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MORPHO-PHYSIOLOGICAL AND ANATOMICAL RESPONSES OF WHEAT PLANTS TO MICRONUTRIENTS AND NITROGEN FERTILIZATION

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ABSTRACT

Two field experiments were conducted in 2005/2006 and 2006/2007 in the region of Tag AL-Ezz, Agricultural Research Station Farm, Dakahlia Governorate, Agricultural Research Center, Egypt to evaluate the effect of nitrogen rates and micronutrients fertilizers on wheat plant growth, some physiological and anatomical parameters and yield.

Application of micronutrients combined mixture under high nitrogen rate produced the highest values of plant growth, yield and physiological characteristics compared with other treatments. The second rank best treatment was observed with applications of Zn, then Mn treatment. Cu and Fe treatments were equal in its effect and the differences between them were insignificant in the most cases. Application of nitrogen rate up to 90 kg/fed increased gradually all studied characters.

Anatomically, application of combined mixtures of micronutrients under high nitrogen rate increased significantly all anatomical characteristics of flag leaf or culm, in particular, number of vascular bundles and thickness of mechanical tissue in culm as well as dimension of vascular bundles, diameter of metaxylem vessels, and thickness of epidermis in both flag leaf and culm. Furthermore, thickness of mesophyll parenchyma and midrib region in flag leaf was also increased.

In conclusion, the obtained results show that foliar application of combined mixture of studied micronutrients (Cu, Mn, Fe and Zn) at the rate of 500 ppm from each under moderate (recommended rate) or high rate of nitrogen fertilizer (70 or 90 kg N/fed) can be recommended to maximize wheat growth and grain yield per main spike and per plant.

Key words: wheat, micronutrient, nitrogen, culm anatomy, leaf anatomy, growth

INTRODUTION

Fe, Mn, Cu and Zn are important essential micronutrients for both plant and human. Deficiency of these microelements in human body will result in a series of severe adverse consequences, such as anemia, low immunity function, skin disease, children response slowness and intelligence stunt, and so on. Currently, about one third of world population, in particular, in developing countries, are facing malnutrition problem due to the deficiencies of Fe/Zn or vitamin A (WHO, 2007). Traditional nutrient food fortification or medication cannot thoroughly solve this problem due to them only has a temporal role to malnutrition but with expensive costs. However, the micronutrient biofortification of staple food crop is regarded as an effective way to solve this problem (Welch and Graham, 2004). Wheat is a staple food crop feeding of the population in Egypt, which also is one of the important approaches for human taking nutrient and microelements from nature. Accordingly, the problem about micronutrient deficiency of human can be solved economically and sustainability by increasing microelements contents in wheat grain and improving their bioavailability. Biofortification, the process of enriching the nutrient content of crops as they grow, provides a sustainable solution to malnutrition in the world (Jeong and Guerinot, 2008). The concept of biofortification is attractive not only for improving the growing conditions of crops but also for exploiting a plant's potential for micronutrient mobilization and utilization. There are several recent reviews on the current strategies for the biofortification of crops, including mineral fertilization, conventional breeding and transgenic approaches (Zhu *et al*., 2007, Mayer *et al*., 2008).

During the past three and half decades, micronutrients have occupied an important position in Egyptian Agriculture and have become indispensable to the productivity of crops. Micronutrients have been known to increase the yield and improve the quality of different cereals including wheat (Sienkiewicz-Cholewa, 2008, Seadh *et al*., 2009). Using soil organic matter leads to a significant and direct impact on the availability of micronutrients as Zn, Fe and Mn (Zhang *et al*., 2001). However, the soil application of micronutrients fertilizers in the cultivar may not meet the crop requirement for growth and nutrient use, thus the alternative effective approach is to apply these micronutrients as foliar spray (Seadh *et al*., 2009). Foliar application with differed micronutrients could be equal or more effective the soil applied and used effectively to overcome the problem of micronutrient deficiency in subsoil (Sienkiewicz-Cholewa, 2008).

Rapid increase in wheat consumption outpaced domestic production due to population growth. The area of wheat in Egypt was estimated at about 2841795 fedden in the 2008/2009 season, which produced about 8.000 million tons. Over all agriculture production from wheat has tended to increases increscent years, but even this is not enough to keep up with population growth. Accordingly, there are still many efforts are underway to improve wheat productivity of unit area such as using optimum nitrogen fertilizer level. Nitrogen fertilization is a key input for increasing wheat productivity in Egypt. Although, the introduction of high yielding cultivars has significantly contributed to the prospect of increasing yields, this increase was often associated with intensive cultivation, in which a high level of N was applied. This high rate of application might negatively affect the agro-environment through nitrate leaching and ground water pollution. Therefore, it is necessary to improve the management of nitrogen fertilization to maintain the growth and productivity of wheat and develop a more sustainable production system.

Our investigations aim to evaluate the efficiency of micronutrients foliar application on improving wheat growth and yield as well as physiological and anatomical responses under nitrogen fertilizer

MATERIALS AND METHODS

Two field experiments were conducted, during the principal 2005/2006 and 2006/2007 seasons at the region of Tag AL-Ezz, Agricultural Research Station Farm, Dakahlia Governorate, Agricultural Research Center, to study the effect of foliar application with micronutrients and nitrogen rates, as well as, their interactions on growth, anatomical and physiological characters as well as grain yield of wheat plant.

The field experiments were laid out in a split–plot design with three replicates. The main plots were allocated to seven foliar treatments as follows: $CuSO_4$, Mn-EDTA, Fe-EDTA and Zn-EDTA at the rate of 500 ppm of each as well as the combined mixture of these micronutrients together at the rate of 500 ppm of each and water spraying beside control plants (without spraying). The foliar solution was at the rate of 200 liter/fed and spraying was done until saturation point twice at the formerly rate after 30 and 50 days from sowing.

The sub plots were assigned to three nitrogen rates (50, 70 and 90 kg N/fed). The nitrogen fertilizer in the form of urea (46.5 % N) was applied as soil addition in two equal doses prior to the first and the second irrigations. Before planting, both physical and chemical analysis for the soil under investigation was undertaken according to Jackson (1973) and corresponding data are presented in Table 1. Each experimental unit was 3 m X 3.5 m occupying an area of 10.5 m^2 (i.e. 1/400 fed). The preceding summer crop was rice (*Oryza sativa* L.) in both seasons.

Table 1: Physical and chemical soil characteristics at the experimental sites during the growing 2005/2006 and 2006/2007 seasons.

Physical characteristics									
Properties Seasons	Soil texture	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	EC dsm^{-1}	Field capacity (%)	Real density (g/cm^3)
2005/2006	Clay loam	6.2	32.6	24.7	35.5	2.45	2.2	34.3	2.66
2006/2007	Clay loam	5.8	33.2	25.3	35.7	2.54	2.4	35.2	2.65

Chemical characteristics						
Properties Seasons	pH soil paste	Organic matter (%)	CEC meq/100g	Available nutrients (ppm)		
				N	P	K
2005/2006	7.6	1.72	53.2	32.3	14.4	315
2006/2007	7.8	1.83	50.7	36.4	15.3	324

From Table (1), it could be seen that soil contents from available P and K are adequate enough for wheat crop. The cultivation took place in November 23rd and 26th in the first and second seasons, respectively. Wheat grains cultivar Giza 168 at the rate of 60 kg/fed were sown by using broadcasting Afir method. The common agricultural practices for growing wheat according to the recommendations of Ministry of Agriculture were followed, except the factors under study.

At 90 days after sowing (DAS) nine plants were harvested from each replicate for determination some growth parameters (flag leaf area, number of leaves per plant, shoot dry weight) and some physiological characteristics (photosynthetic pigments, total carotenoids, total carbohydrates, and soluble protein and nitrogen contents in the flag leaf). Moreover, culm and flag leaf anatomical features of the best selected treatments and both moderate and high nitrogen rates were studied. At harvest, the grain yields per plant and grain weight per main spike were determined.

Flag leaf area (cm^2) was calculated by the following formula a = L×W × 0.75 (Gardner *et al*., 1985). Photosynthetic pigments were extracted for 24 hr. at room temperature in methanol after adding traces of sodium carbonate. Photosynthetic pigments concentrations were determined spectrophotometrically according to Lichtenthaler and Wellburn (1985). Soluble protein concentration was measured at 595 nm using bovine serum albumin as standard according to the method of Bradford (1976).

For ion content, dry flag leaf samples were digested with $HClO_3/H_2SO_4$ until the solution was clear, cooled, and brought to volume at 50 ml using deionized water. Nitrogen was determined by microkjeldahl methods (Jackson, 1973). Total carbohydrates content were estimated using the anthrone method as described by Sadasivam and Manickam (1996).

The best treatments as compared to moderate (recommended rate) rate were anatomical studied in the second season. Flag leaf segment and small blocks about 1 cm in thickness from the middle portions of the basal internodes of the mature wheat culms were taken. The samples were fixed immediately in formalin-acetic-alcohol for 48 h, and then dehydrated in a series of n-butanole series. The completely dehydrated specimens were immersed into paraffin wax (52-54°C melting points). Cross sections of 15-17 µm thick were prepared by a rotary microtome, stained with erythrosin/crystal violet and mounted in canada balsam for discriminating leaf or culm internal structures. The sections were examined microscopically for determining the anatomical changes.

All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split–plot design by means of “MSTAT-C” computer software package as published by Gomez and Gomez (1984).

RESULTS

Plant growth and yield

Foliar application with micronutrients treatments, in particular, combined mixture of micronutrients at the rate of 500 ppm from each, increased significantly all studied growth and yield characters i.e., flag leaf area, number of leaves per plant, shoot dry weight, grain weight per main spike and grain yield per wheat plant in both seasons (Table, 2) as compared with water or control plants. The combined mixture of micronutrients produced the highest values of plant growth and yield characters in both seasons followed by either zinc or manganese; meanwhile both iron and copper gave the lowest value of studied characters followed by water spraying and control plants. The data in the same Table clearly indicate that application of nitrogen fertilizer rates from 50 to 90 kg N/fed increased significantly and gradually all growth and yield characters of wheat plant in the two growing seasons.

Table 2: Flag leaf area (cm^2), number of leaves per plant, shoot dry weight (g), grains weight/main spike (g) and grain yield per plant (g) as affected by N-rates and foliar application with micronutrients as well as their interactions during 2005/2006 and 2006/2007 seasons.

Characters / **Treatments**		**Flag leaf area (cm^2)**		**No of leaves per plant**		**Shoot dry weight (g)**		**Grains weight/ main spike (g)**		**Grain yield per plant (g)**	
		2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007
A-Foliar application with micronutrients:											
Untreated		33.65	30.42	16.88	16.33	12.24	12.09	2.30	2.20	8.514	8.587
Water		35.41	33.05	17.00	16.66	12.45	12.19	2.46	2.36	8.713	8.557
Cu 500 ppm		37.84	35.68	16.77	16.66	12.50	12.17	2.65	2.58	8.711	8.599
Mn 500 ppm		38.79	36.56	17.77	17.33	12.97	12.57	2.73	2.63	9.089	8,930
Fe 500 ppm		38.05	34.98	17.55	17.33	12.82	12.51	2.67	2.61	8.997	8.866
Zn 500 ppm		39.90	37.43	17.66	17.11	12.81	12.50	2.80	2.67	9.033	8.828
Combined mixture		42.46	40.24	18.55	17.55	13.23	12.79	2.96	2.86	9.248	9.047
LSD 5 %		0.57	0.70	0.339	0.240	0.130	0.078	0.05	0.05	0.114	0.070
50 kg N/fed		33.18	29.70	15.52	15.33	11.86	11.57	2.45	2.36	8.177	8.127
70 kg N/fed		39.24	37.42	17.52	17.14	12.67	12.43	2.70	2.60	8.915	8.799
90 kg N/fed		41.63	39.33	19.33	18.52	13.62	13.21	2.80	2.71	9.610	9.399
LSD 5 %		1.33	2.21	0.222	0.157	0.085	0.051	0.15	0.10	0.075	0.046
Untreated	50 kg N/fed	26.59	23.01	13.66	13.33	10.63	10.51	2.05	1.98	7.020	7.423
	70 kg N/fed	35.90	32.52	18.00	17.00	12.76	12.59	2.41	2.25	8.920	8.843
	90 kg N/fed	38.46	35.72	19.00	18.66	13.33	13.17	2.45	2.38	9.603	9.493
Water	50 kg N/fed	28.28	25.86	16.00	16.00	12.19	11.84	2.25	2.10	8.433	8.327
	70 kg N/fed	37.83	35.81	19.00	18.00	13.20	13.03	2.59	2.45	9.430	9.230
	90 kg N/fed	40.13	37.49	16.00	16.00	11.97	11.72	2.56	2.53	8.277	8.113
Cu	50 kg N/fed	33.30	30.13	17.00	17.00	12.60	12.29	2.46	2.39	8.787	8,697
	70 kg N/fed	39.34	38.00	15.00	15.00	11.80	11.43	2.69	2.63	8.123	8.033
	90 kg N/fed	40.89	38.91	18.33	18.00	13.10	12.79	2.78	2.72	9.223	9.067
Mn	50 kg N/fed	35.30	31.43	14.00	14.00	11.26	10.75	2.54	2.44	7.707	7.560
	70 kg N/fed	39.50	38.30	18.00	18.00	12.91	12.75	2.76	2.67	9.067	9.027
	90 kg N/fed	41.57	39.95	21.33	20.00	14.75	14.22	2.89	2.77	10.49	10.20
Fe	50 kg N/fed	33.83	28.52	16.00	16.00	12.30	12.05	2.48	2.41	8.550	8.427
	70 kg N/fed	39.22	38.03	19.66	19.00	13.65	13.31	2.71	2.65	9.803	9.647
	90 kg N/fed	41.10	38.40	17.00	17.00	12.50	12.19	2.82	2.77	8.637	8.557
Zn	50 kg N/fed	36.45	32.60	17.66	17.00	12.63	12.52	2.60	2.49	8.850	8.740
	70 kg N/fed	40.48	38.94	15.00	15.00	11.64	11.25	2.82	2.71	8.047	7.893
	90 kg N/fed	42.78	40.75	20.33	19.33	14.16	13.72	2.98	2.83	10.20	9.850
Combined Mixture	50 kg N/fed	38.51	36.33	14.33	14.00	11.42	11.04	2.78	2.71	7.893	7.713
	70 kg N/fed	42.41	40.33	18.00	18.00	12.77	12.69	2.95	2.87	9.017	8.920
	90 kg N/fed	46.48	44.07	23.33	20.66	15.50	14.63	3.16	3.00	10.83	10.50
LSD 5 %		0.98	1.22	0.587	0.415	0.225	0.135	0.08	NS	0.198	0.122

Regarding to the interaction effects, the data in Table (2) reveal that application of micronutrient, in particular, combined mixture of micronutrients under all nitrogen rates increased significantly all studied characters as compared with nitrogen levels alone or application of water as foliar application in the two growing seasons. The highest values of all studied growth and yield characters were obtained due to application of combined mixture of micronutrients followed by zinc under high rate of nitrogen fertilizer (90 kg N/Fed).

Physiological characters:

Application of micronutrients, in particular, the combined mixture of micronutrients at the rate of 500 mg/l from each, increased significantly all physiological parameter i.e. total chlorophyll, total carotenoids, total carbohydrates, total soluble protein and total nitrogen content in flag leaf in both growing seasons, followed by iron or manganese, zinc and finally copper (Table 3). The data presented in the same Table reveal that raising nitrogen fertilizers rates significantly increased all studies physiological characters. The highest content of all physiological components were obtained with high nitrogen level.

As regard to the interactions between micronutrients and nitrogen rates the data presented in the same Table indicate that application of any micronutrients alone or the combined mixture of micronutrients improved significantly all physiological characters in both seasons. The best interaction treatment which produced the highest values of these characters was foliar application with the combined mixture of each micronutrients at the rate of 500 ppm under high rate of nitrogen fertilizer (90 kg N/fed).

Anatomical studies

Leaf anatomy:

There is the tremendous variety shown in leaf blade structure from species to species, yet the basic organization of internal tissues and the developmental pathways are similar. The epidermis, mesophyll, and veins are three basic tissues of a leaf blade of most vascular plants including wheat (Figure 1). Leaf anatomical characters, such as thickness of upper epidermis (UE), thickness of lower epidermis (LE), thickness of leaf through midrib (TL), tangential dimension of midrib vascular bundle (TDMVB), tangential dimension of big xylem vessel (TDBXV), and thickness of mesophyll tissue (M) of flag leaf were studied in the best selected treatments as compared with moderate and high nitrogen rates.

Cross section of wheat flag leaves (Figure 1) showed that there were significant changes in leaf anatomical characteristics induced by application of micronutrients combined mixture or both zinc and manganese under both moderate and high nitrogen rates as compared with moderate and high nitrogen rates (Table, 4; Figure, 1). Application of combined mixture of micronutrient or Zinc and Mn under high rate of nitrogen increased the thickness of wheat flag leaf blade, due to the increase in the thickness of mesophyll tissue as well as thickness of both lower and upper epidermis cells as compared with moderate or high nitrogen rates alone. In addition, the thickness of flag leaf blade through midrib region was also increased respectively, due to the increase in the midrib vascular bundle, as well as tangential and radial dimensions of big metaxylem vessel as compared with moderate nitrogen rate (Table 4 and figure 1). Moreover the same table and figure indicate that application of combined mixture of micronutrient under moderate nitrogen rate increased all anatomical characteristics as compared with moderate nitrogen rate.

Table 3: Total chlorophyll (mg/g FW), Total carotenoids (mg/g FW), Total carbohydrates (mg/g DW), Soluble proteins (mg/g FW) and Nitrogen content (mg/g DW) in flag leaf as affected by foliar application with micronutrients and nitrogen rates as well as their interactions during 2005/2006 and 2006/2007 seasons.

Treatments	**Characters**	**Total chlorophyll (mg/g FW)**		**Total carotenoids (mg/g FW)**		**Total carbohydrates (mg/g DW)**		**Soluble proteins (mg/g FW)**		**Nitrogen content (mg/g DW)**	
		2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007
A-Foliar application with micronutrients:											
Untreated		1.231	1.193	0.340	0.326	12.33	12.17	7.051	6.953	37.21	37.26
Water		1.265	1.218	0.325	0.319	12.62	12.54	7.390	7.308	36.43	36.32
Cu 500 ppm		1.260	1.211	0.340	0.338	12.59	12.57	7.347	7.264	37.26	37.25
Mn 500 ppm		1.254	1.233	0360	0.359	12.86	12.65	7.607	7.429	40.63	39.05
Fe 500 ppm		1.291	1.263	0.353	0.340	12.91	12.87	7.614	7.560	39.15	38.82
Zn 500 ppm		1.269	1.250	0.354	0.348	12.83	12.69	7.579	7.491	38.93	38.54
Combined mixture		1.290	1.254	0.365	0.358	13.18	13.02	7.948	7.781	40.68	39.72
LSD 5 %		NS	NS	NS	0.017	0.168	0.103	0.058	0.051	1.158	0.552
50 kg N/fed		1.145	1.100	0.281	0.267	11.91	11.77	6.621	6.520	33.38	33.49
70 kg N/fed		1.291	1.248	0.365	0.361	12.83	12.76	7.507	7.443	39.09	38.80
90 kg N/fed		1.360	1.347	0.399	0.395	13.54	13.40	8.387	8.230	43.37	42.13
LSD 5 %		0.062	0.056	0.018	0.011	0.110	0.068	0.038	0.078	0.758	0.361
Untreated	50 kg N/fed	0.987	0.931	0.198	0.189	10.47	10.18	5.360	5.213	28.31	28.43
	70 kg N/fed	1.300	1.273	0.386	0.360	12.91	12.84	7.530	7.450	39.91	40.01
	90 kg N/fed	1.407	1.377	0.436	0.428	13.60	13.49	8.263	8.197	43.41	43.36
Water	50 kg N/fed	1.203	1.164	0.278	0.274	12.32	12.20	7.070	7.050	33.80	33.86
	70 kg N/fed	1.411	1.339	0.428	0.426	13.43	13.44	8.103	8.057	42.49	42.43
	90 kg N/fed	1.181	1.151	0.269	0.258	12.13	11.97	6.997	6.817	33.00	32.67
Cu	50 kg N/fed	1.269	1.212	0.343	0.337	12.63	12.65	7.383	7.360	38.51	37.94
	70 kg N/fed	1.154	1.084	0.259	0.255	11.86	11.86	6.750	6.623	30.61	31.70
	90 kg N/fed	1.356	1.337	0.418	0.421	13.29	13.2	7.907	7.810	42.67	42.11
Mn	50 kg N/fed	1.015	0.952	0.231	0.209	11.23	10.84	5.567	5.467	28.63	29.52
	70 kg N/fed	1.337	1.304	0.403	0.409	13.11	13.05	7.720	7.693	43.69	41.31
	90 kg N/fed	1.409	1.442	0.447	0.458	14.24	14.08	9.533	9.127	49.57	46.33
Fe	50 kg N/fed	1.208	1.186	0.307	0.288	12.45	12.44	7.213	7.130	35.44	35.66
	70 kg N/fed	1.418	1.397	0.441	0.435	13.72	13.64	8.347	8.327	44.88	44.40
	90 kg N/fed	1.247	1.207	0.311	0.296	12.56	12.54	7.283	7.223	37.12	36.40
Zn	50 kg N/fed	1.278	1.250	0.373	0.357	12.81	12.65	7.457	7.430	39.44	39.20
	70 kg N/fed	1.083	1.049	0.246	0.251	11.76	11.58	6.477	6.410	30.72	30.84
	90 kg N/fed	1.445	1.452	0.445	0.436	13.93	13.84	8.803	8.633	46.65	45.58
Combined Mixture	50 kg N/fed	1.057	1.008	0.240	0.215	11.51	11.44	6.297	5.993	29.54	29.80
	70 kg N/fed	1.336	1.287	0.390	0.393	13.00	12.95	7.623	7.543	41.35	40.93
	90 kg N/fed	1.476	1.465	0.464	0.467	15.03	14.69	9.923	9.807	51.15	48.43
LSD 5 %		0.165	0.149	0.048	0.030	0.292	0.180	0.100	0.136	2.007	0.957

Table (4): Leaf anatomical characteristics (µ) of flag leaf of wheat plants at 90 days after sowing as affected by the best treatments as compared with moderate and high nitrogen rates in the second season.

Treatment	Thickness of upper epidermis (UE)		Thickness of lower epidermis (LE)		Thickness of leaf through midrib (TL)		Tangential dimension of midrib vascular bundle (TDMVB)		Tangential dimension of big xylem vessel (TDBXV)		Thickness of mesophyll tissue (M)	
	µ	%	µ	%	µ	%	µ	%	µ	%	µ	%
70 Kg N / Fad (recommended)	3.96	100	3.42	100	113.30	100	63.98	100	6.41	100	78.42	100
90 Kg N / Fad	4.10	103	3.84	112	128.35	113	69.11	107	7.48	116	86.75	110
Mn+ 90 Kg N / Fad	4.22	106	3.93	114	153.82	135	69.83	109	8.03	125	91.15	116
Combined mixture + 70 Kg N / Fad	4.61	116	4.07	119	193.32	170	72.01	112	9.90	154	96.15	122
Zn + 90 Kg N / Fad	4.92	124	4.13	120	223.22	197	74.71	116	9.45	147	102.95	131
Combined mixture + 90 Kg N / Fad	5.20	131	4.42	129	233.04	205	75.44	117	9.01	140	109.21	139

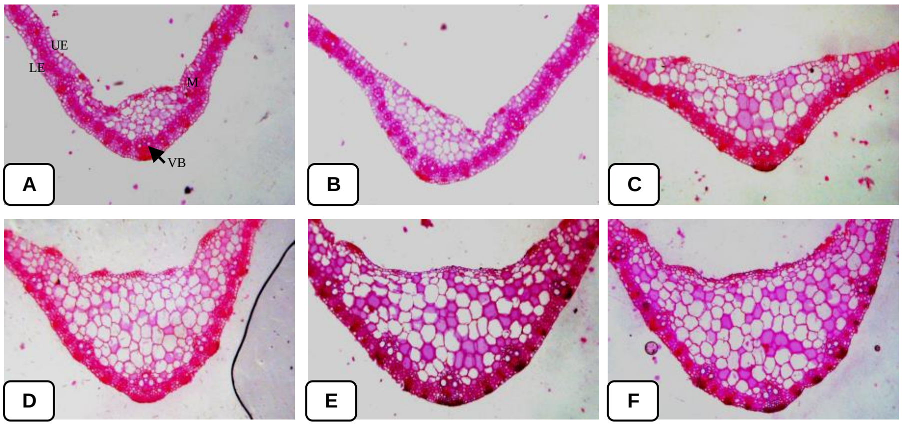

Figure 1: Leaf anatomical characteristics (µ) of flag leaf of wheat plants at 90 days after sowing as affected by the best treatments as compared with moderate nitrogen rate in the second season. (A: 70 Kg N / Fad, B: 90 Kg N / Fad; C: Mn+ 90 Kg N / Fad; D: Combined mixture + 70 Kg N / Fad; E: Zn + 90 Kg N / Fad; F: Combined mixture + 90 Kg N / Fad; UE: upper epidermis; LE: lower epidermis, VB: vascular bundles), 40x

Culm structure:

The anatomical characteristics among the best selected treatments as compared to moderate nitrogen fertilizer rate are summarized in Table (5) and figure (2). It is evident that there was an increase in the diameter of culm associated with increasing the thickness of culm wall, mechanical tissue thickness and the thickness of big metaxylem vessel as well as number of vascular bundles due to application of micronutrients, in particular, combined mixture of micronutrient as compared with moderate nitrogen rate. Meanwhile, application of nitrogen rate up to 90 kg N/fed increased the thickness of vascular bundles as compared with all selected treatments.

Table (5): Culm anatomical characteristics (μ) of wheat plants at 90 days after sowing as affected by the best treatment as compared with moderate nitrogen rate in the second season.

Treatment	Diameter of Culm (mm)		Thickness of culm wall (μ)		Mechanical tissue thickness (μ)		Number of vascular bundles		Thickness of big vascular bundles (μ)		Thickness of big metaxylem vessel (μ)	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
70 Kg N / Fad	3.73	100	553.38	100	79.24	100	22.66	100	55.61	100	7.64	100
90 Kg N / Fad	4.00	107	594.43	107	84.68	106	27.33	120	78.89	141	8.32	108
Mn+ 90 Kg N / Fad	4.78	128	734.61	132	91.80	115	34.33	151	73.70	132	8.73	114
Combined mixture + 70 Kg N / Fad	5.28	141	768.98	138	69.07	87	38.33	169	61.82	111	9.04	118
Zn + 90 Kg N / Fad	5.82	156	915.24	165	54.39	68	40.66	178	67.87	122	9.65	126
Combined mixture + 90 Kg N / Fad	6.40	171	937.47	169	98.31	124	46.33	204	65.22	117	10.1	132

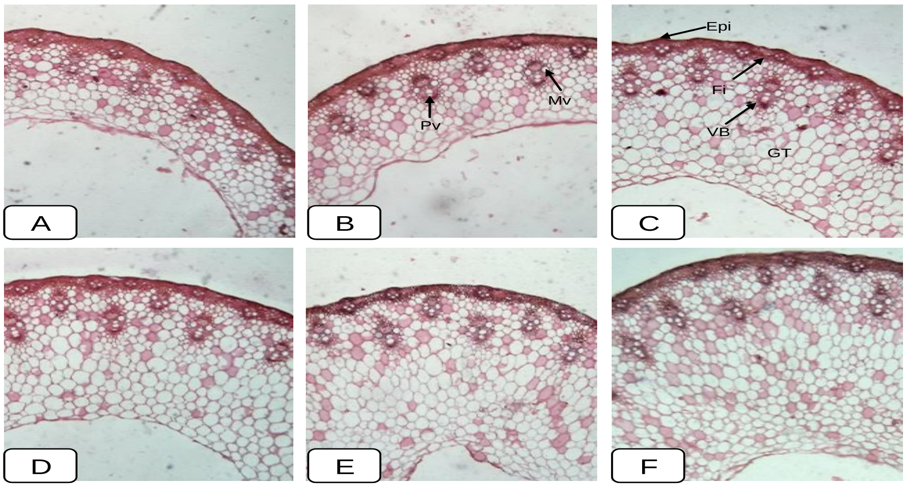

Figure 2: Culm anatomical characteristics (μ) of wheat plants at 90 days after sowing as affected by the best treatment as compared with moderate nitrogen rate in the second season. (A: 70 Kg N / Fed, B: 90 Kg N / Fed; C: Mn+ 90 Kg N / Fed; D: Combined mixture + 70 Kg N / Fed; E: Zn + 90 Kg N / Fed; F: Combined mixture + 90 Kg N / Fed; PV: protoxylem vessel; MV: metaxylem vessel; Epi: epidermis; Fi: fiber; VB: vascular bundle; GT: ground tissue), 40x.

DISCUSSION

The roles of micronutrients, in particular, combined mixture of micronutrients on stimulating plant growth and increasing grain yield in our investigation, may be due to increasing nitrogen content in flag leaf, which playing an important role in increasing photosynthetic rate due to its role in increasing the activity of key enzyme of CO_2 fixation; ribulose-1,5-bisphoosphate carboxylase/oxygenase (RuBisco, EC 4.1.1.39) (Sage and Monson, 1999), in addition to the role of nitrogen in increasing photosynthetic pigments as well as total carbohydrates content in flag leaf. Also, from this study it could be concluded that application of micronutrient under moderate and high nitrogen rates increased significantly all anatomical characteristics specially the number of vascular bundles in culm, thickness of vascular bundles, and big metaxylem vessel in both leaf and culm as well as thickness of mesophyll tissue in flag leaf. Leaf thickness and mean mesophyll cell size were found directly related to light-saturated photosynthesis per unit leaf area in C_3 grasses (Nobel *et al*., 1975). A better photosynthetic rate was also demonstrated in C_3 plants of thicker leaves and higher ratio of total surface area of the mesophyll cells to leaf area (Araus *et al*., 1986). These results provide a clear picture that CO_2 movement through stomata to the carboxylation sites (mesophyll cells) is facilitated in conditions of large area ratios of mesophyll cells to leaf and thicker leaves, and thus enhance photosynthetic rate and increasing the content of photoassimilate which translocate from leaves to growing grain through vascular bundles resulted in increasing grain yield.

The most promising results from this study indicate that application of combined mixture of micronutrients increased significantly the culm diameter as well as the thickness of culm wall and mechanical tissue and the number of vascular bundles leading to increasing grain yield per plant and grain weight per spike. Literatures are numerous focused on the culm anatomical features in relation to grain yield of wheat (Wang *et al*., 1995). Wang *et al* (2000) concluded that culm diameter, thickness of mechanical tissue and the number of vascular bundles are important features of high yield of wheat cultivar. In addition, vascular bundles, the essential conducting tissue, form the most complicated network in the plant body. They transmit signals and transport materials from roots to stems and leaves, and also from storage-tissues to other tissues. The extent of vascular bundle development affects the transportation of assimilates and inorganic nourishment, and sometimes it is a vital factor for improving wheat yield. The conducting systems of various yield-potential cultivars were analyzed and the results showed that their degree of complexity is in agreement with the yield potential of the cultivar. So, to some extent, different sizes and distribution of vascular bundles are the positive factors for spike weight. In this investigation, the number and thickness of vascular bundle and thickness of big metaxylem vessel in best treatment confirmed this conclusion.

The specific effect of each micronutrient may be summarized as fellow. Foliar spraying with zinc encouraged the vegetative growth and increased the plant capacity for building metabolites. Such response may be due to that zinc is known to play an activator of over 300 enzymes in plants (Fox and Guerimot, 1998) and is directly involved in the biosynthesis of growth substances such as auxin, Indole acetic acid in particular (Maischner, 2002) which produces more plant cells and more dry matter. These stimulating effects of zinc might be due to better utilization of available zinc by the plants because of exogenous supplementation. Zinc application have favorable effect in pollen germination, tube elongation and in decreasing the number of ruptured pollen which results in better fertilization, higher seed set and increased seed yield. Zinc plays a vital role as activation of carbohydrate and protein synthesis as well as their transport to the site of seed formation. The present results were in conformity with Manoharan *et al* .(2001) in rice and Seadh *et al*. (2009) in wheat. Moreover, Abd El-Monem *et al*. (2009) found that treated broad bean and lupin plants with zinc significantly stimulated the most of the growth and yield characteristics, increases in the contents of photosynthetic pigments, soluble carbohydrates and soluble proteins as well as gibberellic acid (GA_3) and indole acetic acid (IAA). Moreover, application of Zn increased significantly photosynthetic pigments contents as well as total carbohydrate contents. This increase could be ascribed to the effect of this element on increasing the biosynthesis of photosynthetic pigments and/or retarding their degradation. The results of (Tobbal, 2006) proved these observations. Concerning soluble protein the present investigation indicate that application of Zn increased significantly soluble protein. These results were confirmed with Gamal El-Din (2005).

Very extensive studies have been made on the forms and behavior of Cu in plants. All findings described in a number of outstanding textbooks can be summarized as follows: 1) Cu is mainly complexes with organic compounds of low molecular weight and with proteins; 2) Cu occurs in the compounds with no known functions as well as in enzymes having vital functions in plant metabolism; 3) Cu plays a significant role in several physiological processes—photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein metabolism, and cell wall metabolism; 4) Cu influences water permeability of xylem vessels and thus controls water relationships, and finally 5) Cu controls the production of DNA and RNA, and its deficiency greatly inhibits the reproduction of plants (reduced seed production, pollen sterility). In cereal crops, copper is required for anther and pollen development, and deficiencies can lead to pollen abortion and male sterility (Wojnarowiez *et al*., 2002). In addition, copper deficiency limits the activity of many plant enzymes, including ascorbate oxidase, phenolase, cytochrome oxidase, diamine oxidase, plastocyanin, and superoxide dismutase (Walker and Webb, 1981), also depresses carbon dioxide fixation, electron transport, and thylakoid prenyl lipid synthesis relative to plants receiving full nutrition (Bussler, 1981). The most important practical implications are related to deficiency and toxicity of Cu.

Manganese is involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases, transferases, hydroxylases, and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations (Burnell, 1988). Manganese is involved in oxidation–reduction (redox) reactions within the photosynthetic electron transport system in plants (Amesz, 1993). Manganese is also involved in the photosynthetic evolution of O_2 in chloroplasts (Hill reaction). Owing to the key role in this essential process, inhibition of photosynthesis occurs even at moderate manganese deficiency. The increase in soluble proteins due to manganese application is thought to be due to the role of this element in activating RNA polymeraze enzyme (Ness and Woolhouse, 1980). Lerer and Bar-Akiva (1976) likewise reported that increasing manganese application would mobilize nitrate formation processes, which causes more carbohydrate to be converted to proteins.

Concerning the effect of iron, there are an increase in the plant growth, yield and some physiological parameters. The increase in this parameter can be attributed to the favorable effects of active Fe on the synthesis of chlorophyll, and on the photosynthesis (Rao *et al*., 2001). As well as being a constituent of the heme group, iron is required at two other stages in its manufacture. It activates the enzymes aminolevulinic acid synthetase and coproporphorinogen oxidase. The protoporphyrin synthesized as a precursor of heme is also a precursor of chlorophyll, and although iron is not a constituent of chlorophyll this requirement, and the fact that it is also required for the conversion of Mg protoporphyrin to protochlorophyllide, means that it is essential for chlorophyll biosynthesis (Marschner, 1995). However, the decreased chloroplast volume and protein content per chloroplast (Terry and Abadia, 1986) indicate that chlorophyll might not be adequately stabilized as chromoprotein in chloroplasts under iron deficiency conditions, thus resulting in chlorosis. Along with the iron requirement in some heme enzymes and its involvement in the manufacture of heme groups in general, iron has a function in Fe-S proteins, which have a strong involvement with the light-dependent reactions of photosynthesis. Ferredoxin, the end product of photosystem I, has a high negative redox potential that enables it to transfer electrons to a number of acceptors. As well as being the electron donor for the synthesis of NADPH in photosystem I, it can reduce nitrite in the reaction catalyzed by nitrite reductase and it is an electron donor for sulfite reductase. All these parameters might have contributed for optimum growth and finally for yield attributes. Apart form this increased concentration of active Fe in the plants with these treatments enhanced the concentration of nitrogen in the plants. As physiologically active Fe play many roles in the metabolism of nitrogen within the plants by affecting the activities of nitrate reductase which are directly involved in the assimilation of N (Hewitt and Notton, 1980). The increased availability and assimilation of nutrients at cellular level together with higher biomass of the plants enhanced the concentration of nutrients in the plants.

Nitrogen is one of the most important nutrients for plant growth regulation and biomass production. The present investigation indicate that increasing nitrogen rate significantly increased nitrogen content in flag leaf accompanied with increasing all physiological characters as total chlorophyll, soluble proteins and total carbohydrates. These increases resulted in increasing plant growth and yield. Nevertheless, four major roles for N have been proposed for attaining high yields of cereal plants like maize (Hageman *et al*., 1990), and these roles appear to be valid for many crops: 1) Establishment of photosynthetic capacity; 2) Maintenance of photosynthetic capacity; 3) Establishment of sink capacity (the number and potential size of seeds) and 4) Maintenance of functional sinks throughout seed development. Within limits, and if no other restrictive factors are present, an increase in N supply increases the growth, the composition of N and chlorophyll, and the photosynthetic capacity of leaves (Huber *et al*., 1989). Nitrogen supply has also been shown to regulate the synthesis of photosynthetic carboxylating enzymes, in particular, a key enzyme of CO_2 fixation in photosynthesis, ribulose-1,5- bisphoosphate carboxylase/oxygenase (RuBisco, EC 4.1.1.39) in leaves (Sage and Monson, 1999), by affecting transcription and/or the stability of messenger RNA (Sugiharto and Sugiyama, 1992). Collectively, these effects result in greater light interception, higher canopy photosynthesis, and higher yield. To achieve high yields, plants must not only establish photosynthetic capacity but also continue photosynthesis throughout the grain-filling period. Thus, once established, sufficient N must be available to maintain the photosynthetic apparatus. This role is particularly important because dry matter accumulation in cereal grains is dependent on current photosynthesis (Swank *et al*., 1982). Most of the N in the leaf is associated with proteins in the chloroplast—60% in C_4 plants and up to 75% in C_3 plants (Hageman *et al*., 1990) and these proteins are subject to breakdown and remobilization of the resultant amino acids.

Another important role for N in assuring high productivity of crop plants is establishment of reproductive sink capacity. Sink capacity of a cereal plant is a function of the number and the potential size of grains. Grain number is dependent on the number of ears per unit area, the number of florets per ear, and the proportion of florets that develop into grain and the potential size of individual grains depends on the number of endosperm cells and starch granules (Singh and Jenner, 1984). In either case, reproductive initials, like all growing tissues, are characterized by high concentrations of N and high metabolic activities. This need could indicate that sufficient amounts of both C and N assimilates are required for full expression of the genetic potential for initiation and early development of grains. For cereal crops, grain number is usually more closely related to yield than other yield components. Consequently, many studies have shown that N-induced yield increases are the result of more grains per plant (Jacobs and Pearson, 1992). For wheat, this enhancement is related to an increase in tiller production and survival and to a lesser extent to a decrease in floret abortion (Thomas*et al*., 1978). Because vegetative development in cereal crops is negligible after flowering, the N subsequently acquired, or remobilized from the vegetation, is used exclusively for grain development. This need for N is demonstrated by the fact that adequately fertilized cereal crops typically contain from 9 to 13% protein in the grain. Indeed, some workers have suggested that the deposition and/or accumulation of storage protein by the kernel are a factor regulating grain development (Tsai *et al*., 1978, Tsai *et al*., 1980). This suggestion is based on the positive correlation between storage protein, kernel weight, and grain yield (Tsai *et al*., 1978). Other needs for N by developing kernels could include embryo growth and the initial and continued synthesis of enzymes needed for energy generation and the deposition of storage products in the kernel. Embryo development could affect the kernel's hormonal balance because a large portion of kernel phytohormones are produced by the embryo (Michael and Beringer, 1980). Because several of the key classes of phytohormones either contain N (auxins, cytokinins, polyamines) or are synthesized from amino acids (auxins, ethylene, polyamines), an adequate supply of N may be needed for their production.

It is well noted that, soluble proteins represents the first nitrogenous compounds lost during grain filling (Martignone *et al*., 1987) leading to a higher decrease in the yield of cereal plants, on the other hand as indicated from the present study, application of nitrogen fertilizers increased the soluble proteins in flag leaf resulted in increasing grain yield per plant. This is in conformation with the finding of Manian *et al*. (1987).

In conclusion, the obtained results show that foliar application with combined mixture of studied micronutrients (Cu, Mn, Fe and Zn) at the rate of 500 ppm from each under moderate or high rate of nitrogen fertilizer (70 or 90 kg N/fed) can be recommended to maximize wheat grain per main spike and per plant.

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إستجابة نباتات القمح المورفوفسيولوجية والتشريحية للمغذيات الصغري والتسميد النيتروجيني

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أجريت تجربتان حقبليتان خلال موسمي الزراعة ٢٠٠٥/ ٢٠٠٦ و ٢٠٠٦/٢٠٠٧ في المزرعة البحثية لمحطة تاج العز، مركز البحوث الزراعية بمحافظة الدقهلية، وذلك لدراسة تأثير مستويات التسميد النيتروجيني والتسميد بالمغذيات الصغري والتداخل بينهما علي نمو ومحصول نباتات القمح بالإضافة إلي بعض التغيرات الفسيولوجية والتشريحية.

إستخدام مخلوط العناصر الصغري تحت مستوي النيتروجين المرتفع أعطي أعلي قيم لنمو ومحصول القمح بالإضافة لبعض التغيرات الفسيولوجية مقارنة ببقية المعاملات المختلفة. وكان يقع فى المرتبة الثانية من حيث تحسين النمو والمحصول هو الزنك، يلية المنجنيز، أما إستخدام كل من الحديد والنحاس فأعطو أقل القيم وكانت الفروق بينهما غير معنوية. لوحظ أن إستخدام المستوي المرتفع من النيتروجين يعمل علي زيادة جميع صفات النمو والمحصول والصفات الفسيولوجية

تشريحيا، إستخدام مخلوط العناصر الصغري تحت مستوي النيتروجين المرتفع يزيد ويحسن جميع الصفات االتشريحية المدروسه لكل من ورقة العلم وقاعدة الساق وخاصة، عدد الحزم الوعائية وسمك النسيج الدعامي لقاعدة الساق، سمك الحزم الوعائية وقطر وعاء الخشب التالي وسمك البشرة لكل من ورقة العلم وقاعدة الساق، هذا بالإضافة الي سمك النسيج المتوسط بورقة العلم.

نخلص من الدراسة أن رش نباتات القمح بمخلوط العناصر الصغري بتركيز ٥٠٠ ملليجرام لكل لتر تحت مستويات النيتروجين المتوسطة تُزيد نمو ومحصول القمح.

قام بتحكيم البحث

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