Original Paper Open Access

Comparison of biomass production, growth and solar energy utilization in specialty vs normal maize genotypes at different developmental stages

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Abstract

A great deal of research has been carried out to evaluate dry matter accumulation and solar radiation utilization in normal maize genotypes whereas limited information is available on special genotypes such as high oil and high protein maize. In this paper, we made a comparative analysis on biomass production, solar radiation utilization and growth at five different stages before and after flowering in normal (B73 and Mo17) and specialty maize (IHO and IHP) lines. Specialty maize genotypes were from 70th cycle of Illinois Long Term Selection. We used 12 directly-measured traits, 10 energy calculations and 6 time interval computations obtained from measurements in five developmental stages. A nested design was used to compare temporal changes in the observed traits.

We found significant differences between specialty and normal maize genotypes for most variables throughout the developmental stages. Normal genotypes had higher plant height than special ones. IHP strain had higher expanded leaf area than others, especially around the flowering. IHO produced higher dry matter per leaf area faster in the early stages and stayed green for longer, resulting in high values for total dry matter production and calculated energy equivalent. Radiation use efficiency (RUE) was higher in specialty maize compared to normal maize genotypes. The highest RUE was observed in IHP (1.36 g Mj plant^{-1}) around flowering stage. Overall, specialty and normal genotypes showed significant differences for some agromorphological and physiological traits as well as energy utilization and conversion into dry biomass.

Keywords: radiation use efficiency, energy accumulation, growth indices, high oil maize, high protein maize

Introduction

Maize is an excellent model crop for physiological research thanks to its high dry matter production. There have been intensive studies to uncover the physiological metabolism of dry matter production in this crop. To explain the changes in dry matter production and allocation in plants, researchers have used various measurements and calculations. Direct measurements, growth analysis and energy calculations are good examples of them.

Despite being less informative, direct measurements are more commonly used to describe changes in dry matter production in maize. Several direct measurements such as plant height, leaf area, leaf pigment concentration, and source-sink ratio were used to evaluate dry matter production and grain yield in earlier studies (Rajcan and Tollenaar, 1999; Vitale et al, 2009; Wang et al, 2009). Examples of studies using growth indices in maize research are also abundant. Several indices such as unit leaf rate (ULR), relative growth rate (RGR), specific leaf area (SLA), leaf weight fraction (LWF) and leaf area ratio (LAR) have been used to investigate the plant growth (Poorter and Garnier, 1996). Maize growth has been investigated on the basis of how much it is affected by fertilization and plant density, as well as temperature regimes and genetic factors, using the growth indices in previous studies (Soldati et al, 1999; Rasheed et al, 2003; Adebo and Olayeo, 2010). Dry matter production is directly related to energy utilization in plants. Efficiency of energy utilization, conversion of incoming solar energy into biological forms, is referred to as RUE (radiation use efficiency) (Monteith, 1977). RUE potential depends on plant species (Kiniry, 1989), agricultural practices (Tsubo et al, 2001), and environmental conditions (Lindquist et al, 2005). Energy utilization of plants is mostly measured by RUE; however, more detailed computations are available to decipher energy allocation in plant metabolism. Research has been conducted to compute the conversion potential of light energy and its partitioning into biochemical components, e.g. starch, oil, protein (Transeu, 1926; Penning De Vires et al, 1974), as well as plant parts such as leaves, stalk and kernels in maize (Girardin, 1985; Hedin et al, 1998; Salah et al, 2011).

Although there is a wealth of information in the literature dealing with the use of growth parameters or direct measurements to investigate dry matter production in normal maize genotypes, special types of maize (high oil and high protein, etc) have not been subject to such scrutiny. Similarly, the literature lacks

 RECEIVED 4/18/2016

information on the radiation use efficiency and solar energy conversion of special types of maize. It has been suggested that RUE values may vary significantly among the species depending on their biochemical composition (Sadras and Calderini, 2014). Similarly, special maize types differ distinctly from normal maize genotypes as they contain much higher levels of protein and oil in their kernels (Jugenheimer, 1961; Lambert, 2001). Strains of the Illinois Long-Term Selection Experiment (Dudley, 2001) are good examples of such genotypes. Use of these strains may provide a better analysis of dry matter production and energy storage into different biochemical forms (e.g., oil, protein, and carbohydrate) as well as different plant parts in maize.

We hypothesized that specialty and normal maize genotypes should be different in terms of dry matter production, growth and energy utilization as they significantly differ for their plant biochemical structure. To our knowledge, there has been no in-depth, systematic analysis that compares the changes in dry matter production, solar energy utilization and growth of specialty maize genotypes through developmental stages. Here, we attempt to compare normal and specialty maize lines and to demonstrate their genetic and physiologic differences for dry matter production, growth, and solar energy utilization in the course of plant development. For such a comparison we utilized direct measurements, time interval calculations and estimations on energy utilization of genotypes used.

Materials and Methods

Plant material and field trials

Four maize genotypes were used as plant material in this study (IHO, IHP, B73 and Mo17). The specialty maize lines, Illinois high oil (IHO, GRIN number: NSL20262) and high protein (IHP, GRIN number: NSL20624) strains, were from the 70th generation of the Illinois Long-Term Experiment, and obtained from the North Plant Genetic Introduction Center, Ames, Iowa. The normal lines, B73 and Mo17, are well-known representatives of two important maize heterotic groups: Stiff Stalk and Lancaster Sure Crop, respectively. The seeds were planted in 2011 and 2012 in a Randomized Complete Block Design with three replicates at Dardanos Agricultural Research Station of Çanakkale Onsekiz Mart University, Turkey. Plant density was approximately 70,000 plant ha^{-1}. Each genotype was planted in 2-row plots with 70 × 20 cm apart, 4 m in length. Information about the field applications and study area are given in Table 1. Daily temperature and monthly total precipitation of experimental years are summarized in Figure 1. Temperature and precipitation values were generally higher in the second year.

The plant materials used in this study were similar values (B73: 79 day, Mo17: 77 day, IHO: 73 day and IHP: 75 day) in terms of days to silking. Therefore, sampling was done on five different stage designated by days after sowing (DAS). The first two samplings were in pre-flowering stage (DAS40, DAS60), one of them was around flowering (DAS82) and the other two sampling (DAS100 and DAS122) were made in post-flowering stage. Nine plants were sampled at each date from each genotype (three plants per replicate), and totally 360 plants were sampled during experiment. Sampled plants were pollinated by hand to prevent pollen contamination, which could have resulted in unwanted changes.

Measurement of Plant Traits

Plant height was measured and the plants were cut at soil level. Before each sampling date, the central leaves of the plant samples were tagged. Ten leaf discs (each 0.6 cm^2) were taken from these tagged leaves of the sample plants. Chlorophyll was extracted with dimethyl sulphoxide, and the chlorophyll a (Chl A) chlorophyll b (Chl B) and total chlorphyll (Chl Total) content were computed according to the following equations proposed by Hiscox and Israelstam (1979). Calculated values were converted to mg g^{-1}.

$$\text{Chl A} = [(12.7 \times A663 - 2.69 \times A645) / 10] / Wdisc \quad [1]$$

$$\text{Chl B} = [(22.9 \times A645 - 4.68 \times A663) / 10] / Wdisc \quad [2]$$

$$\text{Chl Total} = [(20.2 \times A645 - 8.02 \times A663)] / 10 / Wdisc \quad [3]$$

where Wdisc: weight of leaf discs, A645: absorbance of sample at 645 nm, and A663: absorbance of sample at 663 nm. Fresh weight of the plant parts (stalk, leaf, and ear) was immediately recorded upon dissection in the field. To estimate leaf area, the leaf blades were scanned on a HP scanner and the pictures were saved in bmp format (at least 200 dpi resolutions). All pictures were downloaded onto CompuEye analyzing software (Bakr, 2005) to compute the total and green leaf area per plant. These values were used to

Table 1 - Crop husbandry details, soil properties of experimental field.

		2011	2012
Sowing	Drill	18 May	13 May
Fertilization	Hand	170 kg ha^{-1} N	170 kg ha^{-1} N
Irrigation	Drip	422.6 mm	420.2 mm
Observation and Sampling Dates		27 June, 17 July, 8 Aug, 25 Aug, 7 Sep	22 June, 12 July, 3 Aug, 21 Aug, 12 Sep
Soil Properties		pH:7.93; E.C: 0.62 mS/cm; Lime: 11.1%; Org. Matter: 1.26%; P: 38.2 kg ha^{-1}; K: 557.8 kg ha^{-1}	pH:7.82; E.C: 0.60 mS/cm; Lime: 13.7%; Org. Matter: 1.28%; P: 37.4 kg ha^{-1}; K: 524.1 kg ha^{-1}

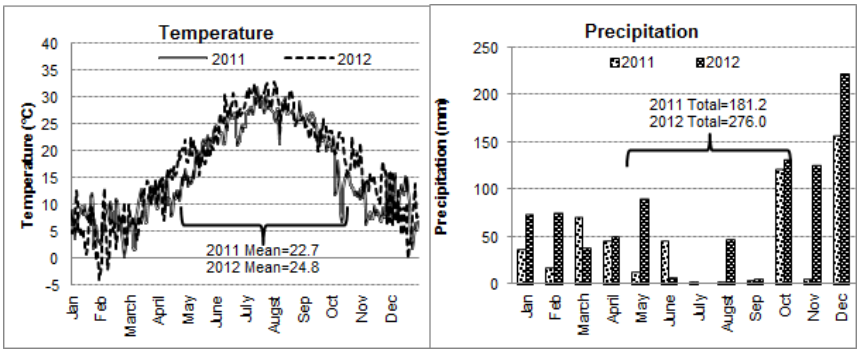

Figure 1 - Changes in daily temperature and monthly total precipitation in experimental years.

determine the total leaf area (TLA), senesced leaf area (LSA) and leaf area index (LAI). Then, all plant parts (leaf, stalk, and ear) were dried at 80°C for 72 hours (Wyss et al, 1999), to calculate dry matter per part and dry matter per plant. Dried ears were shelled to get kernel samples. The samples were weighed, and then stored at + 4°C for further analyses.

Calculation for energy accumulation and storage

To make energy calculations, the biochemical composition of plant samples were determined. For this purpose; the protein, carbohydrate and oil ratios of the stalk, leaf and kernel samples were estimated using a SpectrastarTM 2400 NIR spectrometer (Unity Scientific, USA). Grinding of stalk and leaf samples was achieved with a cutting mill (Retsch SM100, Germany), while kernel samples were ground with a laboratory mill (Fritsch pulverisette 14, Germany). Samples were milled using 0.5 mm sieves in both instruments. Ground samples were put into NIR powdered sample cups (74 and 93 mm diameter) and samples were scanned at 1 nm intervals between 1,200 - 2,400 nm. UnistarTM software was used for NIR analysis (Unity Scientific, USA). Other biochemical components (fiber, ash) were computed by subtracting oil, protein and carbohydrate from the total dry matter of the samples.

NIR data and the dry weight of plant parts were used to calculate carbohydrate, protein and oil yield per plant. Energy equivalents of carbohydrate, protein, oil, and other compounds were calculated according to the following equations suggested by Hanson et al (1960).

Carbohydrate Energy = 3.95 x CRpart x Wpart [4]

Protein Energy = 4.57 x PRpart x Wpart [5]

Other Energy = 3.95 x OtRpart x Wpart [6]

Oil Energy = 9.40 x ORpart x Wpart [7]

where Wpart: dry weight or plant part, CRpart: carbohyrate ratio of plant part, PRpar: protein raito of plant part; OtRpart: other components ratio of plant part and ORpart: oil ratio of plant part. These values were summed up for each plant part (i.e., stalk, leaf, kernel) to calculate the energy equivalents for different parts of the plant. The total of the energy for all plant organs gives us the stored energy (SE) per plant. Energy ratios of the organs and biological compounds were calculated by dividing the respective values by SE. Stored energy ratio (SER) refers to the stored energy/intercepted solar energy by the canopy. To calculate intercepted photosynthetically active radiation

Table 2 - Results of variance analysis for agromorphological traits.

Source of Variation	df†	Plant Height	Tot. Leaf Area	Leaf Senescened Area	Green Leaf Area
Replication (Y)	4	782.6**	128456	93218	30784.3
Y	1	7307.8**	2094498**	404318**	781499.8*
S	4	72554.8**	30334516**	18453442**	38169336.3**
Y × S	4	970.2**	279273*	172570**	141317.9
G (S)	15	803.4**	1421403**	240988**	921637.0**
G×Y (S)	15	52.2**	435339**	96913**	270191.4*
Error	76	86.1	114849	42741	128129.8
Source of Variation	df†	LAI	Chl A	Chl B	Chl Total
Replication (Y)	4	0.06	0.05	0.001	0.06
Y	1	1.08**	1.00**	0.042**	1.50**
S	4	15.5**	3.56**	0.095**	4.93**
Y × S	4	0.14	0.41**	0.012**	0.58**
G (S)	15	0.71**	0.05*	0.003	0.07
G×Y (S)	15	0.22**	0.05*	0.004*	0.09*
Error	76	0.06	0.03	0.002	0.04

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, † df, degrees of freedom. Y: Year, S: Stage, G: Genotype.

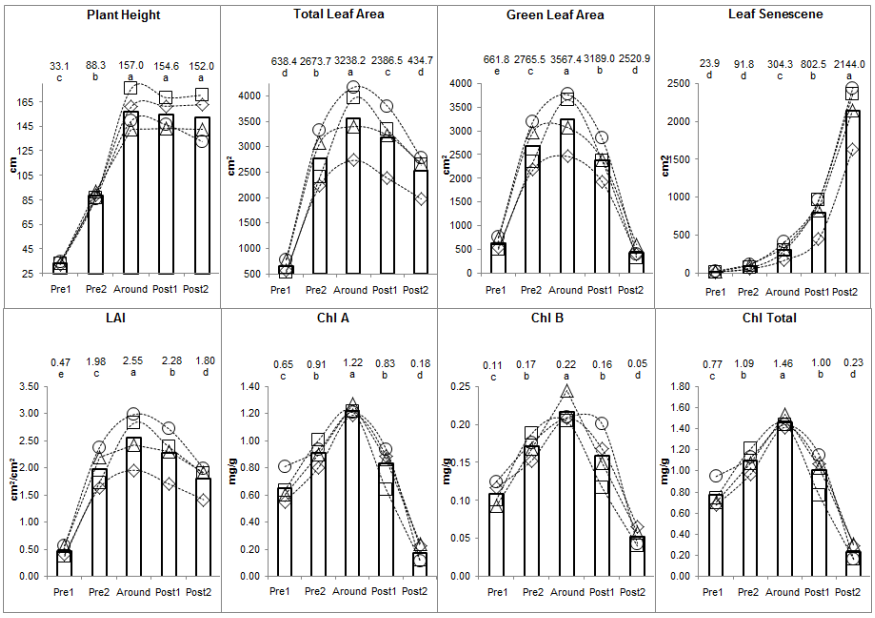

Figure 2 - (a) Changes of plant height, (b) leaf are development, (c) LAI and GLAI and (d) pigment content by genotype and developmental stage. Different letters indicate significant differences between means of stages. Bars show the means of developmental stages; while symbols with lines indicate the genotypes (IHO: △, Mo17: ◊, IHP: ○, and B73 □).

(IPAR), incoming solar energy reaching to the top of the canopy was recorded with a pyronometer sensor (Campbell Scientific Inc, USA). Total daily solar radiation (W m^2 s^{-1}) was converted to Photosynthetically Active Radiation (PAR) using the canopy extinction coefficient (k) of the genotypes. Then IPAR values were calculated based on the formula in Lizaso et al (2003). For the purpose of matching the units of energy calculations, IPAR values of the genotypes were converted to kcal by multiplying by 238.89 (Botu et al, 2012).

$$SRAD = \sum(SRAD \times 3600) / 1000000 \quad [8]$$

$$PAR = 0.429 + 0.12 - SRAD / 2.8 \quad [9]$$

$$IPAR = (PAR / PLTPOP) x^1 - e^{-k \times LAI} \quad [10]$$

$$k = 1.5 - 0.768 (ROWSPC2 - PLTPOP) 0.1 \quad [11]$$

where SRAD: solar radiation, PAR: photosynthetically active radiation, IPAR: intercepted photosynthetically active radiation, k: canopy extinction coefficient, ROWSPC: row spacing, PLTPOP, plant population in a square meter.

Time Interval Calculations

Five growth indices were computed using the directly-measured plant traits, as described by Hunt et al (2002). Relative Growth Rate (RGR), Unit Leaf Rate (ULR), Specific Leaf Area (SLA), Leaf Weight Fraction (LWF), and Leaf Area Ratio (LAR) were calculated for four time intervals. These computations were made using the plagrowanalysis package in R 2.15.1 software (R Development Core Team, 2012) by the following equations:

$$RGR = (logeW2 - logeW1) / (T2 - T1) \quad [12]$$

$$ULR = (W2 - W1) / (T2 - T1) \times [(loge\ LA2 - loge\ LA1) / (LA2 - LA1)] \quad [13]$$

$$LAR = [(LA1 / W1) + (LA\ 2 / W2)] / 2 \quad [14]$$

$$SLA = [(LA1 / LW1) + (LA\ 2 / LW2)] / 2 \quad [15]$$

$$LWF = [(LW1 / W1) + (LW2 / W2)] / 2 \quad [16]$$

where W: plant dry weight, T: time (day), LA: leaf area, LW: leaf weight. Radiation use efficiency (RUE) of the genotypes was computed as the ratio of plant growth rate to the Intercepted Photosynthetically Active Radiation (IPAR) of that genotype. The estimation of IPAR was described above. RUE was computed for each genotype as suggested by Monteith (1977).

$$RUE = (W2 - W1) / (IPAR2 - IPAR1) \quad [17]$$

where W: plant dry weight and IPAR: intercepted photosyntetically active radiation value.

Statistical Analysis

Data were analyzed with SAS V8 (SAS Institute Inc, 1999) using the PROC GLM procedure based on the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \gamma_{k(j)} + (\alpha\gamma)_{ik} + (\alpha\gamma)_{ik(j)} + r_{il} + \varepsilon_{ijkl}$$

where Y_{ijkl}l: observed value, μ: grand mean, α_i: year effect i (i = 1, 2), β_j: effect of plant stage j (j = 1, 2, 3), $(\alpha\beta)_{ij}$: effect of year × plant stage interaction, γ_k: effect of genotype k (k = 1, 2,…8), $\gamma_{k(j)}$: effect of genotype k within plant stage j (k = 1, 2,…8; j=1,2,3), $(\alpha\gamma)_{ik}$: effect of year × genotype interaction, $(\alpha\gamma)_{ik(j)}$: effect of year × genotype interaction within plant stage, r_{il}: block effect l within year i (l = 1, 2, 3; i = 1, 2), and ε_{ijkl}: random error term. Block effect within year was considered as a random factor in this model, whereas the other effects were fixed. Significant differences between the genotype means were detected by LSD (Least Significant Difference) test.

Table 3 - Results of variance analysis for traits related dry matter production allocation

Source of Variation	df†	Total Dry Weight	Leaf Dry Weight	Stalk Dry Weight	Ear Dry Weight
Replication (Y)	4	562.1*	6.45	271.2**	53.0
Y	1	10880.2**	190.9**	2879.6**	2276.2**
S	4	73471.8**	1604.6**	15289.3**	3399.7**
Y × S	4	1322.0**	12.9	517.1**	97.4
G (S)	15	909.7**	83.6**	326.4**	348.6**
G×Y (S)	15	445.7*	20.0**	68.5	326.8**
Error	76	203.6	7.23	63.0	76.1

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, †df, degrees of freedom. Y: Year, S: Stage, G: Genotype.

Results

Comparison for Agromorphological Traits

Variance analysis indicated G × Y interaction within stage was found to be significant for almost all agromorphological traits (Table 2). Genotype within stage effect was of importance in explaining developmental changes in genotype means by time and is therefore discussed below.

Differences in plant height, leaf growth, senescence and pigment content based on developmental stages are shown in Figure 2. The highest mean value for plant height (157 cm) was observed at around flowering stage. Normal maize genotypes (B73 and Mo17) were taller than specialty maize genotypes (Figure 2). Total leaf area (TLA), leaf area index (LAI), and green leaf area (GLA) changed in harmony over the course of plant development. The highest values for those traits (TLA = 3,238.2 cm^2, GLA = 3,567.4 cm^2, and LAI = 2.55) were measured at around the flowering stage. There was only small variation among the genotypes at the first sampling date; however, genotypic differences became apparent over time. IHP and B73 were superior by a wide margin in terms of leaf area to IHO and Mo17, especially around flowering, thereby giving a higher LAI (Figure 2). IHO had higher leaf development during post flowering period. Leaf senescence in IHP increased rapidly in the last period (2,144.0 cm^2), and the GLA lessened accordingly. In the last sampling, IHP had the highest LAI, while the GLA of IHO was consistent with changes in leaf area and green leaf area values. The highest values for chlorophyll a (1.22 mg g^{-1}) chlorophyll b (0.22 mg g^{-1}) and total chlorophyll (1.46 mg g^{-1}) were recorded in around the flowering. IHO, IHP and Mo17 had higher pigment content than B73 (Figure 2).

Comparison for Biomass Production and Energy Utilization

The results of variance analysis for biomass production and biochemical composition of the plant parts showed that Stage, Stage × Year and Genotype (Stage) were significant sources of variation for most of the traits (Tables 3 and 4). Total dry biomass of the genotypes throughout the vegetation ranged between 4.24 g and 126.9 g. In the first stage after flowering, total dry biomass reached the highest mean (125.1 g) value (Figure 3). The highest mean values of stages were 23.6 g, 55.9 g and 48.5 g, for dry weight of leaf, stalk and ear parts, respectively (Figure 3). There were significant differences between the genotypes within stages for total, leaf, stalk and ear

Table 4 - Results of variance analysis for traits related dry matter production allocation

Source of Variation	df†	IPAR	Stored Energy	Energy Efficiency	Leaf Energy Ratio	Stalk Energy Ratio
Replication (Y)	4	6707121	7023.0**	0.05**	0.008**	0.008**
Y	1	231838517**	96140.8**	0.36**	0.024**	0.017**
S	4	6126264434**	587279.6**	1.31**	1.022**	0.401**
Y × S	4	25763473**	10585.6**	0.04**	0.003	0.003
G (S)	15	57641984**	11140.5**	0.03**	0.008*	0.016**
G×Y (S)	15	36174757**	5734.6**	0.01	0.001	0.001
Error	76	5602596	1952.9	0.01	0.001	0.002
Source of Variation	df†	Kernel Energy Ratio	Protein Energy Ratio	Carb. Energy Ratio	Oil Energy Ratio	Other Energy Ratio
Rep (Year)	4	0.0010	0.0001	0.0001	0.0005	0.0002
Year (Y)	1	0.0012	0.0017**	0.0022**	0.0009	0.0009*
Stage (S)	4	0.4575**	0.0157**	0.0241**	0.0045**	0.0335**
Y × S	4	0.0031	0.0019**	0.0002	0.0006	0.0017**
G (S)	15	0.0388**	0.0005**	0.0029**	0.0055**	0.0011**
G×Y (S)	15	0.0026	0.0001*	0.0001	0.0004	0.0002
Error	76	0.0033	0.0001	0.0001	0.0005	0.0002

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, † df, degrees of freedom. ‡ ns, nonsignificant at $p < 0.05$. Y: Year, S: Stage, G: Genotype.§ df values are 1, 2, 2, 9, 9 and 4 for Y, S, Y × S, G (S), G×Y (S) and Rep (S), respectively, ¶ DF values are 1, 1, 1, 6, 6 and 4 for Y, S, Y × S, G (S), G×Y (S)and Rep (S), respectively.

Table 5 - Results of variance analysis for traits related dry matter production allocation.

Source of Variation	df†	RUE	RGR	SLA	ULR	LAR	LWF
Replication (Y)	4	0.11	0.0003	172.1	20.5	209.9**	0.004**
Y	1	0.91**	0.0001	4416.5**	18.0	3349.1**	0.009**
S	3	5.48**	0.0459**	14942.9**	1292.2**	49228.3**	1.063**
Y × S	3	2.05**	0.0005**	2180.9**	202.1**	962.6**	0.001
G (S)	12	0.16*	0.0001	270.9**	38.4**	91.7	0.003**
G×Y (S)	12	0.11	0.0001	145.7	51.7**	55.9	0.001
Error	60	0.07	0.0001	106.9	13.3	57.2	0.001

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, † df, degrees of freedom. Y: Year, S: Stage, G: Genotype.

dry weight. After flowering, B73 and IHO had higher values than others. Specialty and normal genotypes were not separated in terms of dry weight of total as well as plant parts.

Stage effect was a significant source of variation for all variables related to energy calculations (Table 4). Genotypes had significant differences within stages in terms of energy-related traits. Energy calculations showed that the genotypes differed in their ability to capture incoming energy (Figure 3). Interestingly, IHP had lower potential for converting this energy into dry biomass while it seemed to have higher potential for capturing solar energy (IPAR). Indeed, IHP had lower stored energy and energy efficiency values than Mo17, even which had the lowest leaf area. Small differences (~1-2%) in energy efficiency resulted in significant changes in dry matter production (Figure 3). The ratio of stored energy to total captured energy was no more than 2% in aboveground plant organs, excluding husk and cob (Figure 3). This figure also shows the distribution of energy stored in aboveground parts by the genotypes on the basis of biochemical components and plant parts. Leaves had the highest portion of energy (66%) in the first stage, but progressively decreased through the vegetation. Stalk increased its share (68%) and became the most energy containing organ at around flowering, thereafter kernel share started to increase (29%) in energy allocation. A large part of the energy in IHO was in the kernels, while the major part of the energy was in stalks and leaves in others (Figure 3). Based on the biochemical components, carbohydrate > others (fiber + ash) > protein > oil ranking was valid for all genotypes at all growth stages. As expected, the energy ratio of oil was the highest in IHO (Figure 3).

Comparison for Growth and Radiation Use Efficiency

Stage effect was found to be significant for all time interval calculations. Genotype (Stage) effect was also significant for all growth indices, except for RGR and LAR (Table 5).

RUE of the tested hybrids ranged between 0.32 and 1.36 g MJ plant^{-1}. Both IHO and IHP had the highest value of RUE around flowering time, while, IHO was superior in the first post-flowering stage.

Growth indices showed significant differences in terms of developmental stage and genotype within developmental stages. The relative growth rate was progressively decreased during the plant development and no significant differences were observed among the genotypes. Net assimilation rate varied between -4.98 and 19.69 g cm^{-2} d^{-1}. IHO had higher numbers than others around the flowering stage (Table 6). The net assimilation rate of IHO was around 20 mg (Table 6). Our calculations gave negative values for RUE, RGR and ULR in the last post-flowering period (Table 6).

SLA was high in the pre-flowering stage in almost all genotypes; then showed a decrease in later stages. SLA values ranged between 198.5 and 208.6 cm^2 g^{-1} pre-flowering, while they declined to 132.2-151.6 cm^2 g^{-1} in the last sampling. IHO and Mo17 had higher SLA, associated with thinner leaves, compared to B73 and IHP. LAR and LWF values, which indicate leaf development, decreased with the progression of plant development. Specialty maize genotypes had higher LAR and LWF values compared to normal ones in all developmental stages, though some differences were not statistically significant at 5% level (Table 6).

Discussion

The results revealed significant differences between normal and specialty maize genotypes in terms of agromorphological traits. Plant height, leaf area and LAI reached the highest values around flowering. Since internode formation and the vegetative stage stop with the onset of flowering (Kiesselbach, 1949), no increase in plant height and leaf development is expected after the time the generative stage is reached (Abendroth et al, 2011). In our study, normal genotypes had significantly higher values for plant height than high oil and high protein strains. This finding showed that plant height decreased by the pressure of selection in specialty maize genotypes. We observed significant differences among genotypes in terms of leaf formation and development. Total leaf area and LAI are products of leaf number per plant and area per leaf. These traits have a direct effect on capturing solar energy (Lafarge and Hammer, 2002). LAI values of 3-4 have been shown to be associated with high yields in maize (Lindquist et al, 1998). In the present study, LAI values of IHP and B73 at the third stage were less than 3 (Figure 2). Those genotypes

Table 6 - Changes in RUE and growth indices by genotype within interval of phenological stages.

		RUE	RGR	ULR	SLA	LAR	LWF
Pre-Flowering							
	B73	0.32 a†	0.092	9.18 a	191.2 a	118.1	0.64 a
	Mo17	0.33 a	0.091	8.94 a	200.2 a	118.5	0.60 a
	IHO	0.40 a	0.094	8.86 a	208.6 a	128.4	0.64 a
	IHP	0.36 a	0.091	8.04 a	198.5 a	127.4	0.65 a
	Mean	0.35 C‡	0.092 A	8.75 B	199.6 A	123.1 A	0.63 A
Around-Silking							
	B73	1.12 a	0.066	13.20 ab	163.4 a	69.2	0.42 a
	Mo17	1.07 a	0.055	11.54 b	172.4 a	65.2	0.38 a
	IHO	1.23 a	0.052	19.69 a	168.5 a	67.4	0.40 a
	IHP	1.36 a	0.058	11.55 b	167.7 a	72.9	0.42 a
	Mean	1.19 A	0.058 B	14.00 A	168.0 B	68.7 B	0.41 B
Post-Flowering							
	B73	0.77 a	0.015	3.29 a	143.2 b	31.6	0.22 ab
	Mo17	0.57 a	0.013	3.27 a	159.7 a	28.7	0.18 c
	IHO	0.80 a	0.014	6.49 a	153.3 ab	30.9	0.20 b
	IHP	0.57 a	0.007	1.12 a	148.9 b	33.8	0.23 a
	Mean	0.68 B	0.012 C	3.54 C	151.3 C	31.3 C	0.21 C
Post-Flowering							
	B73	0.09 ab	0.000	0.10 a	132.2 c	21.7	0.17 b
	Mo17	0.46 a	0.001	-3.13 a	151.6 a	20.9	0.14 c
	IHO	-0.07 b	-0.004	-4.98 a	146.1 ab	24.7	0.17 b
	IHP	-0.14 b	-0.013	-4.70 a	143.1 b	29.4	0.21 a
	Mean	0.08 D	-0.004 D	-3.18 D	143.2 D	24.2 D	0.17 D

† Lower-case letters in columns indicate statistically significant differences between means ($p = 0.05$). ‡ Significant differences between means of developmental stages are shown by upper-case letters ($p = 0.05$).

were found to have higher values for leaf area and LAI compared to IHO and Mo17. IHO stayed green for longer, and therefore, leaf senescence took place at a slower pace (Figure 2). Leaf senescence is associated with protein and amino acid decomposition (Thomas and Stoddart, 1980) and chlorophyll breakdown (Leopold, 1980). High protein maize appeared to have a faster breakdown, especially later on, compared to other genotypes in the current study (Figure 2). Moose et al (2004) stated that leaf senescence was faster in high protein strains, and attributed this to the fact that respective genes were collectively selected together during the selection process.

Chlorophyll controls the green color in leaves and a decrease in its concentration is associated with leaf senescence. Our results indicated that leaf chlorophyll concentration was higher in IHO and Mo17, from flowering onwards (Figure 2). It was previously reported in previous studies that high oil maize genotypes had higher leaf chlorophyll content (Wang et al, 2009). We observed that chlorophyll content of the genotypes increased until flowering, and decreased thereafter. Suryanarayana Reddy et al (2001) reported that the highest level of chlorophyll content in standard maize genotypes was reached at preflowering (DAS60) and then decreased. The special maize genotypes seem to retain their leaf chlorophyll for a longer time. This variation affected dry biomass production in those genotypes.

B73 and IHO had higher total dry matter production than others (Figure 3). Total dry matter production is related to dry matter production at organ level. Dry matter production in later stages was affected by dry matter storage in ear and stalk. Previous studies demonstrated that ear (61%) and stalk (28%) were the major contributors to total dry weight in the later stages (Pordesimo et al, 2005). Our results were in agreement with these findings. Distribution of dry matter into biochemical components plays an important role in weight gain. Undoubtedly, the differences in biochemical composition of the genotypes at organ level also affected their dry matter production. IHO strain had higher oil content in their kernels, while IHP strain had higher protein content in their kernels, as well as in stalks and leaves. Doehlert and Lambert (1991) reported that IHP (Illinois High Protein) genotypes had higher N transportation. In addition to having been selected for kernel protein, this may be one reason why IHP contains higher protein in leaf and stalk, as well. High oil contentof IHO has been associated with embryo size (Doehlert and Lambert, 1991). In the current study, IHO had higher kernel oil content, as expected. The differences in dry matter production of the genotypes were clearly affected by the variation in their biochemistry. Dry matter production is also closely associated with source-sink relationships (Lee and Tollenaar, 2007). The genotypes with high efficiency dry matter allocation may also have also higher dry matter production, because of faster transportation of photosynthetic products.

The conversion of energy into dry matter is calculated in various ways. These calculations determine

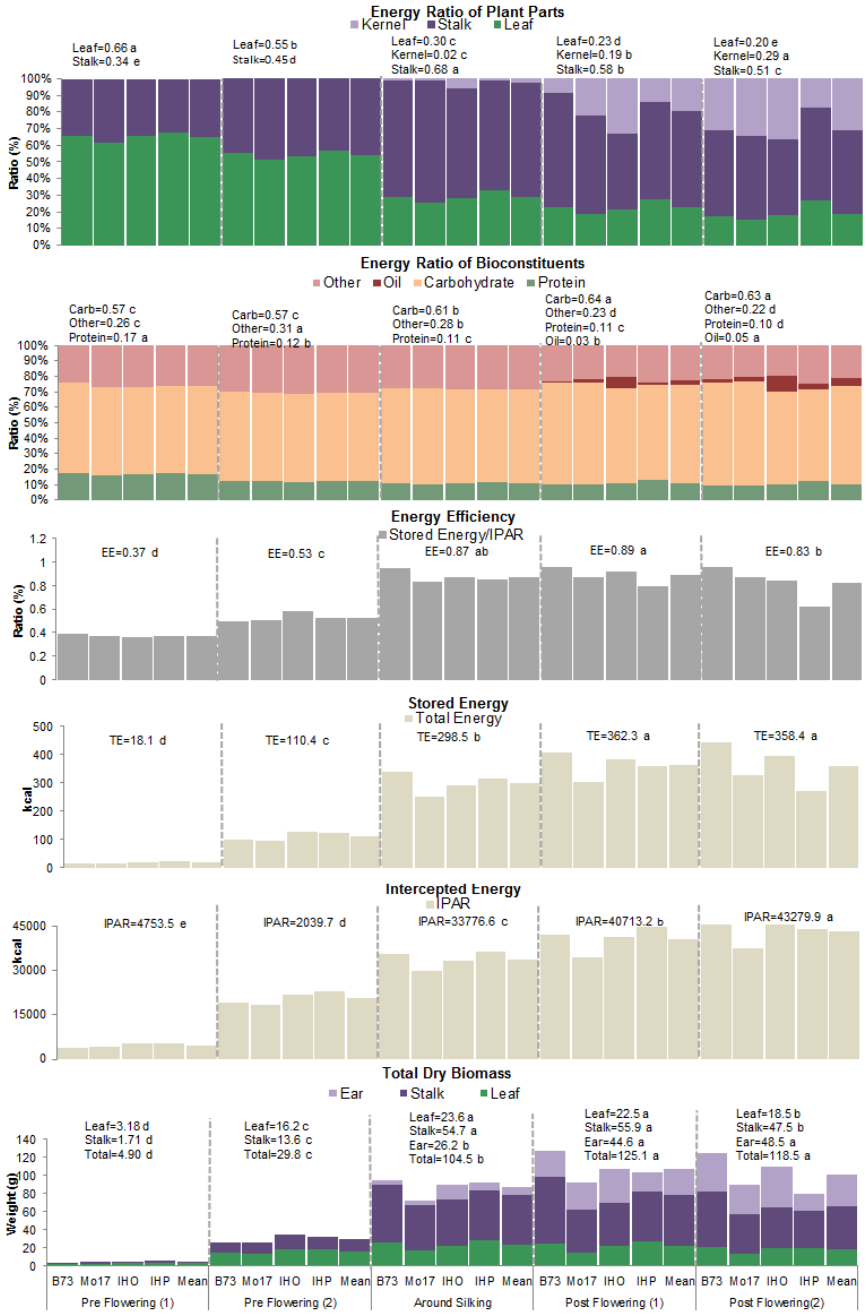

Figure 3 - Genotypic differences for several physiological measurements as observed in five developmental stages. Measured traits are shown in different colors in the plots except total dry weight.

the amount of energy retained, stored and converted in different forms. In our study, we used a retroactive method based on the biochemical composition of dry matter. Oil contains more energy than protein (Lambert et al, 1998), thus the total stored energy values of the IHO strain was higher than the other genotypes (Figure 3). We found that the average energy conversion efficiency of the genotypes was below 1% in all off the developmental stages. Transeu (1926) reported energy efficiency values in normal maize hybrids as about 1.6%. Relatively low values in our study may be a result of using inbred rather than hybrids and/or having special genotypes. Differences in genotypes for stored energy in various parts and biochemical components enabled us to make inferences when comparing different types of maize. Leaf energy value was higher (> 50%) in the early stages, while the energy values of the sink parts (stalk and kernel) increased in later stages. Hedin et al (1998) reported that kernel, the main sink component, contained 45.8 - 47.8% of the total energy in a mature hybrid maize plant. We found this ratio to be around

30% in the last stage (Figure 3). Low figures may be a result of genetic factors (hybrids vs inbreds) and the self-pollination treatment utilized in this study, both are limiting factors in kernel set. The proportion of proteins and other components (fiber, etc.) in total stored energy decreased as the plant grows and matures. In contrast, the share of carbohydrate and oil increased in total stored energy. Our results demonstrated that energy deposition in biochemical constituents showed high variability among the genotypes (Figure 3). This reveals that biochemical differences among the maize genotypes had an important effect on the energy utilization and storage.

Time interval calculations allowed us to understand both changes in dry matter production and energy utilization more clearly. RUE was higher in IHO in post-flowering stages (Table 6). Also, RUE values were lower after flowering than around the flowering stage for all genotypes. Decrease in RUE during grain filling is probably due to sink limitation and/or leaf senescence (Fischer, 1983). Earlier studies reported RUE values for maize in the range of 2.1-4.9 g MJ^{-1} (Kiniry, 1989; Tsubo et al, 2001; Lindquist et al, 2005). The low values in the current study may be due to the fact that our calculations were on a plant basis rather than area basis (Table 6), and the plant material consisted of current study consisted of inbred lines. One may except that specialty maize genotypes should be low RUE values than the normal maize genotypes, because they had more protein and oil content (Penning de Vries et al, 1974). However, IHO and IHP strains had generally higher RUE values than normal genotypes, except in the last sampling date. Minute changes in the genotypes in terms of ULR, RGR and RUE resulted in significant changes in their dry matter production. The most striking difference among the genotypes was in the dry matter produced per unit leaf area (Table 6). IHO produced more dry matter per unit area around silking and the first post-flowering stage (Table 6). However, its total dry weight was higher than that of IHP strain, especially in the last sampling date. IHP lost the dried leaves in the last sampling date (data not shown) resulting in a decrease of its total dry matter. Around 50% of total dry matter is produced after flowering in maize (Lee and Tollenaar, 2007). In fact, kernel sink potential is set in the lag phase (5-15 days after pollination) before the effective grain filling period starts (Borras et al, 2009). Therefore, genotypic differences in the lag phase are important. IHO had a higher mean ULR around flowering stage, suggesting that it produced dry matter faster in the lag phase (Table 6). Besides having an indirect effect on dry matter production, LAR and LWF are also indicators of dry matter partitioning. LAR is a product of SLA and LWF, which provide information about leaf thickness and the ratio of leaf weight/total weight, respectively (Lafarge and Hammer, 2002). LAR also gives idea information on allometric relationships between organs (Williams et al, 1965). In our study, IHP had higher values of LAR and LWF and it produced more leaf area for dry matter production (Table 6). SLA value is an indicator of leaf thickness and leaf density (Wilson et al, 1999). SLA values implied that IHO and Mo17 had thinner leaves, or had less dry matter in their leaves, compared to other genotypes. Overall, RGR, SLA, LAR and LWF decreased with time in all genotypes. Previous studies on normal maize genotypes showed similar results (Lafarge and Hammer, 2002; Karadavut et al, 2010). IHO and IHP had negative RGR, RUE and ULR values in the last sampling date. There may be two reasons for obtaining negative numbers. Fist leaf decay at later stages was high in specialty maize genotypes. Second, our measurements were based on destructive sampling which may distort the time interval calculations.

Physiological effects of SLA on leaf aging may need special consideration. Leaf senescence of the high protein genotypes was faster partly due to the thicker structure of leaves (lower SLA). Chlorophyll breakdown is faster in shaded leaves than fully illuminated ones (Causin et al, 2009). Also, in the last stages of vegetation, the photosynthetic quality of light (red:far red ratio) is low. These factors might have accelerated chlorophyll breakdown and leaf senescence in IHP. IHO would be expected to have higher dry matter production, partly because they stayed green for longer. Previous studies revealed that staying green in the reproductive period for a long time could increase dry matter production in maize (Szulc, 2012). Lee and Tollenaar (2007) stated that functional stay green is more important than visual stay green in maize productivity. IHO and B73 may be photosynthetically more active compared to the other genotypes. Zhang et al (2012) reported that photosynthetically-active hybrids gave superior performance in terms of photosynthesis in the phase of chlorophyll decay. IHP strain possessed more leaves but they senesced earlier compared to others.

In conclusion, biomass production and physiological attributes of normal versus specialty maize genotypes were compared in this study. Despite having fewer leaves IHO was more effective in dry matter production and allocation. This suggests that high oil genotypes may be superior in capturing solar energy and converting it into dry matter. The effective elements in dry matter production were energy distribution at organ level and differences in the structure and weight of the kernel. From the biochemical point of view, the differences among the genotypes in total plant energy arised from the variation in the energy equivalents of carbohydrate and other components (fiber and ash). When considered at organ level, these differences may be attributed to the variation in energy of kernels and stalks. RUE and growth indices discussed here could be used in modeling studies, specifically for high oil and high protein lines. Further studies using non-destructive sampling methods

and/or calculations based on calorimeter analysis may offer additional and more detailed information about dry matter production and energy utilization to understand the differences between normal and special maize genotypes.

Acknowledgements

The authors acknowledge the financial support of the ÇOMÜ Scientific Research Projects Commission (ÇOMÜ BAP-2011/56) for this study.

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