

Maize (*Zea mays* L.) Genotypes Differ in Phenology, Seed Weight and Quality (Protein and Oil Contents) when Applied with Variable Rates and Source of Nitrogen

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Abstract

Field experiments were conducted during summer 2008-09 (Y1) and 2009-10 (Y2) at the Agronomy Research Farm of The University of Agriculture Peshawar-Pakistan to investigate the impact of different N-fertilizer sources [urea, calcium ammonium nitrate (CAN) and ammonium sulphate (AS)] and their levels (50, 100, 150 and 200 kg ha⁻¹) on phenology, seed weight and composition (seed protein, oil and starch content) of maize (*Zea mays* L.) genotypes "Local cultivars (Azam and Jalal) vs. hybrid maize (Pioneer-3025)". The results revealed that the rest (all the experimental plots applied with N) had delayed phenological development and had produced heavy grains with higher protein, oil and starch contents over control (N not applied). In both years, the maturity was delayed, seed weight and seed protein content increased with the application of two higher N rates (150 and 200 kg ha⁻¹) as compared with the two lower N rates (50 and 100 kg ha⁻¹). Applications of N in the form of ammonium sulphate enhanced maturity and decreased seed weight but increased seed oil content over urea and calcium ammonium nitrate. The maize hybrid (P-3025) with delayed maturity produced heavy seeds and higher seed protein and oil contents as compared to the two local cultivars (Azam and Jalal). The hybrid (P-3025) was considered the more efficient maize genotype that responded very well to different sources and rates of N than the local maize cultivars.

Keywords: *Zea mays* L; Nitrogen; Seed composition; Seed weight; Calcareous soils

Introduction

Nitrogen (N) source and rates influence grain yield and profitability [1], leaf area index and total dry matter accumulation [2], agronomic N-use efficiency and harvest index [2] of different in maize (*Zea mays* L.) genotypes. There is lack of published research on the influence of different N-sources and rates influence on grain quality (protein and oil contents, and grains weight) of different maize genotypes. Grain quality is a major issue especially in low input farming systems. Assimilate supply can be altered by N availability and by genotype, and that both can effect yield and grain composition [3]. Grain composition is a result of the genetic make-up of the endosperm sink, the maternal plant, and the environment [3]. Efficient use of N is considered one of the most important inputs needed for increasing maize grains weight and protein concentration [4] and profitability [5]. Nitrogen fertilization increased corn yield, protein content and test weight, but decreased corn oil and starch content [6].

Genotype also plays a large role in determining the kernel's ability to utilize the available assimilates for storage product deposition, as providing high levels of N cannot overcome a genetic tendency towards low kernel protein [3]. Maize varieties clearly differ in how efficiently they utilize N for storage product formation, and as a result yield [3], and much of this variation appears to be due to genetically determined processes within the maternal plant [7]. According to Fageria et al. in the 21st century, nutrient efficient plants will play a major role in increasing crop yields compared to the 20th century, mainly due to limited land and water resources available for crop production, higher cost of inorganic fertilizers, declining trends in crop yields globally, and increasing environmental concerns [8].

Though the existing maize genotypes have a high yield potential, soil and climatic conditions in the North-West Pakistan are very ideal for maize production, yet grain yield and seed quality of maize genotypes is very low. The causes of yield and seed quality gap include injudicious

use of N fertilizer and growing of low yielding maize genotypes by the growers. In order to bridge this gap in maize productivity and seed quality, the package of latest production technology involving the use of high yielding maize genotypes and low cost N fertilizers at appropriate level needs to be find out and used to increase maize productivity and grain quality. There is lack of research on the interactive effects of maize genotypes, level and source of N fertilizers in various maize growing areas in the world. For sustainable maize production, research on the interactive effects of N-fertilizer source into genotypes (NS × G), N levels into genotypes (NS × G), and N source into N levels (NS × NL) is indispensable. This experiment was therefore designed with an objective to investigate impact of different N fertilizer sources applied at various levels on the days to physiological maturity (PM) and grain quality i.e., seed protein content (SPC), seed oil content (SOC), seed starch content (SSC) and thousand seed weight (TSW) of maize hybrid "Pioneer-3025" in comparison to the two local high yielding cultivars "Jalal and Azam" as check.

Materials and Methods

Site description

Field experiments were conducted at the Agronomy Research Farm of the University of Agriculture Peshawar, Pakistan during

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summer 2008-09 (year one) and 2009-10 (year two). The experimental farm is located at 34.01° N latitude, 71.35° E longitude at an altitude of 350 m above sea level in Peshawar valley. Peshawar is located about 1600 km north of the Indian Ocean and has continental type of climate. The research farm is irrigated by Warsak canal from Kabul River. Soil texture is clay loam, low in organic matter (0.87 %), extractable phosphorus (6.57 mg kg⁻¹), exchangeable potassium (121 mg kg⁻¹), and alkaline (pH 8.2) and is calcareous in nature [9]. Weather data for the maize growing periods in 2008 and 2010 are given in Table 1.

Experimentation

A 4 × 3 × 3 factorial experiment was conducted in randomized complete block (RCB) design with split-plot arrangement using three replications. Factorial experimental treatments were four N (nitrogen) levels [N₁=50 kg ha⁻¹, N₂=100 kg ha⁻¹, N₃=150 kg ha⁻¹ and N₄=200 kg ha⁻¹] and three N-fertilizer sources [S₁=Urea (46 % N), S₂=Calcium Ammonium Nitrate (26 % N) and S₃=Ammonium Sulphate (21 % N)] applied to main plots, while three maize genotypes [G₁=Jalal and G₂=Azam (local OPV cultivars) and G₃=Pioneer-3025 (hybrid maize)] were kept in sub plots. One control plot (N not applied) was also used in each replication as check. A sub-plot size of 4.2 m by 5 m, having 6 rows, 5 m long and 70 cm apart was used. A uniform basal dose of 60 kg P ha⁻¹ as single super phosphate (SSP) having 18 % P₂O₅ and 60 kg K ha⁻¹ as sulphate of potash (50 % K₂O) was applied and mixed with the soil during seedbed preparation. Nitrogen at various rates in the form of various sources (urea, CAN and AS) was applied in two equal splits i.e., 50 % at sowing and 50 % at 2nd irrigation (30 days after emergence). Data was calculated on various parameters including phenology, growth analysis, dry matter partitioning, yield and yield components, harvest index, shelling percentage, grain quality, nitrogen use efficiency and profitability (economics). This paper presents the data on days to

physiological maturity (PM) and grain quality i.e., seed protein content (SPC), seed oil content (SOC), seed starch content (SSC) and thousand seed weight (TSW) only.

Appearance of black layer in seeds was used as criteria for physiological maturity, and was calculated as difference between date of physiological maturity and date of emergence [9]. Protein concentration in the grain was determined from total N in the grain using the Kjeldahl method [10]. Grain protein concentration was then determined using the formula: Protein concentration=% N × 6.25 [4]. Percent oil and starch contents in dried grains were determined with the help of Near Infra-red Reflectance Spectroscopy (Foss-6500; FOSS, Hillerød, Denmark) system at Oilseed Quality Lab, Crop Breeding Division, NIFA-Peshawar.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) according to the methods described by Steel et al. and means between treatments was compared by least significant difference (P ≤ 0.05) [11]. Analysis of variance of the data for the two years is given in Table 2.

Results and Discussions

Days to physiological maturity

In both years the rest (treated) plots (average of all the experimental plots that received N) had delayed physiological maturity (PM) than the control plots (the average of all the experimental plots which did not received N) as shown in Table 3. Nitrogen application might have increased the rate of photosynthesis that resulted in the leaf longevity and delayed PM in maize [9,12]. Crop photosynthesis is closely associated with leaf N content depending strongly on the availability

Weather Data	Growing Season 2008 (year one)				Growing season 2010 (year two)			
	July	August	Sept	Oct	July	August	Sept	Oct
Mean Temperature °C	31	30	28	25	31	29	24	24
Max Temperature °C	36	35	34	32	34	33	34	32
Min Temperature °C	26	25	22	19	26	26	21	19
Precipitation(mm)	37	274	38	1	409	125	4	0
Mean Humidity (%)	66	71	63	60	75	80	63	65
Wind Speed (km/h)	19	14	11	6	15	13	11	5

Table 1: Weather data of maize growing periods in 2008 and 2010 at Peshawar- Pakistan.

Source of variance	Degree of freedom	Probability (P ≤ 0.05)									
		2008 (year one)					2010 (year two)				
		¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g
Replications	2	-	-	-	-	-	-	-	-	-	-
Treatments	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
N sources (NS)	2	0.00	0.07	0.00	0.23	0.04	0.00	0.50	0.16	0.05	0.72
N levels (NL)	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.08
NS × NL	6	0.05	0.00	0.82	0.29	0.12	0.41	0.00	0.02	0.10	0.43
Control vs. rest	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Error I	24										
Genotypes (G)	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NS × G	4	0.02	0.00	0.42	0.01	0.56	0.00	0.08	0.71	0.12	0.47
NL × G	6	0.00	0.00	0.55	0.27	0.95	0.00	0.04	0.00	0.01	0.02
NS × NL × G	12	0.85	0.00	0.06	0.42	0.91	0.42	0.31	0.00	0.00	0.08
Error II	52										
Total	116										

¹PM=days to physiological maturity, ²SPS=seed protein content, ³SOC=seed oil content, ⁴SSC=seed starch content and ⁵TSW=1000-seeds weight.

Table 2: Analysis of variance table for statistical analysis of the data during 2008 and 2010 at Peshawar-Pakistan.

of nitrogen [13]. In both years, PM was delayed when N was applied at the two higher rates (150 and 200 kg N ha⁻¹) as compared with the two lower rates (50 and 100 kg N ha⁻¹) which enhanced PM (Table 3). In Amanullah et al. Observed more time to tasseling, silking and PM in plots that received the highest rate of N (180 kg N ha⁻¹) in four to five splits than 60 and 120 kg N ha⁻¹ [9]. Days to PM was delayed by three days in year two (Y2) as compared with year one (Y1). The delay in the phenological parameters (days to teaselng, silking and PM) in the first year compared to the second year was attributed to the fluctuation in the amount and distribution of rainfall in the two years [9]. Application of AS (ammonium sulphate) enhanced PM significantly (P ≤ 0.05) than urea and CAN (calcium ammonium nitrate) in both years (Table 3). According to Chien et al. AS is the best N-fertilizer source which contains frees sulfur and had many potential agronomic and environmental benefits over urea and ammonium nitrate [14]. In Lloyd et al. reported that urea (£100 per ton) is a less expensive form of N fertilizer than ammonium nitrate (£130 per ton) [15]. However, urea has been considered to be less effective than other N fertilizers,

due to N loss by ammonia volatilization, especially when used on soils of high pH or low CEC [16]. Among the maize genotypes, the maize hybrid (P-3025) had significantly (P ≤ 0.05) delayed PM (99.7 days in year one and 101.7 days in year two) than the two local cultivars (Azam and Jalal) in both years (Table 3). Between the two local cultivars, Jalal had delayed PM (87.8 and 90.6 days) as compared with Azam (85.8 and 88.7 days) in year one and year two, respectively. The difference in the PM of the three maize genotypes could be due to the difference in their genetic makeup. It studied the heritability, genetic advance, correlation and path analysis of 25 winter type rapeseed varieties. Among all the parameters studied, they found highest heritability of 0.903 for days to maturity, followed by flower duration (0.662). In Y1, increase in N rate delayed PM but the delay was noticed more with application of urea and CAN as compared to AS resulting into a significant (P ≤ 0.05) N-level × N-source interaction (Table 4). In both years, increase in N level extended the growth period and the extension (delay) was more in the hybrid than the two local cultivars that resulting in N-level × genotype interaction (Table 5). In Frank et al. found a significant cultivar × N

Planned Mean Comparison	2008 (year one)					2010 (year two)				
	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g
Control	87.7	7.9	5.1	70.7	237.7	90.7	8.3	5.5	68.8	240.5
Rest	91.2	9.4	4.9	68.8	243.5	94.0	8.8	5.1	68.1	268.4
N rate (kg ha⁻¹)										
50	88.4	9.0	5.0	69.4	240.9	91.4	8.5	5.1	68.3	252.9
100	90.4	9.5	4.9	69.0	242.8	92.7	8.8	5.1	68.5	278.6
150	92.1	9.6	4.8	68.7	245.0	95.1	8.9	5.2	68.1	271.9
200	93.7	9.6	4.8	68.0	245.3	96.8	9.0	4.9	67.5	270.1
LSD (P ≤ 0.05)	0.51	0.10	0.04	0.42	1.75	0.63	0.19	0.14	0.25	20.3
N Fertilizer Source										
Urea	91.6	9.4	4.9	68.6	244.4	94.5	8.8	5.0	68.3	264.5
CAN	91.8	9.4	4.8	68.8	243.7	94.6	8.8	5.1	68.1	269.6
AS	90.0	9.5	4.9	68.9	242.5	92.9	8.8	5.2	68.0	271.1
LSD (P ≤ 0.05)	0.44	ns	0.03	ns	1.52	0.54	ns	ns	0.21	ns
Maize Genotypes										
Azam	85.8	8.6	4.8	67.9	234.7	88.7	8.5	5.0	67.7	258.4
Jalal	87.8	8.9	4.8	68.3	236.4	90.6	8.6	5.0	68.0	264.5
Pioneer-3025	99.7	10.8	5.0	69.8	259.5	101.7	9.3	5.2	68.7	282.3
LSD (P ≤ 0.05)	0.51	0.08	0.03	0.38	1.68	0.44	0.17	0.11	0.18	10.8

¹PM=days to physiological maturity, ²SPS=seed protein content, ³SOC=seed oil content, ⁴SSC=seed starch content and ⁵TSW=1000-seeds weight.

Table 3: Control (N not applied) vs. rest (all N applied plots), N rate, N source and genotypes influence on physiological maturity (PM), SPC (seed protein content), SOC (seed oil content), SSC (seed starch content) and 1000-seeds weight (TSW) of maize in the two years.

N rates (kg ha ⁻¹)	N source	2008 (year one)					2010 (year two)				
		¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g
50	Urea	89.0	8.7	5.0	69.6	241.5	92.0	8.3	5.1	68.3	233.9
	CAN	88.7	9.1	4.9	69.2	241.1	91.7	8.7	5.1	68.2	252.9
	AS	87.4	9.2	5.0	69.5	240.3	90.4	8.5	5.2	68.4	271.9
100	Urea	90.3	9.5	4.8	69.0	242.6	92.8	8.8	5.0	68.7	288.7
	CAN	91.0	9.5	4.9	68.8	242.1	93.3	8.8	5.2	68.5	274.7
	AS	89.8	9.6	5.0	69.3	243.8	92.1	8.9	5.1	68.2	272.5
150	Urea	92.3	9.6	4.8	68.6	246.9	95.7	8.9	5.3	68.1	265.9
	CAN	92.7	9.5	4.8	69.0	246.1	95.7	8.9	5.0	68.3	280.3
	AS	90.9	9.7	4.9	68.9	242.1	93.9	9.1	5.3	67.9	269.5
200	Urea	94.4	9.8	4.8	67.7	246.6	97.4	9.3	4.8	67.9	269.3
	CAN	94.8	9.6	4.8	68.2	245.7	97.8	8.7	5.2	67.3	270.5
	AS	92.0	9.5	4.9	68.0	243.7	95.3	8.9	4.9	67.4	270.3
LSD (P ≤ 0.05)		0.88	0.18	⁶ns	⁶ns	⁶ns	⁶ns	0.33	0.25	⁶ns	⁶ns

¹PM=days to physiological maturity, ²SPS=seed protein content, ³SOC=seed oil content, ⁴SSC=seed starch content, ⁵TSW=1000-seeds weight and ⁶ns=non-significant at P ≤ 0.05

Table 4: Interactive effects of nitrogen rates x sources on physiological maturity and different seed parameters of maize during summer 2008 and 2010 at Peshawar-Pakistan.

rate interaction at all sites for turfgrass visual quality and color [17], and that these ratings decreased with decrease in N rates. In Amanullah et al. Reported that the higher average yield in the Punjab Province than Khyber Pakhtunkhwa Province in Pakistan is due to the use of hybrid maize and efficient fertilizer use by the farmers in Punjab than Khyber Pakhtunkhwa [18]. In both years, the delay in PM was more in the hybrid than the two local cultivars, and the delay was more with CAN and urea as compared with AS resulting in N-source × genotype interaction (Table 6). The S content in AS could be responsible for the early maturity in the maize genotypes as compared with urea and CAN having no S in them. In our previous study Amanullah et al. confirmed that the free S in SSP enhanced PM in maize as compared to DAP (Di-ammonium phosphate) and NP (nitrophos) having no free S [19].

Seed protein content

Seed protein content (SPC) was higher for the treated than the control plots in the two years (Table 3). The fluctuation in the weather data between the two years (Table 1) could be responsible for variation in SPC. In Argentina, even for the same genotype cropped under non-limiting N conditions in the field, grain protein concentration varied drastically among the years from 60 to 120 g kg⁻¹ [20]. According to Thomison et al. grain protein concentration, averaged across hybrids and N treatments, in maize were 9.2, 7.5, and 9.4% in 2000, 2001, and 2002, respectively [21]. The relatively low percent grain protein in 2001 was attributed to protracted wet soil conditions during the spring, which favored loss of N through denitrification and leaching. Drier and

warmer than normal conditions during late vegetative development and grain fill contributed to the higher grain protein composition in 2002. Concentration of kernel protein could be altered by genotype or the N supply, but remained constant along the length of the ear [7]. In our previous study Amanullah et al. did not found significant differences in the SPC of maize “cv. Azam” in the two years although there was much fluctuation in the precipitation and temperature of the two years [4]. In both years, SPC was increased when N was applied at the two higher rates (150 and 200 kg N ha⁻¹) as compared with the two lower rates (50 and 100 kg N ha⁻¹) as shown in Table 3. According to Amanullah et al. the highest grain protein concentration (93 g kg⁻¹) were observed in the plots to which the highest rate of N (180 kg N ha⁻¹) had been applied and lowest grain protein concentration (82 g kg⁻¹) were recorded for those plots which received the lowest rate of N (60 kg N ha⁻¹) [4]. Different sources of N had no significant effects on the SPC in both years indicating that using same level of N while using different N-fertilizer could not improve or decrease SPC. Among the maize genotypes, the maize hybrid (P-3025) had significantly (P ≤ 0.05) increased SPC (10.8 and 9.3% in Y1 and Y2, respectively) than the two local cultivars (Azam and Jalal) in both years (Table 3). Between the two local cultivars, Jalal had higher SPC (8.9 and 8.6%) than Azam (8.6 and 8.5%) in Y1 and Y2, respectively. Differences in the SPC in different maize genotypes were reported by earlier. In both years, application of any N source increased SPC when applied at higher rates resulting in N-level × N-source interaction, and the increase in SPC was generally more in Y1 than Y2. As compared to AS and CAN, application of urea at

N rates (kg ha ⁻¹)	Genotypes	2008 (year one)					2010 (year two)				
		¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g
50	Azam	84.0	8.1	4.9	68.6	232.9	87.0	8.2	5.1	67.8	247.0
	Jalal	84.7	8.8	4.9	69.3	235.9	87.7	8.3	4.9	68.2	236.8
	P-3025	96.4	10.0	5.1	70.3	254.1	99.4	9.0	5.3	68.9	274.9
100	Azam	85.4	8.8	4.8	68.0	233.8	87.9	8.6	5.2	68.2	280.4
	Jalal	87.4	9.1	4.8	69.1	235.7	89.7	8.5	5.0	68.4	275.3
	P-3025	98.2	10.7	5.1	69.9	258.9	100.7	9.4	5.1	68.7	280.3
150	Azam	86.2	8.7	4.8	68.2	236.5	89.2	8.4	5.1	67.8	250.9
	Jalal	88.9	9.2	4.8	68.3	238.2	91.9	9.0	5.2	67.9	284.2
	P-3025	101.1	10.9	5.0	69.8	260.4	104.1	9.4	5.3	68.7	280.6
200	Azam	87.8	8.6	4.8	66.7	235.8	90.8	8.7	4.7	66.8	255.1
	Jalal	90.3	8.8	4.8	67.7	235.8	93.3	8.6	5.0	67.5	261.6
	P-3025	103.1	11.6	4.9	69.4	264.4	106.4	9.7	5.3	68.3	293.4
LSD (P ≤ 0.05)		1.01	0.16	⁶ ns	⁶ ns	3.50	0.92	0.35	0.22	0.38	22.6

¹PM=days to physiological maturity, ²SPS=seed protein content, ³SOC=seed oil content, ⁴SSC=seed starch content, ⁵TSW=1000-seeds weight and ⁶ns=non-significant at P ≤ 0.05

Table 5: Interactive effects of nitrogen rates x genotypes on physiological maturity and different seed parameters of maize during summer 2008 and 2010 at Peshawar-Pakistan.

N Source	Genotypes	2008 (year one)					2010 (year two)				
		¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g	¹ PM days	² SPC %	³ SOC %	⁴ SSC %	⁵ TSW g
Urea	Azam	86.2	8.6	4.8	67.4	234.6	88.9	8.7	4.9	67.9	261.1
	Jalal	88.1	8.8	4.8	68.5	237.6	90.9	8.5	4.9	68.1	255.8
	P-3025	100.6	10.8	5.0	69.9	261.1	103.6	9.4	5.2	68.7	276.6
CAN	Azam	86.1	8.5	4.8	67.8	234.3	89.0	8.4	5.1	67.6	260.8
	Jalal	88.6	9.1	4.8	69.0	236.4	91.4	8.6	5.0	67.9	264.9
	P-3025	100.6	10.6	4.9	69.5	260.5	103.4	9.3	5.3	68.8	283.2
AS	Azam	85.3	8.7	4.9	68.4	235.4	88.2	8.3	5.1	67.4	253.2
	Jalal	86.8	9.0	4.9	68.3	235.3	89.6	8.7	5.2	68.1	272.8
	P-3025	97.9	10.8	5.0	70.1	256.7	101.0	9.5	5.3	68.5	287.2
LSD (P ≤ 0.05)		0.92	0.14	⁶ ns	0.68	⁶ ns	0.80	0.35	⁶ ns	0.38	22.6

¹PM=days to physiological maturity, ²SPS=seed protein content, ³SOC=seed oil content, ⁴SSC=seed starch content, ⁵TSW=1000-seeds weight and ⁶ns=non-significant at P ≤ 0.05

Table 6: Interactive effects of nitrogen source x genotypes on physiological maturity and different seed parameters of maize during summer 2008 and 2010 at Peshawar-Pakistan.

the lowest N rate (50 kg N ha⁻¹) had the lowest SPC but had the highest SPC when applied at the highest level (200 kg N ha⁻¹). In Fageria et al. found that the higher and lower N rate of AS produced higher LAI and most of the plant growth and yield components, while the intermediate N rates (125 to 275 mg N kg⁻¹) of urea was slightly better compared to AS for grain production [22]. The SPC increased with increase in N level and the increase was more in the hybrid than the two local cultivars resulting in N-level × genotype interaction (Table 5), and the increase in SPC of maize hybrid was more in Y1 than Y2. In Frank et al. found a significant cultivar × N rate interaction at all sites for turfgrass visual quality and color, and that these ratings decreased with decrease in N rates [17]. In addition to benefits related to feed or food utilization, selection of high protein genotypes is advantageous because high protein is related to high seed quality [23]. In Munamava et al. suggested that protein concentration in the seed of different *B. oilseed* crops may be affected by multiple factors including genetic differences within a crop species [24]. In Uribealrrea et al. found that abundant nitrogen supply stimulated protein synthesis in high protein genotypes and that protein and oil had a positive correlation. However, reported that higher inputs of nitrogen did not result in higher protein in maize in the conventional cropping system [3]. In both years, the hybrid has more SPC when applied with urea and AS than CAN resulting in N-source × genotype interaction (Table 6). According, maize genotype influences the protein content of the seed. Protein concentration in maize seeds can be altered by the N supply, or by the source-to-sink ratio, these grains still adhere to the negative relationship between starch and protein [7].

Seed oil content

The seed oil content (SOC) was higher for the rest than the control plots (Table 3). In both years, SOC was decreased when N was applied at the two higher rates (150 and 200 kg N ha⁻¹) as compared with the two lower N rates (50 and 100 kg N ha⁻¹) as shown in Table 3. Different sources of N had no significant effects on the SOC in maize seeds (Table 3). In Welch et al. reported that nitrogen applications increased the oil concentration of corn grain slightly, but more important was that the increased grain yield resulted in greater oil production per unit of land area [25]. In Jellum et al. found that increasing nitrogen application rate had no influence on the oil concentration of corn grain [26]. According to Thomison et al. grain oil concentration, averaged across hybrids and N treatments, in maize were 6.0, 6.5 and 6.4% in 2000, 2001, and 2002, respectively [21]. Among the maize genotypes, the maize hybrid (P-3025) had significantly ($P \leq 0.05$) higher SOC than the two local cultivars (Azam and Jalal) in both years (Table 3), although the differences in the SOC of two local cultivars were not significant. Differences in the SOC in different maize genotypes were earlier reported. Application of AS at the lowest rate (50 kg N ha⁻¹) improved SOC in Y2, but the increase in SOC was more with CAN at the rate of 100 and 200 kg N ha⁻¹, and application of both urea and AS produced higher SOC in maize when applied at the rate of 150 kg N ha⁻¹ resulting in N-source × N-level interaction (Table 4). In case of rice crop, In Fageria et al. found that the higher and lower N rates of AS produced higher LAI and most of the plant growth and yield components, while the intermediate N rates (125 to 275 mg N kg⁻¹) of urea was slightly better compared to AS for grain production [22]. The discrepancies in our results and that of Fageria et al. was due to the differences in the crop species used, amount of N sources applied, and the difference in the climatic conditions [22]. The hybrid produced more SOC (5.0 and 5.2%) than Azam and Jalal at all levels of N, and the SOC in the two local cultivars drastically decreased at the highest rate of N resulting in N-source × genotype interaction (Table 5). In Frank et al. found a

significant cultivar × N rate interaction at all sites for turfgrass visual quality and color, and that these ratings decreased with decrease in N rates [17]. It reported that higher inputs of nitrogen did not result in higher oil content in maize in the conventional cropping system. In Malhi et al. suggested that oil concentration in the seed of different *B. oilseed* crops may be affected by multiple factors including genetic differences within a crop species [24].

Seed starch content

The rest plots had higher seed starch content (SSC) than the control plots (Table 3). In both years, SSC was decreased significantly when N was applied at the two higher rates (150 and 200 kg N ha⁻¹) as compared with the two lower N rates (50 and 100 kg N ha⁻¹) at $P \leq 0.05$ (Table 3). In Seebauer et al. found that without fertilizer N promoted the greatest concentration of kernel starch, which was on average 16 g kg⁻¹ greater than kernels grown with maximum N supply [7]. The SSC decreased and SPC increased with each increment in N supply indicating negative relationship between SSC and SPC. Different sources of N had no significant effects on the in maize seeds in both years indicating that using same level of N while using different N-fertilizer could not increase or decrease SSC in maize (Table 3). Among the maize genotypes, the maize hybrid (P-3025) had significantly ($P \leq 0.05$) higher SSC than the two local cultivars (Azam and Jalal) in both years (Table 3). Between the two local cultivars, Jalal had higher SSC (68.3 and 68.0%) in than Azam (67.9 and 67.7%) in year one and year two, respectively. Concentration of kernel starch could be altered by genotype or the N supply, or by the source-to-sink ratio, and there was a negative relationship between starch and protein content in seeds but remained constant along the length of the ear [7]. In both years, hybrid has more SSC with urea and AS than CAN. Earlier evidence for a negative starch/protein relationship in maize grains comes from evaluations of the Illinois Protein Strains, which have been selected for extremes in grain protein concentration [3]. Nitrogen fertilization increased corn yield, protein content and test weight, but decreased corn oil and starch content [6].

Seed weight

In both years the rest plots had heaviest thousand seeds weight (TSW) than the control plots (Table 3). The use of N fertilizers considerably influenced 1000-grain weight in maize and resulted in 30-40% higher yields in comparison with the unfertilized plot [27]. Nitrogen application delayed PM [9,12] and increase grain weight [4]. The TSW was increased by 14 g in year two (Y2) as compared with year one (Y1). The fluctuation in the weather data between the two years (Table 1) could be responsible for variation in TSW. In our previous study [4], however, did not found any significant differences in the TSW of maize “cv. Azam” in the two years although there was much fluctuation in the precipitation and temperature of the two years. In both years, TSW was increased when N was applied at the two higher rates (150 and 200 kg N ha⁻¹) as compared with the two lower N rates (50 and 100 kg N ha⁻¹) which decreased TSW (Table 3). According to Amanullah et al. the heaviest kernels weighing 235.35 g per 1000 kernels was observed in the plots to which the highest rate of N (180 kg N ha⁻¹) had been applied and the lowest weight (222.58 g per 1000 kernels) was recorded for those plots which received the lowest rate of N (60 kg N ha⁻¹) [4]. Elevated rate of N level increased kernel weight [6,28]; the increase in maize seed weight with increasing N rates was attributed to the increase in the activity of enzyme [29]. In Y1, application of AS decreased TSW significantly indicating that early maturity could decrease seed weight in maize. In Y2 the TSW was increased with

application of AS but the differences were not significant among the N-sources. According to Amanullah et al. AS is the best N-fertilizer source which contains free sulfur and had many potential agronomic and environmental benefits over urea and ammonium nitrate [14]. In Northwestern Pakistan where most of the soils are calcareous in nature, AS because of its free sulfur content, could be the most beneficial N-fertilizer in terms of soil improvement, higher crop growth, increase in number of leaves plant⁻¹, mean single and leaf area plant⁻¹ and more dry matter partitioning to leaves. However, because of its highest N cost (PKR 191 kg⁻¹ N, one USD=90 PKR) as compared to other sources of N i.e., CAN (PKR 97 kg⁻¹ N) and urea (PKR 55 kg⁻¹ N) the poor growers in the area can't afford to use AS. Moreover, the transportation charges of AS was more than urea and CAN (unpublished data). In Lloyd et al. reported that urea (£100 per ton) is a less expensive form of N fertilizer than ammonium nitrate (£130 per ton) [15]. However, urea has been considered to be less effective than other N fertilizers, due to N loss by ammonia volatilization, especially when used on soils of high pH or low CEC [16]. Our previous research work [19] on maize response to different phosphorus (P) fertilizers sources indicated that plots applied DAP (di-ammonium phosphate) produced heaviest seeds than SSP (single super phosphate) and NP (nitrophos), while application of SSP resulted in early PM and lowest TSW than urea and NP. Among the maize genotypes, the maize hybrid (P-3025) had significantly ($P \leq 0.05$) higher TSW (259.5 g in Y1 and 282.3 g in Y2) than the two local cultivars (Azam and Jalal) in both years (Table 3). But between the two local cultivars, Jalal had the higher TSW (236.4 g in Y1 and 264.5 g in Y2) than Azam (234.7 g in Y1 and 258.4 g in Y2). These results confirmed that delay in PM of genotypes could be the possible cause of higher TSW than the genotypes with early PM. In both years, increase in N level extended the growth period and increased the TSW; and the extension (delay) and increase in TSW was more in the hybrid than the two local cultivars (Table 5) resulting in N-level \times genotype interaction. Maize delayed its PM and produced heaviest seeds in the Y2 than Y1. In Frank et al. found a significant cultivar \times N rate interaction at all sites for turfgrass visual quality and color, and that these ratings decreased with decrease in N rates [17]. Thousand grains weight significantly varied in maize genotypes, however different forms of N-fertilizers did not caused differences in 1000-grains weight in different maize genotypes [27].

Days to PM, SPC, SSC and TSW increased, while the SOC decreased in the rest plots than control in the two years. The higher rate of N application delayed PM and increased both SPC and TSW but decreased SOC and SSC in maize seeds indicating negative relationship of SPC with SOC and SSC. Earlier in sunflower, In Amanullah et al. found that seed protein concentration had negative association with seed oil concentration [30]. The SSC decreased and SPC increased with each increment in N supply indicating negative relationship between SSC and SPC [7]. The early PM in maize increased both SOC and SSC indicating positive relationship between SOC and SSC. Earlier evidence for a negative starch and protein relationship in maize grains comes from evaluations of the Illinois Protein Strains, which have been selected for extremes in grain protein concentration [3]. As compared to urea and CAN, application of AS enhanced PM and decreased TSW, but increased SOC in maize seeds. According to Swift et al. and Amanullah et al. urea gave 3% less total DM production than ammonium nitrate and produced on average 7% less DM in the spring, and 12% less in autumn [31,32]. However, urea could be a suitable alternative to ammonium nitrate, and when used in spring must be about 20% cheaper per kg N. In Lloyd et al. found that grain yield was unaffected by the type of N-fertilizer N, but grain N concentration was usually less from urea than ammonium nitrate [15]. The early maturity

and higher SOC in maize due to AS application could be due to presence of free S in it that helped the maize plants to achieve early PM and increased SOC. In Amanullah et al. found that S fertilized plots might helped the plants to uptake balanced amounts of N and P, and that may have enhanced the plant development and hence early flowering, pod formation, seed fill duration and maturity in *Brassica* species as compared to the S control plots [30]. Among the maize genotypes, the maize hybrid (P-3025) had delayed PM, increased SPC, SOC, SSC and TSW than the two local cultivars (Azam and Jalal) and was confirmed the most efficient genotype than the two local cultivars because of its positive response to the all three sources and levels of N. Jalal stood second, but Azam because of its early PM, ranked in bottom in terms of its lowest SPC, SOC, SSC and TSW and was considered the least efficient genotype in this study. Grains of maize hybrids containing nutritionally enhanced genetics may exhibit higher oil and crude protein profiles than normal field maize [21]. In Fageria et al. suggested that in the 21st century, nutrient efficient plants will play a major role in increasing crop yields compared to the 20th century, mainly due to limited land and water resources available for crop production, higher cost of inorganic fertilizers, declining trends in crop yields globally, and increasing environmental concerns [8].

Conclusions

Our results confirmed that the rest plots (average of all the experimental plots that received nitrogen) had delayed physiological materiality, and had produced heavy grains with higher protein, oil and starch contents over control (N not applied). The physiological maturity was delayed, seed weight and seed protein content increased with the application of two higher N rates (150 and 200 kg N ha⁻¹) as compared with the two lower N rates (50 and 100 kg N ha⁻¹). Applications of nitrogen in the form of ammonium sulphate had early physiological maturity and had decreased seed weight but increased seed oil contents as compared with other two N-fertilizers (urea and calcium ammonium nitrate). The maize hybrid (P-3025) had delayed physiological maturity and produced heavy seeds with higher seed protein and oil contents as compared to the two local cultivars (Azam and Jalal). The hybrid (P-3025) was considered the more efficient maize genotype that responded very well to different sources and rates of N than the local maize cultivars. Application of N-fertilizer either as CAN or AS at the rate of 200 kg N ha⁻¹ was found most beneficial for the high yielding hybrid maize (P-3025) but 150 kg N ha⁻¹ was most suitable for the two local OPV cultivars (Azam and Jalal) in the study area.

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