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EFFECTS OF NITROGEN AT DIFFERENT GROWTH STAGES ON PHENOLOGY AND GRAIN FILLING PERIOD OF MAIZE (*ZEA MAYS* L.)

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Abstract

An experiment was conducted to see the effects of nitrogen at different growth stages on phenology, rate and grain filling period of maize. Nitrogen rates (0, 75, 150 and 225 kg/ha) were arranged in main plot and time of nitrogen application in sub plots [($^{1}/_{3}$ at sowing + $^{1}/_{3}$ at $V_{8\text{-}10}$ + $^{1}/_{3}$ at tasseling), (½ at sowing + ½ at tasseling) and (½ at sowing+ ¼ at $V_{8\text{-}10}$ + ¼ at tasseling) as T_1, T_2 and T_3, respectively]. A two part linear model was used to quantify the grain filling parameters. Application of high N rates as T_1 resulted 33% increase in potential rate of grain filling compared with the control. Maximum rate (0.00904 g/day), grain filling period (42.64 days), effective grain filling period (37.72 days), maximum grain yield (7700 kg/ha), days to 50% anthesis (63.85 days), days to 50% silking (68.2 days) and days to physiological maturity (128.9 days) were observed in the plots receiving 225 kg N/ha as T_1. It was *vice versa* in tasseling-silking interval. Results suggested that application of 225 kg N/ha in three equal splits can be recommended for profitable maize production in the study area.

Introduction

Maize is usually considered to have a high soil fertility requirement to achieve maximal yields (Paponov *et al*. 2005, Uribelarrea *et al*. 2009). Time of nitrogen (N) application plays a very important role in maize yield. Maize obtains 35 - 5% of kernel N from post silking uptake between the 8-leaf (V_8) and dough (R_3) growth stages. Pre-anthesis uptake is necessary in order to accumulate N in the vegetative sink for later remobilization to the ear. Post-silking uptake supplements remobilized N and prevents excessive N relocation from the vegetative sinks to the ear, which is essential for maintenance of appropriate N partitioning between grain and stover to maintain photosynthesis and grain yield formation. Post silking uptake is also critical for many physiological processes including spikelet differentiation and kernel formation (Paponov *et al*. 2005). It is reported that maximum N uptake by maize occurs during the month prior to tasseling and silking. Mariga *et al.* (2000) and Scharf *et al.* (2002) reported that grain yield in maize increases with increase in rate and split application for N. Nitrogen is vitally important during grain filling period (Bruns and Ebelhar 2006, Subedi and Ma 2005). Final grain weight was related to grain filling rate, grain filling duration, and their interaction (Sadras and Egli 2008). Under N stress conditions, increased amounts of N remobilization in the leaves occurs for filling the grain which leads to the accelerated leaf senescence and the reduced duration of grain filling.

Crop phenology is one of the most important aspects of crop yield. Dolan *et al*. (2006) reported that higher nutrients availability and favourable soil conditions due to organic source of N may delay phenology. Most of the farmers use high amount of N fertilizer haphazardly due to lack of knowledge in crop phenology. Thus, an experiment conducted to investigate the effect of N at different growth stages on phenology, rate and grain filling period of maize.

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Materials and Methods

Four rates of N (0, 75, 150 and 225 kg/ha) applied as urea were used as main plot factor, and three rates of N ($^{1}/_{3}$ at sowing + $^{1}/_{3}$ at V_{8-10}+ $^{1}/_{3}$ at V_T stage or last branch of the tassel was completely visible and the silks were not yet emerged), (½ at sowing + ½ at V_T stage) and (½ at sowing+ ¼ at V_{8-10} + ¼ at VT stage,) as T_1, T_2 and T_3, respectively) as subplot factor. The field experiment was arranged in split plot way in RCBD using three replications at the research farm of University of Mohaghegh Ardabili. The area is located at 38º15′ N latitude and 48º15′ E longitude with an elevation of 1350 m above mean sea level. Climatically, the area placed in the semi arid temperate zone with cold winter and hot summer. Average rainfall was about 254.3 mm that most rainfall concentrated between winter and spring. Mean temperature and precipitation maize growing season. The field was prepared well before sowing by plowing twice with tractor followed planking to make a fine seed bed. Phosphorous (100 kg/ha as TSP) and potassium (100 kg/ha as K_2SO_4) were applied as basal dose.

In each plot there were 4 m long 5 rows. Plots and blocks were separated by 0.75 m unplanted distances. Seed placement was done by hand in individual hills at inter-row and intra-row spacing of 75 cm ×13.3 cm. Maize seed (var. Cordona) was planted in the second week of May. Two seeds were sown per hill and two week after emergence and at 4 - 5 leaves stage thinned to one plant per hill. The field was immediately irrigated after planting. Three manual weeding were done at the maize at 4 - 6 leafy stage. Number of grains in rows, grains per ear row and grains per ear were measured with ten randomly selected plants from the three central rows and then average was calculated.

Three central rows each 2 m long were harvested from each plot. The ears were dehusked, dried and then threshed for grain yield (kg/ha). Days to silking, tasseling and anthesis-silking interval: Silking refers to the stage which silk emerged on 50% of the observed plants. Tasseling reached when 50% of the plants shed pollen from the main branch of the tassel and from few other branches. Silking date was recorded when 50% of the plants had extruded silks. The anthesis-silking interval (ASI) is the number of days from anthesis to silking. Appearance of black layer in seeds was used as criteria for physiological maturity. It was determined by counting number of days from planting date to physiological maturity. In each sampling, three ears in each plot were taken for the investigation of grain filling parameters. The first sampling was taken on the 10th day after silking and other samples were taken in 5 day intervals for determining the grain weight accumulation. At each sampling, grains were removed by hand from three upper, middle and lower parts of each ear were dried at 80ºC for 48 hrs. Grain dry weight and number were used to calculate the average grain weight for each sample. Total duration of grain filling was determined for each treatment combination by fitting a bilinear model (Eqs. 1 and 2):

$$GW = \begin{cases} a + gfr\,(daa),......if\ ...daa\ <\ p_m \\ a + gfr\,(p_m),.......if\ ...daa\ \geq\ p_m \end{cases} \qquad (1)$$

where, GW is the kernel dry weight, a is the GW-intercept, gfr is the slope of grain weight indicating grain filling rate, daa is the days after silking and p_m is physiological maturity. Effective grain filling duration (EGFD) was calculated using a bilinear model:

EGFD = The highest kernel weight (g)/ratio of seed filling (g/day) (2)

In the other hand, increase of kernel weight in filling period was calculated by using equation in SAS statistical soft were using Proc NLIN DUD method.

The data recorded were statistically analyzed by using computer software SAS ($Ver_{9.1}$) for LSD at 5% probability level.

Results and Discussion

N rate and their application time had significant effects on phenolgy, rate and grain filling period, yield and some agronomic traits of maize. The response of number of grains/ear to N rates and time was significant (Table 1). Maximum number of grains/ear (472.9) was recorded at the highest N rate (225 kg N/ha). Mean comparison of N rates × N application time indicated that maximum of the number of grains/ear (502) was recorded with 225 kg N/ha when applied in three equal splits. Increase in number of grains/ear at higher N rates might be due to conversion of more photosynthesis into sinks resulting in more grains/ear. This suggests that N stress on grain number occurs indirectly through effect on photosynthesis and also via its effect on phenology stages such as physiological maturity, silking date and anthesis-silking interval. So, maize took more time to silking and physiological maturity in plots that received the highest rate of N (225 kg N/ha) and it was *vice versa* in anthesis-silking interval (Table 1). Increasing N application up to the maximum tested rate of 200 or 314 kg N/ha (Bruns and Ebelhar 2006) resulted in the increase of both grain number and grain weight. These results are in conformity with the results obtained by Mariga *et al*. (2000). The greater number of grains/ear with higher N rate might have been resulted from the greater assimilates partitioning to the seeds as a result of longer growth period, delay in phenology stages such as physiological maturity and decrease in anthesis-silking interval and more photosynthates availability during the grain filling period (Amanullah *et al*. 2009).

It seems that environmental factors have a lesser influence on the number of grain rows and this trait is significantly affected by genetic factors than the other sources. Maximum and significant number of grains per ear row (30) was recorded at 225 kg N/h (Table 1). Mean comparison of rates×N application time indicated that, significantly maximum value (32.2) was recorded at of 225 kg N/ha as T_1 compared to control (Table 1). The greater number of grains per ear row with higher N rate might have been resulted from the greater assimilates partitioning to the seeds as a result of longer growth period and more photosynthates availability during the grain filling period (Amanullah *et al*. 2009). Decrease the number of grains per ear row under lower N application might be attributed to poor development of sinks and reduced translocation of photosynthates. Gungula *et al*. (2003) reported that the effect of N on grain number occurs indirectly through N effect on photosynthesis and also via its effect on anthesis and silking interval and silking date. The high N rates increased time to silking (67.28 days) and physiological maturity (127.33 days) and it seems that can be one of reasons for increase the number of grains per ear row. Similar results have been reported by Mariga *et al*. (2000).

Increasing N rates increased days to 50% tasseling, significantly. Maximum of the days to 50% tasseling (63.85 days) was recorded at 225 kg N/ha as T_1 (Table 1). Gungula *et al*. (2003) suggested that increase in N rate and number of split application might have increased the rate of photosynthesis that resulted in the leaf longevity and delayed phenological characteristics such as tasseling in maize. Increasing N level increased days to 50% silking (Table 1). Similar trend (as for tasseling) for silking was observed regarding N fertilization and was delayed by all N treatments as compared to control. Means comparison indicated the maximum days to 50% silking (68.2 days) was recorded at 225 kg N/ha as T_1 (Table 1). Present findings are in line with the finding of Shrestha (2007) who stated that phenological events like tasseling, silking and maturity in maize were significantly delayed by increasing rate of mineral N than the other sources.

Table 1. Means comparison of N application time and rates on phenology and some agronomic traits of maize (*Zea maize*).

Treatments	NGR	NGER	NGE	50% tasseling (day)	50% silking (day)	ASI (day)	Physiolo-gical maturity (day)	GY (kg/ha)	Duration of grain filling (day)	Rate of grain filling (g/day)	Effective grain filling period (day)	Fitted equations
Nitrogen rates (kg/ha)												
$N_0 = 0$	14.3 c	24.7 c	348.4 c	58.8 d	63.57 d	5.3 d	121.5 d	4744.8 c	34.45 d	0.006 d	33.21cd	-
$N_1 = 75$	15 b	25.9 b	392.6 b	59.66 c	64.61 b	4.95 a	123.63 c	5535.3 b	36.65 c	0.00628 c	33.706 c	-
$N_2 = 150$	15.7 a	29.6 a	464.8 a	60.1 b	64.86 b	4.76 b	126.25 b	7160.9 a	37.08 b	0.00661 b	34.72 b	-
$N_3 = 225$	15.8 a	30 a	472.9 a	62.73 a	67.28 a	4.5 c	127.33 a	7355.5 a	37.5 a	0.00736 a	35.29 a	-
LSD ($p < 0.05$)	**0.1477**	**1.058**	**22.07**	**0.3418**	**0.293**	**0.0543**	**0.4106**	**506.38**	**0.1089**	**0.0001**	**0.1896**	
Significance	**	**	**	**	**	**	**	**	**	**	**	
Application time												
T_1	15.3 a	28.5 a	438.9 a	61.96 a	66.41 a	4.5 c	126.73 a	6598.3 a	38.13 a	0.00711 a	34.795 b	-
T_2	15 b	26.9 b	405.4 c	59.8 c	64.76 c	4.96 a	124.22 c	5884.4 c	37 b	0.00716 a	36.165 a	-
T_3	15.2 ab	27.2 b	414.7 b	60.78 b	65.58 b	4.79 b	126.26 b	6114.7 b	36.12 c	0.00599 b	32.76 c	-
LSD ($p < 0.05$)	**0.1693**	**0.385**	**7.84**	**0.3418**	**0.293**	**0.0543**	**0.4106**	**165.2**	**0.108**	**0.0001**	**0.189**	
Significance	**	**	**	**	**	**	**	**	**	**	**	
Interaction (N × T)												
Control		24.7 e	354 g	56.2 g	61.56 h	5.36 a	121.5 g	4800 f	37.6 i	0.006 h	32 g	Y = 0.0263+0.00628X
$\mathbf{N_1T_1}$	-	27.5 d	420 e	60.7 d	65.35 de	4.65 ef	124 e	6500 e	39.13 h	0.00658 fg	33.71 f	Y = –0.00129+0.0064
$\mathbf{N_1T_2}$	-	25.6 e	387 f	58.5 f	63.7 g	5.2 b	122.7 f	5000 ef	39.9 fg	0.00623 g	34.21 e	Y = –0.0085+0.0068X
$\mathbf{N_1T_3}$	-	25.6 e	391 f	59.8 e	64.8 ef	5 c	124.2 e	6250 d	40.604 de	0.00681 f	34.38 e	Y = –0.0237+0.00656X
$\mathbf{N_2T_1}$	-	32 a	500 ab	61.2 cd	65.7 d	4.5 f	127.3 bc	7500 b	39.82 g	0.00723 e	34.53 e	Y = –0.00166+0.00729X
$\mathbf{N_2T_2}$	-	28.5 c	442.3 d	59.2 ef	64.2 fg	5 c	124.67 de	6800 c	40.25 ef	0.0078 c	35.22 d	Y = –0.00098+0.00787X
$\mathbf{N_2T_3}$	-	30 b	478 c	59.9 e	64.7 f	4.8 d	126.8 c	7250 bc	41.23 bc	0.00837 b	35.78 c	Y = –0.00104+0.00835X
$\mathbf{N_3T_1}$	-	32.2 a	502 a	63.85 a	68.2 a	4.35 h	128.9 a	7700 a	40.9 cd	0.0068 f	36.01 c	Y = –0.00139+0.00678X
$\mathbf{N_3T_2}$	-	30.2 b	479 c	61.7 c	66.4 c	4.7 d	125.3 d	7100 c	41.608 b	0.0075 d	36.97 b	Y = –0.00094+0.000745X
$\mathbf{N_3T_3}$	-	30.3 b	483 bc	62.66 b	67.25 b	4.59 fg	127.8 b	7300 bc	42.64 a	0.00904 a	37.72 a	Y = –0.0392+0.00901X
LSD ($p < 0.05$)	-	**0.931**	**18.13**	**0.747**	**0.645**	**0.1097**	**0.808**	**365.28**	**0.374**	**0.0003**	**0.475**	
Significance	ns	**	*	**	**	**	**	**	**	**	*	

Grain yield, GY; The number of grains rows, NGR; Number of grains per ear row, NGER; Number of grains per ear, NGE; Anthesis silking interval, ASI. $T_1 = (^1/_3$ at sowing + $^1/_3$ at $V_{8\text{-}10}$ + $^1/_3$ at tasseling,); $T_2 = (½$ at sowing + ½ at tasseling,); $T_3 = (½$ at sowing+ ¼ at $V_{8\text{-}10}$ + ¼ at tasseling) - Means with similar letters in each column are not significantly different-*, ** and ns showed significant differences at 0.05, 0.01 probability levels and not significant, respectively.

N application time and rates significantly affected anthesis and silking interval. Maximum ASI was recorded at T_2 (4.96 days) and minimum of it was recorded at T_1 (4.5 days). Control plots took more days (5.36 days) to ASI. The shorter ASI with higher N was because of inducing early and rapid growth. Gungula *et al*. (2007) reported that there will be more synchrony in flowering with higher N. An asynchronous flowering may limit grain production per ear due to lack of pollen, lost of silk receptivity or early kernel abortion caused by dominance of early formed ovaries from the base of the ear on the late formed from the tips. The results indicated that minimum of ASI (4.35 days) was recorded at 225 kg N/ha as T_1 and maximum of it (5.36 days) was recorded at control treatment (Table 1).

The data along with means comparison are presented in Table 2 which indicate that time and rate of N application had significant effect on days to maturity. Process of physiological maturity was delayed by time and rate of N application and their combinations as compared to control. Days to maturity was delayed significantly with the higher levels of 150 and 225 kg N/ha (Table 1). The mean's comparison of N application time effect on days to maturity showed that N splits as T_1 delayed maturity as compared with T_2 and T_3 (Table 1). These results are in line with N application time and rates effects on days to tasseling and silking. The prolonged stages of days to maturity due to rates and time of N application were reported by Dolan *et al*. (2006) who observed that higher nutrients availability and favourable soil conditions due to N could be possible reason for delayed phenology in N treated plots.

Fig. 1 and Table 1 demonstrate the greater variations of the grain filling period. Means comparison about rate of grain filling showed that there is a significant difference between rates, N application time and interaction between rates × N application time on rate of grain filling at $p \leq 0.05$ (Table 1). Application of high N rates (225 kg N/ha) as T_1 resulted in 33% increase in potential rate of grain filling over the control (Table 1). In other words, peak grain filling rates maintained longer in the treatment receiving of high N rates in application time as T_1 over the control. Increasing the rate of N fertilizer also delayed the occurrence of maximum grain filling rates and grain weight in this compound treatment was more than control (Fig. 1). Final grain weight was related to grain filling rate, grain filling duration, and their interaction (Sadras and Egli 2008). It is suggested that under N stressful conditions, increased amounts of N remobilization in the leaves for filling the grain will lead to the accelerated leaf senescence and the reduced duration of grain filling. Bruns and Ebelhar (2006) reported that maize yield and grain weight increased with N application. It seems that high grain filling rate in plot treated with 225 kg N/ha could result from sufficient assimilate supply and large partitioning capacity.

The data presented in Table 1 indicate that time and rate of N application had significant effect on grain filling duration and effective grain filling period. Grain filling duration was delayed by time and rate of N application and their combinations as compared to control. This trend was similar to process of physiological maturity. Among the N rates, the highest grain filling duration was observed in rates of 150 and 225 kg N/ha (Table 1). Among the N application times, increase in grain filling duration was observed with T_1 as compared with T_2 and T_3 (Table 1). Means comparison showed that grain filling duration increased in plots that received the highest rate of N application in three equal splits (Table 1). Similar results were obtained in effective grain filling period. These results are in line with N application time and rates effects on days to tasseling, silking and physiological maturity. Hamidi and Nasab (2001) reported that increases in N rates significantly delay the duration of the vegetative and reproductive period and could be possible reason for lengthening of grain filling duration.

Grain yield is the main target of crop production. The grain yield was significantly affected by both N application time and rates. Among the N rates, the grain yield varied between 4744.8 kg/ha in without N application and 7355.5 kg/ha in application of 225 kg N/ha (Table 1). Maximum grain yield was produced by T_1 (6598.3 kg/ha) while minimum by T_2 (5884.4 kg/ha). Results of interaction rates and N application time indicated that maximum of it (7700 kg/ha) was obtained in application of 225 kg N/ha and minimum (4800 kg/ha) was recorded at control treatment (Table 1). Mariga *et al.* (2000), Scharf *et al.* (2002) reported that grain yield in maize increases with increase in rate and spit application for N. The increase in grain yield at the high N rate application might be due to the delay in maturity period, decrease in number of barrenness plants, decrease in anthesis-silking interval and increase in number of seeds per ear.

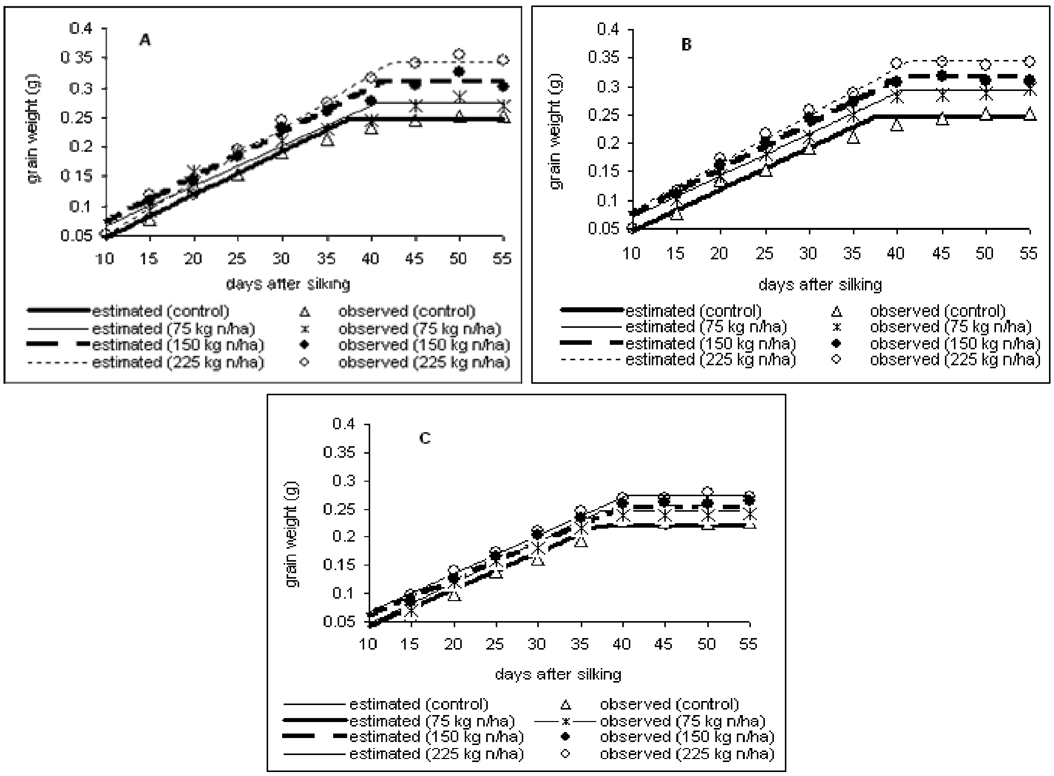

Fig. 1. Effect of N at different growth stages on grain filling (A = Application of N as T_1; B = Application of N as T_2 and C = Application of N as T_3) in maize.

Gungula *et al.* (2007) suggested that decrease in anthesis-silking interval or close synchrony between male and female inflorescence is desirable to improve grain yield and number of grains/ear. The greater grain yield with higher N rate might have been resulted from the greater assimilates partitioning to the seeds as a result of longer growth period (Table 1), increasing of grain filling period (Fig. 1 and Table 1) and more photosynthates availability during the grain filling period. Dolan *et al.* (2006), Hamidi and Nasab (2001) who reported that higher nutrients availability and favourable soil conditions due to N could be the possible reason for increasing of grain yield and delayed phenology in N treated plots.

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