CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH

EFFECT OF NITROGEN ON ACHENE PROTEIN, OIL, FATTY ACID PROFILE, AND YIELD OF SUNFLOWER HYBRIDS

Amjed Ali[1*], and Sami Ullah[2]

Seed yield and achene oil yield are the main determinants for N application rates rather than seed composition. Nitrogen plays a critical role in producing unsaturated fatty acids (oleic and linoleic acids), which are the main factors determining sunflower oil quality (*Helianthus annuus* L.). Studies were conducted on the effect of N fertilization on seed yield, achene oil yield, and quality parameters of sunflower hybrids for two successive years (2010 and 2011) in a split plot arrangement under a randomized complete block design. The hybrids (Hysun-33 & S-278) and N levels (0, 75, 150, and 225 kg ha^{-1}) were allotted in main and sub-plots, respectively. Increasing N levels resulted in steady increases in yield, protein contents and linoleic acid, whereas oil contents and percentage of oleic acid responded negatively during both years. At the same time, crop oil yield was positively related to increased N supply with higher achene yield (AY). Palmitic acid varied from 5.27 to 6.42 % and stearic acid ranged from 2.27 to 2.95%. Hybrid S-278 exhibited significantly ($P < 0.05$) higher AY (3380 kg ha^{-1}), oil content (42.11%) than Hysun-33 (2968 kg ha^{-1} and 40.75%, respectively), while the opposite was true for protein content. Oil yield varied in response to N fertilizer, with a range of 34 to 37% providing the best quality traits in both seasons.

Key words: Fertilization, linoleic acid, oleic acid, palmitic acid, *Helianthus annuus*.

Global demand for vegetable oils/fats, both for food and other industrial purposes, is anticipated to expand vigorously. Pakistan is facing a severe shortage of edible oil because its domestic production is far below the consumption level. Consequently a major share of foreign exchange is expended on oil imports, which is increasing every year. Sunflower oil is gaining popularity in European and East Asian countries for salad and cooking oil and margarine production, which are based on oil composition and the absence of cholesterol. In oil seed crops, quality criteria are fatty acid composition of the seed oil and the intended use of the oil. Polyunsaturated cooking oils have been the driving force for the sunflower industry. The oleic acid (a monounsaturated fatty acid) content of oil seeds has important implications for product performance and consumer health. High oleic varieties have provided the opportunity for repositioning sunflower products at the premium end of the growing monounsaturated market. Ryland (2003) compared different vegetable oils and found that sunflower oil to be the healthiest due to its high oleic acid content. Sunflower a non-conventional oil seed crop that can improve edible oil production due to its high oil contents and wide adaptability to soils and climatic conditions. Bakht *et al*. (2006) concluded that one of the reasons for low sunflower seed production is the sowing of exotic hybrids that are not well suited to our environmental conditions. Zahoor *et al*. (2010) reported that sowing newly developed hybrids and optimum N rates are important management practices to increase edible oil production and reduce import bills.

Pakistanis farmers believe that more fertilizer and high grain yields are synonymous. A better understanding of the relationship between crop yield and N rate could help agronomists match fertilizer rates with plant requirements (Gao *et al*., 2012). Abbadi and Gerendas (2009) noted that optimal supply of N fertilizer in sunflower result in grain yield more efficiently than low supply of N. Regina (2008) concluded that N is the most important element to increase grain protein content. Increasing N rates reduced seed oil percentages but increased seed yields and consequently increased oil yield per unit area (Zheljazkov *et al*., 2008; 2009). Therefore, the aim of this study was to evaluate the effect of different levels of N application on yield and quality traits of sunflower in order to achieve the optimum use of resources.

MATERIALS AND METHODS

The experiment was carried out at the Research Area of University College of Agriculture Sargodha (32°05' N, 72°67' E) Pakistan, under irrigated semi-arid conditions during the spring seasons of 2010 and 2011. Table 1 presents weather data for sunflower growing season (March-June in 2010 and 2011), and Table 2 presents physical and chemical analysis of experimental soil. The experiment was laid out in a Split plot arrangement under randomized complete block design with three replicates,

[1]Washington State University, Prosser, Washington 99350, USA.
[*]Corresponding author (ali.amjed@wsu.edu).
[2]University of Sargodha, University College of Agriculture, Sargodha, 40100, Pakistan.
Received: 16 March 2012.
Accepted: 17 September 2012.

keeping net plot size 4.20 × 8 m. Sunflower hybrids (Hysun-33, S-278) were kept in main plots and N levels in sub plots. Nitrogen treatments were 0, 75, 150, and 225 kg ha^{-1}, i.e. N_0, N_1, N_2, and N_3, respectively.

The crop was sown by the dibbler method on 70-cm-spaced ridges and at 22.5 cm plant spacing using a recommended seed rate of 7 kg ha^{-1}. Phosphorus and K was applied at the rate of 100-50 kg ha^{-1}, respectively. Nitrogen, P, and K were applied in the form of urea, single super phosphate and sulfate of potash, respectively. Full doses of P and K and 1/3 of N were applied at sowing and the remaining 2/3 of N was applied in two equal splits, at the first irrigation and R_3 stage (immature bud elongates). All other cultural practices, such as weeding, water application and plant protection measures were standard for the crop.

The harvested crop was sun dried, and threshed and achene yield was recorded at 15% moisture contents, then total achene yield was multiplied with oil contents to calculate achene oil yield. Protein and oil contents of seed samples were determined with the nuclear magnetic resonance (NMR) technique (Wamsely, 1998). Sunflower seed oil obtained from each sample was analyzed to determine the relative composition of different fatty acids (oleic, linoleic, palmitic, and stearic acids) with a GC-9A fatty acid analyzer (Model MQA-7005, Oxford Institute, USA). Data collected were statistically analyzed for ANOVA using the Fisher's ANOVA technique. The least significant difference (LSD) test at 0.05 was used to compare means (Steel *et al*., 1997).

Table1. Mean monthly weather data for sunflower growing season March-June in 2010 and 2011.

Months	Mean temperature		Total rainfall		Mean relative humidity	
	2010	2011	2010	2011	2010	2011
	°C		mm		%	
March	22.5	21.3	9.2	7.11	58.5	59.4
April	30.2	25.6	4.06	35.06	44.2	46.3
May	32.7	33.8	2.04	8.89	44.9	38.5
June	33.5	33.7	14.74	126.25	44.6	51.0

Table 2. Physical and chemical analysis of experimental soil.

Soil parameter	Value
Physical analysis	
Sand, %	23
Silt, %	60
Clay, %	17
Textural class	Silty loam
Chemical analysis	
pH	7.6
Total soluble salts, %	15.02
Organic matter, %	0.96
Total N, %	0.06
Available P, mg kg^{-1}	16.8
Available K, mg kg^{-1}	235

RESULTS AND DISCUSSION

Achene protein contents (%)

There was a significant increase in protein contents with the increased N rates (Table 3). In 2010, the highest protein content (19.80%) was recorded in treatment N_3 (225 kg N ha^{-1}) and lowest (15.33%) in N_0 (control). There were similar results in 2011, which are in line with those of Nanjundappa *et al*. (2001) and Munir *et al*. (2007), who observed increased achene protein due to N application. Sunflower hybrids had a significant effect on protein content in achene. 'Hysun-33' attained significantly higher protein content (18.23%) than 'S-278' (17.10%), which might be due to varying genetic potential of the hybrids. These results are in accordance with the findings of Roche *et al*. (2010) and Bukhsh *et al*. (2011) that different sunflower hybrids exhibit the differential response to protein content in achene due to their difference of genetic makeup.

Achene oil contents (%)

Oil content in achene decreased with higher N levels (Table 3). Maximum oil content (42.40%) was observed in treatment N_0 and minimum oil content (39.63%) in treatment N_3 in 2010. Results were similar in 2011. These results are consistent with those of Abdel-Sabour and Abo El-Seoud (1996), Nanjundappa *et al*. (2001), Munir *et al*. (2007), and Nasim *et al*. (2012), who observed decreases in achene oil percentages with increased N application.

Table 3. Effect of nitrogen on achene protein, oil, and oleic and linoleic acid contents of two sunflower hybrids.

Treatments	Achene protein		Oil content		Oleic acid in oil		Linoleic acid in oil	
	2010	2011	2010	2011	2010	2011	2010	2011
	%							
Hybrids								
Hysun-33	18.02a	18.23a	39.97b	40.75b	40.21	40.09	46.30	46.76
S-278	17.10b	17.37b	41.95a	42.11a	40.30	40.25	46.27	46.60
LSD value	0.33	0.33	0.42	0.40	NS	NS	NS	NS
N levels								
N_0 (control)	15.33d	15.60d	42.40a	42.87a	42.60a	42.24a	43.29c	43.77c
N_1 (75 kg ha^{-1})	16.92c	16.98c	41.48b	41.94b	41.17b	41.00b	45.37b	44.98b
N_2 (150 kg ha^{-1})	18.22b	18.53b	40.47c	40.89c	39.98b	39.85b	48.65ab	48.83ab
N_3 (225 kg ha^{-1})	19.80a	20.09a	39.63d	40.02d	38.25c	38.30c	49.00a	49.15a
LSD value	0.86	0.79	0.80	0.77	0.64	0.64	0.42	0.41
Interactions								
Hybrids × N	NS	NS	NS	NS	NS	NS	NS	NS

Any two means not sharing a letter are significantly different at 5% level of probability (least significant difference LSD); NS: non significant.

Sunflower hybrids have had a significant effect on oil content in achene. The S-278 hybrid showed significantly higher oil content (41.95%, 42.11%) than 'Hysun-33' (37.97%, 40.75%) in 2010 and 2011, respectively, which might be due to the varying genetic potential of the hybrids. The effect of the year was also not significant. These results are consistent with the findings of Roche *et al*. (2010) and Bukhsh *et al*. (2011), who found that different sunflower hybrids exhibit distinct responses to oil content in achene due to differences in their genetic makeup.

Fatty acid composition (%)

Oleic and palmitic acid concentrations responded negatively to N application (Tables 3 and 4). In 2010, N_0, had the highest oleic (42.60%) and palmitic acid contents (6.35%), followed by N_1 and N_2 having oleic acid concentrations of 41.17% and 39.18% and palmitic acid 5.95% and 5.90%, respectively. Likewise, N_3 had the lowest oleic (38.25%) and palmitic acid concentrations (5.27%). These results are supported by Nanjundappa *et al*. (2001), Munir *et al*. (2007), and Boydak *et al*. (2010), who observed decreases in the composition of this fatty acid with increased N application.

There was significant variation among different N treatments regarding linoleic acid concentration. N_3 had the highest linoleic acid content (49.00%), which was statistically similar to the 48.85% linoleic acid concentration obtained in N_2, while the plots without N application had the lowest linoleic acid (43.29%) contents. The highest stearic acid content (2.93%) was obtained from N_1, followed by N_3, and the lowest was 1.90% from N_2 with 1.9%. These figures are from 2010, but the tendencies were found in the next year of the experiment. Boydak *et al*. (2010) also observed these results in their experiments. Sunflower hybrids did not respond differentially to varying N levels in either year in terms of fatty acid composition.

Achene and oil yield

The response of achene yield (AY) and achene oil yield to increased applications of N fertilizer was significant (Table 4). In 2010, a maximum AY of 3498 kg ha^{-1} was observed in treatment N_3, which was statistically at par with the AY (3485 kg ha^{-1}) of treatment N_2 and followed by treatment N_1 with an AY of 3192 kg ha^{-1}, while the lowest AY (2485 kg ha^{-1}) was in the control plot where N was not applied. Results were similar in 2011. Sunflower hybrids had a significant effect on AY, 'S-278' had a higher achene (3350, 3380 kg ha^{-1}) than 'Hysun-33' (2980, 2968 kg ha^{-1}) in 2010 and 2011, respectively. These results are in line with the findings of Khaliq and Cheema (2005), Al-Thabet (2006), Nasim *et al*. (2011; 2012) who noted increased AY with different sunflower hybrids with increased N levels. The maximum oil yield (1418, 1433 kg ha^{-1}) was with N_2, followed by N_3 with AY (1395, 1409 kg ha^{-1}) in 2010 and 2011, respectively. The lowest achene oil yield was with N_0, in which increased N levels increased AY to a certain point, but after that achene oil yield was reduced due to a decrease in achene oil contents. These results are supported by Abdel-Sabour and Abo El-Seoud (1996) and Gholinezhad *et al*. (2011). Sunflower hybrids have significant effect on AY. Hybrid S-278 had higher achene oil yield (1405 and 1423 kg ha^{-1}) than Hysun-33 (1191 and 1209 kg ha^{-1}) in 2010 and 2011, respectively, which might be due to genetic characteristics of sunflower hybrids as explained by Flagella *et al*. (2002), Vega and Hall (2002), Thavaprakash *et al*. (2002), Zheljazkov *et al*. (2008), (2009), and Arshad *et al*. (2009), who also reported similar results regarding achene oil yield.

CONCLUSION

Sunflower hybrids exhibited differential genotypic responses to different N levels by increasing seed yields combined with achene oil yields. The hybrid S-278 had the highest achene yield (3350 and 3380 kg ha^{-1}) with more seed oil yield (1405 and 1423 kg ha^{-1}) and a better proportion of saturated and unsaturated fatty acids in both years. Higher achene yield (3485 and 3505 kg ha^{-1}) and oil yield (1418 and 1433 kg ha^{-1}) with good ratios of oleic, linoleic, and palmitic acids in 2010 and 2011, respectively, was in response to the application of 150 kg

Table 4. Effect of nitrogen on palmitic and stearic acids, achene yield, and achene oil yield of two sunflower hybrids.

	Palmitic acid in oil		Stearic acid in oil		Achene yield		Achene oil yield	
Treatments	2010	2011	2010	2011	2010	2011	2010	2011
	%				kg ha^{-1}			
Hybrids								
Hysun-33	5.91	5.98	2.45	2.52	2980b	2968b	1191b	1209b
S-278	5.82	5.87	2.39	2.34	3350a	3380a	1405a	1423a
LSD value	NS	NS	NS	NS	17.2	11.3	39.3	35.4
N levels								
N_0 (control)	6.35a	6.42a	2.27c	2.28c	2485c	2456c	1041d	1052d
N_1 (75 kg ha^{-1})	5.95b	6.00b	2.93a	2.95a	3192b	3215b	1333c	1348c
N_2 (150 kg ha^{-1})	5.90b	5.90b	1.90d	1.91d	3485ab	3505ab	1418a	1433a
N_3 (225 kg ha^{-1})	5.27c	5.38c	2.59b	2.61b	3498a	3521a	1395b	1409b
LSD value	0.375	0.374	0.302	0.301	38.89	49.70	17.7	15.9
Interactions								
Hybrids × N	NS	NS	NS	NS	NS	NS	NS	NS

Any two means not sharing a letter are significantly different at 5% level of probability (least significant difference LSD); NS: non significant.

N ha^{-1}. In contrast, Hysun-33 had a higher protein content (18.02%, 18.23%) than S-278 in both years.

Efecto de nitrógeno en la proteína, aceite, y perfil de ácidos grasos del aquenio y rendimiento de híbridos de girasol. Parece racional que el rendimiento de semilla y rendimiento de aceite de aquenio sigan siendo el foco determinante para tasas de aplicación de N en lugar de la composición de la semilla. El N parece jugar un papel imprescindible en la proporción de ácidos grasos insaturados (ácidos oleico y linoleico), factor principal que determina el aceite de girasol (*Helianthus annuus* L.). Los estudios fueron realizados para evaluar el efecto de la fertilización N en el rendimiento de semilla, rendimiento de aceite de aquenio, y parámetros de calidad de híbridos de girasol por 2 años sucesivos (2010 y 2011) en un diseño de bloques completos al azar con arreglo de parcelas divididas. Los híbridos (Hysun-33 y S-278) y niveles de N (0, 75, 150 y 225 kg ha^{-1}) fueron asignados a parcelas principales y sub parcelas, respectivamente. Niveles crecientes de N aumentaron de manera estable el rendimiento, contenidos de proteína y de ácido linoleico en el aceite, donde contenidos de aceite y porcentaje de ácido oleico respondieron negativamente durante ambos años. Al mismo tiempo, el rendimiento de aceite del cultivo permaneció positivamente relacionado al gran suministro de N dando la ventaja de mayor rendimiento de aquenio (AY). El ácido palmítico varió de 5,27% a 6,42% y el ácido esteárico de 2,27% a 2,95%, respectivamente. 'S-278' exhibió apreciablemente (P < 0,05) mayores AY (3380 kg ha^{-1}) y contenido de aceite (42.11%) que 'Hysun-33' (2968 kg ha^{-1} y 40.75%, respectivamente), mientras se observó lo contrario para contenido de proteína. El rendimiento de aceite varió en respuesta a fertilización N, de 34% a 37% teniendo mejores rasgos de calidad durante ambas temporadas.

Palabras clave: ácido linoleico, ácido oleico, ácido palmítico, *Helianthus annuus*.

LITERATURE CITED

Abbadi, J., and J. Gerendas. 2009. Nitrogen use efficiency of safflower as compared to sunflower. Journal of Plant Nutrition 32(6):929-945.

Abdel-Sabour, M.F., and M.A. Abo El-Seoud. 1996. Effects of organic-waste compost addition on sesame growth, yield and chemical composition. Agriculture, Ecosystems and Environment 60:157-164.

Al-Thabet, S.S. 2006. Effect of plant spacing and nitrogen levels on growth and yield of sunflower (*Helianthus annuus* L.) Journal of Agricultural Science 19:1-11.

Arshad, M., M. Ilyas, and M.A. Khan. 2009. Genetic divergence and path coefficient analysis for seed yield traits in sunflower (*Helianthus annuus* L.) hybrids. Pakistan Journal of Botany 39:2009-2015.

Bakht, J., S. Ahmad, M. Tariq, H. Akbar, and M. Shafi. 2006. Performance of various hybrids of sunflower in Peshawar valley. Journal of Agricultural Science 1(3):25-29.

Boydak, E., D. Karaaslan, and H. Turkoglu. 2010. The effect of different nitrogen and irrigation levels on fatty acid composition of peanut oils. Turkish Journal of Field Crops 15:29-33.

Bukhsh, M.A.H.A., J. Iqbal, S. Kaleem, A. Wasaya, and M. Ishaque. 2011. Qualitative analysis of spring planted sunflower hybrids as influenced by varying nutritional area. Pakistan Journal of Nutrition 10:291-295.

Flagella, Z., T. Rotunno, E. Tarantino, R.D. Caterina, and A.D. Caro. 2002. Changes in seed oil yield and oil fatty acid composition of high oleic sunflower hybrids in relation to the sowing date and water regimes. European Journal of Agronomy 17:221-230.

Gao, Q., C. Li, G. Feng, J. Wang, Z. Cui, X. Chen, and F. Zhang. 2012. Understanding yield response to nitrogen to achieve high yield and high nitrogen use efficiency in rainfed corn. Agronomy Journal 104:165-168.

Gholinezhad, E., A. Aynaband, A.H. Ghorthapeh, G. Noormohamadi, and I. Bernousi. 2011. Effect of drought stress and nitrogen rates on grain yield, quality traits and physiological indices in sunflower hybrid *Iroflor* at different plant density. World Applied Sciences Journal 14:131-139.

Khaliq, A., and Z.A. Cheema. 2005. Influence of irrigation and nitrogen management on some agronomic traits and yield of hybrid sunflower (*Helianthus annuus* L.) International Journal of Agriculture and Biology 7:915-919.

Munir, M.A., M.A. Malik, and M.F. Saleem. 2007. Impact of integration of crop manuring and nitrogen application on growth, yield and quality of spring planted sunflower (*Helianthus annuus* L.) Pakistan Journal of Botany 39:441-449.

Nanjundappa, G., B. Shivaraj, S. Janarjuna, and S. Sridhara. 2001. Effect of organic and inorganic sources of nutrients applied alone or in combination on growth and yield of sunflower (*Helianthus annuus* L.) Helia 24(34):115-120.

Nasim, W., A. Ahmad, A. Bano, R, Olatinwo, M. Usman, T. Khaliq, *et al*. 2012. Effect of nitrogen on yield and quality of sunflower (*Helianthus annuus* L.) hybrids under sub humid conditions of Pakistan. American Journal of Plant Science 3:243-251.

Nasim, W., A. Ahmad, A. Wajid, J. Akhtar, and D. Muhammad. 2011. Nitrogen effects on growth and development of sunflower hybrids under agro-climatic conditions of Multan. Pakistan Journal of Botany 43:2083-2092.

Regina, H. 2008. Influence of macro-and micro nutrient fertilization on fungal contamination and fumonisin production in corn grains. Food Control 19:36-43.

Roche, J., M. Alignan, A. Bouniols, M. Cerny, Z. Mouloungi, F. Vear, and O. Merah. 2010. Fatty acid profile composition in sunflower seeds as affected by genotypes and environmental conditions. Food Chemistry 121:990-995.

Ryland, J. 2003. Manufacturing and food service. Sunflower Conference Proceedings 2003. Session III. 20 May. Australian Oilseeds Federation, Australia Square, New South Whales, Australia.

Steel, R.G.D., J.H. Torrie, and D.A. Dickey. 1997. Principles and procedures of statistics. A biometrical approach. 3rd ed. p. 400-428. McGraw Hill Book, New York, USA.

Thavaprakash, N., S. Kumar, S.D. Raja, and S. Kumar. 2002. Effect of nitrogen and phosphorus levels and ratios on seed yield and nutrient uptake of sunflower hybrid DSH-I. Helia 25:59-68.

Vega, A.J., and A.J. Hall. 2002. Effects of planting date, genotype, and their interactions on sunflower yield: I. Determinants of oil-corrected grain yield. Crop Science 42:1191-1201.

Wamsely, J. 1998. Simultaneous determination of oil and moisture in seed by NMR. Lipid Technology 10:6.

Zahoor, A., M. Riaz, S. Ahmad, H. Ali, M.B. Khan, K. Javed, *et al*. 2010. Ontogeny, growth and radiation use efficiency of sunflower (*Helianthus annuus* L.) as affected by hybrids, nitrogenous regimes and planting geometry under irrigated arid conditions. Pakistan Journal of Botany 42:3197-3207.

Zheljazkov, V.D., B.A. Vick, B.S. Baldwin, N. Buehring, T. Astatkie, and B. Johnson. 2009. Oil content and saturated fatty acids in sunflower as a function of planting date, nitrogen rate, and hybrid. Agronomy Journal 101:1003-1011.

Zheljazkov, V.D., B.A. Vick, M.W. Ebelhar, N. Buehring, B.S. Baldwin, T. Astatkie, and J.F. Miller. 2008. Yield, oil content and composition of sunflower grown at multiple locations in Mississippi. Agronomy Journal 100:635-642.