



# Selenium Supplementation Alters Biochemical Constituents and Improves Yield of Wheat Plants Subjected to Drought Stress

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**Abstract** - The antioxidant properties of selenium (Se) and its usage to mitigate oxidative stress in plants are widely investigated and well-established. Whether here are other non-antioxidant effects of Se that contribute to its stress-mitigating effects is poorly-understood. The aim of the current study was to explore the non-antioxidant effects of selenium (Se) on wheat plants subjected to drought stress. It was hypothesized that Se could cause complementary effects to its antioxidant effects which may protect wheat plants from drought stress effects and improve yield under such stressful conditions. What grains were soaked in either distilled water or 5 or 10 mgL<sup>-1</sup> Se. Plants raised from Se-soaked grains were either well irrigated (WI) or water stressed (WS) through withholding irrigation from 50-70 days after sowing (DAS). Treatments effects were evaluated through determining growth, yield as well as certain biochemical constituents. Results indicated that Se supplementation restored growth and enhanced yield which were depressed in WS plants. Se-induced restoration of growth and yield of WS plants was accompanied with increased leaf photosynthetic pigments, relative water content, flavonoids and anthocyanins contents. On the other hand, total phenols and silicon contents were decreased in Se-pretreated WS plants. In conclusion, pre-soaking with Se could lead to metabolic adjustments which complement its antioxidant effects that alleviate the adverse effect of drought stress on growth and yield of wheat.

**Keywords** – Wheat, *Triticumaestivum*; Selenium; Drought stress.

## I. INTRODUCTION

Global warming and water scarcity could increase the likelihood of plants being affected by drought stress. Drought stress is detrimental to plant growth, development and yield. Increasing plant tolerance to drought stress is pivotal undertaking to overcome yield losses due to drought. Enhancing plant drought tolerance could be achieved through costly and high-technology-demanded transgenic or on-transgenic approaches of biotechnology, which are not affordable in most cases in developing countries. Alternatively, a less-costly approach could be employed through pretreatment with abiotic stress-alleviating agents. Selenium (Se), though not considered as an essential element to higher plants, it was reported to not only promote plant growth and development, but also increased plant tolerance to stressful environment [1, 2]. With respect to wheat Se was reported to enhance plant

tolerance to cold stress [3], UV-B stress [4, 5] and drought stress [2, 6, 7, 8, 9].

The beneficial effect of Se on plant stress tolerance was attributed principally to its antioxidant effects [2, 10, 11, 12]. Besides, it was reported to increase chlorophyll content [5, 13, 14] as well as effective quantum yield of photosystem II (PSII) and stomatal conductance [15]. Plant water relations were also reported to be positively affected by Se addition manifested mainly by a higher relative water content [6, 9].

Flavonoid and phenolic compounds have the ability to scavenge free radicals hence, they are reported to protect against membrane lipid peroxidation in plants exposed to UV-β stress [4, 16]. Nevertheless, their role under drought stress as well as the effect of Se application on their contents under such conditions were less studied.

In the process of ameliorating plant stress through the addition of Se, it was mostly added as a foliar spray [5, 9, 11, 12, 15, 17], or added to the growth medium [2, 3, 4, 6, 7, 14, 18], whereas its effectiveness as a seed-soaking, which facilitate cost-effective large-scale applications, was less-adopted and needs to be affirmed.

The aim of the present study was to test the non-antioxidant effects of Se applied as a seed soaking treatment on wheat plants growing under drought stress conditions. I hypothesize that Se could cause complementary effects to its antioxidant effects through affecting plant water content as well as modulating phenols, flavonoids, anthocyanins and Si contents that may lead to mitigation of drought stress effects on wheat plants.

## II. MATERIALS AND METHODS

### 2.1 Plant materials, cultivation conditions and experimental design

The experiment was conducted in the experimental Farm whereas the biochemical analyses were performed in the Labs. of the Agric. Bot. Dept., Fac. of Agric., Mansoura Univ. during the two going seasons 2011/2012 and 2012/2013. Main physical and chemical properties of the experimental soil are shown in Table (1). Grains of wheat cv. Giza 168 were disinfested by immersion in 2.5% solution of sodium hypochloride for 10 min and washed four times with distilled water and sown on November 5 and 8 during the two growing seasons 2011/2012 and 2012/2013, respectively.

Table (1)\*. Mechanical and chemical analysis of the used soil.

CS %	FS %	S %	C %	CaCO <sub>3</sub> %	OM %	T N%	A P ppm	E K ppm	TSS %	ASe ppm
9.8	28.7	27.1	34.4	2.5	2.2	0.15	18	226	0.18	0.42

\*CS, Coarse sand; FS, Fine sand; S, Silt; C, Clay; OM, Organic matter; TN, total N; AP, available P; EK, exchangeable K; TSS, total soluble solutes; ASe, available selenium

Experiment was designed as a split plot with four replications; irrigation schemes in the main plots whereas subplots were assigned to Se treatments. The experimental unit was 2 x 3 m containing 12 rows, 25 cm-apart. Drought stress was imposed (water-stressed plants; WS plants) by withholding irrigation from 50-70 days after sowing (DAS) in coincidence with late tillering to early flowering growth stages. Control plots (well-irrigated plants; WI plants) were irrigated every 10 days. At the end of irrigation deprivation period, soil water content was 23.2% in droughted plots vs. 50.6% in control plots. Se treatments were applied by pre-soaking grains in sodium selenite (Sigma chemical comp.) at either 5 or 10 mgL<sup>-1</sup> for six h. Control seeds were soaked in double-distilled water. On 71 DAS, flag leaf samples were collected to determine leaf photosynthetic pigments, relative water content, total phenols, flavonoids, anthocyanins, and Si contents. In addition, growth parameters were recorded

### 2.2 Estimation of growth parameters as well as yield and its components

Plant dry weight and leaf area plant<sup>-1</sup> were recorded at 71 DAS and re-estimated one week later to calculate relative growth rate (RGR) and net assimilation rate (NAR) according to equations of Radford, 1967 [19]. RGR was calculated as :  $(\log_e W_2 - \log_e W_1) / (t_2 - t_1)$  whereas NAR was calculated as  $[(W_2 - W_1)(\log_e A_2 - \log_e A_1)] / [(A_2 - A_1)(t_2 - t_1)]$  where W<sub>1</sub>, A<sub>1</sub> and W<sub>2</sub>, A<sub>2</sub> are dry weight and leaf area at time 1 and time 2, respectively. At harvesting, yield and its components was determined. In addition, Se content in mature grains was estimated. Each parameter was estimated using 5 different plants from each replication.

### 2.3 Determination of leaf biochemical constituents

#### Leaf photosynthetic pigments:

Weight of 0.5 g leaf tissues was grounded in 80% acetone and leaf pigments were determined according to Lichtenthaler, 1987 [20].

Relative water content (RWC):The method of Smart and Bingham, 1974 [21] was followed for estimation of RWC and calculated as

$$RWC = \frac{\text{Fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100$$

Total soluble sugars (TSS): TSS were extracted in 80% ethanol and determined according to Homme *et al.*, 1992 [22] by mixing 0.1 mL of the ethanolic extract with 3.0 ml

freshly-prepared anthrone (150 mg anthrone in 100 ml 72% H<sub>2</sub>SO<sub>4</sub>) and boiling the mixture in water bath for 10 min. After cooling, absorbance of samples was recorded at 620 nm.

Total phenols, flavonoids and anthocyanins: were extracted from leaves in acidified methanol (HCl/M-OH, 1:99 v/v). Absorbance of the extract was recorded at 280, 300, and 530 nm for determination of phenols, flavonoids and anthocyanins, respectively according to the methods of Nogues and Baker, 2000 [23] and Peng *et al.*, 2006 [24].

Si determination: For Si estimation, powder of dry samples was digested in a mixture of 3 ml of 62% (w/w) HNO<sub>3</sub>, 3 ml of 30% (w/v) H<sub>2</sub>O<sub>2</sub> and 2 ml of 96% (w/w) hydrofluoric acid. The digested sample was then diluted to 100 ml with 4% (w/v) boric acid and Si concentration in the solution was determined according to Ma *et al.*, 2002 [25] by the colorimetric molybdenum blue method at 600 nm.

Se determination:Se content in harvested grains was determined as described by Proiettiet *al.*, 2013 [12]. A weight of 1.0 g grains powder was digested with a mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> (9:1 v/v), and Se concentration in the digested material was estimated using an atomic absorption spectrophotometry.

#### Statistical analysis:

Data of the two growing seasons were subjected to combined analysis of variance using MSTAT-C software. Significant differences between treatments means were compared with Duncan multiple's range test.

## III. RESULTS

### 3.1 Growth parameters as well as yield and its components

#### 3.1.1 Growth parameters:

Drought stress decreased all growth characters of wheat plants. Se applied to control plants increased RGR and NAR only at 5 mgL<sup>-1</sup> but didn't affect other growth parameters (Table 2). In drought-stressed plants treated with Se, growth attributes were increased compared with drought-stressed only plants, but still below those in unstressed plants. Generally, Se at 5 mgL<sup>-1</sup> was more efficient in this respect. RGR and NAR in drought stressed plants were increased by 45% and 42%, respectively when treated with Se at 5 mgL<sup>-1</sup>.

**Table (2). Effects of selenium on growth parameters of drought-stressed wheat plants.**

Treatments	Plant height (cm)	Leaf No.	Plant fresh weight(g)	Plant dry weight(g)	Leaf area cm <sup>2</sup> plant <sup>-1</sup>	Tillers no. /plant	RGR (mg g <sup>-1</sup> d <sup>-1</sup> )	NAR (mgcm <sup>2</sup> d <sup>-1</sup> )
Cont	93.1±7.6 <sup>ab</sup>	5.4±0.3 <sup>a</sup>	22.7±2.0 <sup>a</sup>	2.12±0.16 <sup>a</sup>	35.8±4.3 <sup>ab</sup>	3.2±0.4 <sup>a</sup>	34.2±5.6 <sup>b</sup>	1.38±0.18 <sup>b</sup>
Se 5mgL <sup>-1</sup>	102.0±6.3 <sup>a</sup>	5.0±0.3 <sup>ab</sup>	23.1±3.1 <sup>a</sup>	2.24±0.15 <sup>a</sup>	38.2±6.0 <sup>a</sup>	3.0±2.6 <sup>ab</sup>	41.3±7.0 <sup>a</sup>	1.5±0.20 <sup>a</sup>
Se 10 mgL <sup>-1</sup>	100.0±7.0 <sup>ab</sup>	5.0±0.2 <sup>ab</sup>	24.0±1.8 <sup>a</sup>	2.16±0.10 <sup>a</sup>	40.6±5.2 <sup>a</sup>	3.3±0.2 <sup>a</sup>	38.3±3.8 <sup>b</sup>	1.41±0.26 <sup>b</sup>
DS	67.3±4.6 <sup>d</sup>	3.7±0.4 <sup>c</sup>	12.8±1.5 <sup>c</sup>	1.67±0.17 <sup>c</sup>	18.9±3.0 <sup>d</sup>	1.7±0.5 <sup>c</sup>	19.1±3.0 <sup>d</sup>	0.72±0.10 <sup>d</sup>
DS+Se 5mgL <sup>-1</sup>	84.5±5.9 <sup>b</sup>	4.5±0.4 <sup>b</sup>	18.3±1.2 <sup>b</sup>	1.90±0.12 <sup>b</sup>	30.4±4.1 <sup>bc</sup>	2.6±0.5 <sup>b</sup>	27.7±3.6 <sup>c</sup>	1.02±0.22 <sup>c</sup>
DS+Se 10 mgL <sup>-1</sup>	75.6±8.8 <sup>c</sup>	4.0±0.5 <sup>bc</sup>	18.8±3.0 <sup>ab</sup>	1.85±0.24 <sup>ab</sup>	18.2±7.2 <sup>b</sup>	2.7±0.4 <sup>b</sup>	26.9±6.0 <sup>c</sup>	0.96±0.12 <sup>c</sup>

Values are means ±SD of four replicates; different letters in each column indicate significant differences at P<0.05.

### 3.1.2. Yield parameters and grains Se content:

Se treatments to well-irrigated plants didn't affect yield and its components, whereas increased Se content in the grains (Table 3 ). Drought stress led to a decrease in spikelets no. / spike, no of grains of spike, 100-grains weight and grain yields plant. Se content in the grains did

not affected in drought-stressed plants not treated with Se. In water-stressed plants treated with Se, grains yield/ plant was increased substantiated with increased no. of spikelets/spike, no of grains/ main spike and 100-grain weight (Table 3). Grains Se content was increased in WS plants treated with Se.

**Table (3)\*. Effects of Se on yield and its components as well as on grains selenium content of drought-stressed wheat plants.**

Treatments	Spikelets No./main spike	Grains No./main spike	100 seed weight (g)	Grain yield gplant <sup>-1</sup>	Grain's Se content µg g <sup>-1</sup> D. Wt.
Cont	18.0±0.9 <sup>ab</sup>	49.7±3.4 <sup>b</sup>	4.3±0.3 <sup>a</sup>	11.4±0.9 <sup>b</sup>	0.062±0.004 <sup>b</sup>
Se 5mgL <sup>-1</sup>	19.2±0.6 <sup>a</sup>	56.0±4.0 <sup>a</sup>	4.5±0.3 <sup>a</sup>	13.6±1.0 <sup>a</sup>	0.170±0.009 <sup>a</sup>
Se 10 mgL <sup>-1</sup>	19.0±0.9 <sup>a</sup>	54.8±5.3 <sup>ab</sup>	4.4±0.4 <sup>a</sup>	12.4±1.2 <sup>ab</sup>	0.183±0.010 <sup>a</sup>
DS	13.6±0.5 <sup>c</sup>	32.6±3.7 <sup>d</sup>	2.8±0.2 <sup>c</sup>	4.9±0.5 <sup>d</sup>	0.057±0.005 <sup>b</sup>
DS+Se 5mgL <sup>-1</sup>	17.2±0.6 <sup>ab</sup>	42.0±4.0 <sup>bc</sup>	3.6±0.5 <sup>b</sup>	8.4±0.7 <sup>c</sup>	0.152±0.008 <sup>a</sup>
DS+Se 10 mgL <sup>-1</sup>	16.0±0.8 <sup>b</sup>	38.2±6.6 <sup>c</sup>	3.8±0.4 <sup>b</sup>	8.9±0.6 <sup>c</sup>	0.164±0.006 <sup>a</sup>

\* Values are means ±SD of four replicates; different letters in the same column indicate significant differences at P<0.05.

### 3.2. Leaf biochemical constituents

#### 3.2.1. Leaf photosynthetic pigments:

In WS plants, chlorophyll a (Chl a), Chl b, total chlorophylls(tchls), total carotenoids (tcarots) as well as chl a/b and tchls/tcarots ratio were decreased (Fig 1). Se increased tchls and tcarots contents, only at 10 mgL<sup>-1</sup>. Both Se levels decreased tchls/tcarotsratio whereas didn't

affect chl a/b ratio. In drought-stressed plants treated with Se, chl a, chl b, tchls, tcarots as well as chl a/b and tchls/tcarots ratio were increased compared with drought-stressed only plants. No significant difference was recorded between the effect of both Se levels, though the values tend to be higher at 10 mgL<sup>-1</sup>.

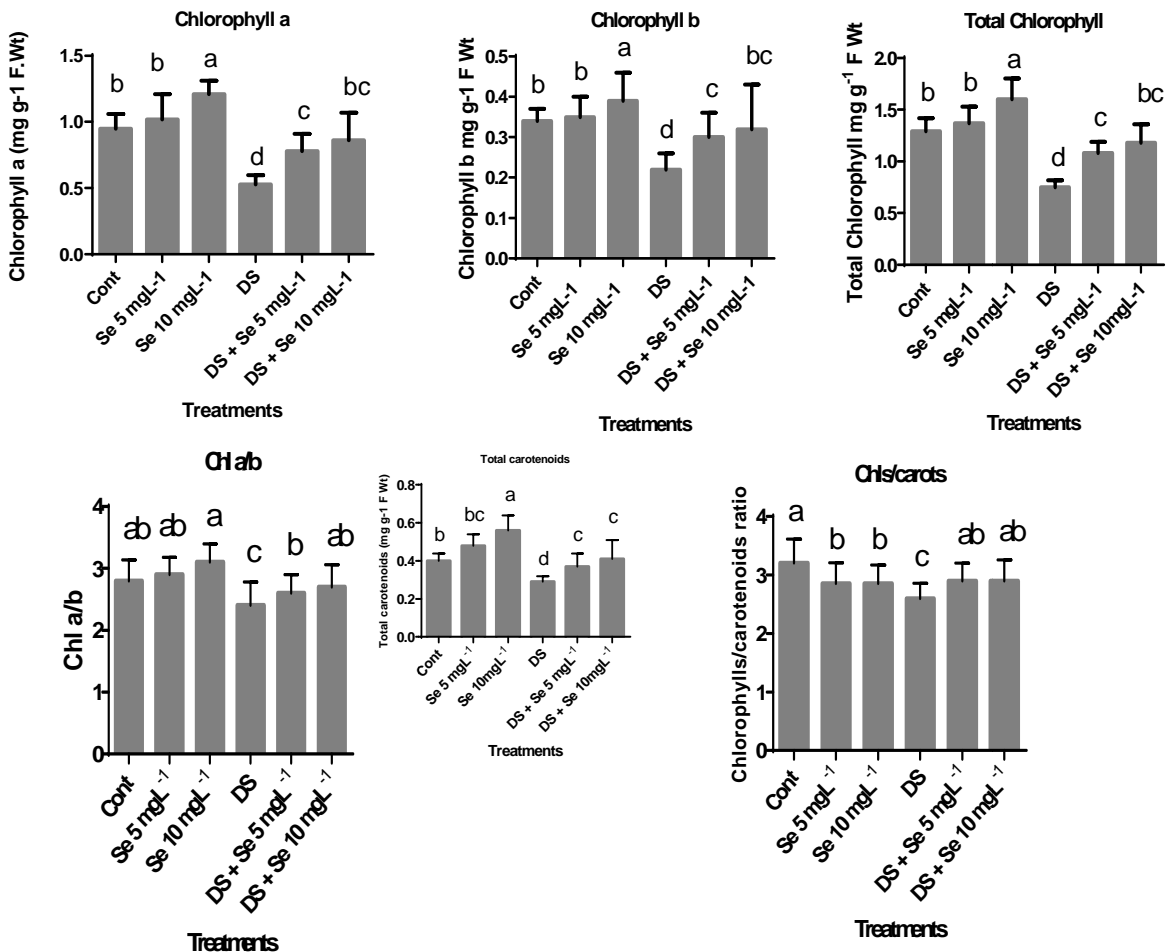


Fig (1): Chlorophyll a (a), Chlorophyll b(b), total chlorophylls (c), chlorophyll a/b ratio (d), carotenoids (e), and total chlorophylls/carotenoids ratio (f) in water-stressed wheat leaves as affected by Se. Mean ( $\pm$ SD) of four replicates. Bars with different letters are significantly different at  $p < 0.05$ .

### 3.2.2. Other leaf biochemical constituents:

Se applied to WI plants didn't affect relative Water content (RWC), total soluble sugars (TSS), total phenols, flavonoids, anthocyanins and Si contents (Fig 2). Drought stress decreased RWC, whereas increased other tested leaf

biochemical constituents. When drought-stressed plants were treated with Se, RWC was increased whereas total phenols and Si contents were decreased. On the other hand, TSS, total flavonoids and anthocyanins contents were further increased in drought-stressed, Se-treated plants.

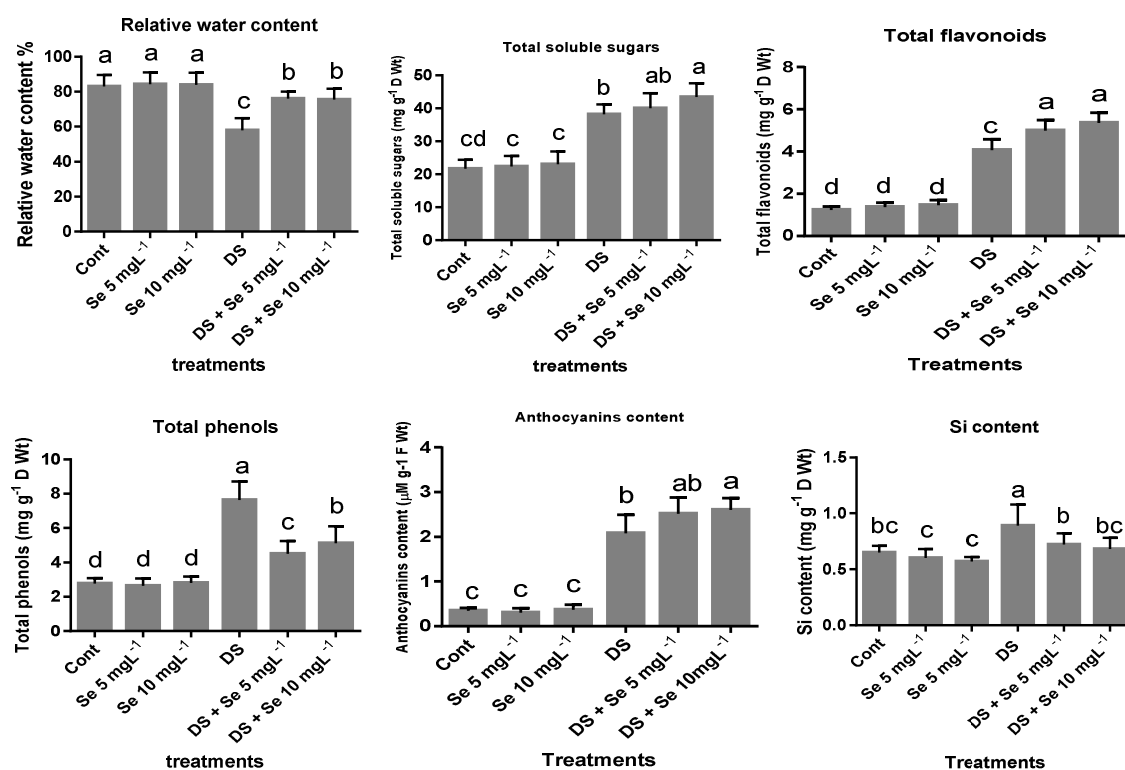


Fig (2): Relative water content (a), total soluble sugars(b), total flavonoids (c), total phenols (d), anthocyanins (e) and Si (f) contents in water-stressed wheat leaves as affected by Se. Mean ( $\pm$ SD) of four replicates. Bars with different letters are significantly different at  $p < 0.05$ .

#### IV. DISCUSSION

The current study test the hypothesis that Se exert complementary effects to its antioxidant effects which contribute to its mitigating affect on drought stress which could contribute to enhancing yield of wheat plants subjected to drought stress. In the present investigation, drought stress decreased RWC (Fig. 2) and leaf photosynthetic pigments (Fig. 1) leading to reduced growth traits (Table 2) as well as yield (Table 3); and Se counteracted these effects. In accordance with these findings, Se was reported to mitigate abiotic stress-induced depressing effect on chlorophyll content [2, 5, 14]. Se-induced chls content under abiotic stress conditions could be attributed to its ROS-Scavenging effect that could otherwise destroy the photosynthetic pigments. ROS-scavenging effect of Se may be due to its enhancing effect on enzymatic antioxidants [1], non-enzymatic antioxidants [26] or both [10, 27]. In addition, a possible regulatory role of Se in chls biosynthesis was suggested by its interaction with 5-aminolevulinic acid dehydratase and porphobilinogen deaminase [28]. Se-improved integrity of cellular organelles and their membrane systems in leaf cells [29] may also contribute to its effect on enhancing photosynthetic pigments under drought stress conditions.

Results indicated that Se partially restored drought-induced decrease in RWC. Similar effect was previously reported [6, 9]. Se-induced RWC under drought stress conditions may be due to its enhancing effect on water uptake capacity of the root system [6], or to decreasing

water loss from water-stressed plants through Se-dependent decreases in stomatal conductance and transpiration rate [18].

Under drought stress, a sugar flux may act as a signal for metabolic regulation [30]. In the current study, drought stress increased TSS concentration which further increased in response to Se treatments. Enhanced TSS accumulation in abiotic stress-affected plants in response to Se supplementation was reported [8, 31, 32]. This effect may be due to an enhanced amylase activity [33] or to an increased chlorophyll content (Fig. 1).

Accumulation of phenols, flavonoids and anthocyanins in drought-stressed plants and exacerbation of flavonoids and anthocyanins due to Se treatments was recorded in the present investigation (Fig. 2). Flavonoids and anthocyanins are important low molecular weight antioxidant compounds in plants [34], which play an important role in stress defense [3]. These results substantiate the hypothesis of the study. Similar results were reported when Se-supplemented wheat plants were subjected to low temperature stress [3] and enhanced UV-B stress [4]. The recorded decrease in total phenols content in drought-stressed plants when treated with Se may reflect a Se-induced general alleviative effect to which plants respond by slowing down their adaptive mechanism.

The present study revealed elevated Si content in plants affected by drought stress, which may be an adaptive mechanism enabling plants to cope with drought stress conditions [35]. Their results suggest that silicon may be involved in metabolic or physiological activities in higher

plants under drought. In drought-stressed plants treated with Se, Si content was reduced compared with WS, Se-untreated plants. This may be due to Se-induced recovery response which reduce drought stress burden on plants and its associated adaptive mechanisms, including Si-dependent responses.

Results of the present investigation revealed that Se had positive effect on yield not only in WS plants but also in WI plants. It was generally reported that Se have beneficial effects on yield of plants under stress conditions [5, 9]. This effect may be due to Se-induced growth (Table 2), photosynthetic leaf pigments (Fig. 1) and RWC (Fig. 2).

## V. CONCLUSIONS

Drought stress decreased leaf photosynthetic pigments and leaf RWC as well as yield components hence, inhibited growth and reduced yield of wheat plants. On the other hand, drought stress increased Si, TSS, phenols, flavonoids and anthocyanins contents. Se supplementation alleviated the adverse effects of drought stress on both growth and yield. Se-induced drought stress mitigation was founded on its enhancing effect on RWC and leaf photosynthetic pigments of drought-stressed plants. Noteworthy is the effect of Se on flavonoids and anthocyanins as they were elevated further in drought-stressed plants treated with Se, indicating that they contribute to and synergize its antioxidative effect. Se pretreatment is therefore recommended in conditions where wheat plants experience periods of water stress. Under such stressful conditions, Se supplementation attenuates negative impacts of drought stress, thus maintains growth and yield.

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