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Tillage systems and nitrogen timings effect on growth, stay green and grain quality in maize (*Zea mays* L.)

Sistemas de preparo do solo e efeitos do tempo de nitrogênio no crescimento, manutenção da qualidade de grãos e verde no milho (*Zea mays* L.)

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Abstract

Nitrogen (N), being mobile in soil is exposed to various losses owing to unwise use of nitrogen fertilizer, and conventional soil and crop management practices which can be minimized by temporal nitrogen application and different tillage practices. This study was conducted to elucidate the effect of different tillage systems and nitrogen timings on growth, stay green and grain quality in maize. Three tillage systems viz. T_1: tillage with cultivator, T_2: mouldboard plough + 2-cultivations, T3: chisel plough + 2-cultivations; and five nitrogen timings viz. N_1: whole at sowing, N_2: ½ at sowing+½ at V5 (5-leaf stage), N_3: ½ at sowing+½ at tasseling, N_4: ½ at V5+½ at tasseling, N_5: 1/3 at sowing+1/3 at V5+1/3 at tasseling). Tillage systems and nitrogen application had significant effect on leaf area per plant, specific leaf area and leaf area ratio. Tillage systems had non-significant effect on stay green and grain quality parameters except for oil contents. However, nitrogen timings had significant effect on chlorophyll a, b and total contents as well as grain quality parameters. The higher a, b and total chlorophyll contents were noted with three splits i.e. 1/3 at sowing+1/3 at V5+1/3 at tasseling compared with other treatments. The results suggest to grow maize by preparing the field through chisel plough and applying N in three splits to improve its growth, chlorophyll contents and grain quality.
Key words: Chlorophyll contents. Leaf area ratio. Specific leaf area. Grain quality. Nitrogen application timings. Tillage systems.

Resumo

Nitrogênio (N), sendo móvel no solo, está exposto a várias perdas devido ao uso imprudente de fertilizantes nitrogenados e práticas convencionais de manejo do solo e das culturas, que podem ser minimizadas pela aplicação de nitrogênio temporal e diferentes práticas de preparo do solo. Este estudo foi realizado para elucidar o efeito de diferentes sistemas de preparo do solo e tempos de nitrogênio no crescimento, permanência e qualidade do grão verde no milho. Três sistemas de lavoura, viz. T1: preparo do solo com cultivador, T2: arado de aiveca + 2-cultivos, T3: escarificador + 2-cultivos; e cinco

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temporizações de azoto, viz. N1: todo na sementeira, N2: ½ na sementeira + ½ na V5 (fase de 5 folhas), N3: ½ na sementeira + ½ no pendoamento, N4: ½ na V5 + ½ na pompa, N5: 1/3 na sementeira + 1/3 em V5 + 1/3 no pendoamento). Sistemas de preparo do solo e aplicação de nitrogênio tiveram efeito significativo na área foliar por planta, área foliar específica e área foliar. Os sistemas de preparo do solo não tiveram efeito significativo na manutenção dos parâmetros de qualidade verde e de grãos, exceto para os teores de óleo. Entretanto, os tempos de nitrogênio tiveram efeito significativo sobre os teores de clorofila a, b e total, bem como parâmetros de qualidade de grãos. Os maiores teores de clorofila a, b e total foram observados com três fendas, isto é, 1/3 na semeadura + 1/3 em V5 + 1/3 no pendoamento comparado com outros tratamentos. Os resultados sugerem o cultivo do milho preparando o campo através do arado de cinzel e aplicando o N em três fendas para melhorar o seu crescimento, o teor de clorofila e a qualidade do grão.
Palavras-chave: Teor de clorofila. Razão Área foliar. Área foliar específica. Qualidade de grãos. Intervalos de aplicação de nitrogênio. Sistemas de manejo.

Introduction

Tillage is one of the important agriculture practices because of its impact on crop growth, and soil health (SHARMA et al., 2011), which on one hand, alter crop yield (LIU; WIATRAK, 2012), including soil physical (D'HAENE et al., 2008), chemical (VILLAMIL; NAFZIGER, 2015) and biological properties (LUPWAYI et al., 2012). Due to soil compaction, water and nutrients supply to plants is restricted which leads towards reduction in crop growth and yield (HAMZA; ANDERSON, 2005). Lower bulk density due to subsoil compaction causes reductions in crop roots due to resistance to penetration (COELHO et al., 2000; YUSUF, 2006; WANG et al., 2009; JABRO et al., 2010) and soil cone index (WANG et al., 2009), hence resulting in poor crop yield. The existence of plough pan prevents penetration of rain water into deep soil layer and affect crop yield (MAO, 2009). However, it has been well documented that mechanical subsoiling efficiently reduced the soil strength thereby increasing root penetration (OLESEN; MUNKHOLM, 2007). Subsoiling improves soil aeration, increase the availability of nitrogen (N) through organic nitrogen mineralization, and thus modifies the soil environment which is favorable for crop growth (HALVORSON et al., 2001; DINNES et al., 2002) and also increase root density as well as its distribution (MOSADDEGHI et al., 2009; SUN et al., 2017). This increase in root distribution helps to delay plant senescence (LIANG et al., 2010) and ultimately improves grain yield (HOU et al., 2013).

Nitrogen is highly susceptible to several losses such as leaching, denitrification, volatilization and immobilization which influence the N dynamics and makes N application timing a best tool to balance N demand for optimum plant growth and to reduce its losses in various forms (DINNES et al., 2002). Thus, practicing best N management can boost up N use efficiency (NUE) and crop yield (MA et al., 2012; MA; BISWAS, 2015). For improving NUE, N application timing is one of the important components, as per-plant application undergoes leaching losses. Split application of N reduces losses (MITCHELL et al., 2000) and results in higher maize yield (BAKHSH et al., 2002; JAMIL et al., 2015; WASAYA et al., 2017a) through improving grain components (WASAYA et al., 2017a). It has been noted that N application can improve photosynthetic rate and photosynthetic NUE (MA et al., 2010; MA; BISWAS, 2016). Increase in the photosynthesis rate with higher N supply in different maize hybrids has been reported (URIBELARREA et al., 2009). Increase in photosynthetic active leaf area due to higher chlorophyll contents results in higher grain yield (VANYINE et al., 2012; WASAYA et al., 2017a, 2018b) and biomass production (WASAYA et al., 2012). Although lot of work has been done on tillage and time of nitrogen application as an individual factor, however little information is available as combined effect of tillage and nitrogen timings in maize. Keeping this in view, the present study was carried out to check the combined effect of tillage and nitrogen application timings on growth, stay green and grain quality in maize.

Materials and Methods

Experiment detail

This two year field experiment was conducted during autumn season of 2008 and 2009 at the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan in order to assess the effect of tillage systems and nitrogen timings on growth dynamics, chlorophyll contents, dry matter production and grain quality of maize. The soil of experimental site is sandy clay loam with organic matter (0.72%) and total N contents (0.04%). The weather data is given in our paper published as Wasaya et al. (2018a).

Maize crop was sown under three different tillage systems viz. conventional tillage; tillage with moldboard plough; and tillage with chisel plough up to 40 cm depth followed by 2-cultivations with cultivator and one planking with five N application timings viz. whole at planting; ½ at planting + ½ at 5-leaf stage (V_5) (30 DAS), ½ at planting + ½ at tasseling (VT) (50 DAS), ½ at V_5 + ½ at VT and N_5: $^1/_3$ at planting + $^1/_3$ at V_5 + $^1/_3$ at VT. The treatments were applied in a split plot design by assigning tillage application and N timings in main and sub plots, respectively with net plot size of 4.5 m × 10 m having 3 replications.

Crop husbandry

Before maize planting to ensure homogenous moisture level, experimental area was irrigated at a depth of 10 cm. Field was prepared according to treatments needs and maize hybrid pioneer-31R88 was sown on August 07, 2008 and August 01, 2009. The crop was planted with the help of dibbler by using 25 kg ha^{-1} seed rate, maintaining line spacing of 0.75 m and a plant spacing of 0.2 m. Initially two seeds were manually planted per hill and then one plant per hill was retained after thinning at 3-leaf stage. Nitrogen (N) was applied @150 kg ha^{-1} while phosphorous (P) and potassium (K) both were applied @100 kg ha^{-1} each. Total P and K were added to soil at the sowing time while N was applied according to the treatments. Single super phosphate (SSP), sulphate of potash (SOP) and urea were used as source of P, K and N. Five irrigations were applied as and when needed by crop.

Observations recorded

Growth parameters

At maturity, 10 plants were cut from each plot, dried for 8 days under sun. After drying, plants were weighed to calculate dry matter yield (g plant^{-1}).

For measurement of leaf area per plant, five (5) plants were harvested from each plot with 15 days interval starting from 40 days after planting up to 100 DAP. Leaves were separated from stem then leaf area was calculated using leaf area meter. On the basis of leaf area, specific leaf area and leaf area ratio were calculated using formulae as given below.

$$Specific\ leaf\ area = \frac{leaf\ area\ per\ plant}{Leaf\ weight\ per\ plant}\ (cm2g-1)$$

$$Leaf\ area\ ratio = \frac{Leaf\ area\ per\ palnt}{weight\ per\ plant}\ (cm2g-1)$$

Chlorophyll contents (µM)

For chlorophyll (a, b and total) content determination, five flag leaves were selected randomly from each experimental plot and were separated from the plant stem. The leaf samples were taken at two reproductive stages viz. at blister (R2) and dough (R4) stages. These leaf samples were cut into small pieces and then frozen for overnight. About 2 g frozen leaves from each sample were ground with the help of mortar and pestle in 80% acetone solution. The grinded samples were then kept in dark for few hours to allow the leaf tissues to be homogenized thoroughly. The extracts of each sample were centrifuged for 10 minutes at 6000 rpm and absorbance of the supernatant was observed using a UV spectrophotometer (Unicam 8620) at 647 and 664 nm, and the chlorophyll a, b and total chlorophyll contents were calculated using the formulae as reported by COOMBS et al. (1987).

Grain quality

For estimation of protein contents, the grain samples were oven dried at 70 °C for 24 h. After drying, the samples were grinded with mechanical grinding machine and N content in maize grain was estimated using micro-Kjeldahl method (AOAC, 1990) and protein contents were calculated using following formula

$$Protein\ content\ (\%)\ =\ N\ concentarion \times\ 6.25$$

Oil contents in maize grain were estimated by Soxhlet method as proposed by Low (1990) and starch contents were estimated by Gluco-amylase method (AOAC, 1990).

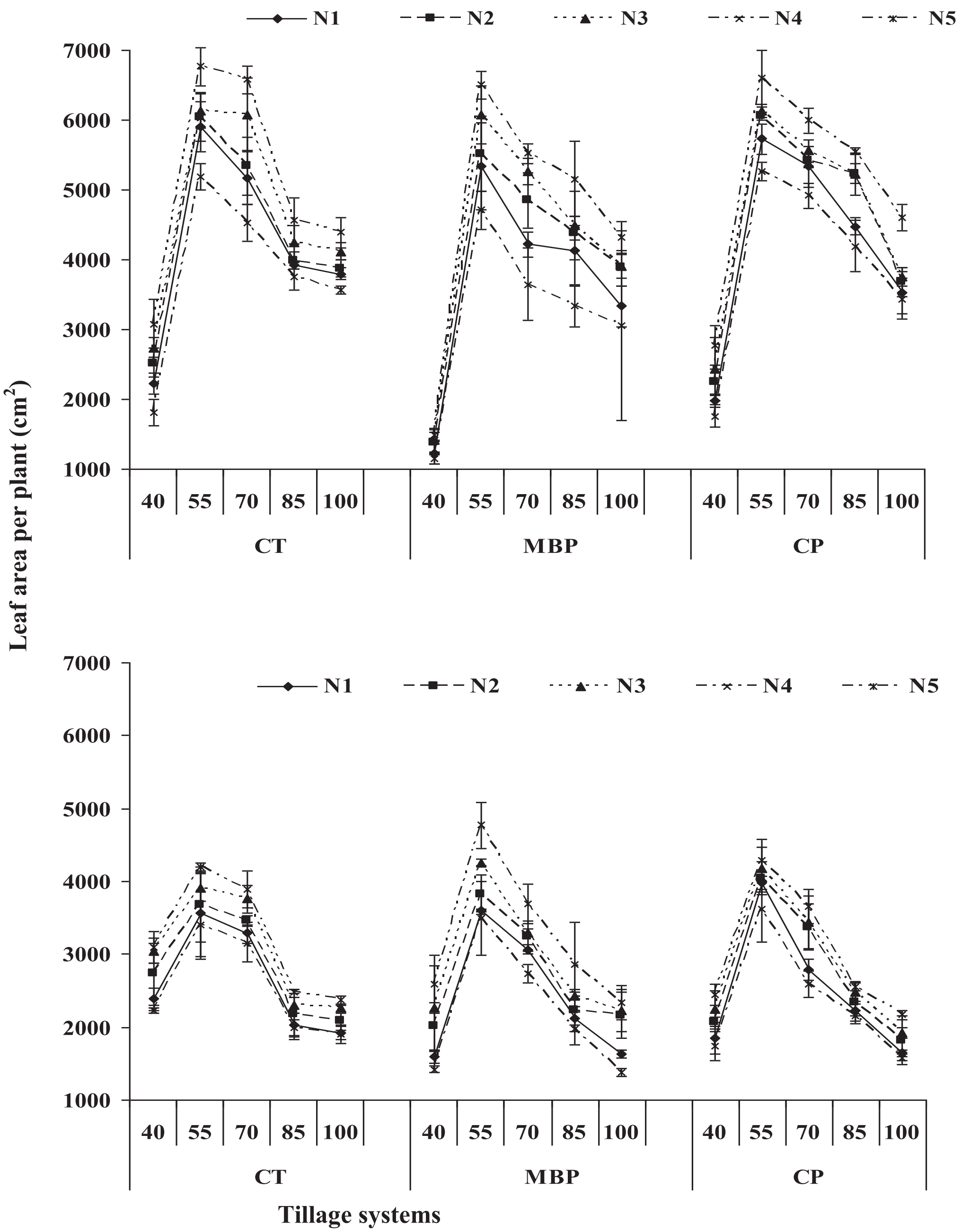
Statistical analysis

Data collected were interpreted using software statistix 8.1. To compare the means of different treatments, analysis of variance technique and LSD test was used at 5% probability level (STEEL et al., 1997).

Results

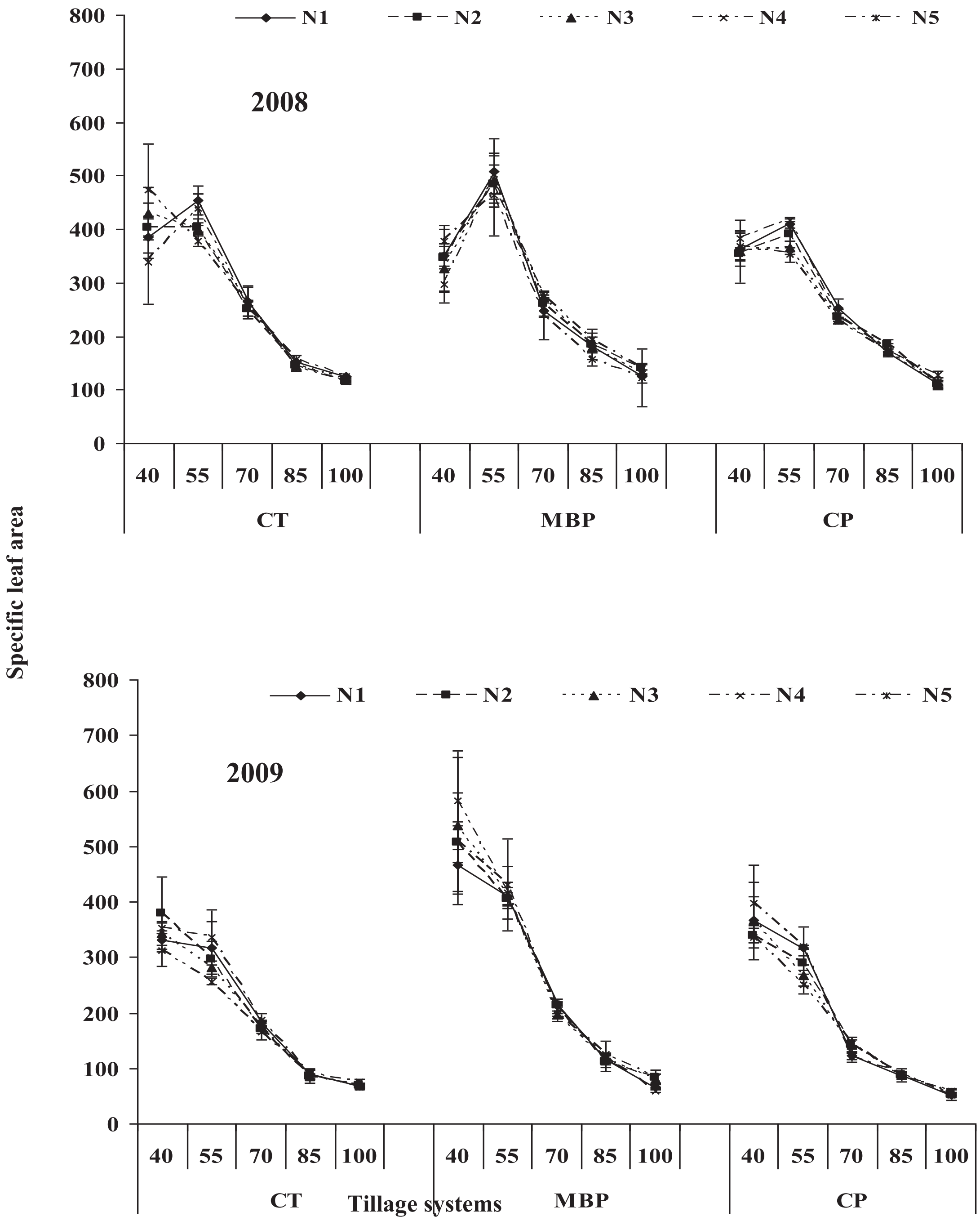
Tillage with chisel plough improved the leaf area per plant (LAPP) while tillage with MBP caused decrease in leaf area per plant during both years of study (Figure 1), but this increase was higher during first year of study compared with second year (Figure 1). Among different N application timings, N_5 (i.e. N in three splits) produced higher leaf area per plant compared with other treatments with minimum leaf area per plant in N_4 (Figure 2). However, specific leaf area showed reverse results than leaf area per plant and higher specific leaf area was noted in MBP compared with other tillage systems with minimum value found in CP during both study years (Figure 2). Maize grown with nitrogen application at sowing time (N_1) produced higher specific leaf area compared with other treatments (Figure 2). Specific leaf area was reduced with the passage of time as the crop grow older it showed reduction with higher value at 40 days after sowing (DAS), while minimum value was specific leaf area was noted at 100 DAS during both years (Figure 2). Similarly, higher leaf area ratio (LAR) was noted in conventional tillage (CT) compared with other treatments during both study years (Figure 3). Nitrogen application timings also had significant effect on LAR and higher LAR was observed in N_5 while lower was noted in N_1 during both years (Figure 3).

Figure 1. Tillage systems and nitrogen timings effect on leaf area per plant in maize.



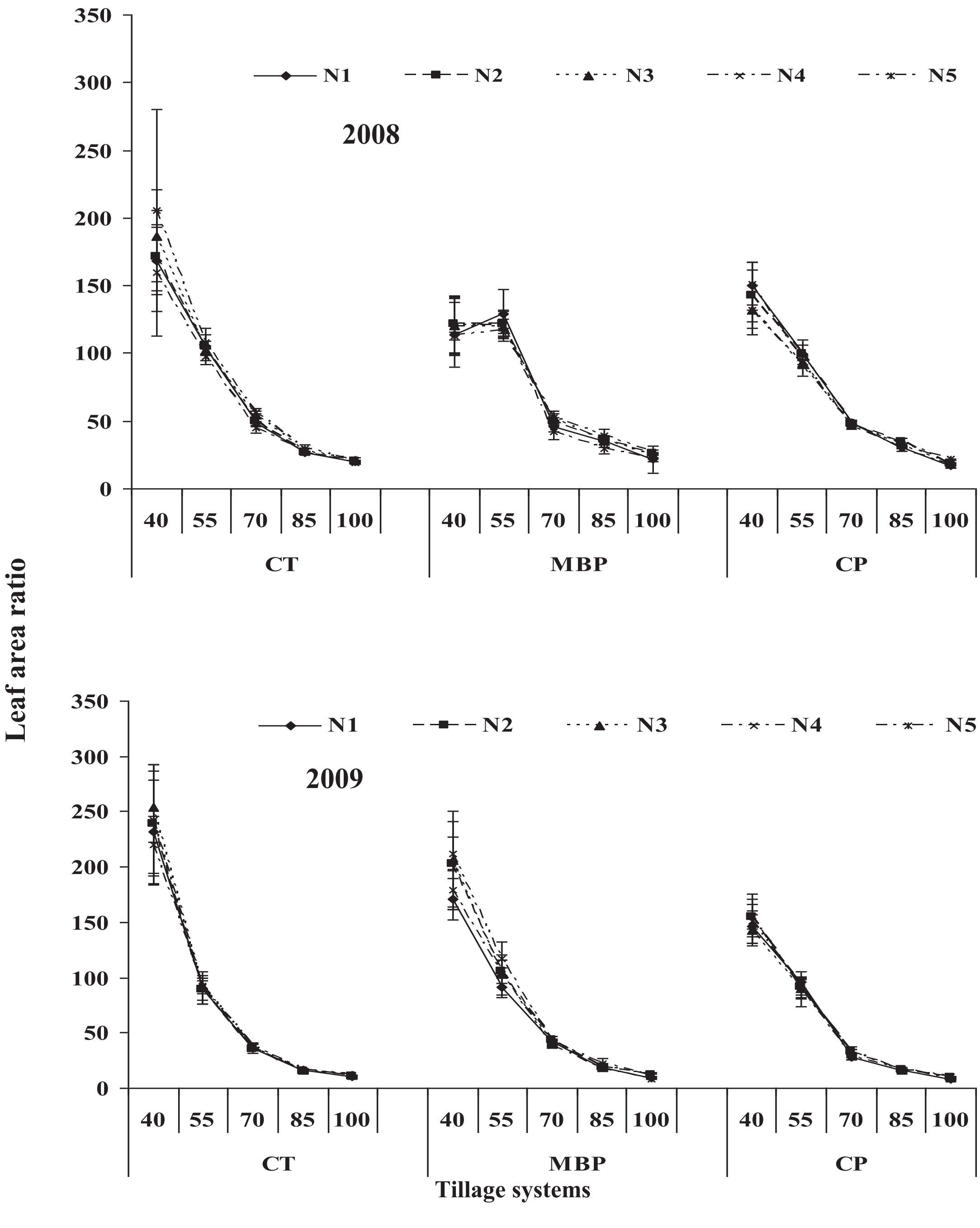
Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Figure 2. Tillage systems and nitrogen timings effect on specific leaf area in maize.



Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Figure 3. Tillage systems and nitrogen timings effect on leaf area ration in maize.



Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Different tillage systems had non-significant effect on a, b and total chlorophyll contents at both blister (R2) as well dough (R4) stages. While nitrogen application timings had significant effect on chlorophyll contents and N_5 showed higher a, b and total chlorophyll contents compared with other treatments with minimum values for N_4 (Tables 1, 2). Various tillage systems had non-significant effect on protein and starch contents while significant effect on oil contents (Table 3) during both years, while N application timing had significant effect on protein, oil and starch contents during both years (Table 3). However, higher protein contents were observed with N_5 compared with other treatments with minimum protein contents in N_1 during both study years. Regarding oil contents, higher oil contents were found in N_1 while lower with N_5. Similarly, higher starch contents were noted for N_1 compared with other treatments during both study years (Table 3). The maximum oil contents were noted in plots where maize was grown by preparing the field with MBP and whole nitrogen was applied at sowing with minimum value in maize grown by ploughing the field with CT and applying N in two splits (i.e. ½ at planting + ½ at V_5) during both study years (Table 4).

Table 1. Effect of different tillage systems and time of nitrogen application on chlorophyll contents at blister stage (R2-stage) in maize**.**

Treatments		Chlorophyll a	Chlorophyll b	Total chlorophyll
Tillage (T)	CT	23.69	16.11	39.810
	MBP	23.11	15.72	38.84
	CP	23.58	16.39	39.97
	LSD (0.05)	NS	NS	NS
Nitrogen (N)	N_1	21.97 c	14.40 d	36.37 d
	N_2	23.03 c	16.30 c	39.33 c
	N_3	24.57 b	18.05 b	42.63 b
	N_4	20.64 d	12.32 e	32.96 e
	N_5	27.10 a	19.30 a	46.41 a
	LSD (0.05)	1.14	1.18	1.669
	T×N	NS	NS	NS

Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Table 2. Effect of different tillage systems and time of nitrogen application on chlorophyll contents at dough stage (R4-stage) in maize.

Treatments		Chlorophyll a	Chlorophyll b	Total chlorophyll
Tillage (T)	CT	18.47	14.23	32.70
	MBP	18.24	13.878	32.11
	CP	19.23	13.66	32.89
	LSD (0.05)	NS	NS	NS
Nitrogen (N)	N_1	16.81 d	12.39bc	29.20 d
	N_2	18.64 c	13.79 b	32.43 c
	N_3	20.15 b	15.38 a	35.53 b
	N_4	15.73 d	11.39 c	27.12 d
	N_5	21.91 a	16.66 a	38.57 a
	LSD (0.05)	1.378	1.472	2.24
	T×N	NS	NS	NS

Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Table 3. Effect of different tillage systems and time of nitrogen application on grain protein, oil and starch contents in maize.

Year			Protein content (%)	Oil content (%)	Starch content (%)
2008	Tillage (T)	CT	8.13	3.24 b	70.94
		MBP	8.09	3.66 a	71.33
		CP	8.13	3.39 b	71.20
		LSD (0.05)	NS	0.15	NS
	Nitrogen (N)	N_1	8.03 d	3.77 a	72.30 a
		N_2	8.06 cd	3.41 b	70.07 c
		N_3	8.15 b	3.31 c	71.82 ab
		N_4	8.10 bc	3.42 b	70.42 bc
		N_5	8.23 a	3.23 d	71.16 abc
		LSD (0.05)	0.06	0.08	1.51
		T×N	NS	*	NS
2009	Tillage (T)	CT	8.07	3.29 b	71.21
		MBP	8.04	3.70 a	71.73
		CP	8.05	3.42 b	71.40
		LSD (0.05)	NS	0.17	NS
	Nitrogen (N)	N_1	7.97 c	3.79 a	72.74 a
		N_2	8.00 c	3.45 b	70.39 b
		N_3	8.09 ab	3.34 c	72.11 a
		N_4	8.03 bc	3.49 b	70.59 b
		N_5	8.16 a	3.28 c	71.39 ab
		LSD (0.05)	0.08	0.10	1.44
		T×N	NS	*	NS

Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Table 4. Interactive effect of different tillage systems and time of nitrogen application on grain oil contents.

Tillage	Nitrogen	**Oil content (%) (2008)**	**Oil content (%) (2009)**
CT	N_1	3.45 bc	3.48 bc
	N_2	2.98 g	3.06 g
	N_3	3.19 ef	3.26 def
	N_4	3.06 fg	3.13 efg
	N_5	3.51 b	3.51 b
MBP	N_1	3.95 a	3.98 a
	N_2	3.91 a	3.96 a
	N_3	3.51 b	3.47 bc
	N_4	3.92 a	4.00 a
	N_5	3.00 g	3.10 fg
CP	N_1	3.93 a	3.91 a

continue

continuation

	N_2	3.35 cd	3.32 cd
	N_3	3.22 de	3.28 de
	N_4	3.27 de	3.35 bcd
	N_5	3.17 ef	3.22 defg
	LSD (0.05)	0.13	0.17

Here CT = Conventional tillage; MBP = Mouldboard plough; CP = Chisel plough; N_1= Whole N application at sowing, N_2= ½ at sowing + ½ at V5 (5-leaf stage), N_3= ½ at sowing + ½ at tasseling, N_4= ½ at V5 + ½ at tasseling, N_5= 1/3 at sowing + 1/3 at V5 + 1/3 at tasseling.

Discussion

Tillage systems had significant effect on soil properties and root distribution (HAMZA; ANDERSON, 2005; HOU et al., 2012) and had direct impact on leaf area per plant and specific leaf area (Fig1-3). In present study, increase in leaf area per plant in chisel ploughed plots might be due to higher root proliferation (SUN et al., 2017), which helped to delay leaf senescence (LIANG et al., 2010) and ultimately improved leaf area per plant (Figure 1) (SUN et al., 2017). However, less leaf area per plant under mould board ploughed plots was due to higher bulk density and lower porosity of soil which restricted roots to upper soil and reduced uptake of nutrients and water (BENGOUGH et al., 2011; EKELOF et al., 2015). Similarly, N application in three splits produced higher leaf area per plant compared with other treatments which might be due to availability of nitrogen at all plant growth stages which ultimately improved leaf area per plant. This increase in leaf area per plant resulted in increased LAR (Figure 3) under split application (AMANULLAH et al., 2007). This increase in leaf area per plant led to increase in leaf is index (LAI) as well as grain and biomass production (WASAYA et al., 2017a,b).

Different tillage systems had non-significant effect on chlorophyll a, b and total chlorophyll contents at both blister (R2) as well dough (R4) stages (Tables 1, 2). The same non-significant effect of tillage systems on chlorophyll SPAD value was also reported by Liu and Wiatrak (2012) in maize crop. However, nitrogen application in splits significantly improved stay green which was visible through improved values of chlorophyll contents (Tables 1, 2). This increase in stay green might be due to N availability at all growth stages which improved chlorophyll contents as nitrogen is important for chlorophyll synthesis and photosynthetic activity (TURNBULL et al., 2007).

Grain quality is an important parameter with reference to product quality as well as nutrient composition. The results of current study indicated that different tillage systems had no significant effect on grain protein and starch contents while significant effect on oil contents. Grain oil contents were significantly influenced by tillage operations and highest oil contents were produced in grain obtained from plots treated with board plough followed by chisel plough and conventional tillage. Cociu and Alionte (2011) stated that grain contents in a zero tilled sown crop were significantly higher than those were grown in moldboard and disc plough tillage systems. Similarly decrease in oil contents under chisel ploughed plots might be due more grain protein contents as these are contrary to each other as increase of one component lead to decrease in other nutrient. This might be due more uptake of nitrogen from deeper soil layer which is an important constituent of protein and hence led to decrease in oil contents (VITA et al., 2007; BOOMSMA et al., 2009; ANDRIJA et al., 2009).

In present study, timing of nitrogen application had significant ($p \leq 0.05$) effect on grain protein content. It has been documented that nitrogen fertilization at reproductive stage increases N contents in grains (SILVA et al., 2005) and results in increased grain protein contents (AMANULLAH; SHAH, 2010). Similarly, decreased protein contents in N_1 (whole N application at sowing) might be due to higher losses of nitrogen at early growth stages (GALLAIS; BERTIN, 2008). Reduced concentration of grain oil in N_5 (1/3 at sowing+1/3 at V5+1/3 at tasseling) might be due to supply of N at all growth stages which resulted in reduction of oil contents. These results might be due to higher negative relation between N and oil content. However, grain oil contents were significantly higher in plants having sole application of N compared with those fertilized with split doses of N. Increase in starch contents under splits application might be due to availability of N for whole crop season which led to healthy crop and ultimately resulted in higher grain starch contents (Table 3) (MUTHUKUMAR et al., 2005).

Conclusion

Tillage systems and nitrogen timings had significant effect on leaf area per plant, specific leaf area and leaf area ratio. Tillage systems had non-significant while nitrogen timings had significant effect on stay green and grain quality parameters. The higher a, b and total chlorophyll contents were noted in treatment where N was applied in 3-splits compared with all other treatments. Similarly, higher protein contents were noted with N_5 compared with other treatments while, higher oil and starch contents were noted with N_1. Chiseling the subsoil is a useful tool for improving growth of maize crop. Similarly, N should be applied in three splits to improve its growth, stay green as well as grain quality in terms of proteins contents which is major constituent of maize grain.

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