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Performance of winter maize (*Zea mays*) hybrid to planting methods and nitrogen levels*

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ABSTRACT

The experiment was conducted during the crop years 2006–07 and 2008–09 to study the effects of various doses of nitrogen and planting methods on various physiological processes associated with nitrogen-use efficiency and productivity of winter maize (*Zea mays* L.). The treatments comprised 3 planting methods (bed, ridge and flat) and six nitrogen levels (0, 175, 200, 225, 250 and 275 Kg N/ha) with planting methods in the main plot and nitrogen levels in sub-plots. Biometrical parameters, viz plant height, leaf area, dry matter accumulation, crop growth rate, relative growth rate were maximum among the bed planted crop and at 250 and 275 kg N/ha. Yield attributes namely, bundle weight, straw yield, shelling percentage, harvest index, total grain yield, 1000-grain weight, were significantly higher in the bed planted crop and in the crop supplied with higher doses of nitrogen. Crop supplied with 250 and 275 kg N/ha took fewer days to anthesis and silking than the crop supplied with lower nitrogen doses. Biochemical parameters, viz chlorophyll content and activities of various enzymes (nitrate reductase, glutamine synthetase and acid invertase) estimated from the leaves at pre- and post-anthesis stage were also found to be maximum among the bed planted crop and the crop supplied with higher doses of nitrogen. Activity of these enzymes was negatively co-related to anthesis-silking interval (ASI), whereas a positive corelation was found with yield.

Key words: Acid invertase, Glutamine synthetase, Growth parameters, Maize, Nitrate reductase, Nitrogen levels, Planting methods

Maize (*Zea mays* L.) is the third most important cereal crop next to rice and wheat and has the highest production potential among the cereals (Muthukumar *et al.* 2005). Maize growth and productivity/unit area depends upon the genetic make up of the crop, plant density, appropriate planting methods and supply of essential nutrients. Planting of maize on raised beds and ridges provides a good option for managing water, nutrients and weeds (Freeman *et al.* 2007).

Nitrogen is a critical input in agriculture and is a powerful tool for increasing grain yield in cereals. However, excessive use of nitrogen fertilizer in intensive agricultural areas causes serious environmental problems, such as nitrate leaching and nitrous oxide emissions, especially where there is heavy rainfall during maize growing season (Ju *et al.* 2004). It is, therefore, necessary to identify the critical steps associated with nitrogen-use efficiency. Winter maize due to its vigorous plant growth and longer duration has proved to be more responsive to fertilizer application and is therefore one of the best model plants to combine physiological and agronomic studies on nitrogen nutrition. Hence, a study was conducted to study the effects of various doses of nitrogen and planting methods on productivity of winter maize and to study the physiological processes associated with nitrogen use-efficiency under varying nitrogen supplies and planting methods.

MATERIALS AND METHODS

Field experiment was carried out at the Students' Research Farm, Department of Agronomy, Punjab Agricultural University, Ludhiana (36° –54□N; 75° –48□E; 247 m above sea level). The treatments consisted of three planting methods (bed, ridge and flat) and six nitrogen levels (0, 175, 200, 225, 250 and 275 kg N/ha.) with planting levels in the main plot and nitrogen levels in sub-plots (7.0 m×3.4 m). The treatments were replicated four times. The crop was sown by dibbling in November 2006 and 2007, respectively. Nitrogen was supplied as per treatment in three splits, ie 1/3 at sowing, 1/3 in mid January and 1/3 at pre-tasseling stage. At sowing 60 Kg P_2O_5, 30 Kg K_2O and 25 Kg $ZnSO_4$/ha was also added. The crop rows were oriented in east to west direction. In case of bed and ridges, the seeds were sown on

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the southern slopes 6–7 cm above the base. In case of ridges and flat method row-to-row spacing was 60 cm, while in case beds the row-to-row spacing was 67.5 cm. The plant-to-plant spacing was 20 cm in case of ridges and flat method, whereas it was 15 cm in case of beds. The crop husbandry was as per recommended package of practices (PAU 2005).

Five plants were randomly selected from each plot and tagged. The plant height (from the base of the stem to the base of the topmost unfolded leaf) was recorded periodically at 30 days interval starting from the day of sowing. The mean plant height of five tagged plants was computed. The periodic dry matter accumulation and leaf area was determined by randomly selecting two plants/plot at 30 days interval. Both crop growth rate (CGR) and relative growth rate (RGR) were calculated as per formulae given by Radford (1967).

Days to anthesis and silking were determined by selecting and tagging 15 plants from each plot and the number of days taken for initiation of anthesis and silking were recorded. Five cobs were selected at random from each plot for measuring cob length, girth, shelling percentage and grain yield.1000–seed weight was determined by randomly selecting a sample from the pool of harvested seeds from each plot. Grain yield (tonnes/ha) was determined by recording the seed yield after shelling. The shelling percentage was calculated by dividing the grain weight of five cobs by total weight of five cobs and multiplying it by 100.

All the physiological and biochemical parameters were estimated both at pre- (approx 125 days after sowing) and post-anthesis (approx 150 days after sowing) stages. Chlorophyll content was determined from the middle leaf by Dimethyl sulphoxide (DMSO) method (Hiscox and Israelstam1979) and calculated as per Anderson and Boardman (1964). Hill reaction activity was measured by the method given by Cherry *et al* (1973). Nitrate reductase activity was estimated according to the method by Nicholas and Nason (1957). The glutamine synthetase activity was determined as per Lea *et al.* (1990). The activity of acid-invertase was determined by the method of Dey (1986).

RESULTS AND DISCUSSION

Growth parameters

Leaf area and dry matter accumulation was maximum among the bed planted plants (Table 1). Nitrogen application also had significant effect on leaf area and dry matter accumulation being maximum among the bed planted plants supplied with 275 kg N/ha which is statistically at par with 250 kg N/ha but significantly higher over control. Dry matter accumulation (DMA) is one of the important parameters reflecting the growth of a crop. The optimum accumulation of dry matter, followed by adequate partitioning of assimilates to the sink leads to higher grain yield. Increase in nitrogen level produced more number of leaves/plant, more LAI resulting in more DMA (Bangarwa *et al.* 1988). An increase in leaf area with increasing nitrogen level is attributed to better crop growth with higher nitrogen availability (Vedivel *et al*. 2001).

Crop growth rate (CGR) showed an increasing trend up to maturity. Bed planting showed the maximum CGR, followed by ridge and least being in flat at the harvest stage (Table 1). Application of nitrogen showed an increasing trend in the CGR, being maximum at 275 kg N/ha. Like CGR, relative growth rate (RGR) too showed an increasing trend up to maturity. Among the planting methods bed showed the maximum RGR, followed by ridge and flat. Application of nitrogen also recorded an increase in RGR being maximum at 275 kg N/ha (Table 1).

Regarding initiation of anthesis and silking bed planted crop recorded fewer days than the ridge and flat sown crop. Nitrogen application also significantly influenced days to anthesis and silking. Maximum number of days for anthesis and silking were taken by control grown plants and minimum by the plants grown at 275 kg N/ha (Table 2). Abundant nitrogen is known to stimulate flowering in maize because it is a nitro-positive crop (Tollenaar *et al.* 1997).

Yield attributes and yield

Significant increase in total grain yield, harvest index and shelling percentage was observed in bed planted crop supplied with higher nitrogen doses (Table 2). Yielding ability is one of the most important quantitative characters in a crop and it depends upon the development of other plant characters, viz leaf area, chlorophyll content, photosynthesis and dry matter accumulation, which in turn resulted into higher growth parameters, viz LAI, RGR and CGR etc. (Sahoo and Guru 1998). Significant increase in plant height, LAI, DMA and yield-attributing characters, viz cob length, cob width and 1000-grain weight with the application of nitrogen were mainly responsible for improvement in grain yield. Singh *et al.* (2003) have also observed a significant increase in grain yield at high N- levels. Grain yield of maize is function of yield attributes which are favourably influenced by N-application (Singh *et al.* 2000). High yield in bed planted crop (Table 2) could be attributed to better access of roots to nutrients and water which in turn led to better growth of the plants.

Harvest index and shelling percentage showed a similar trend as that of other yield attributes being maximum among the bed planted plants and the most effective nitrogen dose was 275 kg N/ha which was statistically at par with 250 kg N/ha.

Likewise, cob length and girth were maximum among the bed planted plants-followed by ridge and flat grown plants. Cob length and girth were maximum among the cobs obtained from the plants supplied with 275 kg N/ha (Table 2). High levels of nitrogen produced cobs having better length and girth. Enhanced N supplements led to an increase in leaf area, photosynthesis etc. which in turn result in the formation of healthy cobs.

Table 1 Effect of planting methods and nitrogen levels on leaf area, dry matter accumulation, crop growth rate and relative growth rate in winter maize

Treatment	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS
			Leaf area (cm^2)			
Planting method						
Bed	69.17	89.69	191.94	421.38	558.83	532.39
Ridge	66.45	89.02	189.71	417.65	557.62	530.31
Flat	65.22	87.98	189.39	416.66	556.06	528.38
CD (*P*=0.05)	2.180	0.606	2.070	1.250	NS	NS
Nitrogen levels (kg/ha)						
0	61.60	83.04	185.64	414.42	552.57	526.44
175	65.12	86.39	188.34	416.73	555.61	528.43
200	66.83	87.97	190.45	417.57	557.01	529.4
225	68.14	89.72	191.42	419.23	558.06	530.89
250	69.34	92.53	192.28	421.07	560.32	532.20
275	69.64	93.74	192.94	421.31	560.74	532.79
CD (*P*=0.05)	1.490	0.835	1.390	3.260	1.390	1.900
Interactions P×N	NS	NS	NS	NS	NS	NS
			Dry matter accumulation (g/plant)			
Planting method						
Bed	0.11	2.11	9.78	90.80	141.73	162.09
Ridge	0.11	2.13	9.58	87.71	138.71	162.67
Flat	0.098	2.11	9.50	86.00	132.21	145.86
CD (*P*=0.05)	0.003	NS	NS	NS	1.700	4.040
Nitrogen levels (kg/ha)						
0	0.09	2.09	9.14	80.44	126.61	142.79
175	0.10	2.10	9.38	85.73	132.11	150.02
200	0.10	2.14	9.67	87.38	136.59	153.77
225	0.11	2.11	9.76	89.29	139.89	160.44
250	0.11	2.12	9.83	92.66	143.77	166.66
275	0.12	2.12	10.07	94.61	146.33	167.56
CD (*P*=0.05)	0.005	NS	0.400	1.570	1.980	2.320
Interactions P×N	NS	NS	NS	NS	NS	NS

Treatment	30–60 DAS	60–90 DAS	90–120 DAS	120–150 DAS	150 –180 DAS
		Crop growth rate (g/plant/day)			
Planting method					
Bed	0.067	0.260	0.800	1.700	2.710
Ridge	0.067	0.250	0.680	1.700	2.610
Flat	0.067	0.250	0.450	1.520	2.600
CD (*P*=0.05)	NS	NS	0.130	0.110	NS
Nitrogen levels (kg/ha)					
0	0.066	0.240	0.540	1.540	2.380
175	0.066	0.240	0.570	1.550	2.550
‘200	0.067	0.250	0.600	1.640	2.590
225	0.067	0.260	0.680	1.690	2.670
250	0.067	0.260	0.710	1.700	2.760
275	0.068	0.260	0.720	1.720	2.820
CD (*P*=0.05)	NS	0.010	1.000	0.060	0.060
Interactions P×N	NS	NS	NS	NS	NS
		Relative growth rate (g/plant/day)			
Planting method					
Bed	0.004	0.015	0.051	0.074	0.102
Ridge	0.005	0.015	0.051	0.073	0.100
Flat	0.003	0.014	0.050	0.073	0.098
CD (*P*=0.05)	0.001	NS	NS	NS	0.001
Nitrogen levels (kg/ha)					
0	0.0038	0.0144	0.049	0.072	0.097
175	0.0042	0.0147	0.050	0.074	0.098
200	0.0039	0.0147	0.050	0.074	0.100
225	0.0045	0.0149	0.051	0.074	0.101
250	0.0047	0.0150	0.051	0.075	0.103
275	0.0048	0.0150	0.052	0.075	0.104
CD (*P*=0.05)	0.001	NS	0.020	0.001	0.001
Interactions P×N	NS	NS	NS	NS	NS

DAS, Days after sowing; P, planting method; N, nitrogen levels
Data represent mean value of two years

Table 2 Effect of planting methods and nitrogen levels on days to anthesis and silking, yield and yield attributes in winter maize

Treatment	Days to anthesis	Days to silking	Total grain yield (tonnes/ha)	Harvest index	Shelling percentage	1000-seed weight (g)	Cob length (cm)	Cob girth (cm)
Planting method								
Bed	140.39	148.94	10.6	33.9	84.3	296.7	16.3	16.6
Ridge	141.89	150.44	10.4	31.4	81.2	295.2	15.1	16.3
Flat	143.06	151.56	7.6	29.3	79.5	270.2	14.5	15.5
CD (*P*=0.05)	1.52	1.53	0.36	NS	NS	3.74	0.61	0.12
Nitrogen levels (kg/ha)								
0	144.33	151.44	7.64	26.9	80.2	270.0	12.7	15.4
175	144.00	151.0	8.34	28.2	80.5	275.0	13.5	15.6
200	142.67	150.67	8.84	29.4	81.2	283.0	14.5	16.0
225	141.11	150.11	9.17	30.5	81.9	289.0	15.4	16.3
250	140.22	149.44	9.79	31.5	82.5	285.5	16.7	16.6
275	139.33	149.22	9.85	32.0	83.8	297.0	16.9	16.8
CD (*P*=0.05)	1.94	NS	2.48	1.2	NS	3.53	0.67	0.19
Interactions P×N	NS	NS	NS	NS	NS	NS	NS	NS

P, Planting methods; N, nitrogen levels
Data represent mean values of two years

Chlorophyll content, hill reaction activity and enzyme activity

At pre- and post-anthesis stages, among the planting methods the highest chlorophyll content was obtained in the leaves of bed, followed by ridge and least being in flat-sown crop. Similar trends were obtained for the hill reaction activity (Table 3). Nitrogen application showed an increase in the chlorophyll content and hill reaction activity. The highest chlorophyll content and hill reaction activity were obtained at 275 kg N/ha at pre- and post-anthesis stages, respectively which is significantly higher over control but statistically at par with 250 kg N/ha and 225 kg N/ha at pre-and post-anthesis stages, respectively (Table 3). Nitrogen is a structural component of chlorophyll. Hence high N-levels promote chlorophyll synthesis (Lawlor 2002) and consequently hill reaction activity.

The activity of enzymes, nitrate reductase and glutamine synthetase and acid invertase were found to be maximum among the bed planted crop, followed by ridge and flat (Table 3). Application of nitrogen also had significant effect on the activity of these enzymes which was found to be maximum at 275 kg N/ha which is statistically at par with 250 kg N/ha (Table 3). All inorganic nitrogen is first reduced to $NH^{+}_{4,}$this reduction is carried out by the activities of nitrate reductase (NR) and nitrite reductase. The NH^{+}_{4} ions are converted into glutamine synthetase and glutamate dehydrogenase (GOGAT) system. This metabolic system is regulated at different levels by many factors including the nitrogen source and light. Hence, high levels of nitrogen led to an increase in the activity of nitrogen assimilating enzymes (Forde 2000). Carbohydrate distribution within a plant is also affected by the nitrogen supply which strongly influences the processes of carbon assimilation, allocation and partitioning. Sucrose is a transport and storage sugar in plants. Acid invertase cleaves the sucrose into glucose and fructose. Hence, high activity of acid invertase in the leaves during pre- and post-anthesis stages provide ample glucose for respiration, which in turn provides fuel for all the metabolic processes going on in the plant (Druege *et al.* 2004). Correlation analysis of the enzymes (nitrate reductase, glutamine synthetase and acid-invertase) at both pre- and post-anthesis stages were found to be negatively correlated to anthesis silking interval (ASI) (Table 4) which implies that higher the value of enzyme activity, lower the ASI. High ASI increases the interval between anthesis and silking. Excessive silk delay leads to poor fertilization and hence reduced yield (Hirel *et al.* 2001). Therefore higher activity of these enzymes needs to higher yield. Further, pre- and post-anthesis activity of these enzymes has been found to be positively correlated with total grain yield. Among the enzymes the activity of acid-invertase was highly correlated to grain yield than that of nitrate reductase and glutamine synthetase (Table 4).

In conclusion, in the present investigation, bed planted crop gain higher yield than the ridge and flat methods of planting. Therefore cultivation of winter maize on raised beds may be adopted for better resource conservation after further detailed studies. Application of higher doses of nitrogen

Table 3 Effect of planting methods and nitrogen levels on chlorophyll content, Hill reaction activity and enzyme activity (nitrate reductase, glutamine synthetase and acid invertase) in winter maize

Treatment	Chl a	Chl b	Total Chl	Hill reaction activity	Chl a	Chl b	Total Chl	Hill reaction activity	Nitrate reductase*		Glutamine synthetase**		Acid invertase***	
	Pre-anthesis				Post-anthesis				Pre-anthesis	Post-anthesis	Pre-anthesis	Post-anthesis	Pre-anthesis	Post-anthesis
Planting method														
Bed	0.975	0.136	1.120	0.123	2.370	0.174	2.550	0.218	0.330	0.369	0.370	0.500	2.740	3.460
Ridge	0.861	0.128	0.994	0.109	1.870	0.165	2.060	0.190	0.327	0.360	0.350	0.470	2.570	3.300
Flat	0.810	0.124	0.941	0.128	1.610	0.160	1.780	0.176	0.310	0.348	0.350	0.450	1.740	2.720
CD (*P*=0.05)	0.02	0.01	0.023	0.034	0.055	0.0012	0.020	0.020	0.01	NS	0.007	0.03	0.109	0.150
Nitrogen levels (kg/ha)														
0	0.610	0.058	0.676	0.081	1.090	0.158	1.270	0.147	0.272	0.300	0.310	0.410	1.900	2.130
175	0.770	0.073	0.850	0.102	1.780	0.162	1.940	0.172	0.290	0.340	0.330	0.440	2.800	2.160
200	0.910	0.016	1.070	0.115	1.950	0.166	2.110	0.187	0.310	0.370	0.360	0.470	3.600	2.220
225	0.950	0.161	1.120	0.127	2.050	0.168	2.230	0.197	0.340	0.390	0.380	0.500	4.200	2.280
250	1.020	0.163	1.190	0.140	2.340	0.170	2.530	0.220	0.360	0.420	0.400	0.530	5.300	2.340
275	1.030	0.165	1.200	0.141	2.370	0.171	2.550	0.221	0.370	0.420	0.410	0.540	5.500	2.350
CD (*P*=0.05)	0.02	0.01	0.010	0.059	0.043	0.002	0.020	0.020	0.020	0.010	0.010			
Interactions P×N	0.012	NS	0.097	0.06	0.011	NS	0.118	0.024	NS	NS	NS	NS	NS	NS

P, Planting methods; N, nitrogen levels, Data are mean values of two years

Chl a, Chlb and total chlorophyll expressed as mg chlorophyll/g leaf fresh weight: Hill reaction activity expressed as change in OD/mg chl/h

*nitrate reductase expressed as μ moles $N0^{-}_{3}$ reduced/h/g leaf fresh weight

**glutamine synthetase expressed as μ moles glutamine formed/min/g leaf fresh weight

***acid invertase expressed as μ moles reducing sugars formed/min/g leaf fresh weight

Table 4 Correlation analysis between enzymes (nitrate reductase, glutamine synthetase and acid-invertase at pre- and post-anthesis stages) with days to anthesis and silking, amino-acid content and yield

Enzyme	Days to anthesis	Days to silking	Total free amino-acids	Grain yield	Anthesis-silking interval (ASI)
Nitrate reductase (pre-anthesis)	4.0**	3.63**	14.50**	5.72**	–0.891
Nitrate reductase (post-anthesis)		2.49*	12.01**	5.06**	–0.925
Glutamine synthetase (pre-anthesis)	3.65**	2.07*	13.40**	5.14**	–0.893
Glutamine synthetase (post-anthesis)		1.10 NS	6.11**	5.45**	–0.773
Acid-invertase (pre-anthesis)	0.711**	–0.859**	4.03**	10.34**	–0.523
Acid- invertase (post-anthesis)		–3.09**	5.46**	10.44**	–0.656

*P=0.05; **P=0.01

Correlation analysis between mean data of two years

fertilizers over recommended dose (175 Kg N/ha) enhanced the growth and yield-attributing characters during all the stages of crop growth leading to increased yield. However, results obtained with nitrogen application of 250 kg/ha were at par with those obtained with 275 kg/ha. Therefore a fertilizer dose of 250 kg N/ha may be used to obtain the desired results. Days taken to anthesis and silking were minimum for bed planted crop and also crop supplied with 250 and 275 kg N/ha. The reduced ASI and consequently higher yield were positively co-related to higher activities of nitrate reductase, glutamine synthetase and acid invertase.

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