

Grain yield and quality responses of durum wheat (*Triticum turgium* L. var. durum) to nitrogen and phosphorus rate in Yilmana Densa, Northwestern Ethiopia

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ABSTRACT

Proper rate of fertilizer application is the major factor that affects yield and quality of durum wheat. Location specific fertilizer recommendation is lacking for durum wheat, therefore, nitrogen (N) and phosphorus (P) field experiment on durum wheat was carried out on farmer's field in 2017 and 2018 cropping seasons at Yilmana Densa district, Northwestern Ethiopia. The objective was to examine the response of durum wheat to N and P application and determine the optimal rate of nitrogen (N) and phosphorus (P) for durum wheat production in the district and similar agro ecology areas. Factorial combinations of four N levels (0, 90, 180 and 270 kg ha⁻¹) and four P levels (0, 17.5, 35 and 52.5 kg ha⁻¹) were evaluated in a randomized complete block design with three replications per site, over two sites in each year. Results indicated that N significantly affected most of the crop parameters including grain yield, plant height, total number of tillers and effective tillers, spike length and seeds per spike, and the maximum value of the parameters recorded at the highest rate of N while application of phosphorus affected grain yield, plant height and spike length. The interaction effect of N and P significantly affected grain yield, plant height and seeds per spike. Grain yield showed quadratic and linear response to N and P application, respectively. The maximum grain yield (5182 kg ha⁻¹) was obtained from the highest rate 270/52.5 N/P kg ha⁻¹. The economic analysis revealed application of 270/52.5 N/P kg ha⁻¹ gave the highest net benefit of 169741 ETB ha⁻¹ with marginal rate of return 1453% and full fills the standard for quality of durum wheat production and hence can be recommended in Yilmana Densa District and other similar agro-ecologies in Ethiopia. The study revealed the need to further investigation for optimal N and P rates for different durum wheat varieties as they differ in protein content under the same management practices.

1. Introduction

Durum wheat (*Triticum turgium* L. var. durum) is the second most important cultivated wheat species in the world next to bread wheat [1]. Ethiopia is the center of diversity for durum wheat [2] also the leading producer in sub-Saharan Africa countries. In Ethiopia, durum wheat is traditionally grown on heavy black clay (Vertisols) on the residual moisture and recently being grown on

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light soils under rainfed conditions. Although the primary use of durum is in the manufacture of pasta products (macaroni, spaghetti, and noodles), it is also used in making fermented leveled bread, called *injera* and other indigenous food preparations in Ethiopia [3]. According to Ref. [4], bread and durum wheat were produced in Ethiopia by approximately 4.94 million households during the “meher” and “belg” (rain and dry) seasons on an estimated 2.13 million ha of land, with an annual production of 6.23 million tons and a mean national yield of 3.05 t ha^{-1} . Regardless of the long history of durum wheat cultivation and its importance in Ethiopian agriculture, little emphasis was given on improving the quality and grain yield of durum wheat in the past and its average productivity remains far below the world average (3.5 t ha^{-1}) and very low in Amhara region as compared to the national average (3.046 t ha^{-1}) [4, 5].

Low soil fertility due to continuous nutrient uptake of crops, mono-cropping system and slow progress in developing wheat cultivars with durable resistance to disease are considered the most important constraints limiting wheat production in Ethiopia [6]. The soils of Ethiopian highlands are mainly deficient in organic matter, nitrogen and phosphorus. These deficiencies are due to nutrient mining by cereal mono-cropping and leaching losses which resulted reduction in yield and quality of crops [7]. Mineral fertilizers or combined organic and inorganic fertilizers have been found to have a significant beneficial effect on food production worldwide, and are an indispensable component of many agricultural systems [8]. The low productivity also allied with low fertilizer use (nitrogen and phosphorus) and insufficient organic matter application [9]. Currently, there is a high demand to both commercial and small-scale farmers for durum wheat with high grain yield and better end-use quality. The yield and end-use quality of durum wheat depend upon the protein and gluten content which is largely influenced by the genotype and environment especially the nitrogen element available in the soil [10]. On the other hand most of the quality parameters of durum wheat increased as nitrogen application increased beyond 120 kg N ha^{-1} [9]. Phosphorus (P) is the second most important element for crop production, it is known to be involved in many physiological and biological processes of plants [7].

Consequently, low volumes and poor quality of the national durum wheat production in Ethiopia leads the pasta industries to import the required raw material from abroad [3]. These pasta producers used to rely on massive importation of durum wheat grains, which was not a sustainable long-term business strategy due to high and volatile costs. Pasta import increased two-fold between 2011 and 2015, when it reached 50,000 t at a cost of about €40 million [11]. The food crises all over the world and increasing population pressure demand urgent need to increase the quantity and improve the quality of grains. To reverse this trend, the Ethiopian Millers Association has eagerly explored the possibility to procure the needed raw material directly from local farmers to reduce production costs and increase competitiveness against foreign pasta imports. Increasing domestic quality produce could mitigate not only the shortage but also substitutes the import of huge industrial raw materials. The national research institutes and pasta processing industries also jointly working to improving durum wheat grain quality. There is also a plan to establish pasta and macaroni industries by public developmental agencies and unions in various parts of the Amhara region. However, there is limited information on the effect of NP fertilizer rate on the production and quality of durum wheat, as a result, farmer's even researchers use the bread wheat NP rate recommendation for durum wheat which may have effect on the productivity and quality of durum wheat. Therefore, the objective of

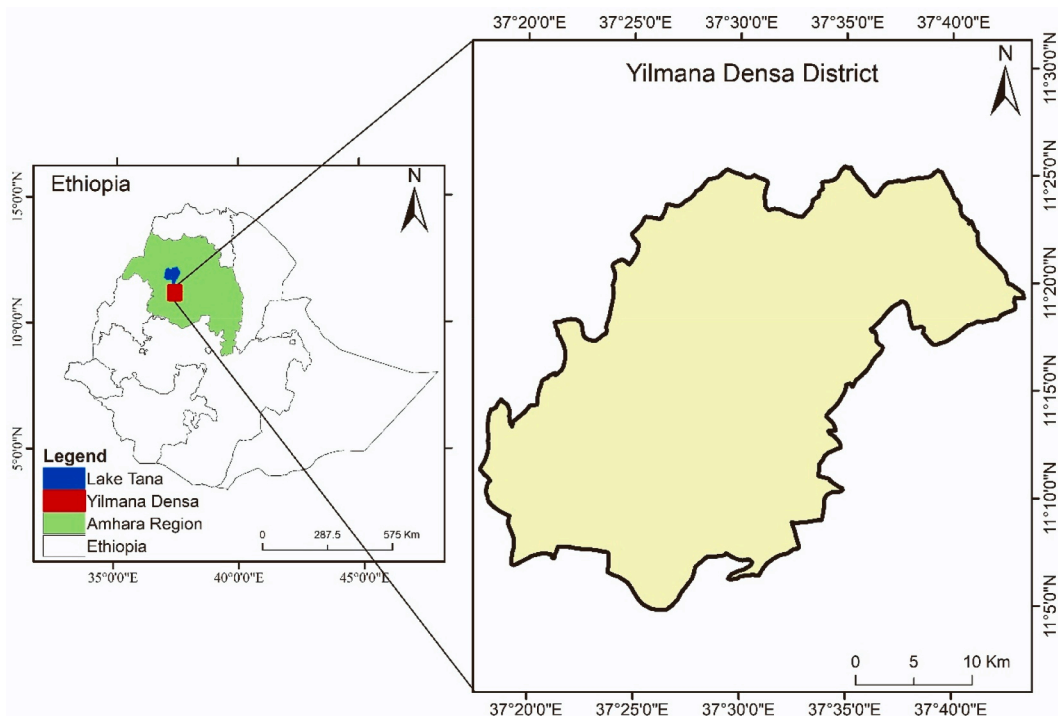


Fig. 1. Map of the study area.

the experiment was to examine the response of durum wheat to N and P application and determine the optimal rate of nitrogen (N) and phosphorus (P) for durum wheat production in Nitosols of Yilmana Densa district and similar agro ecology areas of northwestern Ethiopia.

2. Material and methods

2.1. Description of the study area

The experiment was conducted in Yilmana Densa district on farmer's field during the 2017 and 2018 main cropping season (two fields in each year). Yilman Densa district is located on latitude of 11°5' to 11°25' N and longitude of 37°20' to 37°40' E at about 42 km far from Bahir Dar with an altitude ranging from 2216 to 2696 m.a.s.l. (Fig. 1). The rainfall pattern of the study area is unimodal, about 79% (1127 mm) of the rainfall received from June to October. During these months the mean minimum and maximum temperature ranged from 8 to 12 °C and 23 to 26 °C, respectively (Fig. 2). Most of the farmers in the study area used cereal based rotation and experimental sites with maize precursor were selected for the study. The soil of the experimental sites was Nitosol and analysis on some of the selected soil physicochemical properties for composite surface soil (0–20 cm depth) samples collected at planting showed pH 4.96, organic carbon 1.6%, total nitrogen 0.16%, available phosphorus 8.49 ppm and cation exchange capacity 28.55 mol (+)/kg soil (NHAc). The soil sample analysis result further revealed the acidic nature of the soil, and available P content was in optimum range according to Ref. [12]. While organic carbon and total N rated low [13]. The overall result of soil analysis showed the need to design for reduce soil acidity, improve organic carbon and total nitrogen through integrated soil fertility management approach.

2.1.1. Treatments, experimental design and procedures

A factorial combination of four N rates (0, 90, 180 and 270 kg ha⁻¹) and four P rates (0, 17.5, 35 and 52.5 kg ha⁻¹) was evaluated in RCBD with three replications in a gross plot size of 4 m in length and 3.2 m in width while the net plot size was 4 m × 2.8 m. All the phosphorous and 1/3 of nitrogen was applied at planting while the remaining 2/3 N was applied during mid-tillering stage of durum wheat. The experimental field was prepared according to the local practice. Thus, the land was ploughed four times using oxen before planting to pulverize the soil and make control of early emerging weeds. Seeds of durum wheat variety 'Mangudo' was uniformly hand drilled at seed rate of 150 kg ha⁻¹ with 20 cm rows spaced and covered by soil on 28th of June to 2nd of July in 2017 and on 3rd of July to 6th of July in 2018. The space between blocks and plots were 1.5 m and 0.6 m, respectively. One hoeing at early tillering stage and hand weeding was carried out to keep the plots free from weeds and to provide better aeration. The next two hand weeding activities were operated at mid-tillering and booting stage of durum wheat.

2.1.2. Data collection and analyses

Data were collected from a total of four experimental sites (two sites in each year) and averaged value of each parameter for the year was considered for analysis. Data on plant height, spike length, number of tillers, effective tillers, seed per spike, thousand kernel weight and dry grain protein content were collected from the net plot using random sampling. Ten randomly selected plants and ten spikes were used to measure plant height and spike length, respectively. Plant height was measured from the bottom of the plant to the tip of spike. Total number of tillers were counted within one metersquare quadrant and those tillers with spikes were considered as effective tillers. Grain yield (Gy) was collected from the whole net plot for the purpose of more accuracy and precision. About 250 g grain samples was taken to measure grain protein content using Infratec 1241 Grain Analyser (Foss, Hilleroed) at Amhara Agricultural Research Institute grain quality laboratory. The Infratec 1241 is a whole grain analyser using near-infrared transmittance technology to test multiple parameters (moisture, protein, oil, starch etc) in a broad range of grain and oilseed commodities.

The data were subjected to analysis of variance (ANOVA) using SAS (statistical analysis System) GLM procedures version 9.0. Combined analysis over years was made considering year as a random variable. The response of wheat to nitrogen and phosphorus rates either for its linearity or quadratic was detected using single degree of freedom orthogonal contrast test. For the quadratic responses, prediction of the optimal level of fertilizer for maximum yield was done using polynomial response equation [14]. Duncan

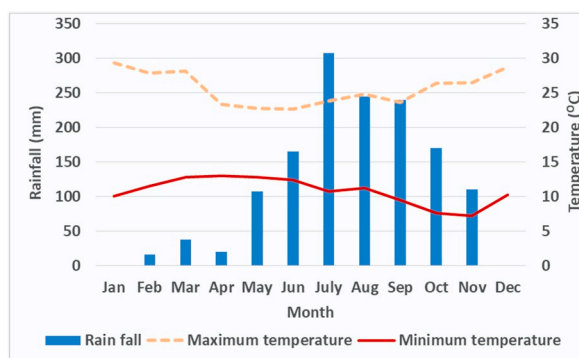


Fig. 2. Monthly average rainfall and monthly mean maximum and minimum temperature during 2017 and 2018 growing seasons in the study area.

multiple range test at 5% of probability levels was used for mean separation when the analysis of variance indicates the presence of significant differences [15]. Correlation analysis also made between grain yield and protein content.

2.1.3. Partial budget analysis

Partial budget analysis was made based on CIMMYT methodology [16]. Besides to the current cost price ratio (CPR), economic return of durum wheat grain yield was performed at the scenario when fertilizer cost increased by double (100%) compared to the current cost while durum wheat grain price remain unchanged. The market prices of durum wheat grain (35.08 ETB kg⁻¹) of the month January and February 2020 was considered for the economic analysis. Similarly, cost of N, ETB 33.33 kg⁻¹ (derived from cost of urea, ETB 15.33 kg⁻¹) and cost of P, ETB 59.75 kg⁻¹ (derived from cost of NPS, ETB 16.25 kg⁻¹) of the year 2020 were used. Fertilizer NPS (19% N, 16.6% P, 7% S) is commonly available in the market and farmers usually used it as source of phosphorus whereas TSP is not easily available and used only for the experiment purpose. The CPRs were calculated and these resulted 0.95 and 1.90 for N cost kg⁻¹ to durum wheat grain price kg⁻¹; and 1.70 and 3.40 for P cost kg⁻¹ to durum wheat grain price kg⁻¹ for the current situation and sensitivity analysis, respectively.

Total cost that varied (fertilizer cost) for each treatment were calculated and treatments were ranked by sorting their total variable cost (TVC) in ascending order and dominance analysis was used to eliminate those treatments costing more but producing a lower net return than the next lowest cost treatment. Net returns were calculated by deducting the variable costs of N and P from the gross return. A treatment that is non-dominated and having the highest net return with minimum acceptable marginal rate of return (MRR) value of 100% is said to be economically profitable [16]. Marginal rate of return was calculated by a change in net return over a change in total variable cost.

3. Results and discussion

3.1. Yield components

Yield components such as plant height, seed per spike, spike length, number of total tillers and effective tillers were analyzed combined over years. Plant height and seeds per spike were significantly affected by main effect of both N and P and their interaction whereas spike length, grain protein content, total and effective tillers were significantly affected by main effect of nitrogen (Table 1). Plant height, total and effective tillers, spike length and seeds per spike increased as N rates increased (Table 2). As the N rate increased from nil to 270 kg ha⁻¹ it had an increment of 41% in plant height and spike length, 89% total tiller, 101% effective tillers and 92% number of seed per spike over the respective control. Increasing in plant height in response to N rate was in agreement with the findings [17] similarly, increasing N rate increased plant height [7]. Spike length increase in response to N and similar result also reported by Ref. [18]. Increasing on number of tillers in response to N rate was also reported by Refs. [7,17]. Increasing in the number of effective/fertile tillers with the increase in nitrogen levels could be attributed to the well-accepted role of nitrogen in accelerating the vegetative growth of plants [18]. An increased application of nitrogen increases the number of kernel per spike [19]. The optimal N and P interaction for the highest plant height and seeds per spike were 270/52.5 N/P kg ha⁻¹ and 270/17.5 N/P kg ha⁻¹ respectively (Table 3). The result indicated that high demand of nutrient especially nitrogen at vegetative and booting stage of the crop to boost the grain yield related components of durum wheat.

3.2. Grain quality parameters

Grain quality was expressed in terms of thousand kernel weight and grain protein content. Thousand kernel weight ranged from 34.8 to 38.3 g (Table 2). As Nitrogen rate increased the TKW increased up to 184 kg ha⁻¹, further increased N decreased TKW. In line with the present result thousand kernels weight ranges of 42.5–49.5 g for durum wheat varieties [20]. The standard thousand-kernel weight for pasta industry is in the range of 35–40 g according to Ref. [21]. Protein content ranged from 7.5 to 11.5%, the highest protein content obtained from high rate of N as protein content is directly influenced by nitrogen rate (Table 2). As the nitrogen rate increased from nil to 270 kg ha⁻¹ the protein content increased by 53%. In agreement with these result the highest grain protein

Table 1

Significance value (P value) on grain yield and yield related parameters of durum wheat at Yilmana Densa district, Northwestern Ethiopia (combined over 2017 and 2018 crop season).

| source of variation | df | GY | TKW | Pht | TT | ET | SPS | SL |
|---------------------|----|-----|-----|-----|----|----|-----|-----|
| Year (yr) | 1 | ns | *** | *** | * | * | ** | *** |
| yr*rep | 4 | ns | ns | ns | ** | ** | ns | ns |
| N | 3 | *** | ns | ** | ** | ** | ** | * |
| yr*N | 3 | ns | * | *** | ** | ** | ns | *** |
| P | 3 | ** | ns | ** | ns | ns | * | ns |
| yr*P | 3 | ns | ns | ns | ns | ns | ns | ns |
| N*P | 9 | * | ns | * | ns | ns | ** | ns |
| yr*N*P | 9 | ns | ns | ns | ns | ns | ns | ns |

*, ** and *** significant difference at 5, 1 and 0.1% level of significance, respectively; ns non-significant difference. GY-grain yield, TKW-thousand kernel weight, Pht-plant height, TT-number of total tillers, ET-number of effective tillers, SPS-number of seed per spike, SL-spike length.

Table 2

Main effect of N rate on some yield components and grain quality parameters of durum wheat at Yilmana Densa (combined over seasons 2017 and 2018, PC only for 2017).

| N rate (kg/ha) | Plant height (cm) | Spike length (cm) | No. of total tiller (m ⁻²) | No. of Effective tiller (m ⁻²) | No. of seeds spike ⁻¹ | TKW (g) | PC (%) |
|----------------|-------------------|-------------------|--|--|----------------------------------|-------------------|-------------------|
| 0 | 56.8 ^c | 3.2 ^d | 34.9 ^d | 31.8 ^c | 19.7 ^c | 34.8 ^a | 7.5 ^c |
| 90 | 76.1 ^b | 4.1 ^c | 50.9 ^e | 49.5 ^b | 29.8 ^b | 37.0 ^a | 7.9 ^c |
| 180 | 79.7 ^a | 4.3 ^b | 62.4 ^b | 60.8 ^a | 35.8 ^a | 38.3 ^a | 9.9 ^b |
| 270 | 80.2 ^a | 4.5 ^a | 65.8 ^a | 63.9 ^a | 37.9 ^a | 36.1 ^a | 11.5 ^a |
| CV (%) | 2.8 | 5.3 | 9.6 | 10.3 | 11.6 | 7.0 | 4.3 |

Numbers with different letter within the column are significantly different at 5% level of significance using Duncan multiple range test. TKW-thousand kernel weight, PC-protein content.

Table 3

The interaction effect of NP fertilizer on plant height and no. of seeds per spike of durum wheat at Yilmana Densa (combined over 2017 and 2018).

| N rate (kg/ha) | Plant height (cm) | | | | Number of seed per spike | | | |
|----------------|-------------------------------|--------------------|-------------------------------|---------------------|-------------------------------|----------------------|-------------------------------|----------------------|
| | P rate (kg ha ⁻¹) | | P rate (kg ha ⁻¹) | | P rate (kg ha ⁻¹) | | P rate (kg ha ⁻¹) | |
| | 0 | 17.5 | 35 | 52.5 | 0 | 17.5 | 35 | 52.5 |
| 0 | 56.8 ^f | 56.05 ^f | 56.66 ^f | 57.65 ^f | 21.83 ^e | 19.17 ^e | 19.31 ^e | 19.58 ^e |
| 90 | 72.53 ^e | 75.98 ^d | 76.81 ^{cd} | 79.03 ^{bc} | 23.46 ^e | 34.40 ^{bc} | 29.44 ^d | 32.04 ^{cd} |
| 180 | 75.69 ^d | 79.63 ^b | 80.76 ^{ab} | 82.92 ^a | 33.78 ^{bcd} | 36.07 ^{abc} | 38.10 ^{ab} | 35.65 ^{abc} |
| 270 | 75.76 ^d | 80.29 ^b | 81.53 ^{ab} | 83.06 ^a | 35.20 ^{abc} | 39.66 ^a | 39.20 ^a | 37.87 ^{ab} |

Numbers with the different letter for each of the parameter are significantly different at 5% level of significance using Duncan multiple range test.

(13.09%) was accumulated at the highest N rate (120 kg N ha⁻¹) [19]. But in contrast to this finding, additional nitrogen fertilization did not significantly increased grain protein content of the durum wheat at Debre Zeit Agricultural Research Center, Ethiopia [9]. The quality of durum wheat is highly dependent on the protein content of the grain, which is largely dependent on genotypes and influenced by environment, especially nitrogen (N) availability of the soil [9]. The acceptable Ethiopian standard protein content for whole durum wheat semolina is 11.5% [22]. The protein content of wheat grains may vary between 10% and 18% of the total dry matter [23].

3.3. Grain yield

The combined analysis over years on grain yield showed non-significant difference of year interaction with N, with P and with N*P indicating that wheat yield response for the treatment is similar trend across the years (Table 1). Grain yield significantly responded to the main and interaction effects of N and P. The response for N was quadratic while P was linear (Fig. 3a and b). The equation for predicting agronomic optimum N rate for highest grain yield (Gy) was generated from the quadratic response function (Eq-1). Accordingly, the highest grain yield (4492 kg ha⁻¹) was obtained on the application of 280 kg N ha⁻¹. While the phosphorus rate for highest grain yield couldn't be determined as the response was linear (Eq-3). The linear grain yield response to P application might be due to soil acidity (pH 4.96, as indicated in the description of the study area) which make the applied phosphorus not fully available to the crop and indicate the need to increase phosphorus level more.

$$Gy = -0.04718N^2 + 26.39031N + 801.9698, R^2 = 0.99 \quad (1)$$

$$26.39031 - 0.09436N = 0 \quad (2)$$

derived from Eq-1.

$$Gy = 2622.47223 + 15.41596P, R^2 = 0.9283 \quad (3)$$

The rate for N*P interaction was determined from multiple regression equation:

$$Gy = 686.36731 + 24.24907N + 4.4039P - 0.04718N^2 + 0.08157NP \quad (4)$$

The highest grain yield (5182 kg ha⁻¹) obtained from the application of 270/52.5 kg N/P ha⁻¹ while the lowest yield obtained at the lowest NP rate (Fig. 3c). Application of 270/52.5 kg N/P ha⁻¹ gave a yield advantage of 4496 kg ha⁻¹ (655%) over unfertilized plot. Yields increased with increased NP fertilizer rates. This result in line with the finding of [7,19]. The correlation analysis between grain yield and protein content indicated positive and significant ($P < 0.001$) correlation with correlation coefficient, $r = 0.76$. This implies that high management levels towards increasing a grain yield improves the protein content and then grain quality. The highest grain yield of any crop is the result of positive relationships of most yield components due to nitrogen fertilizer application [17,19].

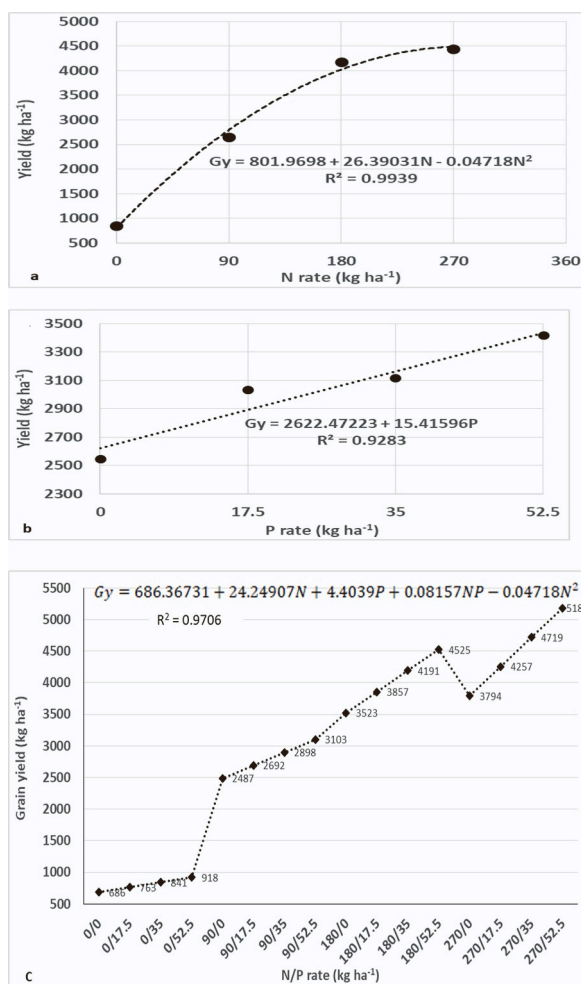


Fig. 3. Grain yield response of durum wheat to applied nitrogen (a) and phosphorus rate (b) and NP interaction (c) in Yilmana Densa district, Northwestern Ethiopia (combined over 2017 and 2018).

Table 4

Partial budget analysis of N and P fertilizer application on durum wheat production in Yilmana Densa district (combined data of 2017 and 2018 cropping season) under two scenarios

| Treatments | | Grain yield (kg ha ⁻¹) | Gross benefit (ETB ha ⁻¹) | Current situation | | | Sensitivity analysis | | |
|-------------------------------|-------------------------------|------------------------------------|---------------------------------------|-----------------------------|----------------------------|---------|-----------------------------|----------------------------|---------|
| N rate (kg ha ⁻¹) | P rate (kg ha ⁻¹) | | | TVC (ETB ha ⁻¹) | NB (ETB ha ⁻¹) | MRR (%) | TVC (ETB ha ⁻¹) | NB (ETB ha ⁻¹) | MRR (%) |
| 0 | 0 | 686 | 24091 | 0 | 24091 | 0 | 24091 | | |
| 0 | 17.5 | 763 | 26797 | 1046 | 25751 | 159 | 24705 | 29 | |
| 0 | 35 | 841 | 29502 | 2091 | 27411 | 159 | 25319 | 29 | |
| 90 | 0 | 2487 | 87281 | 2999 | 84281 | 6262 | 81282 | 3081 | |
| 0 | 52.5 | 918 | 32207 | 3137 | 29070 D | | 6273 | 25933 D | |
| 90 | 17.5 | 2692 | 94495 | 4045 | 90450 | 590 | 8090 | 86405 | |
| 90 | 35 | 2898 | 101710 | 5091 | 96619 | 590 | 10181 | 91529 | |
| 180 | 0 | 3523 | 123642 | 5999 | 117643 | 2315 | 11997 | 111645 | |
| 90 | 52.5 | 3103 | 108924 | 6136 | 102788 D | | 12272 | 96652 D | |
| 180 | 17.5 | 3857 | 135366 | 7044 | 128322 | 1021 | 14089 | 121277 | |
| 180 | 35 | 4191 | 147090 | 8090 | 139000 | 1021 | 16180 | 130910 | |
| 270 | 0 | 3794 | 133176 | 8998 | 124178 D | | 17996 | 115180 D | |
| 180 | 52.5 | 4525 | 158814 | 9135 | 149678 | 1021 | 18271 | 140543 | |
| 270 | 17.5 | 4257 | 149409 | 10044 | 139366 D | | 20087 | 129322 D | |
| 270 | 35 | 4719 | 165643 | 11089 | 154554 | 250 | 22178 | 143464 | |
| 270 | 52.5 | 5182 | 181876 | 12135 | 169741 | 1453 | 24270 | 157606 | |

TVC-total variable cost, NB-net benefit, MRR-marginal rate of return, ETB-Ethiopian Birr, D-dominated treatment.

3.4. Economic benefit

The partial budget analysis indicated most of the treatments gave highest net benefits with marginal rate of return greater than 100% (Table 4). Fertilizer application of 270 kg N with 52.5 kg P ha⁻¹ gave highest net benefit of 169741 Ethiopian Birr (ETB) ha⁻¹ with marginal rate of return (MRR) 1453% followed by fertilizer rate of 270 kg N with 35 kg P ha⁻¹ which gave net benefit of 154554 ETB ha⁻¹ with marginal rate of return 250%. Sensitivity analysis (when the fertilizer cost increased by double while grain price remain unchanged) revealed the first option of the recommendation (270 kg N with 52.5 kg P ha⁻¹) remain stable with net benefit of 157606 ETB ha⁻¹ and MRR 676%. This recommendations can full fills the standard for quality of durum wheat (36.1 g thousand kernel weight and 11.5% protein content).

4. Conclusion and recommendations

Nitrogen and phosphorus fertilizer showed quadratic and linear response to grain yield, respectively. Application of 270/52.5 kg N/P ha⁻¹ gave a yield advantage of 655% over unfertilized and can be recommended as the first option where as depending on fertilizer cost variability fertilizer application of 270/35 N/P kg ha⁻¹ or 180/52.5 N/P kg ha⁻¹ can be considered as alternative option for poor farmers who are in constraint of cash. The linear response of grain yield to phosphorus fertilizer application indicated that optimal agronomic rate couldn't be achieved and implies further investigation in the study area. Moreover, as durum wheat varieties differ in protein content under the same management practices, selection of those varieties with high protein content help a grower to reduce N inputs to achieve high protein content at lower N level should be a research area.

Author contribution statement

Bitwoded Derebe Agegn: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Alemayehu Assefa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Nigatu Gebrie: Agegnehu Shibabaw: Wudu Getahun: Oumer Beshir: Abebe Worku: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The author declared that there is no conflict of interest.

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