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Comparing the effect of organic and chemical nutritional management and intercropping with clover on the quantity and quality of different wheat (*Triticum aestivum* L.) cultivars

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Abstract

Optimal availability of nutrients, particularly nitrogen (N), from authorized resources is one of the most important issues in organic agriculture. Intercropping forage legumes with cereal crops may be a way of providing ecological services such as providing N for companion or following crops by biological N fixation. To determine the effect of biological, chemical, and organic fertilization on the quality and quantity of ten different wheat cultivars, an experiment was conducted during two successive growing seasons. The three experimental factors (F1, F2, F3) were: mix cropping with Persian clover (*Trifolium resupinatum* L.) (F1: biological N fixation, as control); chemical nitrogen fertilizer (F2: 100 kg ha^{-1}, urea), and organic cow manure (F3: 40 t ha^{-1}). The vegetative growth parameters improved by F1 and F2 treatments. The F2 had the highest values of spikelet and seed number per spike, 1000-grain weight, and seed yield, followed by F1. The cv. 'Kabul-13' was the best among all tested cultivars in terms of almost all reproductive parameters. The highest seed yield was obtained by F2 'Kabul-13' (8.02 t ha^{-1}), and F1 'Kabul-13' (7.26 t ha^{-1}). By considering seed yield, 'Chont-1' and 'Kabul-13' were more suitable to be intercropped with clover. The effect of experimental factors was not significant on seed potassium and phosphorus contents, while percentage of seed protein improved by F3 (13%), and 'Mugawim' (13.59%). The highest protein yield was gained by F2 and F1 (820 and 650 kg ha^{-1}) and also by 'Kabul-13' (850 kg ha^{-1}). Overall, mixed cropping of wheat and clover can be suggested as a possible alternative for nutrients providing in wheat production in low input agro-ecosystems.

Keywords: harvest index; mix cropping; protein yield; seed yield

Introduction

In agrosystems efforts to achieve more productivity and promote sustainability has been increased over time (Dhima *et al*., 2007). In recent years, one of the main important problems in agriculture is the high cost

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of fertilizers. Besides that, soil and water pollution imposed mainly by chemical fertilizers, and global warming mainly through emissions of nitrous oxide are the other important problems related to the use of fertilizers (Masclaux-Daubresse *et al*., 2010; Wu *et al*., 2021). Therefore, applying environmentally friendly strategies such as symbiosis relationships of plant with microorganisms to reduce the negative effects of fertilizers is considered beneficial (Rosenblueth *et al*., 2018). In this path, the introduction of leguminous plants into the agroecosystem can be an important part of the path towards sustainable agriculture (Rodriguez *et al*., 2020).

Cereal-legume intercropping offers potential benefits in low-input cropping systems, where nutrients in particular nitrogen and phosphorus are limited (Mouradi *et al*., 2018). Intercropping of cereal and legume crops is a common cropping system, especially in arid and semi-arid areas (Esmaeili *et al*., 2011). This strategy can provide ecological services such as increasing nitrogen availability in the cropping system by biological nitrogen fixation, reducing nitrogen leaching and supplying nitrogen to the following crop (Anil *et al*., 1998; Rodriguez *et al*., 2020). There are also other benefits for intercropping of cereal with legumes, including increase in carbon sequestration, better control of weeds, pests and diseases, elevated land equivalent ratio and reduced soil erosion (Daryanto *et al*., 2020).

Results of a meta-analysis study revealed that cereal-legume intercropping stimulates complementary nitrogen use between legumes and cereals by increasing N_2 fixation by legume crop and increasing soil nitrogen acquisition in cereals (Rodriguez *et al*., 2020). In another study it was reported that legume (faba bean) and cereal (barley) intercropping was beneficial for barley in terms of increasing the growth as well as phosphorus availability and uptake, through the stimulation of APase activity in both rhizosphere and root nodules (Mouradi *et al*., 2018). Dabin *et al*. (2016) found that legume-wheat rotation replaced by average 31% of the applied mineral nitrogen fertilizer in wheat cultivation. Dhima *et al*. (2007) found that vetch–wheat intercropping systems was profitable and had a yield advantage. In another study on wheat, it was concluded that nitrogen chemical fertilizer as single or combined with organic manure increased the grain yield and seed nitrogen content (Yang *et al*., 2020). Nuru Seid and Tarikua Shumi (2020) also reported that integrated application of organic manures and mineral fertilizers leads to increase in wheat yield and grain NPK content.

Another strategy to increase nutrient use efficiency and reduce the negative impact of fertilization is to breed cultivars that can absorb and utilize nutrients, particularly nitrogen, more efficiently. This strategy will reduce environmental pollution, and will meet and improve grain yield and quality (Bahrman *et al*., 2004; Cormier *et al*., 2016). For instance, cultivars with dipper root system will probably uptake nitrogen from deeper soil layers and reduce nitrate leaching (Rasmussen *et al*., 2015). The other key processes in wheat breeding towards enhanced sustainability include nitrogen uptake efficiency, utilization efficiency, partitioning (harvest index) and trade-offs between yield and quality aspects (grain nitrogen content) (Hawkesford and Riche, 2020). Researchers believe that it is possible to employ genetic variation available among modern cultivars to further improve nitrogen use efficiency. However, to speed up the breeding process it requires integration of agronomy, crop physiology, and efficient selection strategies (Bueren and Struik, 2017). In a study on six different cultivars of wheat, it concluded that modern cultivars had higher root nitrogen uptake efficiency than old cultivars (Zhang *et al*., 2020). In another study on wheat, the cultivar with higher root densities and deeper root growth, had more tendency to subsoil nitrogen depletion and produced more grain per nitrogen supply (Rasmussen *et al*., 2015). Noureldin *et al*. (2013) also reported that there were significant differences among the wheat cultivars in terms of vegetative and reproductive traits under different levels of nitrogen application. Tyagi *et al*. (2020) also emphasized the role of genotype and breeding programs in nutrient intake and producing high-yielding wheat varieties for low nitrogen conditions.

The aim of this study was to evaluate the response of ten different cultivars of wheat to application of nitrogen chemical fertilizer, manure and wheat mix cropping with clover as a companion plant with the ability of biological nitrogen fixation.

Materials and Methods

Location of experiment

To determine the effect of chemical, organic and biological fertilization on different wheat cultivars, an experiment was conducted in research field of Faculty of Agriculture, Herat University, Afghanistan, during two successive growing seasons (2018-2019 and 2019-2020). This area is located in the north western of Afghanistan, with 34° 20′ N, 62° 12′ E and 1000 m elevation. The experimental site is characterized with arid-climate with an annual rainfall below 200 mm and mean annual temperature of 16 °C. The main properties of soil in experimental field are shown in Table 1.

Table 1. The properties of soil and manure used in the experiment

Substance	Texture	pH	(dS/m) EC	K (ppm)	P (%)	N (%)	OM (%)
Soil	Loam	8.1	0.49	248	0.061	0.055	0.5
Cow manure	-	7.4	3.9	2586	1.2	1.4	43.4

Experimental design

The experiment was set up on split plot in a randomized complete block design (RCBD) with three replications. The main plot factor was three fertilization types [F_1 or Control: mix cropping of wheat and clover as biological fertilizer (as a treatment in which no chemical or organic fertilizer was used), F_2: nitrogen chemical fertilizer at the rate of 100 kg ha^{-1} Urea, F_3: 40 t ha^{-1} semi-rotten cow manure]. Ten different wheat cultivars that are being cultivated in Herat province (V1= 'Mazar-99', V2= 'Baghlan-09', V3= 'Chont-1', V4= 'Kabul-13', V5= 'Gul-96', V6= 'Mugawim', V7= 'Shoshom-b08', V8= 'Drokhshan-09', V9= 'Lalmi-4' and V10= 'Lalmi-3') were considered as subplot factor. The used cultivars were sent to Afghanistan by the CIMMYT office and after several years of testing and research, they were recommended to the farmers of each region for cultivation. The cultivars selected in this research have the necessary adaptability for cultivation in Herat province and are cultivated every year on a large scale by farmers in this province. The main properties of manure used in the experiment are presented in Table 1.

Agronomic operations

Sowing date was on November 11 of both studied years. Phosphorous (100 kg ha^{-1} DiAmmonium Phosphate) was added to the all plots and mixed with the soil, before cultivation. Before sowing, seeds were treated with Vitawax fungicide with the concentration of 2 kg per 1000 kg seed. Wheat density was considered as 200 plant m^{-2}. The amount of seed used in each plot was determined according to 1000-gain weight of each variety, which was varied between 38-43 g. Each plot area was 4 m^2 (2×2 m), with eight planting rows, and the distance between rows was 25 cm. In plots related to chemical fertilization, urea (100 kg ha^{-1}) was added to in three different growing stage (planting, tillerig and shooting) as top-dressing. Cow manure at the rate of 40 t ha^{-1} was added to the plots before cultivation and mixed with the soil. In biological fertilizer treatment, mix cropping with Persian clover (*Trifoliume resupinatum* L.) was considered and the seeds of clover were sowed at the rate of 40 kg.ha^{-1} (Clover and wheat were planted at the same time). Clover is well adapted and is being cultivated in Herat from the distant past. Accordingly, symbiont bacteria are present in sufficient quantities in the arable soils of this region. To ensure symbiosis between clover root and symbiotic bacteria, the presence of nodes on the clover root system were evaluated during late winter. The release of pink liquid from the nodes after cutting or squeezing was considered as the sign of the beginning of nitrogen fixation. The plots were irrigated after sowing. Irrigation was applied 6 times during growing season at the rate of 0.1 m^3 m^{-2}. Weeds were hand controlled one time, at tillering stage of wheat.

Sampling

At the end of each growing season (around May 25), 10 plants were selected randomly in each plot. Then the mean values of vegetative (plant height, stem and leaf weight, total dry matter i.e., all aerial parts of the plant, spike length and spike weight) and reproductive (number of spikelet per spike, number of seed per spike, seed weight based on g m^{-2}, 1000-seed weight) traits were measured in each plot. To determine the dry matter of aerial parts, the plant samples were placed in oven at 65 °C for 48 hours. After removing the marginal effect (two side rows plus 25 cm from the beginning and the end of each plot), the remained plants were harvested from 1 m^2, to determine seed yield. Then, harvest index was calculated by seed yield/ biologic yield × 100.

Seed quality measurement

Seed nitrogen content was determined by Kjeldahl method (Bremner and Mulvaney, 1982). Seed potassium and phosphorous contents were determined by Owen (1992) and, Murphy and Riley (1962) methods, respectively.

Date analysis

The analysis of variance (ANOVA) was performed by using GLM proc. in SAS version 9.1 (SAS, 2003) and the least significant different test (LSD) was used for mean comparison.

Results and Discussion

Vegetative growth

Fertilization had a significant effect on the plant height, plant dry weight and spike weight during both growing seasons (Table 2). The highest values for all mentioned parameters were gained at F2 (chemical N fertilizer), followed by F1 (biological N fixation by symbiotic bacteria), while F3 (manure) had the lowest values (Table 3). The production of plant growth-promoting hormones due to the activity of symbiotic bacteria has been suggested as a possible reason for improving the vegetative growth of wheat (Moradi *et al*., 2016). In a similar study, wheat in mixed cropping with white clover (*Trifolium repens*) obtained the sufficient amount of nitrogen, through biological nitrogen fixation from clover, because the amount of wheat biomass in mixcropping was nearing the level of the fertilized treatment, and higher than the unfertilized sole cropping system (Kintl *et al*., 2018). The better vegetative growth of the plant in F2 compared to the F1 was probably due to the nitrogen availability throughout the growing season in F2, because nitrogen was top-dressed in three times, while in the F1 the nitrogen fixation process started in late winter. The use of starter fertilizer from chemical source in the stage before the beginning of biological fixation can solve this problem (Kavian Athar and Aboutalebian, 2019). Moreover, combined application of nitrogen fixing bacteria and manure may increase the efficiency of symbiotic microorganisms (Moradi *et al*., 2016). In a study on winter wheat, nitrogen top-dressing introduced as a favourable strategy to improve plant growth and yield. It was also concluded that compared with top dressing at flowering and filling phases, early top dressing at jointing stage was more effective in increasing plant growth and yield (Kubar *et al*., 2021). Slow release of nutrients from semi-rotten cow manure was the probable reason for slow growth response of wheat to organic manure (F3). It seems that the nutritional and non-nutritional benefits of manure will become more apparent in the years after consumption date. In a study on wheat, it was recommended that single application of organic manure cannot meet the plant's requirement for nutrients, so the use of chemical fertilizers, especially nitrogen as a supplement is essential (Barahimi *et al*., 2009).

The effect of variety was significant on the plant height, plant dry matter, spike weight (first growing season) and spike length (in both years) (Table 2). 'Kabul 13' and 'Gul-96' were the best varieties in term of plant height and dry matter production. The spike length and weight for 'Kabul 13' were also some higher (Table 3). The interaction effect of fertilization and variety was significant on spike length and weight in the first studied year (Table 2). The highest spike length for F1, F2 and F3 were gained with 'Kabul-13' (10.80 cm), 'Drokhshan-09' (11.26 cm) and 'Shoshom-b08' (10.73 cm) varieties, respectively. 'Chont-1' (1302 g m^{-2}) and 'Kabul-13' (1239 g m^{-2}) for F1, 'Kabul-13' (1421.66 g m^{-2}) for F2 and 'Mazar-99' (1173 g m^{-2}) for F3, were the best varieties in terms of spike weight (Table 4). Overall, 'Kabul-13' (29th ESWYT #124) was the best cultivar in terms of growth parameters, which is in line with the recommendations of the Herat Agricultural Research Center. This variety is a new high yielding wheat variety introduced by CIMMYT, with a potential height of 104 cm and medium-sized spike (Obaidi *et al*., 2015).

Table 2. Analysis of variances (F) for the effect of fertilization and different wheat varieties on vegetative parameters

S.O.V	df	Plant height (cm)		Stem & leaf weight (g m^{-2})		Total dry matter (g m^{-2})		Spike length (cm)		Spikes weight (g m^{-2})	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Block	2	0.47 ns	0.37 ns	1.66 ns	0.76 ns	0.17 ns	0.05 ns	0. 3 ns	1.75 ns	0.01 ns	0.25 ns
Fertilizer (F)	2	11.68 **	0.13 ns	14.39 **	3.63 *	23.16 **	27.66 **	1.34 ns	1.14 ns	43.51 **	11.68 **
Error (MS)	4	64.14	1.21	155695.2	1.01	181146.3	1.14	0.56	3.03	37025.83	0.84
(V) Variety	9	2.25 *	0.81 ns	1.83 ns	1.06 ns	2.20 *	0.54 ns	3.39 **	2.57 *	3.21 **	0.75 ns
V x F	18	1.40 ns	1.33 ns	1.78 ns	0.86 ns	1.47 ns	0.76 ns	2.21 *	1.09 ns	2.51 **	0.59 ns
Error (MS)	54	76.78	85.51	89959.7	91649.56	238336.1	96526.09	0.53	1.77	19234.84	41325.54
CV (%)	-	11.64	11.26	30.81	10.53	23.13	18.87	7.27	14.45	13.22	25

ns, * and **: are non-significant and significant at α=5 and 1% probability levels, respectively

Table 3. Means comparison for the effect of fertilization and different wheat varieties on vegetative parameters

Treatments	Plant height (cm)		Stem & leaf weight (g m^{-2})		Total dry matter (g m^{-2})		Spike length (cm)		Spikes dry weight (g m^{-2})	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Fertilizers										
F_1: mix cropping of	75.15	82.78	1138.60	790.2	2252.9	1556.57	10.04	9.05	1063.17	782.33
F_2: Chemical	80.80	81.61	1041.60	1284.4	2449.9	1979.32	10.06	9.51	1208.07	951.71
F_3: Manure	69.87	81.85	740.23	650.7	1628.4	1403.33	9.79	9.08	874.97	703.42
LSD $_{\%5}$	4.53	4.78	155.26	495.57	252.72	160.83	0.37	0.69	71.79	105.23
Wheat varieties										
V_1: 'Mazar-99'	72.9	80.11	1047.3	791.1	2327.0	1536.0	10.38	8.12	1126.4	732.24
V_2: 'Baghlan-09'	77.9	81.88	1107.2	849.3	2211.0	1620.7	9.31	9.46	1017.1	735.41
V_3: 'Chont-1'	79.2	84.25	1114.4	863.1	2282.7	1696.1	9.81	8.59	1114.4	858.72
V_4: 'Kabul-13'	79.3	87.91	1006.8	1819.7	2366.6	1683.7	10.49	9.70	1184.2	763.44
V_5: 'Gul-96'	82.5	80.07	1135.0	953.2	2396.2	1792.9	9.50	8.78	1055.9	824.69
V_6: 'Mugawim'	74.5	80.40	1015.1	815.0	2110.8	1702.8	9.93	9.22	1096.3	891.14
V_7: 'Shoshom-b08'	70.3	79.03	835.7	754.4	1978.9	1606.6	10.20	9.41	1044.3	815.50
V_8: 'Drokhshan-09'	68.6	84.17	891.4	682.3	1840.2	1623.2	10.32	8.70	956.4	889.83
V_9: 'Lalmi-4'	74.6	79.81	799.2	776.3	1770.6	1649.6	10.34	9.49	947.6	835.72
V_{10}: 'Lalmi-3'	72.9	83.19	782.6	779.9	1820.0	1552.4	9.38	10.67	944.6	778.17
LSD $_{\%5}$	8.28	8.74	283.47	904.79	461.4	293.63	0.68	1.25	131.08	192.13

Table 4. Interaction effect of fertilization and variety on some vegetative and reproductive parameters of wheat

Fertilizer	Variety	Spike length (cm)		Spikes weight ($g\ m^{-2}$)	Spikelet /spike (No.)	Seed/ Spike (No.)	1000-seed weight (g)		Seed yield ($t\ ha^{-1}$)		Harvest index (%)	Seed protein (%)
		2019	2020	2019	2020	2020	2019	2020	2019	2020	2020	2019
F1: Mix cropping of clover & wheat	'Mazar-99'	10.10	9.14	1022.00	17.6	31.50	33.40	39.40	4.74	4.92	32.05	12.91
	'Baghlan-09'	9.93	9.06	1070.66	15.33	30.43	32.67	38.27	5.54	4.64	31.20	11.58
	'Chont-1'	9.66	8.81	1302.00	16.66	28.87	29.90	35.73	6.20	5.37	30.19	12.89
	'Kabul-13'	10.80	9.15	1239.00	17.00	28.19	32.91	44.37	7.26	5.30	35.09	12.22
	'Gul-96'	10.13	8.48	1081.33	18.00	33.54	29.13	35.81	4.78	5.36	31.86	11.33
	'Mugawim'	10.06	9.40	1168.66	17.66	26.76	30.61	35.92	5.66	3.79	26.81	12.85
	'Shoshom-b08'	9.90	8.47	972.66	16.00	28.17	30.67	34.40	4.64	4.58	32.16	12.31
	'Drokhshan-09'	9.96	8.70	1079.00	16.66	28.28	30.81	36.28	5.14	4.31	28.79	12.19
	'Lalmi-4'	10.36	9.63	853.33	18.00	25.44	29.16	35.19	3.89	4.83	28.66	12.47
	'Lalmi-3'	9.53	9.63	843.00	16.33	26.45	32.50	40.91	4.63	5.20	34.16	12.64
F2: Chemical	'Mazar-99'	10.33	6.93	1184.33	16.66	27.21	35.44	39.47	6.97	4.87	26.51	13.53
	'Baghlan-09'	9.90	9.76	1091.66	17.00	26.53	45.62	39.56	5.94	5.99	27.51	14.30
	'Chont-1'	9.16	8.55	1159.33	18.33	26.40	37.68	36.76	6.98	6.14	30.55	13.84
	'Kabul-13'	10.46	9.95	1421.66	31.00	35.28	40.21	38.34	8.02	6.22	33.71	12.37
	'Gul-96'	9.76	8.87	1214.00	15.00	25.81	35.90	38.89	6.61	5.58	25.25	12.15
	'Mugawim'	9.93	8.86	1257.33	14.33	25.31	32.02	36.55	6.20	5.93	29.06	12.75
	'Shoshom-b08'	9.96	10.88	1380.00	16.66	32.04	35.05	35.28	7.20	6.22	30.78	11.12
	'Drokhshan-09'	11.26	10.00	970.33	19.00	28.45	31.75	39.01	4.87	6.41	31.19	12.09
	'Lalmi-4''	10.83	9.44	1239.33	16.66	25.03	34.18	42.43	6.12	6.98	35.32	11.00
	Lalmi-3'	9.00	11.88	1162.66	18.00	33.86	31.90	40.68	6.41	6.26	35.96	12.02
F3: Manure	'Mazar-99'	10.70	8.30	1173.00	17.00	28.13	37.94	41.64	5.94	3.16	25.52	11.76
	'Baghlan-09'	8.10	9.56	889.00	18.50	32.16	45.04	43.89	5.63	4.00	32.67	12.03
	'Chont-1'	10.60	8.41	882.00	16.50	29.65	25.91	39.30	3.28	4.10	30.92	13.18
	'Kabul-13'	10.20	9.99	892.00	18.00	35.29	35.92	45.24	5.10	5.83	34.64	12.92
	'Gul-96'	8.60	9.00	872.33	16.00	29.36	32.72	41.20	3.59	4.75	32.79	12.71
	'Mugawim'	9.80	9.40	863.00	16.50	36.29	27.27	34.60	3.12	6.45	38.03	15.15
	'Shoshom-b08'	10.73	8.88	780.33	16.00	17.05	33.16	35.69	4.02	2.81	23.56	13.93
	'Drokhshan-09'	9.73	7.40	820.00	14.50	29.43	29.99	39.11	3.84	4.28	32.98	11.93
	'Lalmi-4'	9.83	9.40	750.00	18.50	36.90	31.62	42.31	3.82	5.26	41.69	13.96
	'Lalmi-3'	9.60	10.50	828.00	18.00	26.43	30.39	39.64	4.43	4.29	31.24	12.44
LSD %5	-	0.68	1.25	131.08	3.80	4.67	3.38	2.64	0.89	1.11	4.14	0.84

Reproductive growth

Effect of fertilizer application was significant on number of seed per spike, harvest index (in the first year), seed weight (in both years) and 1000-grain weight (in the second year) (Table 5). F2 (chemical nitrogen fertilizer) and F3 (manure) were the best and the worst treatments respectively, for the most reproductive parameters, while F1 (biological nitrogen fixation as mixcropping) had an intermediate position, especially in terms of seed yield (Table 6). In a similar study, wheat mixcropping with white clover reduced the applied nitrogen rate, by more than 20%, without any negative impact on grain and biomass yields. In that study, wheat yield in mix cropping was more than sole cropping, but lower than sole cropping plus 140 kg N ha^{-1} (Kintl *et al*., 2018). In another study, wheat fertilization by chemical nitrogen fertilizer, manure and biological nitrogen fixation, improved all reproductive growth parameters (Moradi *et al*., 2016). Overall, yield and yield components of wheat intercropped with clover was acceptable (Table 6). In previous studies reported that white and red clover as cover crops have a residual effect corresponding up to 90 kg ha^{-1} mineral nitrogen fertilizer (Breland, 1996; Schroder *et al*., 1997; Garand *et al*., 2001). However, in mixcropping of wheat with clover, the clover proportion often becomes small due to generating dense main crops and thus its effect on intercropped crop or its residual effect on the subsequent crop will also reduce (Nassiri and Elgersma, 2002).

In a study, when no or 60 kg ha^{-1} nitrogen was applied to the winter wheat, the total grain yields of wheat and barley using mixed cover crops were similar to the yield with clover cover crops in pure stand, but the effect of the mixtures was smaller at high N rates (Bergkvista *et al*., 2011). Therefore, it can be concluded that mixed cropping of wheat and clover can be suggested instead of chemical fertilizer application as alternative options for sustainable wheat production.

The effect of variety was significant on number of spikelet per spike, number of seed per spike, seed weight and seed yield (in the first growing season), 1000-grain weight and harvest index (in both studied years) (Table 5). The highest amounts of all mentioned parameters belonged to 'Kabul-13' variety (Table 6). One main reason for this finding is because 'Kabul-13' is an awned cultivar. Awns are lemma-derived and photosynthetically active organs. The production of these organs although consumes some assimilates, but can improve plant yield because they have some merits including photosynthesis ability, carbohydrate storage and increased water-use efficiency (Guo and Schnurbusch, 2016). Owns are near to photosynthetic reservoirs (kernels), consequently the pathway for assimilation movement from them to the kernels is minimal. In addition, awns possess a large surface area and are located in an ideally place for light interception and CO_2 uptake (Li *et al*., 2010). In addition, 'Kabul-13' is an early maturing genotype (days to maturity: 137) compared to other tested cultivars (Obaidi *et al*., 2015), which makes it safe from end-of-season drought stress in arid areas. Other advantages of this cultivar are high 1000-seed weight, and no seed shedding during the last stages of growth (Obaidi *et al*., 2015).

Interaction effect of fertilizer and variety was significant on seed weight, 1000-grain weight, seed yield (in the first growing season), seed number per spike and harvest index (in the second growing season) (Table 5). The highest and the lowest seed number per spike were recorded in 'Lalmi-4' ×F3 and 'Shoshom-b08' × F3, respectively, with 113% difference. In all fertilization treatments, the varieties of 'Mazar-99', 'Baghlan-09' and 'Kabul-13' were superior in terms of 1000-grain weight. 'Chont-1' and 'Kabul-13' had the highest seed yield when mix cropping of wheat and clover was applied. The same varieties plus 'Shoshom-b08' produced the highest seed yield under chemical fertilization treatment. Harvest index was varied between 23.5 ('Shoshom-b08' × F3) and 41.6 % ('Lalmi-4' ×F3) (Table 4). The positive role of nitrogen on yield components of wheat cultivars has been reported previously by some studies such as Oscarson (2000), Bergkvista *et al*. (2011) and Jarecki *et al*. (2017). However, different varieties respond differently to nutrients availability due to their variable physiological behavior.

Table 5. Analysis of variances (F) for the effect of fertilization and different wheat varieties on reproductive parameters

S.O.V	df	Spikelet /spike (No.)		Seed/ Spike (No.)		Seed weight (g m^2)		1000-seed weight (g)		Seed yield (t ha^{-1})		Harvest index (%)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Block	2	0.84 ns	1.09 ns	0.16 ns	0.96 ns	0.17 ns	0.17 ns	0.87 ns	1.68 ns	0.17 ns	0.17 ns	0.01 ns	0.08 ns
Fertilizer (F)	2	0.77 ns	1.09 ns	4.20 *	0.80 ns	42.62 **	14.75 **	13.75 **	6.71 **	42.62 **	14.74 **	3.48 *	1.38 ns
Error (MS)	4	1.14	1.28	23.21	0.28	21689.09	0.37	11.29	0.91	2.17	0.37	1.73	2.06
Variety (V)	9	2.64 *	1.61 ns	3.49 **	1.12 ns	4.30 **	1.39 ns	8.07 **	6.36 **	4.30 **	1.39 ns	2.62 *	2.69 *
V x F	18	0.94 ns	1.47 ns	1.19 ns	2.74 **	2.15 *	1.28 ns	2.08 *	1.67 ns	2.15 *	1.28 ns	1.55 ns	2.38 **
Error (MS)	54	2.14	16.18	25.35	24.49	9022.41	13890.97	12.81	7.83	0.90	1.38	24.87	19.19
CV (%)	-	8.76	23.14	18.15	16.98	17.74	22.96	10.72	7.2	17.73	22.96	19.14	13.96

ns, * and **: are non-significant and significant at α=5 and 1% probability levels, respectively

Table 6. Means comparison for the effect of fertilization and different wheat varieties on reproductive parameters

Treatments	Spikelet /spike (No.)		Seed/ Spike (No.)		Seed weight (g m²)		1000- seed weight (g)		Seed yield (t ha⁻¹)		Harvest index (%)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Fertilizers												
F1: mix cropping with clover & wheat	16.73	16.93	26.79	28.76	525.17	483.23	31.18	37.63	5.25	4.83	24.16	31.10
F2: Chemical	16.93	18.26	29.91	30.07	653.50	606.54	35.98	40.26	6.53	6.06	27..45	32.41
F3: Manure	16.47	16.95	26.52	28.59	427.77	449.57	32.99	38.70	4.28	4.49	26.56	30.58
LSD %5	0.75	2.08	2.6	2.56	49.17	61.01	1.85	1.45	0.49	0.61	2.58	2.26
Varieties												
V1: Mazar-99	16.56	17.11	27.3	28.94	588.44	432.14	35.59	40.17	5.88	4.32	25.91	28.02
V2: Baghlan-09	14.89	16.94	26.7	29.71	570.56	487.93	41.11	40.57	5.71	4.88	27.94	30.46
V3: Chont-1	17.78	17.17	30.1	28.31	548.67	520.69	31.16	37.26	5.49	5.20	24.54	30.55
V4: Kabul-13	17.33	22.00	33.1	32.92	679.44	578.47	36.35	42.65	6.79	5.78	30.21	34.48
V5: Gul-96	17.11	16.33	22.5	29.57	499.67	523.48	32.59	38.63	4.99	5.23	20.99	29.97
V6: Mugawim	16.44	16.17	25.4	29.46	499.56	539.21	29.97	35.69	4.99	5.39	23.56	31.30
V7: Shoshom-b08	17.22	16.22	29.6	25.31	529.11	453.85	32.96	35.12	5.29	4.53	26.86	28.83
V8: Drokhshan-09	16.44	16.72	27.9	28.72	461.89	500.17	30.85	38.13	4.62	5.00	25.31	30.99
V9: Lalmi-4	17.00	17.72	24.5	29.12	461.56	569.62	31.66	39.97	4.61	5.69	26.16	35.22
V10: Lalmi-3	16.33	17.44	30.3	28.92	515.89	525.58	31.60	40.41	5.16	5.25	29.07	33.79
LSD %5	1.38	3.80	4.76	4.67	89.77	111.39	3.38	2.64	0.89	1.11	4.71	4.14

Seed quality

Seed protein percentage and protein yield were affected significantly by fertilizer type and wheat variety. The interaction effect of experimental factors was also significant on protein percentage. However, the simple and interaction effects of fertilizer and variety were not significantly effective on seed potassium and phosphorous contents (Table 7). Percentage of seed protein was higher in F3, but there was no significant difference between F1 and F2 (Figure 1). However, due to higher seed yield (Table 6), protein yield in F1 and F2 was 18.1 and 49.0% higher than F3, respectively (Figure 2). Based on the above-mentioned results, it seems to be an inverse relationship between grain quality and quantity, so that, F3 had the lowest seed yield (Table 6) but the highest protein content (Figure 1). In another study on wheat, it was reported that increases in grain yield is generally accompanied by a decrease in the grain's protein content, which is strongly associated with bread making quality (Abedi *et al*., 2011). Among all studied varieties, seed protein percentage was varied between 12.07 ('Gul-96' and 'Drokhshan-09') and 13.59% ('Mugawim'), but the highest protein yield was obtained by 'Kabul-13' (Figure 2), due to its more seed yield (Table 6). Protein content of 12-13% is suitable for bread making (Kunkulberga *et al*., 2019), and the protein content of all tested varieties was in this range (Figure 1). Based on the interaction results of experimental factors, the highest and the lowest amounts of seed protein content were obtained by 'Mugawim' variety (15.15%) with manure application and 'Lalmi-4' variety (11%) with chemical fertilizer, respectively (Table 4). Nitrogen can increase the plant protein content regardless of the cultivar's inherent protein potential (Fjell *et al*., 1984). The positive effect of nitrogen on seed protein content of two wheat cultivars also reported by Kunkulberga *et al*. (2019), but the amount of nitrogen used to obtain the highest amount of protein was different between tested cultivars.

Table 7. Analysis of variances (F) for the effect of fertilization and different wheat varieties on seed qualitative parameters

S.O.V	df	Seed K content (%)	Seed P content (%)	Seed protein content (%)	Protein yield (t ha^{-1})
Block	2	1.07 ns	0.48 ns	0.72 ns	0.03 ns
Fertilizer (F)	2	0.36 ns	0.65 ns	4.46 *	27.48 **
Error (MS)	4	0.01	0.05	0.28	0.04
Variety (V)	9	1.88 ns	0.92 ns	2.73 *	3.76 **
V x F	18	1.58 ns	0.92 ns	3.71 **	1.19 ns
Error (MS)	54	0.02	0.05	0.78	0.02
CV (%)	-	11.76	17.24	7.03	21.21

ns, * and **: are non-significant and significant at α=5 and 1% probability levels, respectively

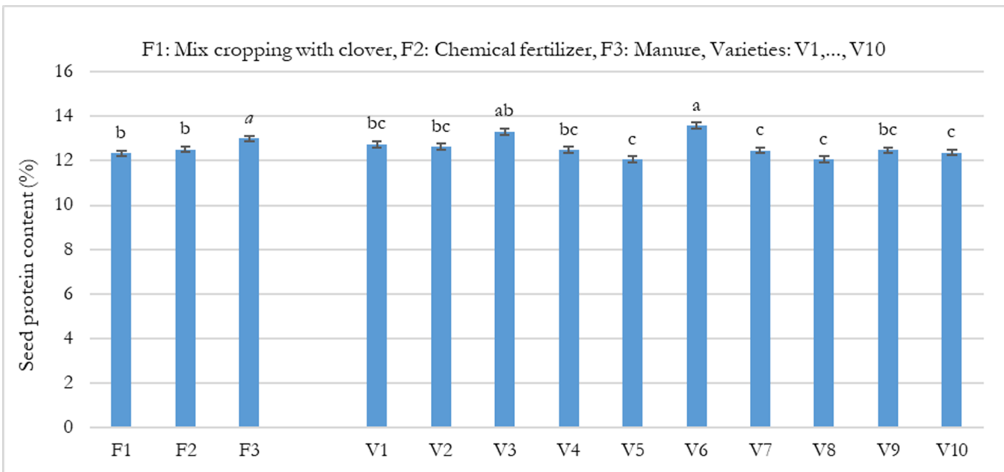

Figure 1. Effect of fertilization and variety on wheat seed protein content

Means followed by the same letter shows no significant differences among treatments at 0.05 level by LSD

V1= 'Mazar-99', V2= 'Baghlan-09', V3= 'Chont-1', V4= 'Kabul-13', V5= 'Gul-96', V6= 'Mugawim', V7= 'Shoshom-b08', V8= 'Drokhshan-09', V9= 'Lalmi-4' and V10= 'Lalmi-3'

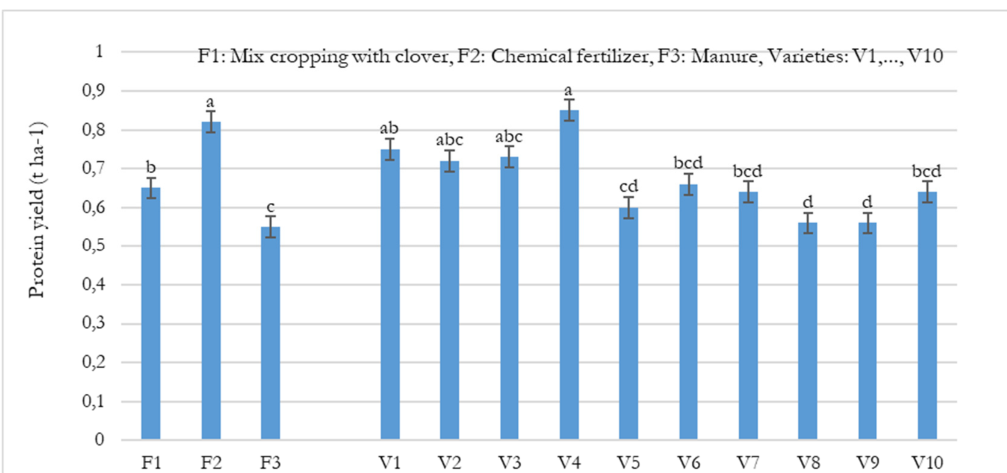

Figure 2. Effect of fertilization and variety on wheat protein yield

Means followed by the same letter shows no significant differences among treatments at 0.05 level by LSD

V1= 'Mazar-99', V2= 'Baghlan-09', V3= 'Chont-1', V4= 'Kabul-13', V5= 'Gul-96', V6= 'Mugawim', V7= 'Shoshom-b08', V8= 'Drokhshan-09', V9= 'Lalmi-4' and V10= 'Lalmi-3'

One of the main aims in sustainable agriculture is to find strategies to reduce the application of chemical fertilizers. The results of this research showed that mix cropping of wheat with legume crops such as clover can provide part of this goal (Table 6; Figures 1 and 2). In the present study, clover was a companion crop for the wheat as the main crop. It has been reported that companion crops play an important role in the establishment of main crop by improving soil fertility, balancing radiation, humidity and temperature, as well as providing nitrogen when a legume crop is used (Koocheki *et al*., 2016a). The residues of companion crop can also improve the physical, chemical and biological properties of the soil and ultimately increase the yield of the main crop (Koocheki *et al*., 2016b).

Conclusions

Wheat mix cropping with clover caused an increase in growth and yield of main crop compared to manure application, and in comparison, with chemical fertilizer also presented acceptable results. Application of starter fertilizer in mixcropping of wheat and clover during autumn and winter, when biological nitrogen fixation does not occur due to cold weather, can increase the usefulness of mixed cultivation of these two species. Among all tested wheat cultivars, the highest seed yield was obtained in 'Kabul-13' (6.79 t ha^{-1}) and 'Mazar-99' (5.88 t ha^{-1}) varieties in the first year and in 'Kabul-13' (5.78 t ha^{-1}) and 'Lalmi-4' (5.69 t ha^{-1}), during the second year. There was no significant different between chemical fertilizer application and clover mix cropping in terms of seed protein percentage. Overall, mixed cropping of wheat and clover can be suggested instead of chemical fertilizer application and, thus could be adopted by the farmers as alternative options for wheat production in low input agroecosystems.

Authors' Contributions

RN and MYJ: Conducting the experiment in the university of Heart; HRF: Writing the main manuscript text; HS: data analysis; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abedi T, Alemzadeh A, Kazemeini SA (2011). Wheat yield and grain protein response to nitrogen amount and timing. Australian Journal of Crop Science 5(3):330-336.

Anil L, Park RH, Phipps RH, Miller FA (1998). Temperate intercropping of cereals of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Science 53:301-317. *https://doi.org/10.1046/j.1365-2494.1998.00144.x*

Bahrman N, Le Gouis J, Negroni L, Amilhat L, Leroy P, Lainé AL, Jaminon O (2004). Differential protein expression assessed by two-dimensional gel electrophoresis for two wheat varieties grown at four nitrogen levels. Proteomics 4:709-719. *https://doi.org/10.1002/pmic.200300571*

Barahimi N, Afyuni M, Karami M, Rezaee Nejad Y (2009). Cumulative and residual effects of organic amendments on nitrogen, phosphorus and potassium concentrations in soil and wheat. Isfahan University of Technology-Journal of Crop Production and Processing 12(46):803-812.

Bergkvista G, Stenberg M, Wetterlind J, Bath B, Elfstrand S (2011). Clover cover crops under-sown in winter wheat increase yield of subsequent spring barley- Effect of N dose and companion grass. Field Crops Research 120:292-298. *https://doi.org/10.1016/j.fcr.2010.11.001*

Breland TA (1996). Green manuring with clover and ryegrass catch crops under sown in small grains: Effects on Soil Mineral Nitrogen in Field and Laboratory Experiments. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 46:30-40. *https://doi.org/10.1080/09064719609413131*

Bremner JM, Mulvaney CS (1982). Nitrogen total. In: Page AL, Miller RH, Keeney DR (Eds). Methods of soil analysis. Part 2. Chemical analysis. American Society of Agronomy and Soil Science Society of American, Madison, Wisconsin. pp 595- 624.

Bueren ETLV, Struik PC (2017). Diverse concepts of breeding for nitrogen use efficiency. A review. Agronomy for Sustainable Development 37:50. *https://doi.org/10.1007/s13593-017-0457-3*

Cormier F, Foulkes J, Hirel B, Gouache D, Moënne-Loccoz T, Gouis JL (2016). Breeding for increased nitrogen-use efficiency: a review for wheat (*T. aestivum* L.). Plant Breeding 135:255-278. *https://doi.org/10.1111/pbr.12371*

Dabin Z, Pengwei Y, Na Z, Changwei Y, Weidong C, Yajun G (2016). Contribution of green manure legumes to nitrogen dynamics in traditional winter wheat cropping system in the Loess Plateau of China. European Journal of Agronomy 72:47-55. *https://doi.org/10.1016/j.eja.2015.09.012*

Daryanto S, Fu B, Zhao W, Wang S, Jacinthe P, Wang L (2020). Ecosystem service provision of grain legume and cereal intercropping in Africa. Agricultural Systems 178:102761. *https://doi.org/10.1016/j.agsy.2019.102761*

Dhima KV, Lithourgidis AS, Vasilakoglou IB, Dordas CA (2007). Competition indices of common vetch and cereal intercrops in two seeding ratio. Field Crop Research 100:249-256. *https://doi.org/10.1016/j.fcr.2006.07.008*

Esmaeili A, Sadeghpour A, Hosseini SMB, Jahanzada E, Chaichia MR, Hashemi M (2011). Evaluation of seed yield and competition indices for intercropped barley and annual medic (*Medicago scutellata*). International Journal of Plant Production 5(4):395-404. *https://doi.org/10.22069/IJPP.2012.749*

Fjell DL, Paulsen GM, Waiter TL, Lawless JR (1984). Relationship among N and P contents of vegetative part and agronomic trials of normal and high protein wheat. Journal of Plant Nutrition 7(7):1102. *https://doi.org/10.1080/01904168409363267*

Garand MJ, Simard RR, MacKenzie AF, Hamel C (2001). Under seeded clover as a nitrogen source for spring wheat on a gleysol. Canadian Journal of Soil Science 81:93-102. *https://doi.org/10.4141/S99-060*

Guarda G, Padovan S, Delogu G (2004). Grain yield, nitrogen use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. European Journal of Agronomy 21:181-192. *https://doi.org/10.1016/j.eja.2003.08.001*

Guo Z, Schnurbusch T (2016). Costs and benefits of awns. Journal of Experimental Botany 67(9):2533-2535. *https://doi.org/10.1093/jxb/erw140*

Hawkesford MJ, Riche AB (2020). Impacts of G x E x M on nitrogen use efficiency in wheat and future prospects. Frontiers in Plant Science 11:1157. *https://doi.org/10.3389/fpls.2020.01157*

Jarecki W, Buczek J, Bobrecka-Jamro D (2017). Response of spring wheat to different soil and foliar fertilization. Journal of Central European Agriculture 18(2):460-476. *https://doi.org/10.5513/JCEA01/18.2.1919*.

Kavian Athar N, Aboutalebian MA (2019). The response of physiological indices of bean (*Phaseolus vulgaris* L.) to application method of phosphate and zinc sulfate fertilizers at different amounts of starter nitrogen fertilizer. Iranian Journal of Field Crop Science 50(3):169-184. *https://doi.org/10.22059/ijfcs.2018.239246.654363*

Kintl A, Elbl J, Lošák T, Vaverková MD, Nedelník J (2018). Mixed intercropping of wheat and white clover to enhance the sustainability of the conventional cropping system: Effects on biomass production and leaching of mineral nitrogen. Sustainability 10:3367. *https://doi.org/10.3390/su10103367*

Koocheki A, Moghaddam PR, Fallahi HR, Aghhavani-Shajari M (2016). The study of saffron (*Crocus sativus* L.) replacement corms growth in response to planting date, irrigation management and companion crops. Saffron Agronomy and Technology 4(1). *https://doi.org/10.22048/jsat.2016.11895*

Koocheki A, Rezvani MP, Fallahi HR (2016). Effects of planting dates, irrigation management and cover crops on growth and yield of saffron (*Crocus sativus* L.). Journal of Agroecology 8(3):435-451. *https://doi.org/10.22067/JAG.V8I3.51323*

Kubar MS, Feng M, Sayed S, Shar AH, Rind NA, Ullah H, … El-Sharnouby M (2021). Agronomical traits associated with yield and yield components of winter wheat as affected by nitrogen managements. Saudi Journal of Biological Sciences 28(9):4852-4858. *https://doi.org/10.1016/j.sjbs.2021.07.027*

Kunkulberga D, Linina A, Ruza A (2019). Effect of nitrogen fertilization on protein content and rheological properties of winter wheat whole meal. 13th Baltic Conference on Food Science and Technology. May 2019.

Li XF, Bin D, Hong-gang W (2010). Awn anatomy of common wheat (*Triticum aestivum* L.) and its relatives. Caryologia 63(4):391-397. *https://doi.org/10.1080/00087114.2010.10589751*

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105:1141-1157. *https://doi.org/10.1093/aob/mcq028*

Moradi M, Soleymanifard A, Naseri R, Ghasemi M, Abromand K (2016). The changes of agronomic traits and harvest index of wheat under the effect of manure and plant growth promotion bacteria at different levels of nitrogen. Crop Physiology Journal 28:73-90.

Mouradi M, Farissi M, Makoudi B, Bouizgaren A, Ghoulam C (2018). Effect of faba bean (*Vicia faba* L.)–rhizobia symbiosis on barley's growth, phosphorus uptake and acid phosphatase activity in the intercropping system. Annals of Agrarian Science 16:297-303. *https://doi.org/10.1016/j.aasci.2018.05.003*

Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36. *https://doi.org/10.1016/S0003-2670(00)88444-5*

Nassiri M, Elgersma A (2002). Effects of nitrogen on leaves, dry matter allocation and regrowth dynamics in *Trifolium repens* L. and *Lolium perenne* L. in pure and mixed swards. Plant and Soil 246:107-121. *https://doi.org/10.1023/A:1021528732218*

Noureldin NA, Saudy HS, Ashmawy F, Saed HM (2013). Grain yield response index of bread wheat cultivars as influenced by nitrogen levels. Annals of Agricultural Sciences 58(2):147-152. *https://doi.org/10.1016/j.aoas.2013.07.012*

Nuru Seid T, Tarikua Shumi T (2020). The effect of organic and inorganic fertilizers on growth and yield of bread wheat (*Triticum aestivum* L.). Current Investigations in Agriculture and Current Research 9(1):1161-1166.

Obaidi MQ, Azmatyar MH, Zamarai A, Qayum A, Mohmand E, Sharma R (2015). Kabul 13: A new Ug99 resistant wheat variety for Afghanistan. Journal of Agriculture and Veterinary Science 8(2):16-17.

Oscarson P (2000). The strategy of the wheat plant in acclimating growth and grain production to nitrogen availability. Journal of Experimental Botany 51:1921-1929. *https://doi.org/10.1093/jexbot/51.352.1921*

Owen CP (1992). Plant analysis reference producers for the southern region of the United States. The University of Georgia 33-45.

Rasmussen IS, Dresbøll DB, Thorup-Kristensen K (2015). Winter wheat cultivars and nitrogen (N) fertilization- Effects on root growth, N uptake efficiency and N use efficiency. European Journal of Agronomy 68:38-49. *https://doi.org/10.1016/j.eja.2015.04.003*

Rodriguez C, Carlsson G, Englund H, Flöhr A, Pelzer E, Jeuffroy M, Makowski D, Jensen ES (2020). Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. European Journal of Agronomy 118:126077. *https://doi.org/10.1016/j.eja.2020.126077*

Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, Reddy PM, Martínez-Romero E (2018). Nitrogen fixation in cereals. Frontiers in Microbiology 9:1794. *https://doi.org/10.3389/fmicb.2018.01794*

SAS Institute (2003). SAS/STAT User's Guide, Version 9.1., Cary, NC., USA

Schroder J, Tenholte L, Janssen BH (1997). Non-overwintering cover crops- a significant source of N. Netherlands Journal of Agricultural Science 45:231-248.

Tyagi BS, Foulkes J, Singh G, Sareen S, Kumar P, Broadley MR, … Singh GP (2020). Identification of wheat cultivars for low nitrogen tolerance using multivariable screening approaches. Agronomy 10:417. *https://doi.org/10.3390/agronomy10030417*

Wu H, MacDonald GK, Galloway JN, Zhang L, Gao L, Yang L, … Yang T (2021). The influence of crop and chemical fertilizer combinations on greenhouse gas emissions: A partial life-cycle assessment of fertilizer production and use in China. Resources, Conservation & Recycling 168:105303. *https://doi.org/10.1016/j.resconrec.2020.105303*

Yang YJ, Lei T, Du W, Liang CL, Li HD, Lv JL (2020). Substituting chemical fertilizer nitrogen with organic manure and comparing their nitrogen use efficiency and winter wheat yield. The Journal of Agricultural Science 158(4):262-268. *https://doi.org/10.1017/S0021859620000544*

Zhang L, Du YL, Li XG (2020). Modern wheat cultivars have greater root nitrogen uptake efficiency than old cultivars. Journal of Plant Nutrition and Soil Science 183(2):192-199. *https://doi.org/10.1002/jpln.201900353*