

## Effect of Stimulating Compounds and Nitrogen Fertilization on Yield, Nitrogen Uptake and Utilization Efficiency

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**Abstract:** A field experiment was conducted at the Experimental Farm, Faculty of Agriculture, Kafr Elsheikh University, Egypt during two consecutive winter growing seasons 2012/13 and 2013/14 to study the response of wheat to stimulating compounds (control, salicylic acid and ascobien) and N fertilizer (0, 57.5, 115, 172.5 and 230 kg N ha<sup>-1</sup>), as well as their interaction on yield traits and N use efficiencies of *Triticum aestivum* L. (cv wheat Sakha 94). The results demonstrated that stimulating compounds of ascobien and N fertilizer level (230 kg ha<sup>-1</sup>) had significant effect on yield traits. Regarding yield and yield components, the interaction between stimulating compounds and N rates was found a progressive increases and the magnitude of increments was much more pronounced in response to salicylic acid and control treatments through enhancing yield components in both seasons. Furthermore, no significant differences between 172.5 and 230 kg N ha<sup>-1</sup> in both seasons. A significant interactive effect on grain N uptake, whole plant N uptake, nitrogen harvest index (NHI), nitrogen utilization efficiency (NU<sub>E</sub>) and nitrogen use efficiency (NUE) between stimulating compounds treatment and N fertilizer rates. Grain N uptake and whole plant N uptake were closely correlated with N fertilizer rates under stimulating compounds, whereas stimulating compounds of ascobien with 172.5 kg N ha<sup>-1</sup> was efficient and greater than stimulating compounds of salicylic acid and control treatment with 230 kg N ha<sup>-1</sup> in both seasons. In addition, it must be noted that there is a “trade-off” between a higher N fertilizer level and NHI, NU<sub>E</sub> and NUE. It could be concluded that, stimulating compounds of ascobien and N level of 172.5 kg ha<sup>-1</sup> was the optimal in our study for N use efficiencies and it can be saved 57.5 kg N ha<sup>-1</sup>.

**Key words:** Wheat • Ascobien • Salicylic acid • Grain N uptake • Nitrogen utilization efficiency

### INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major staple food crop for more than one third of the world population. Wheat is the major cereal cultivated in the Mediterranean region and represent strategic crops for food security across the whole area and the main staple food in Egypt [1]. Wheat has a special importance because the local production is not sufficient to meet the annual demands. FAO [2] indicated that the total cultivated area of wheat reached about 8 mha and the total production exceeded 8.8 million tons [2].

Salicylic acid (SA) has positive effect on plant growth, yield which can also regulate physiological processes in plant [3] may be due to that SA participates in the regulation of several physiological processes such as stomatal closure, nutrient uptake, chlorophyll

synthesis, protein synthesis, transpiration and photosynthesis [4]. Khan *et al.* [4] showed that sprayed plants with SA increased more vegetative growth and yield components.

Ascobien foliar nutrient, contain (13% citric acid, 25% ascobien plus 62% organic materials) acts as a primary substrate and a potential growth -regulating factor which influences many biological processes [5]. Ascobien had a promotion effect on growth and active constituent compounds on various plants [6]. Ascobien have synergistic effect in crops that functions as an antioxidant also has important role in vital processes in plants growth such as cell growth and division, differentiation and metabolism in plants [7] and minimizing the damage caused by oxidative stress [8].

Thus salicylic acid and ascobien might be enhanced the growth and yield components in wheat.

The most effective environmental factor on wheat yield and quality is N fertilization. Nitrogen management has the most impact on final wheat grain size and weight. Applications made early will ensure a canopy that is large, containing high rates of stem carbohydrate that is translocated to the developing grain during maturation. This is particularly important in drought areas where 60% of the grain yield could come from this store. It is important to monitor plant nitrogen rates ensuring the canopy does not senesce early and curtail the grain filling period. Nitrogen use efficiency in wheat grain production may be low owing to losses of N by volatilization, denitrification and leaching [9]. Thus, N application should be the latest possible compatible with the stage of development that still permits rapid N absorption and utilization efficiency, in order to reduce the opportunities for N losses of unused N and improve efficiency of N fertilizer use and to obtain higher productivity [10].

Therefore, the objective of this study was to elucidate of spraying some stimulating compounds and different nitrogen rates on some physiological characters, yield, yield component, nitrogen use efficiency and nitrogen utilization efficiency of wheat (*Triticum aestivum* L.) cv. Sakha 94.

### MATERIALS AND METHODS

**Plant Materials:** This study was carried out at the Experimental Farm, Faculty of Agriculture, Kafr Elsheikh University, Egypt (31°05'54.3"N, 30°57'19.4"E) during two consecutive winter growing seasons in 2012–2013 and 2013–2014, respectively. Climatic data were collected from an agro-meteorological Sakha station located 1 km from the experimental site as shown in (Table 1). Soil samples

were taken from the 0 to 30 cm soil depth using a soil Auger to analyze N content by Kjeldahl method [11]. The soil was clayey and an average bulk density of 1.22 g cm<sup>-3</sup> in upper 30 cm depth. This layer also contained 1.37% total organic matter, 0.15% total nitrogen (N), 35 mg kg<sup>-1</sup> available phosphorus (P) and 255.3 mg kg<sup>-1</sup> exchangeable potassium (K), EC (1.25 ds m<sup>-1</sup>, 1:5), pH (8.1, 1:2.5) and 1.38 cm annual precipitation as average in both seasons.

**Experimental Design:** The experiment was laid out in a randomized complete block design (RCBD) with a split plot with three replicates. Five N fertilizer rates (0, 57.5, 115, 172.5 and 230 kg N ha<sup>-1</sup>) were allocated to the main plots. Subplots were three stimulating compounds applications (control “Tap water”, Salicylic acid and Ascobien). The preceding crop was Maize (*Zea mays* L.) during the growing seasons. N fertilizer was applied as ammonium sulphate (20.6% N) to each plot at three splits; 20% as basal dose at sowing stage, 40% at the beginning of tillering stage and the remaining 40% at end of stem elongation stage. Super phosphate fertilizer (70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) was added during seedbed preparation.

Wheat (*Triticum aestivum* L., Cv. Sakha 94) was planted on November 25<sup>th</sup> during 2012/2013 and November 28<sup>th</sup> during 2013/2014 with arrow spacing of 12.5 cm and a seeding rate 140 kg ha<sup>-1</sup>. The net experimental unit size (plot) was 10.5 m<sup>2</sup> (3 m width x 3.5 m long). Wheat grain yield (14% moisture) obtained by harvesting the center (2 m x 2 m) of the experimental unit, but yield components were determined from two outer rows within each plot.

Plant samples collected at harvest were separated into grain and straw and electric oven-dried at 70°C for 72h till constant dry weight, then were grounded in a mill to produce a fine powder which is needed for the N analysis using the standard procedure of micro-Kjeldahl.

Table 1: Monthly relative humidity (RH,%), wind speed (kg day<sup>-1</sup>), mean minimum and maximum air temperatures (T<sub>max</sub> and T<sub>min</sub>, respectively) during the two winter growing seasons.

Season Month	2012/2013				2013/2014			
	Temperature (°C)		Wind speed (kg day <sup>-1</sup> )	RH (%)	Temperature (°C)		Wind speed (kg day <sup>-1</sup> )	RH (%)
	T <sub>max</sub>	T <sub>min</sub>			T <sub>max</sub>	T <sub>min</sub>		
Dec	17.7	11.6	155.5	37.8	16.9	11.4	156.0	30.7
Jan	22.6	11.0	158.7	41.4	23.5	9.4	142.0	41.4
Feb	23.3	11.9	167.9	45.5	22.0	11.0	152.1	45.1
Mar	22.5	14.7	129.3	49.2	24.0	16.2	123.5	47.9
April	29.1	17.0	89.1	52.5	27.4	17.2	88.2	55.4
May	33.8	18.2	111.7	61.1	31.9	17.8	96.3	64.1

max = maximum, min = minimum, RH = relative humidity

**Morphological and Physiological Measurements:** Plants were sampled at harvesting stage. Plant height (cm.), spike length (cm.), number of spikelets /spike, number of grains per spike, 1000- grain weight, grain yield, straw yield, were counted per both seasons and grain yield was corrected to 14% moisture. Chlorophyll content (SPAD) of flag leaves was determined with a portable chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera, Osaka, Japan) [12] at 120 days after sowing (DAS).

Then, N harvest index (NHI,%) at maturity was calculated [13] and also N uptake (kg N ha<sup>-1</sup>) in the straw or grains was calculated [14] as follows:

$$N \text{ harvest index} = \frac{\text{Grain N uptake (kg ha}^{-1}\text{)}}{\text{Total N uptake (kg ha}^{-1}\text{)}}$$

where, the total N uptake includes all N that accumulated in leaves, stem, shank, cobs, husk organs in addition to the grain N.

$$\text{Stovers N uptake (kg ha}^{-1}\text{)} = \frac{\text{Straw N content (g ka}^{-1}\text{)} \times \text{Straw DM (kg ha}^{-1}\text{)}}{1000}$$

$$\text{Grains N uptake (kg ha}^{-1}\text{)} = \frac{\text{Grains N content (g ka}^{-1}\text{)} \times \text{Grains DM (kg ha}^{-1}\text{)}}{1000}$$

$$\text{Whole plant nitrogen uptake (kg ha}^{-1}\text{)} = \text{Stovers N uptake (kg ha}^{-1}\text{)} + \text{Grains N uptake (kg ha}^{-1}\text{)}$$

N use efficiency (NUE) was calculated as it is described by Michael [14]. In addition, N utilization efficiency was calculated. N utilization efficiency was calculated as described by Haegele [15].

$$NUE \text{ (kg kg}_N^{-1}\text{)} = \frac{GY_t - GY_c \text{ (kg ha}^{-2}\text{)}}{NF_t - NF_c \text{ (kg ha}^{-2}\text{)}}$$

where, GY<sub>t</sub> and GY<sub>c</sub> express the grain yield at different N treatments and control, respectively. While, NF<sub>t</sub> and NF<sub>c</sub> express the N applications for different N treatments and control, respectively.

$$NUE \text{ (kg kg}_N^{-1}\text{ plantN)} = \frac{GY_t - GY_c \text{ (kg ha}^{-2}\text{)}}{N \text{ uptake}_t - N \text{ uptake}_c \text{ (kg ha}^{-1}\text{)}}$$

where, GY<sub>t</sub> and GY<sub>c</sub> express the grain yield at different N treatments and control, respectively. While, N uptake<sub>t</sub> and N uptake<sub>c</sub> express the total N accumulation in whole plant biomass above ground (grains and stover) for different N treatments and control, respectively.

**Statistical Analysis:** Data obtained from the current investigation were subjected to an analysis of variance (ANOVA) procedures according to Gomez and Gomez [16] using the MSTAT-C Statistical Software package. Different Means were compared using Duncan [17], when the ANOVA showed significant differences (P < 0.05).

## RESULTS AND DISCUSSION

**Effect of Stimulating Compounds Application and Nitrogen Fertilizer Rates on Wheat (Sakha 94 cultivar) Yield Components in Both of Winter Seasons 2012/13 and 2013/14:** Stimulating compounds application and nitrogen fertilizer rates caused significant effects on wheat growth and yield attributes in both seasons as shown in Tables 2 and 3. Stimulating compounds by ascorbic acid and salicylic acid significantly increased wheat growth and yield attributes characters as compared with the control treatments in both growing seasons, also results clearly indicated that applying 172.5 or 230 kg N ha<sup>-1</sup> significantly increased wheat growth and yield attributes characters.

Highest growth characters *i.e.* dry weight (410.8 and 427 g m<sup>-2</sup>), chlorophyll content (42.86 and 43.70), plant height (96.02 and 99.12 cm) are presented in Table 2, as well as yield attributes *i.e.* number of spike m<sup>-2</sup> (309.67 and 311.00), spike length, (10.43 and 10.59 cm), number of grains spike<sup>-1</sup> (48.13 and 53.95) and 1000-grain weight (44.43 and 44.42 g) are shown in Table 3 were obtained from stimulating compounds by ascorbic acid in both of winter seasons 2012/13 and 2013/14, respectively. Also it was observed that no significant difference between stimulating compounds by ascorbic acid and salicylic acid in number of grains spike<sup>-1</sup> in winter season 2012/13 in addition to chlorophyll content and plant height in winter season 2013/14. These findings are in good agreement with those obtained by Kowalczyk and Zielony [3], who indicated importance of ascorbic acid may be attributed to that its roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis.

The results presented in Tables 2 and 3 indicated that the effect of nitrogen fertilizer rates on growth and yield attributes was significant in the two growing seasons. It can be stated that all studied growth and yield attributes were significantly steady increased as a result of increasing nitrogen fertilizer rates from 0 to 172.5 and 230 kg N ha<sup>-1</sup> and the differences between them were obvious in both seasons.

The maximum increase was obtained at both of 172.5 and 230 kg N<sup>-1</sup> and no significant difference between N fertilizer rates concerning dry weight, chlorophyll content,

Table 2: Dry weight ( $\text{g m}^{-2}$ ), Chlorophyll content and plant height of wheat as affected by nitrogen rates and stimulating compounds application in both of winter seasons 2012/13 and 2013/14

Treatments	Dry weight ( $\text{g m}^{-2}$ )		Chlorophyll content		Plant height (cm)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
N. fertilizer level ( $\text{Kg ha}^{-1}$ )						
0	189.22e	205.0d	33.39e	34.8d	88.53c	84.27d
57.5	331.67d	296.0c	40.04d	38.66c	90.96bc	92.78c
115	426.44c	395.0b	43.17c	41.91b	94.44b	97.33b
172.5	511.78b	546.0a	46.24b	47.24a	100.13a	102.89a
230	544.67a	562.0a	47.89a	48.6a	102.67a	105.93a
Foliar spraying						
Control	393.13b	376.0c	41.68b	40.55b	94.95	93.33b
Salicylic	398.33b	400.0b	41.90b	42.48a	95.07	97.48a
Ascobien	410.8a	427.0a	42.86a	43.7a	96.02	99.12a
ANOVA						
N	**	**	**	**	**	**
S	**	*	**	*	N.S.	*
N × S	*	*	*	*	N.S.	*

\*,\*\* and N.S. indicate  $P < 0.05$ ,  $P < 0.01$  and not significant, respectively. Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 3: Mean of No. of spikes  $\text{m}^{-2}$ , spike length (cm), number of grains spike $^{-1}$  and 1000-grain weight (g) of wheat as affected by nitrogen rates and stimulating compounds application in of the two winter seasons 2012/13 and 2013/14

Treatments	No. of spikes $\text{m}^{-2}$		Spike length (cm)		No. of grains spike $^{-1}$		1000-grain weight (g)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
N. fertilizer level ( $\text{Kg ha}^{-1}$ )								
0	224.44e	220.0d	9.5d	9.43d	38.56d	39.05d	40.68	40.45
57.5	267.33d	259.0c	9.99c	9.89cd	43.11c	45.59c	41.63	41.18
115	299.56c	299.0b	10.56b	10.38bc	46.22b	54.29b	41.84	42.02
172.5	346.56b	360.0a	10.86a	10.98ab	53.44a	58.51a	42.11	42.75
230	367.78a	368.0a	10.93a	11.29a	54.89a	59.86a	42.22	43.57
Foliar spraying								
Control	293.53c	291.0b	10.28b	10.18b	46.0b	48.72b	43.64b	43.56b
Salicylic	300.2b	301.0ab	10.39ab	10.41ab	47.6a	51.7b	44.22ab	44.00ab
Ascobien	309.67a	311.0a	10.43a	10.59a	48.13a	53.95a	44.43a	44.42a
ANOVA								
N	**	**	**	**	**	**	N.S	N.S
S	**	**	**	**	**	**	**	**
N × S	*	*	*	*	N.S.	N.S.	N.S.	N.S.

\*,\*\* and N.S. indicate  $P < 0.05$ ,  $P < 0.01$  and not significant, respectively. Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

plant height, number of spikes  $\text{m}^{-2}$ , spike length, number of grains spike $^{-1}$  and 1000-grain weight in both seasons, except dry weight, chlorophyll content and number of spikes  $\text{m}^{-2}$  were obtained maximum increase at 230  $\text{N ha}^{-1}$  in the 1<sup>st</sup> season. In this connection, Hafez and Kobata [18], Abou El-Hassan *et al.* [19] and Hafez *et al.* [20] pointed out that N fertilizer application significantly increased grain yield and its components, but the N effect depended on the availability of water for wheat before anthesis. There was an improvement in the plant growth, this might be due to the well utilization of the supplied N in the metabolism and the meristemic activity, which

consequently improve growth characters and yield components. The increase in growth characteristics and yield components contributed to the significant increase in wheat grain yield.

**Effect of Stimulating Compounds and Nitrogen Fertilizer Rates on Some Parameters in Wheat (Sakha 94 cultivar) in Winter Seasons 2012/13 and 2013/14:** The data of grain yield in wheat (Sakha 94 cultivar) plant are shown in Fig. 1. It is obvious from the data that stimulating compounds application of ascobien and salicylic acid with 172.5 or 230  $\text{kg N ha}^{-1}$  promoted wheat grain yield

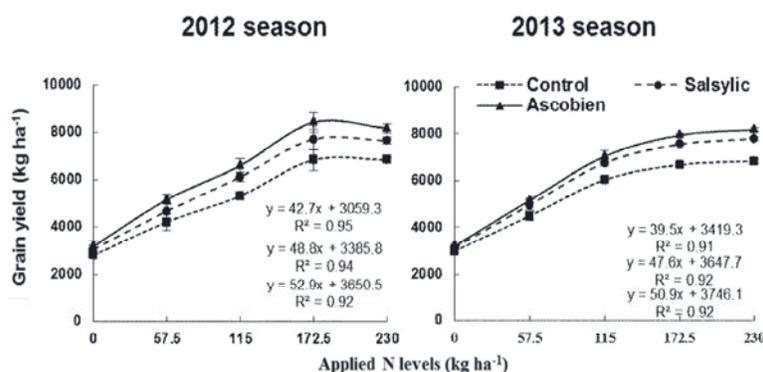


Fig. 1: Grain yield ( $\text{kg ha}^{-1}$ ) in the wheat (Sakha 94 cultivar) supplied with stimulating compounds application of control (■), salicylic acid (□) and ascobien (△) and different amounts of nitrogen fertilizer (0, 57.5, 115, 172.5 and 230  $\text{kg N ha}^{-1}$ ) in 2012/2013 and 2013/2014 seasons. The data are the mean  $\pm$  standard error of three replicates.

compared to control treatment with the same amount of N applied in both growing seasons. Moreover, it was found that the rate of increment decreased with increasing the amounts of applied N and the increase by spraying treatment from ascobien was more effective than that sprayed from control or salicylic acid (Fig. 1) taking into account that no significant difference between the amounts of applied N from 172.5 or 230  $\text{kg N ha}^{-1}$ . It was cleared that the beneficial effect of ascobien on producing vigour plants as well as improving yield components surly reflected on improving production of wheat plants. It has also been observed by Noctor *et al.* [21], who concluded that ascobien and nitrogen fertilizer had promoting effects on grain yield, it may be attributed to the enhancement of cell division and Plant development.

In both growing seasons, the grain N uptake and whole plant N uptake were increased with increasing applied N. The rate of increase was decreased with increasing the amounts of applied N and the increase by stimulating compounds by ascobien was greater than that by salicylic acid and control treatment (Fig. 2). Grain N uptake consists of grain yield and grain N ( $\text{g/kg DM}$ ), while whole plant N uptake consists of grain N uptake and straw N uptake. The difference in both of grain N uptake and whole plant N uptake between the plants treated by stimulating compounds. The grain N uptake and whole plant N uptake reached near maximum when the amount of applied N was 172.5  $\text{kgN ha}^{-1}$  with stimulating compound of ascobien. The grain N uptake and whole plant N uptake per ha at applied N of 172.5  $\text{kgN ha}^{-1}$  with stimulating compounds of ascobien estimated from the regression in (Fig. 2) was 122.21 and 182.20  $\text{kg N ha}^{-1}$ , respectively, for ascobien in the 1<sup>st</sup> season and 116.35 and 163.14  $\text{kg N ha}^{-1}$ , respectively, in the 2<sup>nd</sup> season, but in salicylic acid was 109.81 and 165.57  $\text{kg N ha}^{-1}$ , respectively, in 2012 the

1<sup>st</sup> season and 109.54 and 155.11  $\text{kg N ha}^{-1}$ , respectively, in the 2<sup>nd</sup> season (Fig. 2). Thus, the response of grain N uptake and whole plant N uptake to stimulating compounds was much lower in control treatment than salicylic acid and ascobien treatment was the highest among stimulating compounds applications.

Nitrogen uptake reflects the efficiency of the plant in obtaining N from the soil. Increased N uptake has been noticed as a strategy to increase NUE and NU<sub>E</sub> by Raun and Johnson [22]. Moll *et al.* [23] confirmed that variation in N uptake could be separated from grain yield variation. Moreover, Lopez-Bellido and Lopez-Bellido [24] observed that differences between stimulating compounds application with regard to grain yield, which is clearly related to wheat N uptake. Lee *et al.* [25] showed that N uptake was positively correlated with dry weight and straw nitrogen content. In this connection, there was a positive correlation between N uptake and grain yield (Fig. 2).

The increases in N uptake in grains and whole plant due to stimulating compounds plants with ascobien probably ascribed to its effects on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation [9] as well as the ability of ascobien in induction of endogenous hormones like GA<sub>3</sub> and IAA [26]. The ameliorative effect of ascobien (ascobien + citric acid) on N uptake comes from the fact that they act as an antioxidant.

As shown also in Fig. 1, it has been observed that NHI of wheat varied significantly with different nitrogen rates and stimulating compounds. NHI decreased with increasing N rates. The highest NHI of wheat was associated with the control treatment (no N) regardless of stimulating compounds applications. The difference in NHI between stimulating compounds applications of

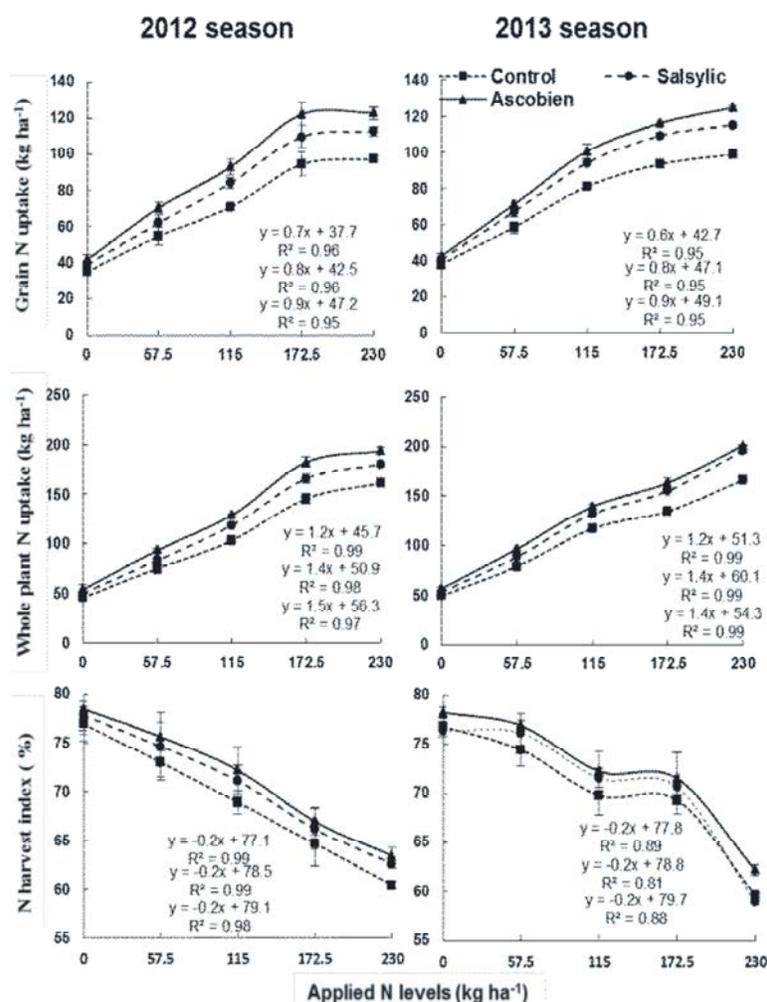


Fig. 2: Grain N uptake (kg ha<sup>-1</sup>), whole plant N uptake (kg ha<sup>-1</sup>) and N harvest index (%) in the wheat (Sakha 94 cultivar) supplied with stimulating compounds application of control (■), salicylic acid (●) and ascobien (▲) and different amounts of nitrogen fertilizer (0, 57.5, 115, 172.5 and 230 kg N ha<sup>-1</sup>) in 2012/2013 and 2013/2014 seasons. The data are the mean ± standard error of three replicates.

ascobien and salicylic acid were smaller than that in control treatment in the first season (Fig. 2). Lopez-Bellido and Lopez-Bellido [24] observed that the increase in wheat N uptake with increasing N fertilizer rates was greater than the increase in grain yield, thus there is less transfer of N to grain when N rates was increased. Montemuro *et al.* [27] noticed that grain N uptake was positively correlated with yield, protein content and total N uptake and a significant positive correlation found in NHI, yield and total N uptake.

The increase in N distribution efficiency (NHI) without N fertilizer confirmed that N translocation to the grain is stimulated by low N availability in the soil, which is strongly associated with Zhang *et al.* [28]. In other words, stimulating compound of ascobien improved both

N uptake and N partitioning efficiency to the grain compared to control treatment, Possibly the reason for an increase of the grain and a decrease of the (leaves and stems), higher sink size induces higher N demand by the grain, stimulating remobilization and N translocation to the grain [23]. When N level in the soil is a limiting factor, vegetative plant parts could have enough N to satisfy gain demand leading to lower NHI and higher grain N uptake [9].

**Nitrogen Utilization Efficiency:** Nitrogen utilization efficiency and its relationship with nitrogen fertilizer rates and stimulating compounds application in wheat are shown in Fig. 3. Nitrogen utilization efficiency reflects the ability of the plant to translocate the N uptakes into grain

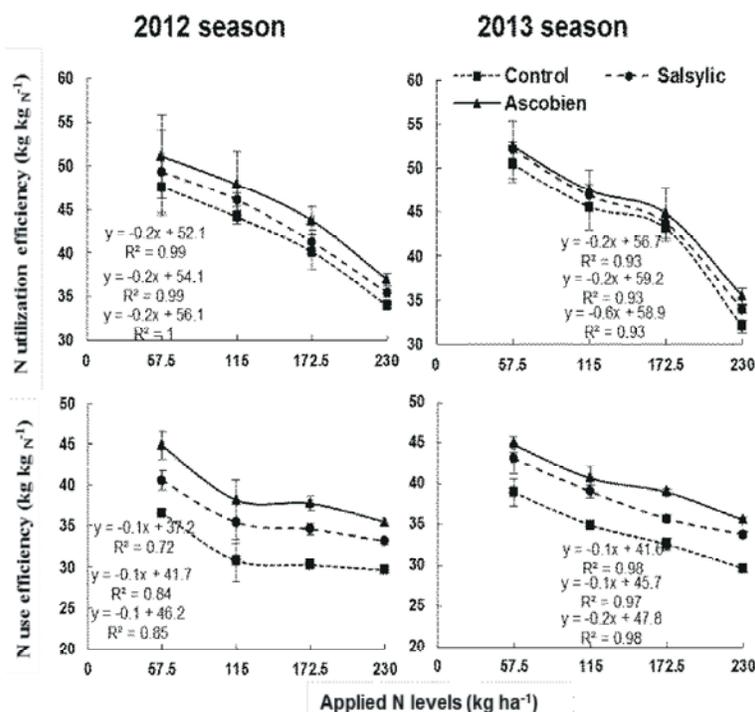


Fig. 3: N utilization efficiency (kg kg N<sup>-1</sup>) and N use efficiency (kg kg N<sup>-1</sup>) in the wheat (Sakha 94 cultivar) supplied with stimulating compounds application of control (□), salicylic acid (○) and ascobien (▲) and different amounts of nitrogen fertilizer (0, 57.5, 115, 172.5 and 230 kg N ha<sup>-1</sup>) in 2012/2013 and 2013/2014 seasons. The data are the mean ± standard error of three replicates.

[29]. It was found that NUtE had significant differences in wheat between N fertilizer rates and stimulating compounds application. This result suggested that utilization efficiency and partitioning of N from vegetative plant parts to the grain respond to N fertilizer level applied in wheat. However, Delogu *et al.* [29] showed that NUtE decreased with increasing N fertilizer rates. The highest NUtE, was obtained when 57.5 kg N ha<sup>-1</sup> applications was applied (Fig. 3). This was similar to Delogu *et al.* [29], who reported that NUtE was highest with the lowest N application. NUtE was reduced from 51 to 36% with stimulating compounds of ascobien, however NUtE was reduced from 49 to 35% with stimulating compounds of salicylic acid in both seasons.

N utilization efficiency was decreased by increasing N fertilizer rates and stimulating compounds of ascobien has the potentiality to exert a suppressive or stimulative impact on NUtE of wheat in both seasons. Stimulating compounds with ascobien was more effective in increase of NUtE, it might be attributed to an increase in stimulation the biosynthesis of chlorophylls, photosynthetic activity and play an important role to enhance the activity of enzymes responsible for drought

resistance [3]. In addition, stimulating compounds application with ascobien significantly increased the number of spikes and grain number (Table 3), ascobien has been found to be closely associated with grain yield in (Fig 1) and grain N uptake in (Fig. 2). According to the aforementioned results, it could be concluded that stimulating compounds with ascobien increased significantly NUtE in both seasons compared with salicylic acid and control treatments. These findings are in good agreement with those obtained by Ercoli *et al.* [9].

Grain N content in wheat depends on uptake of soil nitrate prior to flowering, continued uptake of nitrate during grain fill and remobilization of stored vegetative N accumulated prior to flowering. However, under condition of high N fertility and available soil moisture, the contribution of post anthesis N uptake may increase substantially [9]. Ercoli *et al.* [9] reported that N utilization efficiency decreased, when the amount of N increased. When plants exposed to low nitrogen in the soil, the mechanism of N remobilization was more efficient. Raun *et al.* [10] reported that N utilization efficiency in grain was greater under nitrogen deficiency condition. Raun *et al.* [10] found that most of the N taken up by the

wheat plants was translocated to the grain either directly or by mobilization from other plant parts. One explanation for differences in utilization efficiency is that during grain filling period, the plant retains an amount of N at anthesis that is essential for survival and various biological functions, while the remainder is available for utilization. It appears that N retained depends on cultivars and prevailing growth conditions, although genetic variability in nitrogen utilization has been reported by Jin and Mian [30].

**Nitrogen Use Efficiency:** Nitrogen use efficiency and its relationship with nitrogen fertilizer rates and stimulating compounds in wheat are shown in (Fig. 3). NUE was evaluated as an important parameter for efficient wheat production [30]. From the conventional NUE equation perspective, NUE varied markedly; it ranged from 45 kg kg<sub>N</sub><sup>-1</sup> of stimulating compounds with ascobien in the less fertilizer (57.5 kg N ha<sup>-1</sup>) condition to 35 kg kg<sub>N</sub><sup>-1</sup> in the highest (230 kg N ha<sup>-1</sup>) application and the NUE supplied with salicylic acid was slightly lower (40~33 kg kg<sub>N</sub><sup>-1</sup>) and control treatment was the lowest (36~29 kg kg<sub>N</sub><sup>-1</sup>) among stimulating compounds than that from the plants supplied with ascobien in both seasons.

It was reported that the decrease accumulation of nitrogen before flowering could be caused by nitrogen volatilization from plant tissues and death of partial leaf and organ at post-flowering stage and that nitrogen volatilization was the main cause [30]. The negative effect caused by volatilization of large nitrogen concentration and accumulation at flowering stage was larger than the positive effect of post-flowering dry matter production and N redistribution at high N rate, so NUE decreased with increasing N rates. In addition, a high indigenous N supply of soil (INS) resulted in higher N concentration in rice straw and produce the phenomenon of luxury consumption of N [31].

The interaction between N fertilizer rates and stimulating compounds on NUE in (Fig. 3) were observed, whereas NUE was decreased by increasing nitrogen application level and stimulating compounds with ascobien has the potentiality to exert a suppressive impact on NUE of wheat in both seasons.

Nitrogen use efficiency is also affected by N metabolic processes in which N transporters and enzymes that synthesize N-transport amino acids play critical roles [31]. Recent advances in molecular approaches will facilitate better understanding of mechanisms regulating N uptake and N metabolism, leading to the identification of physiological traits responsible for superior NUE under

stressed condition, although further studies are required to apply these approaches into practical breeding programs.

## CONCLUSION

The current study revealed that both nitrogen fertilizer and stimulating compounds were significantly affected yield, its components and N use efficiencies. The highest effects on yield, its components and N use efficiencies in wheat were obtained by the level of 172.5 kg ha<sup>-1</sup> of nitrogen fertilizer and stimulating compounds of ascobien. In conclusion, data indicated that a high yield and N use efficiencies could be obtained by adding 172.5 kg ha<sup>-1</sup> of nitrogen fertilizer and spraying of 200 ml L<sup>-1</sup> twice during the vegetative growth of ascobien and suggested that ascobien could be used as a potential growth regulator to improve plant growth, yield and N use efficiency and N utilization efficiency.

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