

Nutrients-uptake, nutrients-concentrations and protein-content of bread-wheat as affected by nitrogen, sulfur & phosphorus supplies and interaction

Abstract

Field-experiments were conducted in six farms/sites to investigate the concentrations of nitrogen (N), sulfur (S), and phosphorus (P); their uptake; and the grain protein-concentrations including the grain-yield (GY) of bread-wheat in central Ethiopia. The experiments were laid-out in RCBD in triplicate. The mean values of these elements in the grain responded significantly to their respective nutrient applications. In total, there were (n=144) entries across the locations. Nutrient-concentrations varied spatially ranging between 1.10–2.35% (N); 0.06–0.21% (S); and 0.01–0.16% (P). Similarly, their respective nutrient-uptake ranged between 7.42–137.55 kg/ha; 0.38–11.87 kg/ha; and 0.08–9.88 kg/ha. But, protein-concentrations in the wheat-flour varied between 6.44–13.71%. At specific sites, mean N-concentration in wheat-grain and its uptake responded significantly to N supply ($***P \leq .001$). Meanwhile, S concentration and uptake responded to both N and S supplies ($**P \leq .01$). But P concentration and uptake primarily responded to the interaction-effects N×S×P and N×; and to a lesser extent, to sole-P application ($*P \geq .05$). Similarly, grain protein-concentration was influenced by the interaction-effects N×S×P, N×S, N×P; and by sole-N and sole-S applications, in decreasing order of significance. Nitrogen-concentration and N-uptake in the grain were considerably higher than those of S and P. Furthermore, wheat grain-protein concentration was strongly-correlated with native-soil's N ($r = +0.99$); S ($r = +0.81$); and P ($r = +0.37$), suggesting their degree of significance in protein-synthesis. Under external-supply, the treatment-effects also follow clear order of gradations in the plant-components formation with the $CK \leq S \leq P \leq SP < N < NS < NP \leq NPS$. However, the observed changes in the overall plant-variables are likely to be influenced not only by soil-conditions, but also by plant-factors, environmental-factors, and farming-practices among others, necessitating their further-investigation, even at the partial-factor level. In particular, the effects of N, S, and P; and even other-nutrients on the true-protein composition merit further study.

Keywords: Yield, nutrient-concentrations, nutrients-uptake, bread-wheat, crude-protein, nutrient-interactions, nutritional-quality

1. Introduction

Bread-wheat (*Triticum aestivum* L.), is the dominant-type of wheat that is produced globally, and the most important food-security crop with the production of 752 million tons (MT) on about 220 million hectares (Mha) making it the most widely cultivated crop in the world (FAO, 2020). Its nutritive-value is also fairly-high compared with other cereals. On average it contains 11.80, 1.50, 71.20, 1.50, 0.05 and 0.32 % protein, fat, carbohydrate, mineral-matter, calcium and phosphorus respectively (Swaminathan, et al., 1981).

Ethiopia used to be the leading producer of wheat followed by South Africa, producing 1.7 million hectares, Mha (Tadesse et al., 2018). But, in recent years this figure and ranking has been overtaken by Egypt placing Ethiopia in 2nd place with a production of 5.5 MT annually (FAO, 2020). Based on the report, this is equivalent to 21.7% in the area of production and 18.3% of the harvested produce in Africa.

According to the [CSA, \(2023\)](#) report, Ethiopia produced 9.5 MT of wheat in the same year with the average productivity of 2.8 t/ha, which is still very-low compared with the potential average yield of 5.0 t/ha. This low-productivity is attributable to a complex and interrelated biophysical and socio-economic factors. But, the first most important constraint to crop-production or productivity in our case is that caused by the shortage of plant-nutrients, which is not only due to their continuous-mining and low-availability in soils, but also due to the unbalanced use of imported-fertilizers. For example, for the last half-century, Ethiopia's agriculture depended solely on the purchased fertilizers, mainly urea and DAP, the sources only of N and P. These, indeed are the most limiting nutrients in the arable-soils of the country. But, in recent explorations, ([Menna et al., 2016](#); [Menna, 2017](#); [2018](#); and [2022a](#)) identified severe sulfur and some micronutrients-deficiency in intensively cultivated-soils. This not only threatens the dietary-needs of the people but also the nutritional-quality of consumed food or feed products. It is well recognized that, for health-conscious society, nutrition-security is becoming the most important than the food-security ([Ingram, 2020](#)). In this context, proteins or their specific amino-acids are still the most important, the quantity or quality of which depends on the quality of the harvested grain or their flour-quality. When it comes to the nutritional-quality, crops acquire the needed amounts of essential-nutrients from soils or from mineral-fertilizer inputs.

In proteins-synthesis, the elements N, P and S, however, are perceived to be the most important. For example, in wheat-proteins, N is known for its role as a primary-component of amino-acids. When supplied in adequate amounts, N is recognized to promote plant's photosynthetic-capacity, which is essential for energy-production; and the overall plant-growth and development leading to higher-yields and protein-synthesis. Sulfur is also an essential-constituent of amino-acids such as cysteine and methionine, the constituents of protein in wheat ([Hawkesford & De Kok, 2006](#)). Its adequate supply has also been shown to improve N use-efficiency ([Salvagiotti, et al., 2009](#)). Likewise, P plays a vital-role in several physiological processes *via* photosynthesis, increased dry-matter, respiration, energy-storage and cell-division ([Bakhsh et al., 2008](#)). Phosphorus deficiency reported to result in decrease in chlorophyll-content ([Jacob & Lawlor, 1991](#)) and reduction of photosynthetic capacity of leaves ([Lauer et al., 1989](#)). Ultimately, the nutritional-quality of harvested-produce depends not just on the total-amount of nutrients in the soil or applied, but on their availability, uptake by the plant-parts, and eventual assimilation into the grain. Therefore, the objectives of this study were to: - (1) Evaluate the effects of S and P, individually and in combination with N, on their concentration and uptake in wheat-grain; (2) Estimate grain-protein content and examine the relationship between such plant-variables with soil-pool and mineral-fertilizer inputs.

2. Materials and methods

2.1. Site selection and treatment applications

This study was conducted in randomly-selected six-sites representing three locations, viz. Arsi (Ar); East-Shewa (ES) and West-Shea (WS) zones. It was based on our previous S-response and S-prescription experiments. The sites were Dosh (Do) & Boro-Lencha (BL); Insilale (In) & Bekejo (Bk); and Nano-Kersa (NK) & Dawa-Lafto (DL); two-sites per location respectively.

Sulfur as well the P requirement of wheat ranged between ≤ 5 kg/ha to a little above 20 kg/ha ([Menna, et al., 2016](#)). From this standpoint, eight experimental-treatments were laid-out in RCBD in triplicate, where each block was further divided

into three by five (15 m²) plots. The treatments include, the check-unit (CK) without any fertilizer; sole-sulfur (S); sole-phosphorus (P); sole-nitrogen (N); N & S (NS); N & P (NP); S & P (SP); and N, S & P (NSP). The nutrient rates used were 2-levels of S (S₁= 0= CK, S₂= 20 kg S/ha); 2-levels of N (N₁= 0= CK, N₂= 115 kg/ha); and 2-levels of P (P₁= 0= CK and P₂= 20 kg/ha) which were arranged in a nutrient-omission pattern as gypsum, urea and triple-super-phosphate (TSP). Nitrogen was split-applied; 1/3 just before seeding applied incorporated into soils within rows, whereas the remaining 2/3 its amount was top-dressed at the tillering-stage. But, the entire sources of S and P were applied drilled within rows and incorporating into soils before seeding. Agronomic spacing for wheat, 25 cm by 5 cm (between rows & plants respectively) was used. In total there were 12-rows of plants, from which two-rows were used as borders and another one-row was used for tissue-sampling. In the end, net central-rows, 4 by 1.5 (6m²) per plot were used for data collection. All necessary management-practices pertinent to the wheat-crop were done as per the recommendations until harvest.

2.2. Soil sample collection, preparation and analysis

Pre-sowing soil-samples were collected from 10 points per block (0–20 cm depth), which were then bulked and further composited to make a sample per field. Soil-samples then were air-dried immediately in dry-rooms and later ground and sieved to pass 1-mm size sieve. Selected physico-chemical properties of the soils were then analyzed in laboratories (Lab) as per the methods described in Table 1.

Table 1. Analytical methods of some selected soil parameters of the study Areas

Parameters	Unit of measurement	Extraction methods by	References
pH	pH (1:2.5), soil: H ₂ O	Potentiometrically, 1:2.5 soil: water	Van Reeuwijk(2002)
EC	mS/cm	1:5 soil: water suspension	Klute (1986)
Exch. bases	Cmol(+)/kg	1 M NH ₄ OAc solution at pH =7.00	Van Reeuwijk (2002)
CEC	Cmol(+)/kg	1 M NH ₄ OAc solution at pH =7.00	Van Reeuwijk (2002)
PBS	%	Calculation from exch. Bases	Van Reeuwijk (2002)
TN	%	Kjeldahl as described in	Okalebo <i>et al.</i> , (2002)
OC	%	Walkley-Black as described in	Nelson & Sommers, (1996)
Av. P	mg/kg	Bray-1 & Olsen plant available P.	Bray & Kurtz. (1945); & Olsen, et al. (1954)
SO ₄ -S	mg/kg	Ca-orthophosphate, Turbidimetrically	Rowell (1994)
Soil texture	% (sand, silt & clay)	Hydrometer method	Bouyoucos (1962)

Key: EC = electrical conductivity; Exch. = exchangeable; CEC = cation-exchange-capacity; BS/PBS = % base-saturation; TN = total-nitrogen; OC = organic-carbon; Av.P = available-phosphorus; SO₄²⁻ = available sulfur (sulfate-sulfur).

2.3. Plants-samples collection and analysis

Harvesting was commenced when the average wheat-grain moisture was reached 13.5%. Then, the grain samples were collected from each plot and oven-dried at 65–70°C to a constant weight for about 48hrs. Oven-dried materials, then were finely milled using Tecator-CYCLOTEC-1093 sample mill to a required fineness (1 mm sieve-size) and analyzed for total nitrogen (TN), total sulfur (TS) and total phosphorus (TP). From flours, the TN was extracted by Kjeldahl wet-digestion method using conc.H₂SO₄; whereas TS was extracted by a similar wet-digested digestion, but by using 68%HNO₃-30%H₂O₂. The TP in flour was extracted using the Bray-I methods which involves shaking of the samples with the solutions of HCl & NH₄F. From the solutions, respective-contents were read using spectrophotometer. From the data obtained in Lab, the nutrients uptakes and row-protein concentrations were determined. Nitrogen, S and P uptakes were calculated as the product of dry-wt of the wheat grain, expressed in kg/ha by their respective concentrations, and

dividing the whole by 100. Similarly, the protein-concentration was determined on the basis of TN, as the product of its concentration and a constant value, 5.70.

2.4. Statistical-analysis

The plant variables under considerations were subject to normally (i.e., p-value from Kolmogorov-Smirnov test); and f-tests. In this, the p-values of the variables for all sites were >0.05, implying that there was no significant departure from the normality. Then, ANOVA was performed to evaluate treatment-effects on the GY; nutrient-concentrations; nutrient-uptakes; and the row-protein concentrations using PROC-MIXED generalized-linear-model (GLM) of R-software (R-Core, 2021). When, treatment-differences were found significant, least-significant-difference (LSD) was used to separate the means at 5%, 1% & 0.1% probability-levels. Correlation/regression analyses were also performed to test interrelationships among the soil-plant variables using PROC REG-corr protocol. Also, fertility status of the soils was evaluated based on the established ratings developed by Landon (1991); and Thiagalingam, (2000) using descriptive statistics.

3. Results and discussions

For simplicity of presentations, plant-variables with small-values and narrow-ranges are represented in tables; whereas those with relatively larger-values and wider-ranges are presented in figures. Hence, the effects of N, S and P, and their interaction on their mean S-uptake, P-uptake and the protein-concentrations in wheat-grain are depicted in Figures 1, 2 & 3; whereas the mean concentrations of N, S and P as well as the N-uptake are summarized in the Tables 3-8.

Table 2. Some physico-chemical features of soils of the studied areas cultivated for wheat before planting.

Farmer field (Site)	pH (Soil:H ₂ O)	EC (mS/cm)	Exchangeable Bases				CEC (Cmol(+)/kg)	BS or PBS (%)	TN (%)	OC (%)	Av.P (mg/kg)	SO ₄ -S (mg/kg)	Soil type	Soil texture
			Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺								
			(cmol(+)/kg)											
Dosha	5.0	2.21	6.53	1.42	0.22	1.00	15.00	42.37	0.15	0.92	9.80	11.33	Nitisol	C
B/Lencha	6.8	1.11	12.98	4.42	0.26	1.66	28.96	71.61	0.28	3.00	6.33	5.55	Andosol	SC
Insilale	7.3	1.13	23.23	5.46	0.27	2.06	45.43	90.50	0.13	1.51	11.44	7.56	Chromic-Vertisol	C
Bekejo	7.4	1.11	24.96	5.26	0.45	2.21	47.80	96.52	0.08	1.42	8.62	1.65	Pellic-Vertisol	SC
N/Kersa	6.6	1.02	14.47	3.76	0.28	2.06	40.44	66.87	0.26	1.46	0.50	13.58	Chromic-Vertisol	C
D/Lafto	5.2	0.09	6.12	1.23	0.31	2.20	18.6	41.44	0.18	1.80	8.49	11.82	Nitisol	CL

Key: Soil-texture (SC = Sandy-clay, C = Clay, and CL = Clay-loam). Av.P = available-P (pH >7.0, analyzed by Olsen; & for pH < 7.0, by Bray-I methods).

3.1. Some physico-chemical properties of the studied soils

The was wide-variation in soil-property values across locations/sites owing to their edaphic as well as the environmental factors (Table 2). The investigated soils were salt-free, but major soil-variables like OC, TN, Av.P and SO₄²⁻ were sub-optimal for sustaining crop production/productivity. For example, about 80% of the soils had low OC, and this correlates well with the very-low levels of soil N and S. The sequence of exchangeable-bases was Ca²⁺ > Mg²⁺ > K⁺ > Na⁺, and this was consistent with the high-levels of soil-pH and the percent BS, especially in the calcareous-soils that came from ES-zone. The soils from Arsi-zone were slightly-acidic, whereas those from the West-Shewa zone were strongly-acidic. The pH being a logarithmic function, a unit change in its values is expected to be 10 times more acidic/alkaline than a unit below/above it. This means that, a solution with pH:5 will have 10 times more

hydroxyl-ion (OH⁻) concentration than a solution of pH:4 or 100 times more OH⁻ ion concentration than a solution with pH:6. This is expected to affect primarily the CEC due to the pH-dependent nature of 2:1 clay-minerals, which is expected to affect nutrients' availability and their use-efficiency by crop-plants. The pH values of soils lying between 4.5–8.5 is considered to be typical for agricultural soils. However, according to [Thiagalagam, \(2000\)](#) the values ranging from 5.5 to 7.0 are preferred by most crops/pastures. There was also noticeable change in soil-texture ranging from sandy to clay-loam with clays dominating the textural-class. This, the observed heterogeneity among soil-property-values will be an important tool for implementing site-specific fertilizer prescriptions.

3.2. Nitrogen, S & P concentrations in wheat-grain as affected by their supplies

In the respective sites, mean concentrations (m-c) of N, S and P in wheat-grain was significantly affected by the treatment-effects or nutrients supplies (Tables 3-8). Across-locations, overall, there are a hundred & forty-four entries/observations/plots (n=144); and the N, S and P concentrations in wheat-grain showed wide spatial-variabilities owing to the differences in soil-conditions and/or the external nutrient-supplies.

Table 3. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Dosh-site.

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.208	a	NPS	0.194	a	NPS	106.752	a	NPS	0.140	a
	NP	2.138	b	NS	0.150	b	NP	94.461	b	NP	0.123	b
	NS	2.082	b	SP	0.124	c	NS	89.968	b	P	0.101	c
	N	1.781	c	S	0.116	dc	N	46.127	c	SP	0.101	c
	SP	1.120	d	NP	0.114	dc	SP	12.514	d	NS	0.061	d
	P	1.118	d	N	0.109	d	S	11.146	d	N	0.052	e
	S	1.114	d	P	0.108	d	P	10.511	d	S	0.042	f
	CK	1.106	d	CK	0.065	e	CK	9.437	d	CK	0.012	g
α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.001114	-	-	0.00007	-	-	15.160	-	-	5.613E-6	-	-
CV of t	2.14479	-	-	2.14479	-	-	2.145	-	-	2.14479	-	-
LSD	0.0584	-	-	0.0147	-	-	6.818	-	-	0.0041	-	-

Note: Trt =treatment; m =mean; L= t-grouping(m-separation); TNg =total-nitrogen in grain; TSg =total-sulfur in grain; NU =N-uptake in grain; and TPg =total-phosphorus in grain. Means with the same letter in a column are not statistically-significant.

Table 4. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Boru-Lencha site

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.272	a	NPS	0.189	a	NPS	137.751	a	NPS	0.153	a
	NP	2.206	ba	NS	0.144	b	NP	125.932	b	NP	0.132	b
	NS	2.132	b	SP	0.114	c	NS	118.864	b	P	0.106	c
	N	1.769	c	NP	0.111	c	N	67.418	c	SP	0.104	c
	SP	1.215	d	N	0.104	c	SP	28.568	d	NS	0.064	d
	P	1.211	d	S	0.099	dc	P	21.990	ed	N	0.054	e
	S	1.206	d	P	0.086	de	S	21.767	ed	S	0.044	f
	CK	1.194	d	CK	0.074	e	CK	17.487	e	CK	0.015	g
α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.00229	-	-	0.00008	-	-	23.6702	-	-	6.405E-6	-	-
CV of t	2.14479	-	-	2.14479	-	-	2.14479	-	-	2.14479	-	-
LSD	0.0837	-	-	0.0158	-	-	8.52	-	-	0.0044	-	-

Note: Means with the same letter in a column are not statistically-significant.

These values ranged between 1.10–2.35% for N; 0.06–0.21% for S; and 0.01–0.16% for P with the median-values of 1.47; 0.11; and 0.08 respectively. Nitrogen-concentrations in the grains were significantly higher compared with S and P, expressed symbolically as (Nc>Sc>Pc), elucidating the variations in the nutrient-requirements by the wheat-crop. Indeed, these values comply with the results reported by [Crista et al., \(2013\)](#); and [Randall et al. 1981](#).

Table 5. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Insilale-site.

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.254	a	NPS	0.170	a	NPS	112.921	a	NPS	0.141	a
	NP	2.171	b	NS	0.149	b	NP	81.627	b	NP	0.116	b
	NS	2.086	c	SP	0.113	c	NS	73.994	c	SP	0.105	c
	N	1.785	d	NP	0.111	c	N	49.997	d	P	0.103	c
	SP	1.121	e	N	0.107	c	SP	11.321	e	NS	0.062	d
	P	1.120	e	S	0.095	d	P	9.892	e	N	0.051	e
	S	1.114	e	P	0.084	e	S	9.691	e	S	0.042	f
	CK	1.110	e	CK	0.065	f	CK	9.629	e	CK	0.013	g

α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.0011	-	-	0.00002	-	-	7.0003	-	-	0.00002	-	-
CV of t	2.145	-	-	2.145	-	-	2.145	-	-	2.14479	-	-
LSD	0.058	-	-	0.0069	-	-	4.633	-	-	0.0064	-	-

Note: Means with the same letter in a column are not statistically-significant.

Table 6. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Bekejo-site.

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.237	a	NPS	0.183	a	NPS	133.273	a	NPS	0.147	a
	NP	2.173	b	NS	0.160	b	NP	114.328	b	NP	0.121	b
	NS	2.087	c	NP	0.112	c	NS	91.210	c	P	0.103	c
	N	1.786	d	SP	0.108	c	N	53.216	d	SP	0.101	c
	P	1.122	e	N	0.103	c	SP	15.977	e	NS	0.063	d
	SP	1.122	e	S	0.088	d	P	14.969	e	N	0.052	e
	S	1.117	e	P	0.078	e	S	14.046	e	S	0.044	f
	CK	1.111	e	CK	0.064	f	CK	12.165	e	CK	0.015	g
α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.00084	-	-	0.00003	-	-	26.738	-	-	5.03E-6	-	-
CV of t	2.145	-	-	2.145	-	-	2.145	-	-	2.145	-	-
LSD	0.051	-	-	0.010	-	-	9.0552	-	-	-	-	-

Note: Means with the same letter in a column are not statistically-significant.

Table 7. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Nano-Kersa site.

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.287	a	NPS	0.189	a	NPS	139.474	a	NPS	0.154	a
	NP	2.216	ba	NS	0.153	b	NP	106.081	b	NP	0.126	b
	NS	2.137	b	SP	0.130	c	NS	90.213	c	P	0.105	c
	N	1.774	c	S	0.125	c	N	63.688	d	SP	0.103	c
	P	1.236	d	NP	0.109	d	SP	34.522	e	NS	0.064	d
	SP	1.223	d	N	0.101	ed	P	27.541	e	N	0.054	e
	S	1.207	d	P	0.101	ed	CK	11.364	e	S	0.044	f
	CK	1.199	d	CK	0.096	e	S	10.597	e	CK	0.013	g
α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.00265	-	-	0.000033	-	-	37.3304	-	-	6.899E-6	-	-
CV of t	2.14479	-	-	2.14479	-	-	2.145	-	-	2.14479	-	-
LSD	0.0902	-	-	0.0101	-	-	10.7	-	-	0.0046	-	-

Note: Means with the same letter in a column are not statistically-significant.

Table 8. Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain-N, grain-S, grain-N uptake, grain-P at Dawa-Lafto site.

Stat	Trt	m-TNg	L	Trt	m-TSg	L	Trt	m-NUg	L	Trt	m-TPg	L
	NPS	2.251	a	NPS	0.157	a	NPS	107.305	a	NPS	0.142	a
	NP	2.176	b	NS	0.150	a	NP	96.796	b	NP	0.122	b
	NS	2.091	c	SP	0.113	b	NS	85.296	c	P	0.105	c
	N	1.790	d	NP	0.112	cb	N	58.003	d	SP	0.103	c
	P	1.126	e	N	0.104	c	SP	18.003	e	NS	0.062	d
	S	1.123	e	P	0.089	d	P	13.361	e	N	0.054	e
	SP	1.123	e	S	0.089	d	S	12.655	e	S	0.044	f
	CK	1.115	e	CK	0.072	e	CK	12.624	e	CK	0.012	g
α	0.05	-	-	0.05	-	-	0.05	-	-	0.05	-	-
EDF	14	-	-	14	-	-	14	-	-	14	-	-
EMS	0.0012	-	-	0.000021	-	-	17.894	-	-	-	-	-
CV of t	2.1448	-	-	2.14479	-	-	2.145	-	-	2.145	-	-
LSD	0.060	-	-	0.008	-	-	7.408	-	-	-	-	-

Note: Means with the same letter in a column are not statistically-significant.

Considering specific treatment-effects, the m-Nc was responded mainly to the supplies of N ($P \leq .001$); whereas the m-Sc was responded to the supplies of both S and N ($P \leq .01$). On the other hand, the m-Pc showed response to the P supply and its interactions with N and S ($P \geq .05$). This variation in nutrient-concentrations can be attributable to several factors like the soil-pool and fertilizations; the plant-genetics; the climatic-conditions; and the management-practices among others. Hence, optimization of such factors, esp., the plant-nutrients including their management-practices will be the underlying-principles for enhancing nutrient-accumulations in the plant-parts.

3.3. Nitrogen, S & P uptakes in wheat-grain as affected by their supplies

The grain nutrient-uptake (GnU) values merely reflected the grain-yield (GY) response to applied nutrients. Hence, the mean nitrogen-uptake (m-NU) (Tables 3-8); sulfur-uptake (m-SU); and phosphorus-uptake (m-PU) (Figures 1-3) in wheat-grain were also significantly-affected ($P \leq .05$) by their respective fertilizations.

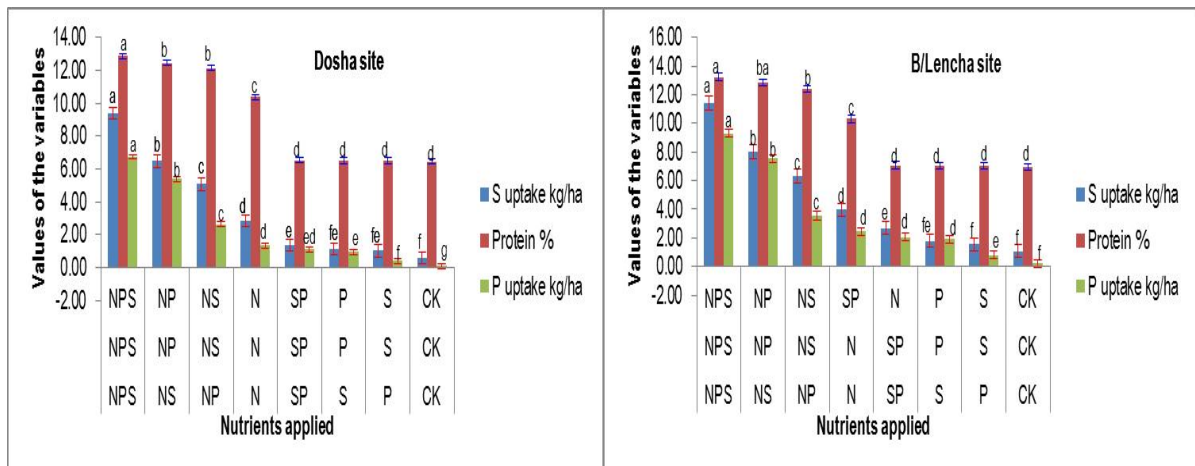


Figure 1: Effect of N, S, and P as sole-nutrients & interacting on mean wheat-grain S-uptake; P-uptake & row-proteins concentration at Arsi-zone (Do- & BL-sites). Values expressed as mean \pm SEM ($n = 24$); * $p \geq 0.05$; ** $p < 0.01$ or *** $p < 0.001$ probability levels compared with control. Means bearing the same letter in the bars are not significantly different at $p < 0.01\%$ of significance analyzed by t-test. **Key:** CK= check/untreated-control; P= phosphorus-alone; S= sulfur-alone; SP= (SxP)-interaction; N= nitrogen-alone; NS= (NxS)-interaction; NP= (NxP)-interaction; NPS= (NxPxS)-interaction effect.

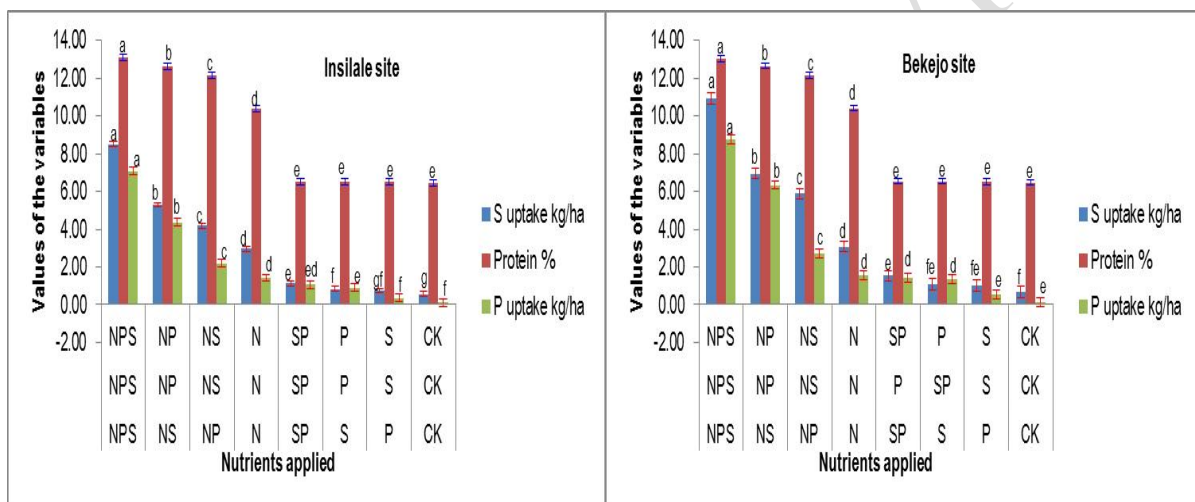


Figure 2: Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain S-uptake; P-uptake & row-protein at East-Shewa zone (In- & Bk-sites).

Nutrient uptake-values across-locations ($n=144$ units) also showed wide spatial-variabilities. And these ranged between 7.42–143.66 kg/ha for N; 0.38–11.87 kg/ha for S; and 0.08–9.88 kg/ha for P with the median-values 41.00; 2.81; and 1.70 respectively. Like the m-Nc, the m-NU in the grains showed a highly significant response ($P \leq .001$) mainly to the N supply; whereas the m-SU was responded to both N & S supplies ($P \leq .01$). On the other hand, the m-PU showed responses to P supply, esp., interacting with N & S ($P \geq .05$). In other words, these nutrient-uptake values showed a strong positive-correlations to their respective-concentrations in grains. These indeed, agreed with the results reported by [ZHU Xin-kai, et al., \(2012\)](#); and [Mahajan, et al., \(2013\)](#).

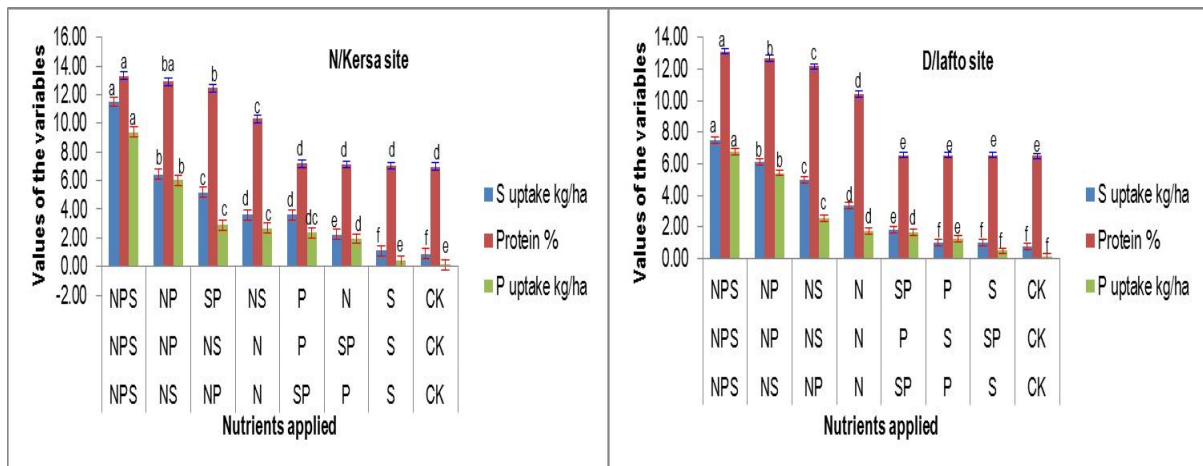


Figure 3: Effect of N, S, and P as sole-nutrients & interacting on mean wheat grain S-uptake; P-uptake and row-protein at West-Shewa-zone (NK- & DL-sites).

As noted, the NU values have come in significantly larger-values, but complies with the values reported in other regions. For example, [Young et al., \(2023\)](#), reported the NU values in wheat-grain ranging between 200–250 kg/ha, that can result from specific growing-conditions. In our case, the presence of adequate variability in soil, esp., the availability of N and S were the main-factors, and these in turn were influenced strongly by soil-texture and the OM contents. The availability of P for plant-uptake, however, was found to be influenced by the soil chemical-process (mainly pH), which may also include some kind of bio-physical processes. Other such conditions, may include the variety-used including higher N management, which can lead to a corresponding higher-yields and grain-proteins. Using such basic parameters, therefore, soil test-based fertilizer prescriptions can be developed for desired target yield/quality.

3.4. Wheat-grain protein-concentration as affected by N, S and P fertilization

The mean protein concentration (m-pc) in wheat-grain also showed significant variations due to the supplies of N, S, and P, with statistical-significance levels of $P \leq .001$, $P \leq .01$, and $P \geq .05$, respectively. Across the studied sites ($n = 144$), overall pc exhibited significant spatial-variation due to treatment-effects, ranging between 6.44–13.71%, with a median-value of 8.55%.

In this study, m-pc was 13.33%, aligning with values reported in the literature. However, for wheat-varieties intended for optimal baking-quality, a minimum pc of $\geq 11\%$ is generally required. This 13.71% in our study was obtained through the application of full-dose NSP, underscoring the importance of balanced fertilizations in achieving higher-proteins. Typically, wheat grain pc ranges between 10% and 19% ([Alomari et al., 2023](#); [Iqbal et al., 2022](#)), depending on the variety and analytical-methods used. The values obtained in our study, determined via Kjeldahl digestion, fall within this range but tend to lie on the lower-side or even slightly below it.

The influences of the applied-nutrients, on wheat grain-protein were found to be in the order: $N > S > P$, highlighting nitrogen's predominant role in protein-synthesis. Phosphorus contributed the least to pc, a finding consistent with [Crista et al. \(2013\)](#), who reported that P alone has minimal impact on wheat grain-protein, though it may enhance the assimilation and metabolism of N when applied together. In our present study, the observed variations in grain pc were primarily attributed to the differences in soil-fertility, both in terms of nutrient-reserves and applied-inputs. Beyond soil-fertility, other factors, including agro-ecological conditions and farming-practices, are

expected to be influencing its levels, pointing to a complex-network of interacting variables that can act independently or synergistically to affect protein-composition, and the potential and opportunities for improving overall-yield, yield-components, and other quality-attributes of harvested-produce. The identification of relevant genes may also advance our understanding of the interactions between factors such as S availability and wheat quality-traits. Results highlight the need for a paradigm-shift in crop-production strategies, emphasizing complex-networks of relationships among production-factors.

In summary, as showed in the figures and tables, min-values for all the plant-variables were recorded in the CK unit, while max-values were observed under full-dose NSP treated-units, the response-trend which can be expressed as: $CK \leq S \leq P \leq SP < N < NS < NP \leq NPS$. To fully understand these effects on various plant variables, further research is needed to evaluate graded-fertilizer-levels across different production-factors and wheat-varieties. Overall, the findings underscore the importance of a holistic-approach to crop-production, one that prioritizes both yield-improvement and nutritional-quality. This approach also offers potential for enhancing nutritional-value of wheat and for informing the development of nutritional and export-standards.

Conclusions

The investigated wheat plant-variables showed significant-variations, particularly in response to N and S supply, relative to their respective soil-test values. However, all plant-variables under consideration, except the P-uptake in the grain, demonstrated differential-changes in response to P supply. As individual nutrient-effects, increases in wheat production-components were mainly influenced by N. In particular, the sole-N application, as well as its interaction with S, had a strong positive-effect on wheat-grain protein-concentration. Although P didn't show as strong an effect as N on protein-composition, it appeared to support the effects of N, likely by enhancing the assimilation or metabolization of absorbed N. However, the interaction-effects of two or three nutrients were found to be more significant than any single nutrient-effect on the production-components of wheat. Among these, the interaction between N and S showed a strong synergistic-effect. In contrast, the interaction between S and P exhibited both synergistic and antagonistic-effects, which may depend on the levels or dosages applied. Beyond quantitative yield-responses, wheat plants that responded to S supply developed deep grayish-green leaves and stems with abundant, vigorous vegetation. In comparison, plants adequately supplied with N developed dark-green leaves/stems or twigs, also with lush and vigorous-growth. Overall, the pronounced fertility-gradient in the native-soil, as well as that introduced through mineral-fertilizers, was strongly-reflected in the observed variations in the plant-variables, esp., in the nutrients-concentrations, protein-concentrations, and nutrient-uptake. However, these variations are not solely attributable to soil conditions; but also, the other factors of production like climate, farming-practices and the genetic make-up of the variety will have their share of influence for the observed variations in the variables. Therefore, further-studies focusing on the quality-attributes of what can be made even taking each at a partial factor-level. Especially, it is of utmost importance to pursue the effects of N, S and P; and even others on the true-protein concentrations. The overall results, emphasize the importance of adopting holistic-approach to crop-production that prioritizes nutritional quality alongside yield improvements.

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