irrigation was applied as described by [15]. Drought stress was imposed by irrigating at an interval of 4 days for three hours, right from germination stage up to 14 days before anthesis (male flowering) when water was withdrawn. Two additional irrigation regimes were applied as follows; the first one was applied, 14 days after 50% anthesis (male flowering) while the second one was applied, 26 days after 80-100% of the plots had completed male flowering [15]. Leaf-rolling during flowering was scored as a complimentary indicator of induced drought stress [15].

Di-ammonium phosphate (DAP) fertilizer was applied during planting at the rate of 150 kg/ha and top dressing was done three weeks after planting with calcium ammonium nitrate (CAN) at the rate of 150 kg/Ha. The data were collected in the middle two rows per plot from a mean of ten plants randomly selected [6] for the following agronomic traits; anthesis and silking dates, ears per plant, 100 kernel weight and grain yield. Anthesis dates were recorded when 50% of the plants had shed pollen whereas silking date was recorded when 50% of the plants had extruded silks. ASI was calculated as the difference between the recorded anthesis and silking dates [6]. Leaf senescence was assessed four times weekly, two weeks after 50% male flowering. This was done through visual estimation of the whole plot and the proportion of green leaves versus senescent dried leaves was scored on a 1-10 scale, where 1 represented the lowest senescence score and 10, the highest. A hundred kernel weight and grain yield was measured as a mean of ten randomly chosen plants in the middle two rows after shelling when the moisture content was 13%.

2.2. Hybridization to generate F1 and F2 populations

The CIMMYT inbred lines CML 440, CML442, CML 444 and CML 445 were designated as the male lines and were crossed to KDV2, KDV4, and K64R designated as the female lines, in the long-rains of 2009 at the Kabete Field Station of the University of Nairobi. Since, the CIMMYT inbred lines were late maturing compared to the female lines, they were planted three weeks earlier than the female lines to ensure that flowering of both male and female plants was well synchronized. The mating design used during crossing was the North Carolina Design II where each male was crossed to each female to generate a total of 12 F1 populations.

The crop was weeded manually, di-ammonium phosphate (150 kg/ha) was applied at planting and top-dressing was done with CAN fertilizer at the rate of 150 kg/ha when the plants were three weeks old. The F1 seed was planted and selfed to obtain the F2 segregating populations. The data were subjected to ANOVA using GENSTAT-5 computer program. The means were separated using LSD. Phenotypic correlations were calculated to determine the relationships among the agronomic and physiological traits.

2.3. Field Evaluation of F2 Populations

A total of 12 different F2 populations generated from inbred lines CML 440, CML442, CML 444 and CML 445 (male lines) and OPVs, KDV2, KDV4, and K64 (female lines) were planted in the KARI Kiboko field station. About 1000 F2 seeds from each population were planted and selfed to generate S1 cobs. Di-ammonium phosphate fertilizer at the rate of 150 kg/ha was applied during planting and top-dressed with CAN fertilizer at the rate of 150 kg/ha. The data collected was entered in the excel application and the days to anthesis were used to select for early and mid-maturing genotypes in the segregating populations. The early maturing genotypes were those genotypes which flowered at less than 57 days from time of planting and matured at 120 days while those that flowered at more than 58 days from planting were regarded as mid maturing genotypes. Only the frequencies of ASI in one of the F2 segregating populations (KDV2 x CML442) are shown here below.

3. Results

Genotypes, KDV1, KDV2, KDV3, KDV4, KDV5, KCB and ZEWA had significantly (P≤ 0.05) larger ASI compared to CML 440, CML 442, CML 444 and CML 445 (Table 1). The KCB genotype had significantly (P≤ 0.05) larger ASI than all the genotypes except that of KDV5. The CML lines had the shortest ASI as compared to the rest of the genotypes (Table 1).

Table 1. Means for anthesis to silking interval, ears per plant, kernel weight, grain yield, days to anthesis and days to silking of parental genotypes planted in 2009 long-rains season at Kiboko Research Centre

Genotype	ASI	Ears plant	Kernel weight	Grain yield	DA	DS
CML 440	2.67c	0.73a	24.20a	2.91a	60.00c	62.67c
CML 442	3.00c	0.58a	18.64b	1.03c	67.00b	70.00b
CML 444	3.00c	0.78a	16.50b	1.41c	70.00a	73.00a
CML 445	4.00c	0.72a	19.97b	1.28c	62.00c	66.00d
KCB	10.67a	0.69a	26.37a	1.17c	47.33e	58.00d
KDV 1	8.33b	0.76a	23.60a	1.64b	51.00d	59.33d
KDV 2	7.67b	0.79a	25.30a	2.42a	49.00e	56.67d
KDV 3	7.67b	0.64a	24.60a	1.45b	49.33e	57.67d
KDV 4	7.67b	0.74a	22.60a	1.98b	53.00d	60.33c
KDV 5	9.33a	0.63a	24.97a	1.33b	51.00d	60.33c
ZEWA	8.00b	0.71a	26.47a	1.98b	51.00d	59.00d
Means	6.55	0.71	23.02	1.69	55.52	62.09
$LSD_{0.05}$	1.53	NS	3.47	0.66	2.26	2.39
CV (%)	2.4	5.9	4.5	5.9	1.1	0.9

Key-Means followed by the same letter are not significant from each other

-NS- Not-significant; DA- days to anthesis; DS- days to silking

There was no significant (P≤ 0.05) difference in ears per plant for all the genotypes. There was significant (P≤ 0.05) difference in the kernel weight of the different maize genotypes. The ZEWA genotype had significantly (P≤ 0.05) heavier kernels than most of the genotypes. The genotype, CML 444 had significantly the least kernel weight among all the genotypes. Genotype, CML 440 had significantly heavier kernels than the other CML lines. There was significant (P≤ 0.05) difference in total grain yield among the different maize genotypes. The CML 440 genotype had the highest grain yield overall. Genotypes, KDV 2 and CML 440 had significant higher yields than the rest. The CML 442, CML 445, CML 444, KCB, KDV3 and KDV 5 genotypes had significantly lower yields than the rest.

There was significant (P≤ 0.05) difference in days to anthesis and days to silking among maize genotypes (Table 2). All the CML lines were late in flowering and were also significantly different in their days to anthesis and days to silking. KDV lines differed significantly in their earliness but were very close to each other.

Key- Means followed by the same letter are not significant from each other Genotype, CML 444 had significantly more days to anthesis than the rest of the genotypes whereas genotypes KCB, KDV1, KDV5 and ZEWA had significantly fewer days to anthesis than the rest. CML 444 showed significantly more days to anthesis and silking as compared to the rest of the genotypes whereas KDV2 from the KARI germplasm was the next earliest flowering genotype after KCB.

Table 2. Means for days to anthesis and days to silking for different maize genotypes grown in 2009 long-rains season at Kiboko Research Centre

Genotype	Days to Anthesis	Days to Silking
CML 440	60.00c	62.67d
CML 442	67.00b	70.00b
CML 444	70.00a	73.00a
CML 445	62.00c	66.00c
KCB	47.33e	58.00f
KDV 1	51.00d	59.33e
KDV 2	49.00e	56.67f
KDV 3	49.33e	57.67f
KDV 4	53.00d	60.33e
KDV 5	51.00d	60.33e
ZEWA	51.00d	59.00e
Means	55.52	62.09
$LSD_{0.05}$	2.26	2.30
CV (%)	1.1	0.9

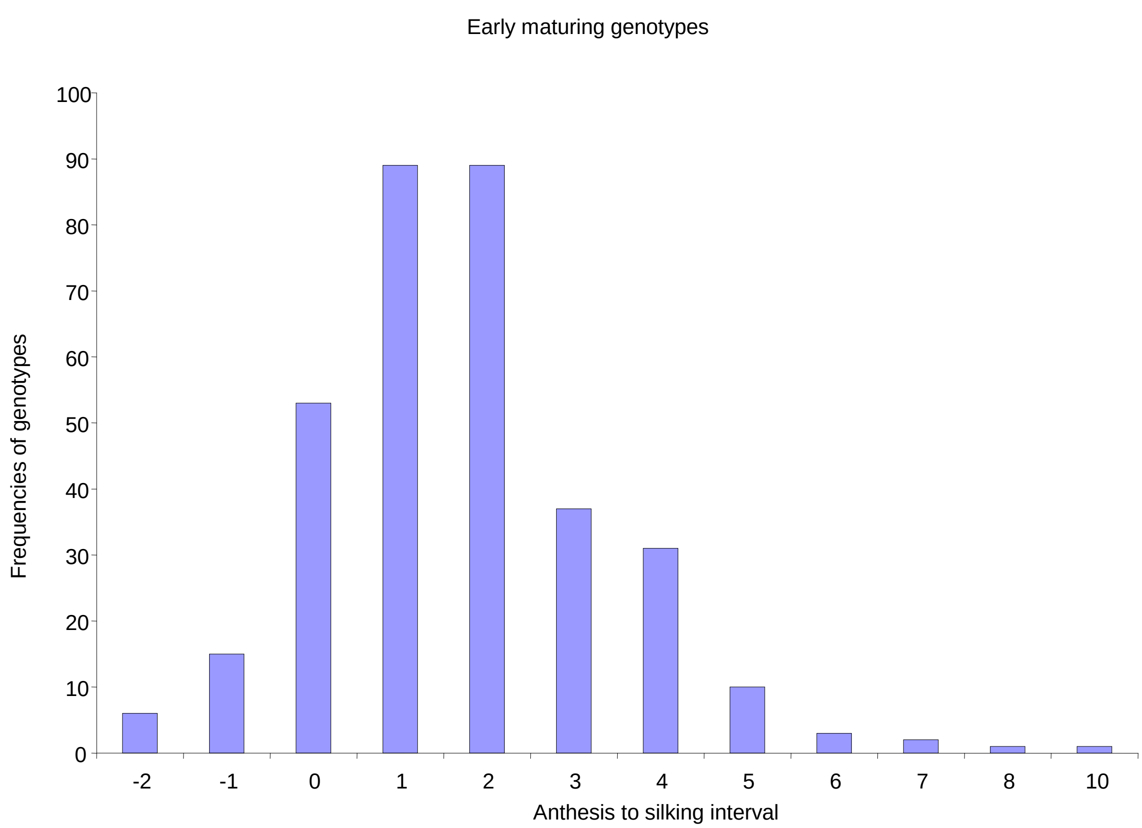

Fig 1. Frequencies of F_2 genotypes from KVD2 x CML442 population flowering at 49-57 days

In the F2 population of KDV2 x CML442, the frequency of early maturing genotypes was 74% with ASI ranging from

-2 to 10 days (Fig 1). The early-maturing genotypes with an ASI of between -2 and 2 days formed 75% of the population while those with an ASI of more than 3 days constituted 25 % of the cross. A negative ASI in this case signaled the occurrence of silking before tassels were produced, a drought escaping phenomenon. Table 3, shows the phenotypic correlations between the traits measured. Grain yield was not significantly correlated with any of the phenotypic traits measured (Table 3).

Table 3. Phenotypic correlation coefficients of the maize agronomic traits measured at Kiboko Research Centre

	ASI	EPP	KW	GY	DA	DS
EPP	-0.035					
KW	0.763**	-0.015				
GY	-0.140	0.544	0.374			
DA	-0.914**	-0.034	0.919**	-0.176		
DS	-0.811**	-0.085	-0.941**	-0.341	0.978**	
LS	0.864**	0.119	0.598	-0.017	-0.823**	-0.746**

*r is significant at $P \leq 0.05$ level; **r is significant at $P \leq 0.01$ level

Key: EPP-ears per plant, KW-kernel weight, GY-grain yield, DA- days to anthesis, DS-days to silking, LS- leaf senescence

However, GY was positively correlated to ears per plant (r=0.54) and kernel weight (r=0.37). The correlation between the ears per plant and the other secondary traits was not significant but the trait was positively correlated with grain yield (r= 0.54). The ASI had significant positive correlations with kernel weight (r=0.76, $P \leq 0.01$) and leaf senescence (r= 0.86, $P \leq 0.01$), but was negatively highly significantly correlated with days to anthesis (r= -91, $P \leq 0.01$) and days to silking (r= -0.81, $P \leq 0.05$) as would be expected. Kernel weight had significant negative correlations with days to anthesis (r= -0.92, $P \leq 0.01$) and days to silking (r= -0.94, $P \leq 0.05$). Days to anthesis had significant positive correlation with days to silking (r= 0.98, $P \leq 0.01$) and a significant negative correlation with leaf senescence (r= -0.82, $P \leq 0.01$). There was a significant negative correlation between days to silking and leaf senescence (r= -0.75, $P \leq 0.01$). Days to anthesis was positively significantly correlated with days to silking as would be expected (r=0.98, $P \leq 0.01$).

Fig 2 shows the regression of F2 ASI on parental ASI. There was no significant regression as shown by the figure mainly because whereas the parental lines were drought-stressed the F2 populations inadvertently received more water from unexpected rainfall during crop growth.

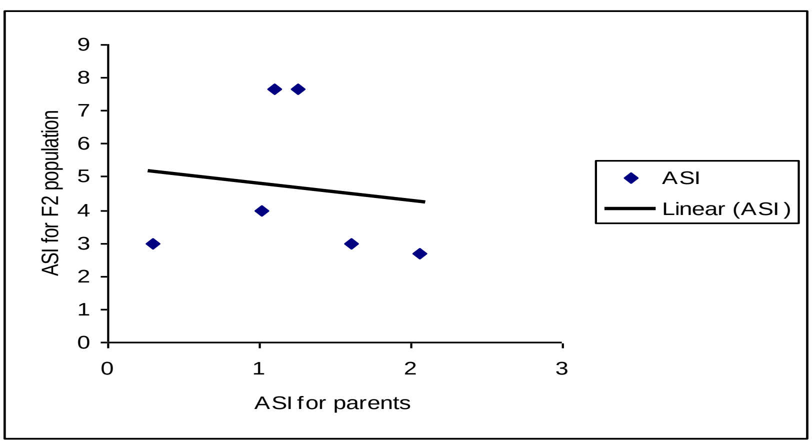

Fig 2. Linear regression (regression equation is y=0.510x+5.290 ; R^2= 0.016) of F_2 population ASI against the ASI of the parental lines.

4. Discussion

As the observations show, the average yield of the inbred lines was 1.7t/ha with a range of 1.03t/ha to 2.9t/ha and that of the OPVs was also 1.7t/ha with a range of 1.17t/ha to 2.42t/ha (Table 1). The OPVs flowered earlier than the inbred lines as would be expected. ASI values for the inbred lines ranged between 2.7 to 4 days and 7.7 to 10.7 days for OPVs. This observation is consistent with that reported by [16] where ASI values of inbred lines ranged between -0.9 to 6.5 days and those of the hybrids ranged between -0.8 to 8.2 days. The long ASI of 7.7 to 10.7 days in the OPVs resulted into lower yields, an observation similarly seen in those inbred lines with a longer ASI. The results reported here are similar to those of [17] who showed that an increase in ASI from 0.4 day to 10 days promoted a decline in grain yield of 8.7% per day. The study done by [16] showed that the inbred lines yielded lower than the hybrids.

Work done by [5,14,16] have shown that short ASI is associated with drought tolerance and therefore this trait together with ears per plant (EPP) are useful as secondary traits for selection of drought tolerance. [5,6,11,12] developed CML lines which have shorter ASI and are also drought tolerant. The segregation frequencies given here from one of the F2 populations (KDV2 x CML442), showed that ASI ranged from -2 days to 10 days, a variation that is not apparent in either of the parental lines or populations. By hybridizing CML inbred lines with KDV OPVs, it was possible to bring together either pleiotropic mechanisms or coupling phase linkage relationships between the alleles for earliness and those of a short ASI. Bolaños and Edmeades [5] showed that maize genotypes that are normally late maturing but have a short ASI produced higher grain yields under drought stress than the genotypes that were early-maturing but had a longer ASI. This study confirmed that drought tolerance mechanisms in maize are conferred by a shorter ASI whereas drought escaping mechanisms are conferred by a longer ASI. Under water limiting conditions, selection of a shorter ASI would therefore result into increased yield [14,11, 12]. Longer ASI results in less partitioning of assimilates to the developing ears, as indicated by the decrease in yield in the OPVs . Bolaños and Edmeades [5] reported that under drought stress, the genetic variation for ASI and kernel weight was large but was lower for EPP and grain yield. In contrast, under field drought stress conditions, [1,16] reported large genetic variation in EPP. Bänziger and Cooper [14] found that genetic variance and heritability of grain yield during the development of maize inbreds are generally lower under stress than under optimal conditions. In this study, the heritability of ASI and grain yield were fairly low, though the degree and level of drought stress could not be ascertained. As shown by [5] it is expected that selection for decreased ASI and increased grain yield will reduce the probability of delay in silk growth and spikelet fertility under stress. Therefore, a short ASI is an important trait in selection for increased yields under water limiting conditions. From the results reported in this study, it was found that the genotypes with a shorter ASI which in this case were from the CML series, gave higher yields than the OPV genotypes which had a larger ASI. This is mostly likely because shorter ASI genotypes utilized the limited moisture more efficiently during flowering.

One can safely conclude that, a shorter ASI is an indicator of drought tolerance. In this study, it was possible to transfer the short ASI trait from the CML lines to the early maturing local OPV genotypes as shown in Figure 1. The F2 populations consequently segregated for both short and long ASI.

The phenotypic correlations between grain yield (GY) and days to anthesis (-0.18), GY and days to silking (-0.34) GY and ASI (-0.140), GY and leaf senescence (-0.02) and GY and kernel weight (0.37) were low and non-significant (Table 3). Similar results of weak correlations between grain yield and other phenotypic traits under stress conditions were reported by [7,18]. The positive correlation between EPP and GY was expected because grain yield is a primary dependent variable of EPP. The highly significantly negative correlations between ASI and days to anthesis and days to silking is an indication that their alleles are linked in repulsion phase relationships whereas the positively significant correlation between ASI and leaf senescence is an indication that these alleles are linked in a coupling phase relationship. From these results, it would appear that ASI loci are closely linked to DA and DS loci but ASI and GY loci are in different chromosomes and it is not therefore easy to select for both traits at the same time.

The heritability of ASI in this study was low (R2= 0.02) (Fig 2). The heritability of ASI was low probably because the parental lines had been subjected to drought stress whereas the F2 populations received unexpected rainfall during growth. It has been argued that, the heritability of ASI is lowly expressed under well-watered conditions but is relatively high under drought stress [1,4]. Bolaños and Edmeades, [5] and Bänziger et al., [6] reported that under stress conditions, the heritability of ASI and EPP is increased while that of grain yield falls mainly because there is a decrease in genotypic variance. Betrán et al., [16] reported that under drought stress, grain yield had a low heritability but was positively correlated with ASI and EPP, suggesting that these secondary traits could be applied in breeding for drought stress tolerance.

5. Conclusion

The study revealed that the OPVs flowered earlier than inbred lines and had a long ASI of up to 11 days while the inbred lines flowered late and had a short ASI of between 2 to 5 days. ASI was highly negatively significantly correlated with days to anthesis, and days to silking but was positively correlated to kernel weight and ears per plant as would be expected.

The segregating populations between long ASI genotypes (KDV, OPVs) and inbred lines (CIMMYT, CML lines) crosses generated early–flowering progenies that flowered between 49-57 days and had an ASI of -2 days to 10 days. There was a strong indication that though the two traits may not be genetically linked to each other, it is possible to combine both drought escape (earliness) and drought tolerance (short ASI) mechanisms in one genetic background.

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