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Agronomic and physiological assessment of nitrogen use, uptake and acquisition in sunflower

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Abstract

A field experiment was conducted to study the effects of N fertilization on uptake, accumulation/remobilization, use efficiency and yield of sunflower grown in alluvial plains of northwestern India comprising four hybrids (PSH 996, PAC 3789, PSH 569 and SH 3322) and five N levels (Control, 40, 80, 100 and 120 kg N ha^{-1}) in split-plot design with three replications. Increased N fertilizer rates significantly prompted sunflower yield only up to 100 kg N ha^{-1}. Every additional kilogram of N taken up increased sunflower yield by 26 kg ha^{-1}. Significant genetic variation for seed yield and NUE traits explicated PSH 569 as the efficient one at sub-optimal N application while PSH 996 outperformed others at N_{80}, N_{100} and N_{120}. Dry matter accumulation pattern revealed average harvest index of 30% with 29% of the biomass as stalk, 19% as leaf and 22% as thalamus. Temporal changes in N acquisition indicated most of the total N uptake upto 50% flowering while maximum remobilization takes place during reproductive phase. Significant correlation between N uptake and N use efficiency parameters with yield indicate the importance of N nutrition in sunflower; LAI (r=0.841**), N uptake (r=0.956**), NU_pE (r= -0.814**), NU_tE (r= -0.787**), NUE (r=-0.802**). Variation in NUE was more closely associated with NU_pE (r=0.996**) than NU_tE (r=0.812**) and linearly decreased with increasing leaf greenness (R^2=0.70) and total leaf area (R^2=0.81). This work will complement other studies to establish a baseline for breeding N efficient sunflower genotypes be grown under semi-arid tropical conditions in India and similar environments.

Keywords: N uptake; Accumulation; Efficiency indices; Yield; Genotypes; Sunflower.

Introduction

Nitrogen is the most essential mineral nutrient for crop growth and development (Sardana and Sheoran, 2011) and is heavily used in modern agriculture to maximize yields (Mulvaney et al., 2009; Zhang et al., 2010). Today, only 30-50% of applied nitrogen is taken up by the crops and a significant amount of it is lost from agricultural fields. These circumstances not only add unnecessary input costs to the farmers, but also lead to pollution of the environment (Arregui and Quemada, 2008; Tilman et al., 2002; Sylvester-Bradley, 1993).

Increased economic cost and environmental concerns augmented the need to manage fertilizer use more judiciously. Subsequently, improvement in nitrogen use efficiency (NUE) has become a desirable goal in crop production enabling efficient N utilization, maximum energy conservation and profitability (Chen et al., 2013; Hirel et al., 2007). There are a number of ways of measuring NUE (Good et al., 2004) and for the purpose of this paper we used the concept of NUE as developed by Moll et al. (1982). It follows that NUE is a complex trait that consists of N uptake efficiency (the genotype's ability to extract soil N) and N utilization efficiency (the genotypes ability to produce grain per unit of N taken up). Selection of improved genotypes adaptable to a wide range of climatic changes has been a major contributor to the overall gain in crop productivity (Montemurro et al., 2007). Effective cultivar's selection with the idea of improving N efficiency necessitates testing at variable N rates and it becomes imperative to measure the crop's response to N supply on traits related to the components of N efficiency, thereby, providing opportunities for genetic improvement of this trait.

Sunflower (*Helianthus annuus* L.) is an important oilseed crop supplying more than 13% of the total edible oil produced globally (Ramulu et al., 2011). The shortage of edible oils has become a chronic problem in India with increasing demographic pressure. It offers tremendous plasticity to adapt contrasting environmental conditions to bridge up the demand-supply gap in the present oil crisis (Hegde and Sudhakarbabu, 2009) because of its desirable attributes viz., short duration with higher per day productivity, photo-insensitivity and quality edible oil rich in linoleic and oleic acids (Sheoran et al., 2013).

The development of N efficient genotypes and improvement of N management strategies will require understanding of the relationship between physiological processes and biomass/yield formation of crop under varying N application conditions (Dreccer et al., 2000). In this backdrop, the present investigation was carried out with the objectives (i) to evaluate yield variations and use efficiency under variable N fertilization in spring planted sunflower hybrids and (ii) to assess and identify specific traits related to improvement in NUE while reducing the fertilizer N use and maintaining/increasing crop yields, yet relatively few studies have investigated this.

Materials and Methods

Site description

The experiment was conducted during *spring* seasons of 2009 and 2010 on well drained typic ustipsamment at Punjab Agricultural University, Ludhiana (30° 56' N, 75° 52' E, 247 m above msl) located in the Indo-Gangetic alluvial plains in the state of Punjab, northwestern India. The climate of the experimental area is semi-arid and monsoonal. Sunflower is usually planted in late January to mid February and harvested by the end of May. The average annual precipitation of the area is about 760 mm with greater distribution (80%) of the rainfall in June to September months. Analysis of the samples showed that the soil of experimental site (plough layer, 0-20 cm) was loamy sand in texture (73% sand; 15% silt; 12% clay), non-saline (EC 0.21 dS m^{-1}) with pH 7.9 containing 0.19% organic carbon, 112 kg ha^{-1} $KMnO_4$-N, 11.5 kg ha^{-1} Olsen's P and 161 kg ha^{-1} NH_4OAc-K in the surface soil.

Experimental design and crop management

A split-plot design with three replications was used for the experimental purpose comprising four sunflower hybrids (PSH 996, PAC 3789, PSH 569 and SH 3322) in the main plots while nitrogen fertilizer application rates viz., N_0 (Control), N_{40} (40 kg N ha^{-1}), N_{80} (80 kg N ha^{-1}), N_{100} (100 kg N ha^{-1}) and N_{120} (120 kg N ha^{-1}) were assigned to the subplots. The seeds of tested sunflower hybrids were hand dibbled putting 2-3 seeds per hill in seven-row subplots measuring 5.1 m long spaced at 0.6 m between rows and 0.3 m within rows. Later on, the plots were hand thinned to one plant per hill when the plants were at the four to six–leaf stages. Each sub-plot was uniformly fertilized with 30 kg P_2O_5 ha^{-1} as di-ammonium phosphate (18% N, 46% P_2O_5) taking into consideration the N supply and 30 kg K_2O ha^{-1} as muriate of potash (60% K_2O). Half dose of N as per treatments and full dose of P and K were drilled at the time of sowing while remaining half dose of N was top-dressed 30-35 days after planting of sunflower. The crop was grown with assured irrigated facilities and other management practices, including insect-pests and weed control, were followed according to local agronomic practices unless otherwise indicated.

Sampling, determination and calculations

Data were collected for plant partitioning, dry matter accumulation, yield and yield attributing characters, quality analysis and nutrient uptake.

Biological measurements

To estimate dry matter accumulation and its partitioning, five representative plants were randomly selected and cut down at the base of the stem at flowering and physiological maturity stages. These plant samples were individually separated by leaves, stems and capitulum and, thereafter, oven dried to reach a constant weight. Values for plant biomass for each component were recorded separately and expressed subsequently on an individual plant basis. At peak flowering, measurements were taken for the leaf area index (LAI) and chlorophyll, which are correlated with plant N status (Wood et al., 1992). The crop was hand harvested at the stage of physiological maturity when the back of the sunflower head turned from green to yellow and the bracts turned brown. The data on seed and straw yield was recorded from the central five rows by discarding two external rows of each sub plot (as borders). The yield samples were dried to a constant weight and threshed manually to determine the seed yield which was then expressed in kg ha^{-1}. Straw yields were expressed on dry weight basis.

Oil content and nitrogen uptake

Oil content in the whole seed was determined by employing non-destructive method of oil estimation (Alexander et al., 1967) using Nuclear Magnetic Resonance Spectroscope (Newport Analyzer, Model MK 111A). Nitrogen content in grain and straw was determined on dry weight basis by micro-Kjeldahl method (Yoshida et al., 1976). Nutrient uptake and accumulation were calculated as the product of concentration and biomass dry weight. The nitrogen concentration in grain plus that in straw was taken as the measure of total plant N uptake (N content above ground x total biomass dry weight).

Nitrogen use efficiency (NUE)

NUE can be advantageously analyzed through some of the commonly used indices as nitrogen uptake efficiency, utilization efficiency, recovery efficiency, harvest index etc. The indicators of nitrogen management and use efficiency used in this study were calculated as per the formulae given in Table 1.

Table 1. Components of nitrogen use efficiency.

Parameter	Unit	Formulae	Definition
Nitrogen uptake efficiency (NU_pE)	kg kg^{-1}	TNU_f / F_{appln}	Total N uptake per unit of N supply
Nitrogen utilization efficiency (NU_tE)	kg kg^{-1}	Y_f / TNU_f	Grain yield produced per unit total shoot N uptake
Nitrogen use efficiency (NUE)	kg kg^{-1}	Y_f / F_{appln}	Grain yield produced per unit of N supply
Nitrogen agronomic efficiency (NAE)	kg kg^{-1}	$(Y_f - Y_0) / F_{appln}$	Increase in grain yield per unit of fertilizer N applied
Nitrogen physiological efficiency (NPE)	kg kg^{-1}	$(Y_f - Y_0)/(TNU_f - TNU_0)$	Increase in grain yield per unit of increased total shoot N uptake
Nitrogen apartment recovery fraction (NARF)	%	$\lfloor (TNU_f - TNU_0) / F_{appln} \rfloor * 100$	Increase in total N shoot uptake per unit of fertilizer N applied
Nitrogen harvest index (NHI)	%	$\lfloor (TNU_s / TNU_p \rfloor * 100$	Ratio of grain N uptake to total shoot N uptake
Nitrogen biomass production efficiency (NBPE)	kg kg^{-1}	$\lfloor (TDM / TNU_p \rfloor * 100$	Ratio of total plant biomass accumulation to total shoot N uptake

Y_f and Y_0 refers to yield obtained in N-fertilized plots and zero-N plots; F_{appln} is the amount of fertilizer N applied; TNU_f and TNU_0 is the total N uptake of N-fertilized plots and zero-N plots; TNU_s and TNU_p is the total N uptake of seed and shoot biomass; TDM is the total dry matter accumulation.

Statistical analysis

Individual parameters were subjected to one-way analysis of variance (ANOVA) technique as per split-plot design to determine the individual treatment effects (genotypic variability and N fertilization) as well as their interaction using statistical programme SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA) and OPSTAT (www.hau.ernet.in/opstat.html). Mean comparison was performed based on Duncan's Multiple Range Test (DMRT) at the 0.05 probability level.

Results

Biomass and Seed yield

The study results resented in Table 2 revealed that sunflower seed and oil yield, above ground plant biomass, harvest index, 100-seed weight varied significantly between N application rates (P<0.01) discerning consistent improvement with N fertilization compared to N_0-N application (unfertilized control). Progressively linear and significant increase in seed yield was recorded with each incremental dose of N over the preceding one only upto N_{100} (100 kg N ha^{-1}). The seed yield of sunflower was increased by 4.4 and 5.7% relative to higher fertilization at N_{100} and N_{120}, respectively in comparison to recommended N application (N_{80}; 80 kg N ha^{-1}).

Table 2. Growth and yield parameters of sunflower in relation to N fertilization and tested hybrids (pooled data).

Parameters	Nitrogen fertilization (kg N ha^{-1})					Significance	Hybrids				Significance
	N_0	N_{40}	N_{80}	N_{100}	N_{120}		PSH 996	PAC 3789	PSH 569	SH 3322	
Seed yield (kg ha^{-1})	1118^{d}	1638^{c}	1958^{b}	2045^{a}	2069^{a}	<0.0001	1858^{a}	1760^{b}	1779^{b}	1665^{c}	0.0004
Dry matter production (kg ha^{-1})	4067^{d}	5480^{c}	6481^{b}	6588^{ab}	6707^{a}	<0.0001	5672^{c}	6067^{a}	5975^{a}	5744^{bc}	0.0003
100-seed weight (g)	4.57^{c}	5.23^{b}	5.68^{a}	5.80^{a}	5.81^{a}	<0.0001	5.86^{a}	4.57^{c}	5.83^{a}	5.41^{b}	<0.0001
Oil production (kg ha^{-1})	440^{d}	644^{c}	766^{b}	799^{a}	808^{a}	<0.0001	741^{a}	706^{b}	682^{c}	636^{d}	<0.0001
Harvest index (%)	27.5^{c}	29.9^{b}	30.2^{a}	31.0^{a}	31.1^{a}	<0.0001	32.3^{a}	28.8^{c}	29.8^{b}	28.9^{bc}	0.0007
SPAD values at flowering	33.76^{e}	37.66^{d}	40.58^{c}	41.58^{b}	42.21^{a}	<0.0001	38.17^{d}	37.58^{e}	41.47^{a}	39.40^{b}	<0.0001
Leaf area index (LAI) at flowering	1.40^{d}	1.68^{c}	2.27^{b}	2.56^{a}	2.65^{a}	<0.0001	2.01^{c}	2.17^{b}	2.28^{a}	1.99^{c}	0.0004

Significance levels are from one-way ANOVA. Data followed by different lower-case letters differs significantly (significance level = 0.05, within row comparison).

Positive association of seed yield is essentially a pattern related to 100-seed weight (4.57 at N_0 to 5.81 at N_{120}) and harvest index (27.5% at N_0 to 31.1 at N_{120}) with increasing N rates (Table 2). Oil production was proportional to seed yield which consistently increased with increasing N fertilization ($P<0.01$) excelling 46.4-83.6% over the control (N_0). N nutrition was greatly beneficial to total dry matter accumulation (Table 2) and N uptake (Table 3) and both varied significantly over plant growth in relation to N application. Consistent and significant improvement in N uptake was noticed upto N_{100}. The observations taken at flowering stage revealed significant and positive response of N fertilization on Chloropyll content (SPAD value) and leaf area index (LAI) values with each successive increase in N rate (Table 2).

N efficiency indices declined as N fertilizer rates increased reflecting a poor crop response (Table 3). Inverse relation was observed between the crop's ability to utilize nutrients relative to applied N and the crop fertilized with lower N had significantly higher NUE than with higher N treatments ($P<0.01$), following the order of $N_{40}>N_{80}>N_{100}>N_{120}$. The average values of NU_tE decreased as the input N rate increased, dropping from 24.5 (N_0) to 21.2 (N_{120}) kg of seed kg^{-1} of N applied. Similarly, the NU_pE significantly decreased from 1.77 (N_{40}) to 0.81 (N_{120}) kg of above ground plant N uptake kg^{-1} of input N applied. The NAE showed diminishing rate of return with increased N fertilization although showed the same results between N_{80} and N_{100}. By contrast, NPE was not significantly different at N_{80}, N_{100} and N_{120} fertilization rates though numerically decreased with increasing N application rates. Inverse relationship was observed between N fertilization and NARF, dropping from 62.4 (N_{40}) to 43.3 (N_{120}) per cent. Consistent decrease in NBPE was observed with increasing N rates, being highest at N_0 (89.36) and lowest at N_{120} (69.05). Variations for NHI were significant at different N rates compared to No-N application (control), with highest value corresponded to application of 100 kg N ha^{-1} (Table 3).

The analysis of variance showed that genotypes included in this study had significant ($P<0.01$) differences for growth and yield components, yield (seed and oil) and NUE indicators owing to variable N fertilization. Seed yield ranged between 1665 kg ha^{-1} (SH 3322) to 1858 kg ha^{-1} (PSH 996) with an overall harvestable yield of 1766 kg ha^{-1} (Table 2). PSH 996 significantly out yielded other hybrids excelling 4.4-11.6% in seed yield and 5.0-16.5% in oil yield. PSH 569 was as good as PAC 3789 but both were significantly superior to SH 3322. Lower above ground biomass (5672 kg ha^{-1}) and higher seed yield in PSH 996 resulted in highest harvest index (32.3%). Contrary to this, highest stover yield in PAC 3789 (6067 kg ha^{-1}) and lowest seed yield in SH 3322 (1665 kg ha^{-1}) culminated in significantly lower harvest index. Hybrids exhibited statistical differences for oil yield following the order of PSH 996>PAC 3789>PSH 569>SH 3322. PSH 569 registered significantly higher values for chlorophyll content (41.47) and leaf area index (2.28).

Highest total N uptake of 83.22 kg ha^{-1} (Table 3) was recorded with PSH 996, outperforming others except PSH 569 (80.86 kg ha^{-1}). Variable response of different hybrids for NUE indicated mean value of 25.78 kg of seed yield per kg of applied N, being highest under PSH 996 (26.99 kg kg^{-1}) and lowest under SH 3322 (24.52 kg kg^{-1}). Non-conspicuous differences for NU_tE were observed for all hybrids except SH 3322. Highest NU_pE was recorded with PSH 996 (1.20 kg above-ground N at harvest per kg N) followed by PSH 569 (1.18), SH 3322 (1.16) and lowest with PAC 3789 (1.13). PSH 996 was found to be the efficient one recording highest NAE (26.99), NPE (20.40), NARF (57.3%) and NHI (75.1%). However, NBPE was found to be significantly higher with PAC (80.64 kg above-ground DM per kg above-ground N) followed by SH 3322 (75.08) and PSH 569 (74.99) compared to the significantly lowest value of PSH 996 (71.21) (Table 3).

Table 3. N uptake and N use efficiency parameters of sunflower in relation to N fertilization and tested hybrids (pooled data).

Parameters	Nitrogen fertilization (kg N ha^{-1})					Significance	Hybrids				Significance
	N_0	N_{40}	N_{80}	N_{100}	N_{120}		PSH 996	PAC 3789	PSH 569	SH 3322	
Total shoot N uptake at maturity	45.68^d	70.69^c	90.13^b	95.42^a	97.62^a	<0.0001	83.22^a	77.23^c	80.86^{ab}	78.32^{bc}	0.0016
N utilization efficiency (NU_tE)	24.48^a	23.19^b	21.74^c	21.44^c	21.20^c	<0.0001	22.62^{ab}	23.11^a	22.38^{ab}	21.54^c	0.0158
N uptake efficiency (NU_pE)	-	1.77^a	1.13^b	0.96^c	0.81^d	<0.0001	1.199^a	1.133^b	1.175^{ab}	1.157^{ab}	0.0413
N use efficiency (NUE)	-	40.96^a	24.48^b	20.45^c	17.24^d	<0.0001	26.99^a	25.73^b	25.88^b	24.52^c	0.0017
N agronomic efficiency (NAE)	-	13.02^a	10.51^b	9.28^b	7.93^c	<0.0001	11.79^a	10.39^b	8.86^c	9.68^{bc}	0.0019
N physiological efficiency (NPE)	-	20.75^a	18.90^b	18.65^b	18.23^b	0.0293	20.40^a	20.03^a	17.59^b	18.51^b	0.0026
N apparent recovery fraction (NARF)	-	62.4^a	55.6^b	49.7^c	43.3^d	<0.0001	57.3^a	51.8^b	49.8^b	52.1^b	0.0018
N harvest index (NHI)	71.4^d	73.1^c	73.8^b	74.3^a	74.2^a	0.0115	75.1^a	73.1^{bc}	73.9^{ab}	72.3^c	0.0116
N biomass production efficiency (NBPE)	89.36^a	77.62^b	72.05^c	69.32^c	69.05^c	<0.0001	71.21^c	80.64^a	74.99^b	75.08^b	0.0021

Significance levels are from one-way ANOVA. Data followed by different lower-case letters differs significantly (significance level = 0.05, within row comparison).

Plant partitioning, accumulation and uptake pattern

Temporal pattern of dry matter acquisition indicated that on an average 27, 49 and 24% of the above ground plant biomass was retained as leaf, stem and capitulum, respectively at 50% flowering stage (Figure 1). With periodic advancement (50% flowering to physiological maturity), mean dry weight of leaf, stem, capitulum and total plant biomass increased from 14.4 to 20.6 g plant^{-1}, 26.1 to 30.4 g plant^{-1}, 13.0 to 52.4 g plant^{-1} and 53.6 to 106.2 g plant^{-1} elucidating 43, 17, 325 and 98% enhancement, respectively (Figure 2a and b). Remobilization of metabolites from vegetative plant parts (leaf and stem portions) to reproductive parts illustrated 19% contribution by leaf, 29% by stem and 51% by capitulum (thalamus + seed) portion relative to total plant biomass at physiological maturity (Figure 2b). Furthermore, improvement in seed dry matter accumulation was noticed upto N_{100} though the thalamus weight increased with increasing N rates. Capitulum partitioning (seed + thalamus) constituted about 61 and 39% contribution on mean dry weight basis, respectively though no definite trend was observed relative to N fertilization (Figure 2b).

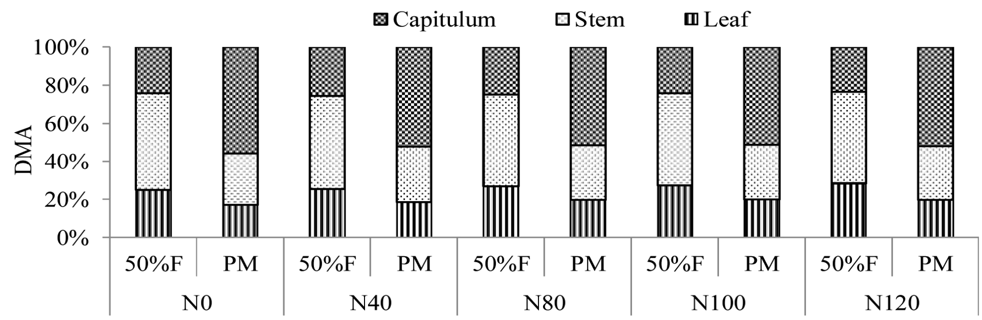

Figure 1. Per cent contribution to total plant biomass accumulation by different plant parts at 50% flowering (50%F) and physiological maturity (PM) relative to N fertilization in sunflower.

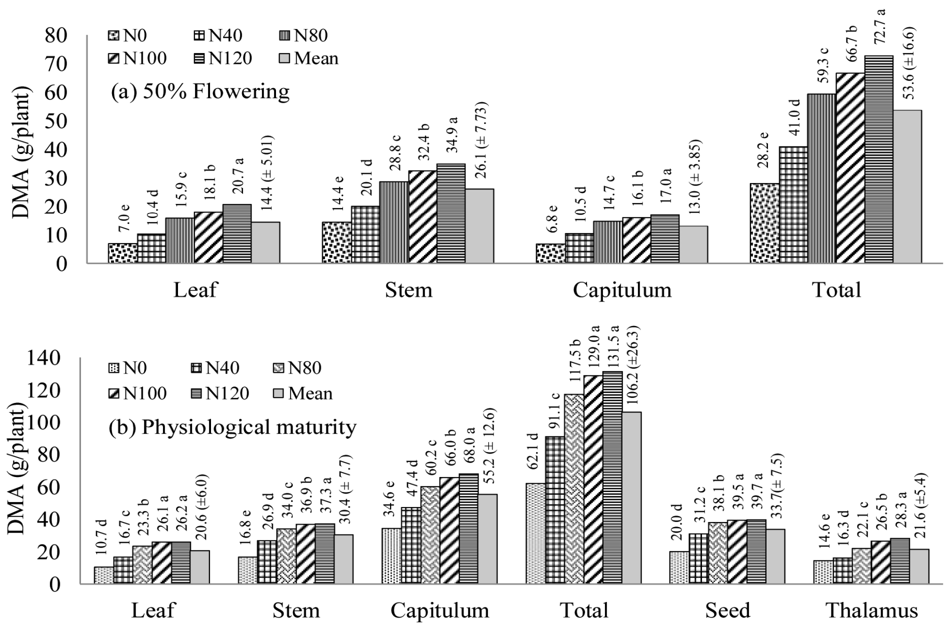

Figure 2. Plant partitioning and dry matter accumulation in relation to N fertilization (across tested hybrids) at (a) 50% flowering and (b) physiological maturity of sunflower [Bars within same group (biomass accumulation by) sharing the same letters do not differ significantly (DMRT, significance level= 0.05); values in parentheses indicate ± s.d.; DMA: dry matter accumulation.

Temporal changes in total N uptake and it partitioning indicated that the majority of the N remained in crop residues and little was translocated to reproductive parts upto initiation of flowering (Figure 3). However, most of the N translocation takes place from source (vegetative tissues) to the sink (seed) during reproductive phase as indicated at physiological maturity.

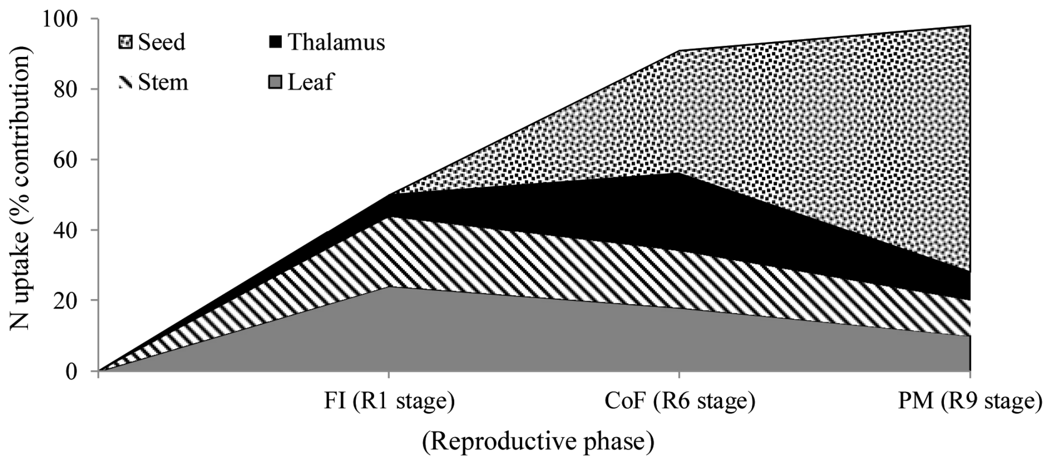

Figure 3. Sunflower N uptake pattern (across N fertilization) and partitioning as a percentage of total uptake for leaf (grey), stem (hatched), thalamus (black) and seed (dotted) at flowering initiation (FI), completion of flowering (CoF) and physiological maturity (PM).

Discussion

The overall findings indicated that the differential yield response of sunflower to N rate is linked to potential growth and yield components, dry matter formation and accumulation (Table 2), varying N uptake and its use capacity (Table 3). In general, increased N fertilizer rates prompted sunflower yield upto a point N_{100} (100 kg N ha^{-1}), beyond which there was no additional response (Figure 4). Eliciting similar yields at 100 and 120 kg N ha^{-1} is attributable to the gradual increase in available soil N for the whole system, deriving from systematic application of N fertilizer, as reported by Campbell et al. (1993). Linear analysis showed that every additional kilogram of N taken up by the crop increased yield by 26 kg ha^{-1}. Contrarily, the observed 16 and 43% reduction in seed yield under moderate N (N_{40}) and No-N application (N_0) compared to recommended N (N_{80}) is comparable with the results of Bertin and Gallais (2000), who also reported significant reduction in crop yield with reduced N fertilization. Delogu et al. (1998) and Sieling et al. (1998) showed that N efficiency indices diminished as N fertilizer rates increased (Table 3), with significant differences among all rates reflecting a poor crop use of fertilizer. The NUE and its two primary component traits (NU_tE and NU_pE) were negatively related to N availability. A mean NUE of 41 kg kg^{-1} at moderate N (N_{40}), 25 kg kg^{-1} at recommended N (N_{80}), 21 kg kg^{-1} at high N (N_{100}) and 17 kg kg^{-1} at very high N (N_{120}) application was recorded indicating progressive decrease in response with increasing N fertilization (Table 3) in accordance with the results of previous studies (De Souza et al., 2008; Uribelarrea et al., 2007). The NUE values at a maximum N fertilizer rate of 120 kg N ha^{-1} were 58, 30 and 16% lower than those obtained with 40, 80 and 100 kg N ha^{-1}, respectively (Figure 4). The decrease in yield with increasing N fertilizer rate is due to the fact that seed yield rises less than the N supply in soil and fertilizer.

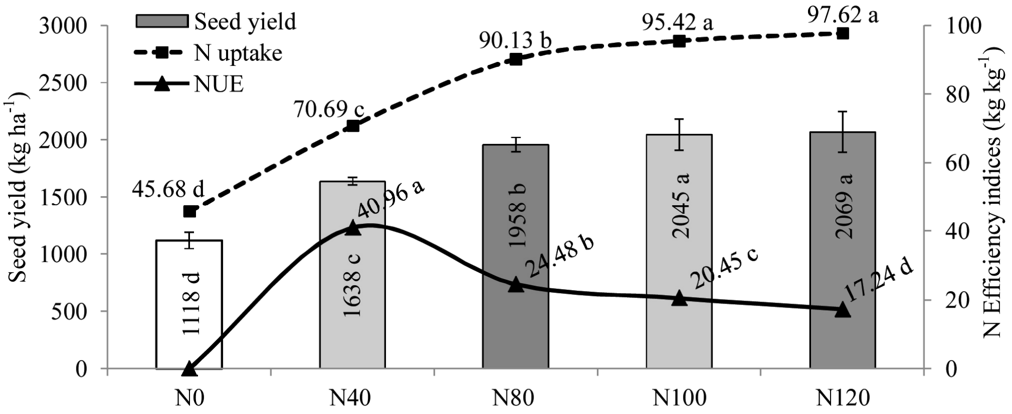

Figure 4. Effect of N fertilization rates (across hybrids) on seed yield (*pri-axis*), N uptake and use efficiency (*sec-axis*) in sunflower. Bars and lines sharing the same lower case letter do not differ significantly (DMRT, *p-value<0.0001*, within treatment comparison).

Increased N fertilizer rates prompted a fall in the NU_tE and NU_pE index (Table 3), a finding also reported by Delogu et al. (1998). Significant reduction in NU_tE was noticed with increasing N rates upto N_{80} only while NU_pE showed diminishing rate of return as the input N increased. This parameter has been extensively used to compare different species or cultivars at different levels of N fertility (Ortiz Monasterio et al., 1997). The chlorophyll content progressively increased with increasing N levels while such a response was noticed only up to N_{100} for leaf area index (Table 2). The regression coefficients based on extent of yield response as a function of fertilizer N applied linearly decreased with increasing leaf greenness and total crop area which turned out to be as high as R^2=0.70 for SPAD and R^2=0.81 for LAI values (Figure 5).

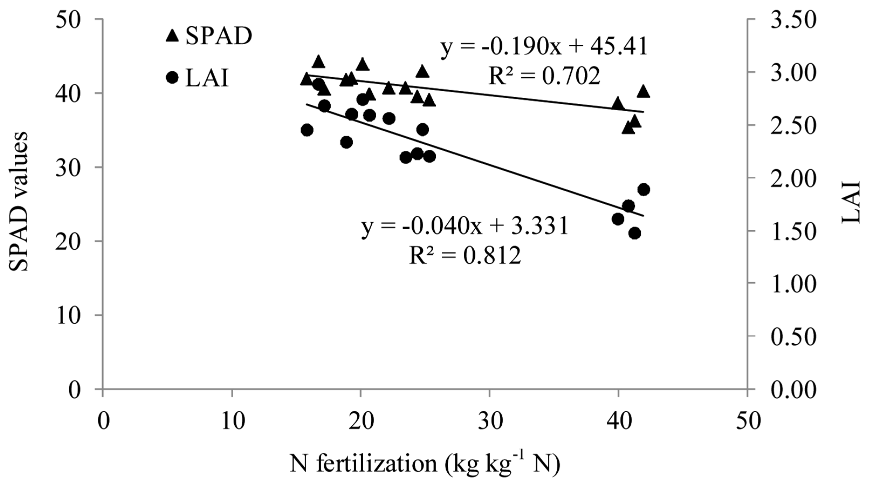

Figure 5. Seed yield response of sunflower hybrids to N fertilization with chlorophyll content (SPAD values)/LAI.

Consistent and significant improvement in total plant biomass and its partitioning and accumulation in different plant parts was observed relative to each successive incremental rate of N fertilization in sunflower both at 50% flowering and physiological maturity (Figure 1). Average harvest index was 30% (% total biomass as seed) with 29% of the biomass as stalk, 19% as leaf and 22% as thalamus (Figure 2). Total N uptake was proportional to yield, being greatest at 100 and 120 kg N ha^{-1}, with no significant difference between these two rates (Figure 4). This mirrors the already-reported response of seed yield to N fertilizer rates (Tables 2 and 3). N harvest index (NHI) is an empirical measure of the amount of N captured in the seed that would be transferred to end-users. As N fertilizer rates increase, there comes a point at which transfer of N to grain ceases to follow N uptake by the plant. In the present experiment, this point is at 100 kg N ha^{-1}, coinciding with an absence of response in yield (Table 3). Increased N fertilizer rates resulted in decline in NAE and NPE, although no significant differences were observed between N_{80} and N_{100} for NAE and beyond N_{80} fertilizer application for NPE. A mean NARF value of 0.53 was obtained with application of N fertilizers. Application of 120 kg N ha^{-1} gave a lower NARF value (0.42) than 40, 80 or 100 kg N ha^{-1} and the significant differences in efficiency was observed among various N rates (Table 3).

The four hybrids included in this study exhibited significant differences in seed yield, NUE and NUE-related traits (Tables 2 and 3). Hybrid PSH 996 had the highest mean seed yield (1858 kg ha^{-1}), harvest index (32.3%) and NUE (26.99 kg kg^{-1} N) showing better physiological competence of this hybrid to translocate the fraction of photo-assimilates to sink (higher seed weight) relative to N nutrition.

The significant hybrid × N rate interaction for seed yield and NUE could result from the differential response pattern of included hybrids to N application (Table 4). PSH 569 was found to be efficient one at lower N rates excelling 10.9-14.7% at N_0 and 1.6-4.9% at N_{40} level. Thereafter PSH 996 showed its superiority with increasing N fertilization outperforming others by 2.0-7.7, 7.4-17.6 and 12.4-21.9% at N_{80}, N_{100} and N_{120}, respectively. The differences in seed yield among hybrids under a particular level of N-fertilizer application could be attributed to differences among the hybrids for NU_pE and NU_tE (Table 5) (Beauchamp et al., 1976; Pollmer et al., 1979). PSH 996 having high N harvest index (75.1%) consequently culminated towards higher N use efficiency (Table 3).

Table 4. Seed yield (kg ha^{-1}) of sunflower hybrids as influenced by N fertilization (pooled data).

Hybrids	Nitrogen fertilization (kg N ha^{-1})				
	N_0	N_{40}	N_{80}	N_{100}	N_{120}
PSH 996	1089^{e}	1650^{d} (41.24)	2022^{bc} (25.28)	2217^{a} (22.17)	2313^{a} (19.28)
PAC 3789	1099^{e}	1630^{d} (40.75)	1950^{bc} (24.38)	2065^{b} (20.65)	2058^{b} (17.15)
PSH 569	1219^{e}	1676^{d} (41.90)	1982^{bc} (24.78)	2013^{bc} (20.13)	2006^{bc} (16.71)
SH 3322	1063^{e}	1597^{d} (39.93)	1878^{c} (23.48)	1886^{c} (18.86)	1898^{c} (15.82)

Data followed by different lower-case letters differs significantly (significance level = 0.05); Figures in parentheses indicate the NUE.

Table 5. Nitrogen uptake and utilization efficiency (kg kg^{-1}) of sunflower hybrids in relation to N fertilization (pooled data).

	Nitrogen fertilization (kg N ha^{-1})								
	N_{40}	N_{80}	N_{100}	N_{120}	N_0	N_{40}	N_{80}	N_{100}	N_{120}
	NU_pE				NU_tE				
PSH 996	1.73	1.15	1.02	0.89	24.17	23.76	21.96	21.65	21.56
PAC 3789	1.73	1.09	0.93	0.78	25.16	23.58	22.54	22.32	21.94
PSH 569	1.80	1.14	0.95	0.81	25.12	23.35	21.60	21.28	20.52
SH 3322	1.81	1.13	0.93	0.76	23.47	22.05	20.86	20.52	20.78

Considering the importance of agronomic traits and efficiency indices (Table 4) towards yield formation, seed yield showed highly significant correlation with LAI (r=0.841**), N uptake (r=0.956**) and utilization efficiencies viz; NU_pE (r= -0.814**) NUtE (r= -0.787**) and finally the NUE (r= -0.802**). The increase in crop N uptake with rising N fertilizer rates is greater than the increase in seed yield (Table 3). Present results showed that variation in NUE was more closely associated with NU_pE (r=0.996**) than NU_tE (r=0.812**). This would explain why there is less transfer of N to seed when N rates are increased.

Conclusions

N use efficiency changes with N fertilization rates, will assist in taking up management decisions considering the profitability and environmental impact. Further investigations on N absorption, its distribution and further utilization will supply the scientific foundation for rational application of N fertilizers and increasing crop production in sunflower. Genetic variation for NUE and its components was present among the sunflower hybrids targeting their suitability for different environmental conditions. This will help to establish a baseline for breeding N efficient sunflower genotypes.

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