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Splitting nitrogen fertilization improves growth, yield and profit of soybean (Glycine max) production in the semi-arid Afghanistan

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Abstract

The objectives of this study were to evaluate the effects of splitting application of 30 kg N ha^{-1} on the growth, yield and economics of soybean (*Glycine max* var. Dea-won) in the semi-arid and sub-tropical Afghanistan. Besides no-N-fertilization control, urea (30 kg N ha^{-1}) was applied to fields in four-splits: S_1, one time basal application at sowing; S_2, two-splits of 50% N at sowing and 10 DAS (days after sowing); S_3, three-splits of 33% N at sowing, 10 and 20 DAS; and S_4, four-splits of 25% N at sowing, 10, 20 and 30 DAS. Aboveground growth and yield parameters were compared at 30, 60, 90 and 127 DAS. Soybean's growth and yields increased in corresponding with the increased frequency of split fertilization. Three- or four-splits significantly increased plant height, leaf area index, aboveground biomass, crop growth rate, net assimilation rate, relative growth rate, pod and seed numbers, 1,000-seed weight, yield production and economics (gross and net returns and benefit cost ratio) than those at one or two-splits N-application at all these four harvests. Positive relationships were observed among growth parameters and yield traits and yield production. Three- or four-splits at tested N rate and growth stages can meet N requirement for soybean's growth and yield while improving N use efficiency in semi-arid Afghanistan.

Keywords: Biomass production, *Glycine max,* N demanding, N use efficiency, N split application, urea.

Introduction

Soybean is the most important oil crop for human beings, animals and the biodiesel industry due to its high contents of oil (19%) and protein (40%) (Mon et al., 2017). Physiologically, soybean's growth has a high nitrogen (N) demand that is required mainly for protein synthesis. For example, a maximal daily uptake of 4.6 kg ha^{-1} is required at the R4 (full pod) stage (Bender et al., 2015) and approximately 300 kg N is needed to produce 3 t ha^{-1} of soybean (Youn et al., 2009). In an environment that is ideal for crop's growth, especially for soybean, biological nitrogen fixation (BNF) can fulfil 50% of soybean's total N demand (Bender et al., 2015). However, high soil NO_3^-, low moisture, low pH, compaction, acidity or drought can inhibit soybean's BNF process, growth and yield production (Mourtzinis et al., 2018). The present 0.6 to 1.2 t ha^{-1} productivity in Afghanistan is substantially low compared to the global 2.77 t ha^{-1} productivity (NEI, 2017). It is most likely due to the lack of indigenous N fixing bacteria and soil residual N, without rhizobial inoculation, excessive or inappropriate N application and low soil fertility in Afghanistan. As a result,

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external N fertilizer has to be supplied to promote soybean growth and productivity. Furthermore, overuse of NO_3^- has always resulted in leaching or denitrification. Even if N was applied as a basal application, plant roots could not possibly absorb all of them at once. In such soil conditions, N fertilization should be carefully managed throughout the crop growth cycle. One of the effective methods of N fertilization management is split application by adjusting the timing of fertilizer application within the plant growth stage. The fertilizer split can also improve the efficiency of crop uptake, re-translocation, and nitrogen use efficiency (Khan et al., 2017). Positive response of different rates and time of N fertilization on soybean's growth, nutrient uptake and seed yield have been studied. For example, fertilization of 30 kg N ha^{-1} or 7.5 mM pot^{-1} as a basal application and at the beginning of flowering (R1) stage increased plant height, leaf area index (LAI), dry matter accumulation and seed yield (Singh and Singh 2013; Bhangu and Virk, 2019; Zhou et al., 2019). The supply of 100, 50, 40, 30 or 10 kg N ha^{-1} as basal, at 25 DAS (days after sowing), the R1, pod initiation (R3) and beginning of seed (R5) stages significantly increased the soybean's aboveground biomass and seed yield (Gan et al., 2002; Bhangu and Virk, 2019).

Taking all above-mentioned information into consideration for rational splitting fertilization to harness maximum productivity, we hypothesized that (1) applying N fertilizer corresponding with plant growth stages could have significant effects on the growth and yield of soybean; and (2) a gradual supplement of the same amount of N fertilizer under split applications could be better than an one-time fertilizer supplement. These hypotheses are based on a fact that soybean can progressively establish its BNF capacity to meet its N-demanding while lessening the N-demand from soil mineralization, particularly in the earlier seedling or vegetative growth stages. A starter N application reportedly improved soybean's biomass accumulation and seed yield (Starling et al., 1998; Hellal et al., 2013). Even though chemical starter fertilizer N tends to lost within a few weeks after planting (Ohyama et al., 2017), subsequent supplementation of N fertilizer via the split application may replenish the soil N pool. Therefore, in this study, a locally practised N fertilization rate of 30 kg N ha^{-1} was employed as four splits application that corresponds to the growth and development stages of a local soybean cultivar to enhance the N use efficiency while increasing its primary productivity.

Therefore, the objectives of this present study were to determine (i) to eveluate the effects of splitting patterns of N fertilizer on growth and yield, and (ii) to eveluate the optimal splitting application of both timing and rates across four splitting times to ensure a higher N-use efficiency, productivity and profitability of soybean in Kandahar, Afghanistan. Findings from the study can provide a foundation for promoting crop production and resource use efficiency in the semi-arid and sub-tropical regions around the world.

Material and Methods

Site specification

A field experiment was conducted between May 13 and September 20, 2017 in the Afghanistan National Agricultural Sciences and Technology University Research Farm in Kandahar (31°26'N and 65°51'E, 1,010 m above mean sea level), Afghanistan. The region has a semi-arid to sub-tropical climate with a mean annual temperature of 5–6°C in winter and of 24.3–35.8°C in summer, and an annual precipitation of 199 mm (most between January and March) (Table 1). The soil is an Aridisol soil (according to the USDA Soil Taxonomy) that has 56.2% sand, 14.3% silt and 29.5% clay. The soil pH (1:2.5; H_2O) was 7.9 with cation exchange capacity of 80.58 meq 100 g^{-1}. Soil organic carbon, available N, P, and K, and total Fe, Zn, Cu and Mn were 9.40 g kg^{-1}, 700, 1.29 and 956.64, and 2.89, 1.35, 0.98 and 3.51 mg kg^{-1}, respectively.

Table 1. Monthly temperature and rainfall during the soybean growing season (2017)

Months (2017)		April	May	June	July	August	September
Temperature (°C)	Maximum	26.65	31.63	35.94	35.56	33.69	29.87
	Average	19.46	24.85	29.55	29.37	27.55	22.87
	Minimum	11.65	17.11	22.41	23.17	20.65	15.84
Rainfall (mm)		0.178	0.001	0.000	0.000	0.000	0.000

Experimental design and treatment

To examine the growth response of soybean to different timing of fertilization, conventional urea (46% N) was applied in four splits for a total rate of 30 kg N ha^{-1} based on the local fertilization rate. In addition, P_2O_5, K_2O and $ZnSO_4$ were applied at sowing to all plots at the rate of 60, 40, and 10 kg ha^{-1}, respectively. The field experiment was designed in a randomized complete block design with three replications. There were a total of 15 plots, each measured at 4.0 m x 3.0 m with 5 numbers of rows spaced at 0.4 m apart. The treatments were consisted of five fertilization rates applied at different days after sowing (DAS): (1) the no-fertilization control, (2) S1 = one-time basal application (100% N) at sowing, (3) S2 = two-times split at 50% N each at sowing and 10 DAS; (4) S3 = three-times split at 33% N each at sowing, 10 DAS and 20 DAS; and (5) S4=four-times split of 25% N each at sowing, 10 DAS, 20 DAS and 30 DAS. The timing of fertilizer application at 10 DAS corresponded to the cotyledon or unifoliate leaves (VC) stage, 20 DAS to the 3rd trifoliate (V3) stage and 30 DAS to the beginning of flower (R1). Soybean (Glycine max cv. Dea-won) was sown at 40 cm x 15 cm without inoculation with a bacterium culture. This cultivar, selected with its wide adaptability in the study region, shows resistance against pest and disease and a productivity of 1.1 to 2.8 t seed ha^{-1}. No information is available to the soil microbiota, to our knowledge. The experiment was conducted under irrigation with wheat as the previous crop and the field was Mouldboard–ploughed once before sowing. In next month, land preparation was started with cross–cultivator to obtain a good soil tilth, followed by two times of rotaries to break soil clods.

Measurements

Five plants were randomly selected as representative samples for a replicated treatment at 30, 60 and 90 DAS corresponding to R1, full pod (R4) and full seed (R6), respectively, to determine (i) plant height, measured from ground level to the tip of the plant, and (ii) leaf area per plant from three sizes of leaves (i.e., small, medium, large). The leaf area was determined using millimetre graph paper and then converted to cm^2 plant^{-1}. The leaf area index was calculated using the following equation:

$$\text{LAI} = \text{leaf area per plant (in cm}^{-2}\text{)/plant ground area (cm}^2\text{)}.$$

Plants were harvested at 127 DAS where the straws (leaf + stem + pod) were sun-dried for several days to a constant weight. The outcome was recorded as g plant^{-1} and then converted to t ha^{-1}. The total pods per plant were determined by the number of pods (containing one or more seeds) from ten randomly selected plants. The average number of pods per plant was derived by dividing the total number of pods by ten. Total seed pod^{-1} was determined by the total number of seeds from ten pods randomly selected from each treatment divided by ten and then averaged to obtain the total seed per pod. 1,000 of clean dried seeds counted randomly from each sample were weighted by using digital electric balance and the results were expressed in grams. The seeds or grains obtained from each net plot were sun-dried for 4-5 days, weighed carefully and recorded in t ha^{-1}. The seed yield was recorded at 10% moisture content. Total aboveground biomass (straw + seed) was determined as the total weight of straw t ha^{-1} plus seed yield t ha^{-1} and harvest index as seed yield t ha^{-1} divided by aboveground biomass and multiply by 100.

Physiological parameters

Net assimilation rate (NAR; g cm^{-2} d^{-1}), crop growth rate (CGR; g cm^{-2}d^{-1}), relative growth rate (RGR; g g^{-1} d^{-1}), and agronomic nitrogen use efficiency (ANUE; kg seed kg N^{-1}) were determined using the following formulas (Delogu et al., 1998; Sun et al., 2019; Díaz-López et al., 2020).

$$\text{NAR}= \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\text{Log}_e L_2 - \text{Log}_e L_1}{L_2 - L_1}$$

Where W_1 and W_2 are the total weight of aboveground biomass of plant at time t_1 and time t_2, respectively and L_1 and L_2 are total leaf area of plant at time t_1 and t_2, respectively.

$$\text{CGR} = \frac{W_2 - W_1}{t_2 - t_1}$$

WhereW_1 and W_2 are total weight of aboveground biomass at time t_1 and time t_2 respectively.

$$\text{RGR} = = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t_2 - t_1}$$

W_1 and W_2 are the total dry weight at times t_1 and t_2, respectively.

$$\text{ANUE} = \frac{\text{seed yield at N treatment-seed yield at zero N treatment}}{\text{applied N at N treatment}}$$

Economic evaluation

All costs and returns per hectare were initially calculated based on Afghan Afghani (AFN) and thereafter converted to US$ based on the exchange rate of 1 AFN=0.015 US$, as of September, 2017, though such conversions only reflected relative values. All the expenses included land lease, labour and farmer segment, land preparation, irrigation, fertilization, weeding, harvesting and threshing, seeds, insecticide and the cost of fertilizers were computed as the costs of cultivation based on individual treatment. The production prices for straw and fallow land wad (t ha^{-1}) were considered as per the average price of domestic market. The seed/grain was counted 395 US$ per tonne as per the FAO international price review for soybean observed in September, 2017. Gross return was calculated as the price of whole plant production (straw + seed t ha^{-1}) and the net return was the difference of gross return and the total cost of all variables. The ratio between gross return to cost of cultivation was computed as benefit/cost ratio (B/C ratio).

Statistical Analyses

Data were subjected to one-way ANOVA. Significant differences among fertilization treatments for the same harvest day or among harvest days for the same fertilization rate were compared by the Duncan's multiple range test at $P < 0.05$ using a SPSS 24 software (Chicago, USA). Microsoft Office Excel (version 2016, Microsoft, Redmond, USA) was used for graphs, correlation, and regression analysis.

Results

Effects of splitting nitrogen applications on growth attributes

Plant heights observed within the same days after sowing (DAS) at 30, 60 and 90 increased with the number of split fertilization compared to control (no-fertilization) (Table 2). At 90 DAS, soybean plants treated with four splits of N (S4, at a rate of 25% N) had the tallest height, which was significantly taller than plants treated with S0-S2 but not significantly different compared to plants treated with S3. In addition, significantly taller plant height for the same fertilization among different growing days patterned as 90 DAS > 60 DAS > 30 DAS (Table 2).

Table 2. Effects of splitting N fertilizations on plant height, leaf area index and aboveground biomass of soybean at 30, 60 and 90 days after sowing (DAS).

Treatment	Plant height (cm)			Leaf area index			Aboveground biomass (g plant^{-1})		
30 kg N ha^{-1}	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
Control	8.1 ± 0.3 (b, z)	29.2 ± 2.8 (b, y)	34.1 ± 2.9 (c, x)	0.24 ± 0.02 (a, z)	3.1 ± 0.22 (c, y)	3.5 ± 0.15 (c, x)	1.63 ± 0.03 (b, z)	12.6 ± 0.48 (c, y)	21.0 ± 2.2 (c, x)
N_{30}-S1	8.6 ± 0.5 (ab,z)	31.8 ± 3.1 (ab, y)	36.7 ± 1.4 (bc, x)	0.27 ± 0.01 (ab, y)	4.3 ± 0.21 (b, x)	4.6 ± 0.57 (b, x)	1.74 ± 0.11 (b,z)	17.6 ± 0.98 (b, y)	35.9 ± 2.7 (b, x)
N_{30}-S2	8.8 ± 0.7 (ab, z)	32.2 ± 3.5 (ab, y)	39.1 ± 1.8 (b, x)	0.27 ± 0.02 (ab, z)	4.3 ± 0.39 (b, y)	4.7 ± 0.52 (b, x)	1.74 ± 0.07 (ab, z)	18.4 ± 1.09 (b, y)	38.0 ± 3.1 (b, x)
N_{30}-S3	9.0 ± 0.7 (a, z)	34.5 ± 3.1 (a, y)	43.8 ± 4.1 (a, x)	0.30 ± 0.07 (a, z)	4.9 ± 0.85 (a, y)	5.6 ± 0.32 (a, x)	1.75 ± 0.04 (ab, z)	23.9 ± 0.95 (a, y)	59.2 ± 2.9 (a, x)
N_{30}-S4	9.0 ± 0.8 (a, z)	34.6 ± 2.8 (a, y)	45.1 ± 2.6 (a, x)	0.32 ± 0.09 (a, z)	4.9 ± 0.42 (a, y)	5.7 ± 0.61 (a, x)	1.77 ± 0.03 (a, z)	24.6 ± 0.83 (a, y)	61.6 ± 4.0 (a, x)

Data are means ± SD (n=3). S1 = One-time basal application at sowing; S2 = two-times split application at 50% N each at sowing and 10 DAS; S3 = three-times split at 33% N each at sowing, 10 DAS and 20 DAS; and S4 = four-times split of 25% N each at sowing, 10 DAS, 20 DAS and 30 DAS. N_{30} indicates 30 kg N ha^{-1} season^{-1}.
Significant differences between N fertilizations for the same day (a, b, c).
Significant differences between growing days for the same N fertilization (x, y, z) at $P < 0.05$.

There were no significant differences in leaf area index (LAI) observed at 30 DAS when the plants were treated with different rates of fertilization (Table 2). However, at 60 and 90 DAS, the LAI increased significantly until the plants were treated with three-times split at 33% N (S3). Increasing the number of split fertilizations to four times (S4) at 25% N did not affect the LAI significantly when observed at 60 and 90 DAS. Moreover, significant higher LAI was observed for the same fertilization among growing days when the plant was progressed toward the growing period between 30, 60 and 90 DAS and pattern as 90 DAS > 60 DAS > 30 DAS (Table 2).

Similar trends were also observed for the aboveground biomass (Table 2). Plants accumulated significantly more aboveground biomass with an increased split application of fertilizers until S3 at 60 and 90 DAS compared to control. However, there was no significant difference in aboveground biomass accumulation in plants treated with S4 of N fertilizer at 60 and 90 DAS compared to S3. Furthermore, significant difference was also observed for the same fertilization among growing days and rank as 90 DAS > 60 DAS > 30 DAS (Table 2)

Table 3 showed the soybean crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR) in response to four different splits application of 30 kg N ha^{-1} compared to non-fertilized plants. The split N application significantly influenced the soybean CGR, NAR and RGR compared to the control. However, increasing the frequency of N supplies in split application to four times (S4) did not influence the CGR, NAR and RGR significantly compared to S3 for both observations at 30-60 DAS and 60-90 DAS. In addition, significant difference was observed when the soybean plants treated within the same fertilizer treatment (in S1, S2, S3 and S4) progressed towards the growing period between 60 and 90 DAS.

Yield attributes and agronomy N use efficiency (ANUE)

The yield attributes and ANUE were significantly affected by the increased frequency of split N applications (Table 3 and 4). In terms of ANUE, there were no significant differences between the fertilizer treatment of S1 and S2. However, ANUE increased significantly as the frequency of split application increased from S2 to

S3. A maximum ANUE was achieved in the S4 treatment but, it was not significantly different from S3 treatment (Table 3). The yield attributes including 1,000-seed weight, total pods plant^{-1}, seed weight plant^{-1} and seed pod^{-1} showed parallel trend toward different split fertilization treatments. However, significantly yield attributes were increased under the S3 and S4 than under the S1, S2 and control (Table 4).

Table 3. Effects of splitting N fertilizations on agronomy N use efficiency (ANUE), crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR).

Treatment 30 kg N ha^{-1}	ANUE (kg seeds kg N^{-1})	CGR (mg cm^2 d^{-1}) 30-60 DAS	CGR (mg cm^2 d^{-1}) 60-90 DAS	NAR (mg cm^2 d^{-1}) 30-60 DAS	NAR (mg cm^2 d^{-1}) 60-90 DAS	RGR (mg g^{-1} d^{-1}) 30-60 DAS	RGR (mg g^{-1} d^{-1}) 60-90 DAS
Control		318.6 ± 39 (c, ns)	329.5 ± 92 (c, ns)	0.42 ± 0.04 (c, ns)	0.16 ± 0.04 (c, ***)	64.00 ± 3.1 (c, ns)	21.11 ± 5.6 (b, ***)
N_{30}-S1	13.6 ± 8.4 b	530.4 ± 34 (b, ns)	608.8 ± 88 (b, *)	0.60 ± 0.05 (b, ns)	0.22 ± 0.03 (b, ***)	77.20 ± 3.4 (b, ns)	23.63 ± 2.6 (b, ***)
N_{30}-S2	17.3 ± 9.4 b	558.3 ± 35 (b, ns)	651.6 ± 97 (b, *)	0.63 ± 0.05 (b, ns)	0.24 ± 0.05 (b, ***)	78.69 ± 2.1 (b, ns)	23.99 ± 2.7 (b, ***)
N_{30}-S3	32.0 ± 10.6 a	738.2 ± 31 (a, ns)	1178.5 ± 99 (a, ***)	0.76 ± 0.06 (a, ns)	0.37 ± 0.04 (a, ***)	87.00 ± 1.5 (a, ns)	30.25 ± 2.0 (a, ***)
N_{30}-S4	38.8 ± 10.8 a	761.2 ± 27 (a, ns)	1234.5 ± 150 (a, ***)	0.75 ± 0.07 (a, ns)	0.38 ± 0.04 (a, ***)	87.66 ± 1.0 (a, ns)	30.55 ± 2.9 (a, ***)

Data are means ± SD (n=3). N_{30} indicates 30 kg N ha^{-1} season^{-1}.
Significant differences between N fertilizations for the same growing period (a, b, c).
Significant differences between growing period for the same N fertilization (*P < 0.05; **P < 0.01; ***P < 0.001 and ns not significant).

Biomass and yield production

At harvest (127 DAS), the total aboveground biomass was significantly influenced by the split application of N (Table 4). Production of straw biomass, aboveground biomass (straw + seed) and seed yield were highest under S4, although they were not significantly different from S3 treatment. The harvest index varied significantly between the no-N fertilization control and N fertilizer treatments. There were no significant changes in the harvest index among the four split fertilization, but a significantly higher harvest index was observed under the no-N fertilization control (Table 4).

Table 4. The effects of splitting N fertilizations on yield attributes and total aboveground biomass of soybean at 127 DAS.

Treatment 30 kg N ha^{-1}	Yield attributes: Total pod (plant^{-1})	Total seed (pod^{-1})	1,000-seed weight (g^{-1})	Seed weight (g plant^{-1})	Total aboveground biomass (straw + seed): Straw (t ha^{-1})	Seed yield (t ha^{-1})	Straw + seed (t ha^{-1})	Harvest index (%)
Control	29.1±0.3 c	1.80±0.05 c	97.4±5.03 c	6.01±0.7 c	1.27±0.04 c	1.42±0.08 c	2.6±0.09 c	52.8±1.8 a
N_{30}-S1	35.6±2.9 b	2.40±0.15 b	108.9±4.6 b	12.00± 0.1 b	1.65±0.08 b	1.64±0.15 b	3.3±0.21 b	49.7±1.9 b
N_{30}-S2	36.3±1.9 b	2.50±0.07 b	112.0±3.4 b	12.00± 0.1 b	1.69±0.04 b	1.71±0.10 b	3.4±0.11 b	50.2±1.5 b
N_{30}-S3	41.5±2.1 a	2.90±0.13 a	122.1±3.5 a	17.10±0.9 a	2.03±0.05 a	1.95±0.17 a	3.9±0.17 a	48.8±2.2 b
N_{30}-S4	42.2± 2.2 a	3.02±0.04 a	122.2±1.5 a	17.40±0.6 a	2.07±0.25 a	2.06±0.14 a	4.1±0.34 a	49.9±2.5 b

Data are means ± SD (n=3). N_{30} indicates 30 kg N ha^{-1} season^{-1}.
Values followed by the same letter within the column are not significantly different at P < 0.05.

Economics

The statistical significance of the soybean productivity that was affected by different rates of fertilizer treatment demonstrated concomitant economic returns. That is, the increasing frequency of split N applications produced higher grain yield, gross and net returns with a higher benefit-cost ratio despite the higher cost of cultivation (Table 5). The maximum values were recorded under S4, although they were not significantly different compared to S3.

Table 5. Effects of splitting N fertilizations on grain yield price, cost of cultivation, gross return, net returns and benefit cost ratio of soybean. Data (means ± SD, n = 3). Abbreviations: N_{30}= 30 kg N ha^{-1} season^{-1}; S_1= One time basal application at sowing; S_2= an equal two times of 50% N split rate at sowing and 10 DAS; S_3= an equal three times of 33% N split rate at sowing, 10 DAS and 20 DAS; and S_4 = an equal four times of 25% N split rate at sowing, 10 DAS, 20 DAS and 30 DAS.

Nitrogen 30 kg ha^{-1}	Economics: Grain yield (US$ tonne^{-1} price)	Cost of cultivation (US$ ha^{-1})	Gross returns (US$ ha^{-1})	Net returns (US$ ha^{-1})	Benefit cost ratio
Control	1084.5 ± 23.2c	641.0 ± 3.1c	829.4 ± 18.5c	188.3 ± 15.4c	0.30 ± 0.03c
N_{30}-S_1	1254.7 ± 126.2b	665.1 ± 13.6b	973.6 ± 69.3b	308.4 ± 58.3b	0.46 ± 0.09b
N_{30}-S_2	1303.0 ± 112.2b	670.2 ± 16.1b	1004.6 ± 46.6b	334.4 ± 46.7b	0.50 ± 0.06b
N_{30}-S_3	1485.9 + 93.6a	694.6 ± 7.7a	1150.7 ± 46.1a	456.1 ± 38.4a	0.66 ± 0.05a
N_{30}-S_4	1623.0 ± 69.4a	708.1 ± 11.2a	1232.0 ± 67.1a	523.8 ± 55.9a	0.74 ± 0.07a

Significant differences between N fertilizations (a, b, c, d, e) at P < 0.05.

Relationships between plant growth parameters and biomass and/or yield production

The aboveground biomass (leaf + stem) showed positive relationships with plant height (r^2= 0.64, P = 0.001) and leaf area index (r^2= 0.74, P = 0.002) at 90 DAS (Figure 1a-b). Similar positive relationships were also observed with the total pod per plant (r^2= 0.66, P = 0.003) and CGR (r^2= 0.98, P = 0.002) (Figure 1 c-d). The seed yield at harvest 127 DAS had a strong positive relationship with aboveground biomass (straw + seed) (r^2 = 0.91, P = 0.002), but fairly positive relationship with total pod plant^{-1} (r^2 = 0.54, P = 0.002), 1,000-seed weight (r^2 = 0.55, P = 0.003) and CGR (r^2= 0.67, P = 0.002) (Figure 1 e-h).

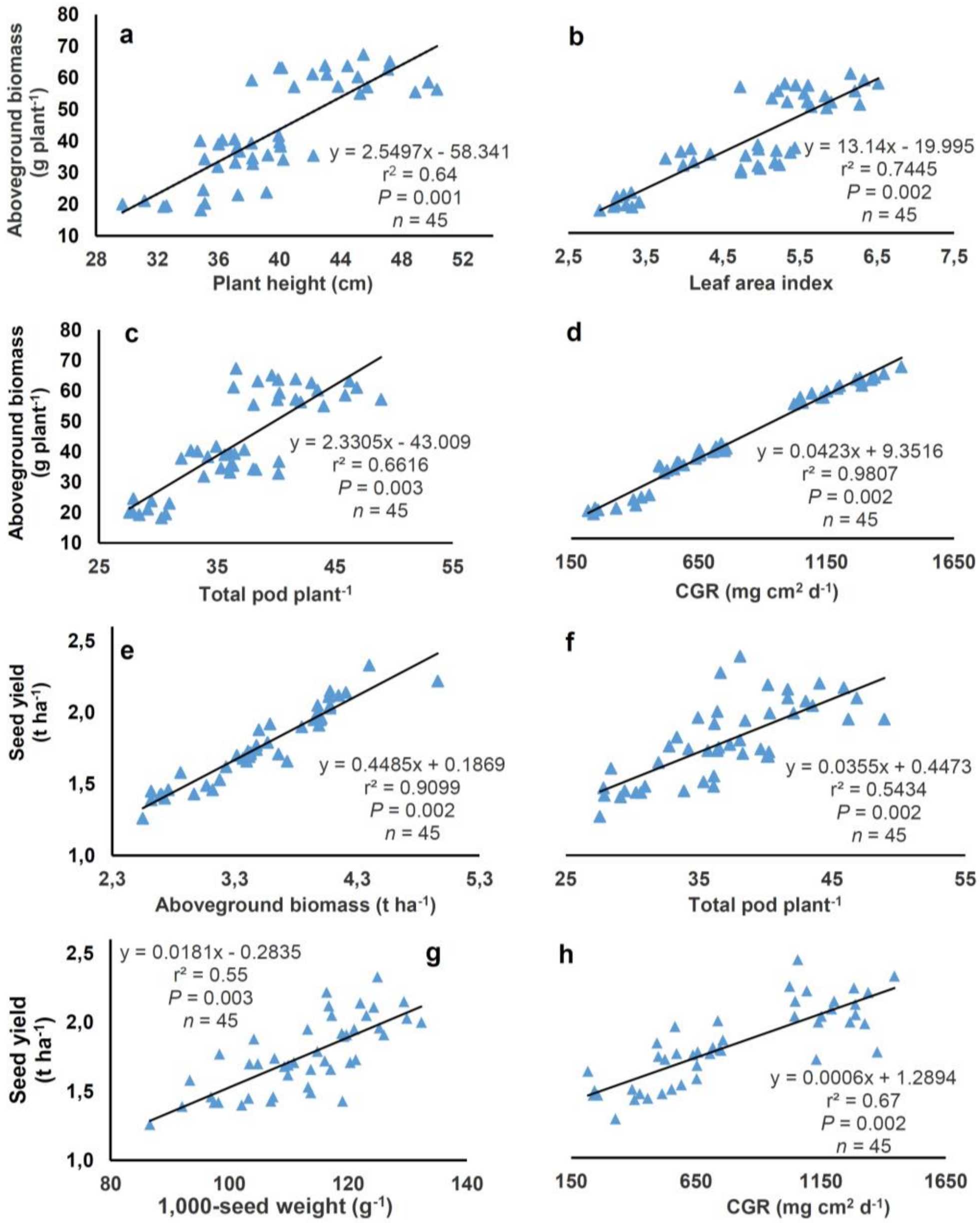

Figure 1. Relationship between aboveground biomass (leaf + stem) with plant height (A) or leaf area index (b) at 90 DAS and total pod plant^{-1} (c) at harvest 127 DAS or CGR (d), between seed yield with aboveground biomass (e) or total pod plant^{-1} (F) and 1,000-seed weight (g) or CGR (h) at harvest 127 DAS.

Positive relationships were also observed between leaf area index (r^2 = 0.69, P = 0.003) and NAR (r^2 = 0.93, P= 0.001) with CGR at 90 DAS (Figure 2 a-b), between seed yield (r^2= 0.88, P = 0.03) and aboveground biomass (straw + seed) (r^2= 0.84, P = 0.004) with agronomy N used efficiency (ANUE) at harvest 127 DAS (Figure 2 c-d), between leaf area index with plant height at 90 DAS (r^2= 0.52, P = 0.002, Figure 2e) and between NAR with RGR (r^2 = 0.83, P = 0.00; Figure 2 f) and seed yield (r^2 = 0.63, P = 0.001; Figure 2 g).

Discussion

In Afghanistan, the occasional cultivation of legume crops, without rhizobial inoculation coupled with certain environmental factors have considerably reduced the number and activities of N_2 fixing bacteria in soil. Hence, the crop growth and yield production mostly depend on the external application of N fertilizer and its management.

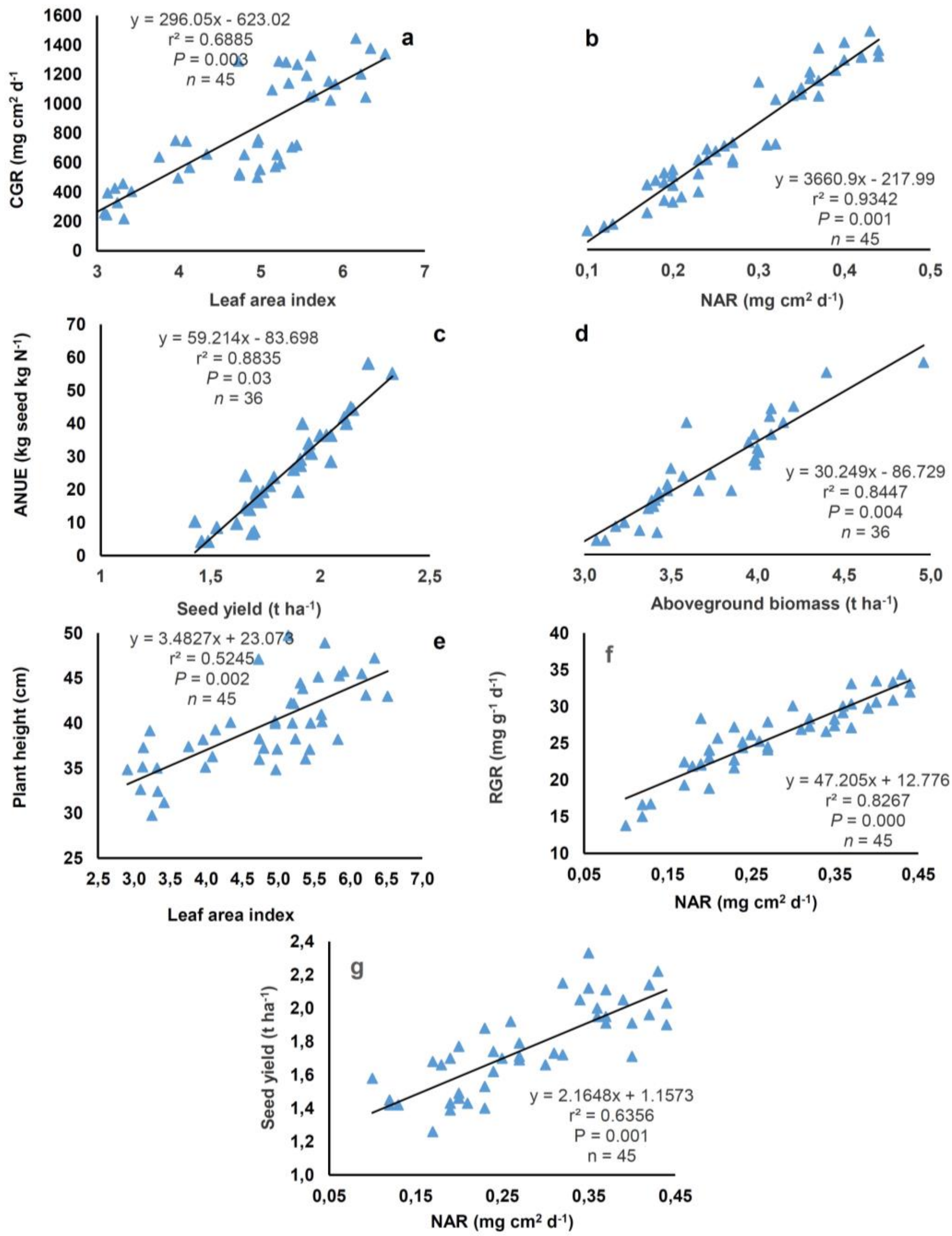

Figure 2. Relationships between CGR with leaf area index or NAR (a or b) at 90 DAS, between Agronomy N use efficiency (ANUE) with seed yield or aboveground biomass (straw + seed) at harvest 127 DAS (c and d), between plant height with leaf area index (e) or NAR with RGR at 90 DAS (f) and NAR with seed yield at harvest 127 DAS (g).

In our field experiment, splitting the 30 kg N ha^{-1} into three and four splits (at 33% and 25% N, respectively) and applied at sowing, 10, 20 and 30 DAS resulted in significantly higher physiological traits of soybean i.e., taller plant height, higher leaf area index and greater aboveground biomass (Table 2). Meanwhile, the application of N fertilizer in fewer splits notably two-times at 50% N each at sowing and 10 DAS as well as the one-time basal application at sowing resulted in significantly lower physiological traits; there were no significant differences compared to plants treated with no-N fertilization (Table 2).

The differences observed could be attributed to N management since the gradual application in splits from sowing to 30 DAS allows the soil to replenish its N pool, timely for plant uptake and thereby increased plant growth and yield. Furthermore, soybean requires less amount of N at its initial growth days since the plant root is not well developed to absorb more N from soil. If all N is applied at sowing, a portion will be taken up by the plant while the remaining will be lost through denitrification, volatilization, leaching and runoff (Li et al., 2018). Hence, the split application of N fertilizer during the crop growth reduces N losses while improving N use efficiency and fulfilling the crop N demand at the early growth and later in the reproductive stage. For example, a frequent rainfall at the early vegetative stage during the spring could often result in N losses due to run-off and/or leaching, while a split fertilization would decrease N leaching in the soil profile (Gan et al., 2002). Higher yield production and reduce N losses to the environment was positively related with the decrease of the basal N ratio while the increase of N split numbers during growth stages of rice (Li et al., 2018). Fertilization of external N at an earlier growth stage to sustain later growth/development and seed yield (Salvagiotti et al., 2009).

The results from our field study further indicate that soybean's growth increased in correspondingly increased with the increased split application of N fertilizers (Table 3). Both S3 and S4 significantly increased CGR, NAR and RGR. In addition to the abovementioned plant height, LAI and aboveground biomass. Gradual supplementation of N fertilizers from sowing to the initial flowering stage enhanced the soybean's growth. Notably, the beginning of the flowering stage is characterized as a rapid stage with the plant transitioned from vegetative growth to reproductive growth. A substantial amount of nutrients are required at this stage to maintain a better canopy for photosynthesis and accordingly, higher biomass production and grain yield.

These above-mentioned findings are in accordance with results from other studies. For example, Begum et al. (2015) applied 25 and 40 kg N ha^{-1} at sowing and 25 DAS respectively while Mohan and Angadi (2016) supplied 60 kg N ha^{-1} in equal split, each at basal and 40 DAS. Both group of researchers reported significantly higher plant height, LAI and biomass. Furthermore, the application of 20, 25, 10 and 66 kg N ha^{-1} as basal, at V3 stage, 25 DAS and R1 stage or 1.8 g N cm^2 at sowing and 4.2 g N cm^2 at R3 of soybean produced a significantly higher LAI (Zhang et al., 2014; Begum et al., 2015; Mon et al., 2017). Significantly higher biomass was also obtained when 30 N ha^{-1} was applied at sowing and 50 kg N ha^{-1} at the R1 stage (Gan et al., 2002) and higher biomass production was reported when 30 kg N ha^{-1} was applied at 42 DAS (Masaka et al., 2007). The application of 50% before sowing and 50% at a full flowering stage at a total rate of 80 kg N ha^{-1} resulted in higher LAI, biomass production and leaf photosynthesis (Caliskan et al., 2008).

RGR, CGR and NAR are the important plant growth parameters that indicate the net increase in dry matter per unit of dry matter, gain in dry matter production on a unit of land area, respectively. Results from our field study showed that these parameters in parallel with an increasing split number of N fertilization (Table 3). A similar increment was also observed in other studies with the application of 60 kg N ha^{-1} in four splits (basal ½ + V3 $^1/_8$ + R1 $^1/_8$ + R3 $^1/_8$, R5 $^1/_8$) (Mon et al., 2017) or the fertilization of 1.8 g N cm^2 at sowing and 4.2 g N cm^2 at the R3 stage (Zhang et al., 2014) and the application of 0.6 g N pot^{-1} as basal and at the R1 stage (Youn et al., 2009).

The accumulation of exogenous N in plant tissue at the vegetative stage is important for higher biomass and yield production. The plant organ including leaf, pod wall and stem at the vegetative period acquire N from BNF and the soil. At the onset of the seed filling stage, plants remobilized their stored N from vegetative tissue to reproductive organ and fulfil most of the N demand for seed. Ortez et al. (2019) demonstrated that 61% of N remobilization from vegetative tissue to seed at the R5 stage in soybean and 12% of more N uptake from the soil at the seed filling stage. It also indicates that split application of N at the vegetative stage remobilize more N compared to a single N application at sowing (Table 4). Salvagiotti et al. (2009) studied the influence of the application of 180 kg N ha^{-1} application in two equal splits on N remobilization i.e., 50% before planting and at the vegetative stage of V6. As a result 164 kg N ha^{-1} was remobilized from vegetative to reproductive components, which was 24% greater than a single application either before planting or at the R5 stage. Accordingly, this also translates to a close relationship between biomass, seed components and seed yield. Moreover, Fageria (2014) has indeed shown that there is a strong positive relationship between plant dry weight and seed yield for soybean and faba bean.

In this study, the effects of splitting the N fertilizer produced a consistent trend in aboveground biomass, yield components and seed yield at 30, 60, 90 and 127 DAS (Table 4). Positive relationships were observed between the aboveground biomass, yield components and seed yield (Figure 1 and 2). Seed yield tended to increase with increasing accumulation of aboveground biomass (r^2 = 0.90, P = 002). A linear regression produced a slope of 0.44 t ha^{-1} of seed yield from 1 t ha^{-1} aboveground biomass. Accordingly, the positive relationship between ANUE with seed yield was observed in this study. This is expected since soybean yield is heavily influenced by N uptake. The data from our field experiment further indicate that split application of 30 kg N ha^{-1} into S4 or S3 significantly increased pod $plant^{-1}$, total seed pod^{-1}, 1,000-seed weight, seed weight $plant^{-1}$ than with fewer splits application (S1 and S2) of the same N rate and the no-N control (Table 4). Our findings are in accordance with results from other studies where 60, 50, 20, 10 and 25 kg N ha^{-1} rates were applied before sowing, at V2, 25 DAS, R1 and R2 stages and significantly increased pod $plant^{-1}$, total seed pod^{-1}, 1,000-seed weight, seed weight (Gan et al., 2003; Singh and Singh, 2013; Begum et al., 2015; Bobrecka-Jamro et al., 2018).

At the harvest at 127 DAS, soybean aboveground biomass and yield production increased with increased frequency of split N fertilization (Table 4). Among the four split treatments, S_4 or S_3 significantly increased seed yield, straw, total aboveground biomass, compared to S_2, S_1 or control (Table 4). Seed yield treated in S4 or S3 significantly increased by 25% and 18% over S1 and 21% and 14%, respectively over S2 and 45 and 37% compared to the control. Similar findings were also reported in the previous studies. For instance, lowering the dose of N fertilization and the timing of application produced a significantly higher seed yield of soybean (Gan et al., 2002; Masaka et al., 2007) or splitting the rate from 60 or 90 to 30 kg N ha^{-1} as a basal, 30 DAS, and at the (R1) stages (Sawyer 2001; Bobrecka-Jamro et al., 2018). Splitting the application of 30, 60, 90 kg N ha^{-1} as basal and topdressing also resulted in higher straw, biological and seed yield (Singh et al., 2001).

Economically, increasing the frequency of split fertilizer treatments incurred a higher cost by 3.7, 4.6, 8.4, 10.5 % from S1 to S4 (Table 5). Compared to the control, the S3 and S4 treatment produced significantly higher gross returns of 38.7 and 48.5 %, respectively and net returns of 142.1 and 178.1 %, respectively. The net returns were mainly due to the grain yield associated with the market price. Despite incurring a higher cost of cultivation with the splitting frequency of fertilizer treatment, the benefits outweighed the cost because higher grain yield and gross net returns were obtained when the soybean plants were treated with an increased split application of N fertilizer (Table 5).

The benefit-cost (BC) ratio is an index of gross return and cost of cultivation (fertilizers, crop management costs, etc.) (Table 5). Similar benefits were also reported in other studies. For example, Ali et al. (2015) applied 90 kg N ha^{-1} in two equal split as ½ at sowing + ½ at 30 DAS in addition to 90 P_2O_5 + 60 K_2O kg ha^{-1} at sowing. As a result, the researchers obtained a higher BC ratio (1:6.05) as compared to the application of 120 kg N ha^{-1} in two equal split as ½ at sowing + ½ at 30 DAS + 90 P_2O_5 + 90 K_2O kg ha^{-1}. In another study, Chowdhury et al. (2014) conducted a field study on soybean and applied three doses of 17, 25, 28 N kg ha^{-1} along with other macro- and micro-nutrients. Each N dose was applied as in two equal splits at sowing and 22 DAS, and the results revealed that the application of 17 kg N ha^{-1} achieved significantly higher gross return (1347 US$ ha^{-1}), net returns (726 US$ ha^{-1}) and BC ratio (2.16) compared to other treatments in the study.

In the present study, however, it is important to highlight that the S3 treatment produced favourable outcomes compared to the S4 treatment since there were no significant differences in the agronomic performance between the two treatments. Besides, the S3 treatment is also more cost-effective and produced fairly decent economic returns compared to S4. This will bring relief to the farmers since a farmer who is taking care of a specific crop only received 1/6 of the total yield from her or his landlord at the end of a crop season in Afghanistan.

Conclusion

Both treatments in the three (33%) and four (25%) times of N split fertilization for a total rate of 30 kg N ha^{-1} have improved the N use efficiency and met the N requirement of soybean's growth and yield production. They also produced significantly positive effects on the growth and yield of soybean than those at one (100%) or two (50%) times of N split fertilization. Although splitting the N application four times would produce maximum agronomic performance – highest ANUE and seed yield, it was also costly with a disadvantage to the farmers. Results from this present study demonstrate that splitting the N fertilizer up to three times is both agronomically and economically optimum and therefore recommended for practice in Afghanistan.

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