Drought Stress Mitigation By Foliar Application Of Salicylic Acid In Two Linseed Varieties Grown Under Newly Reclaimed Sandy Soil

Bakry, B.A., D.M. El-Hariri, Mervat Sh. Sadak and H.M.S. El-Bassiouny

Field Crops Res. Department, Botany Department National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT

A field experiment was conducted at the experimental farm of National Research Center, Nubaria, El-Beihair Governorate, Egypt, during two successive seasons. The aim of this work was to investigate drought stress mitigation by foliar application of salicylic acid (SA) at four levels (0, 25, 50 and 75 mg/l) on linseed two varieties (Olin and Amon, of oil purpose types) grown under newly reclaimed sandy soil. SA was applied twice as foliar spraying and skipping the irrigation at 45 and 60 days after sowing in two linseed varieties. Application of 25, 50 and 75 mg/l SA to flax two varieties. SA treatment improved morphological criteria of linseed varieties (shoot and root height, fresh and dry weights) compared to untreated plants when exposed to drought stress under newly reclaimed sandy soil. Application of SA increased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments), total soluble sugars, polysaccharides, total carbohydrates, proline and free amino acids, indole acetic acid, phenol contents. Meanwhile, decreased lipid peroxidation as malonaldehyde contents as compared with untreated controls. SA application increased yield and yield components as plant height, technical length, fruiting zone length, number of fruiting branches/plant, number of capsules/plant, also, biological yield/plant, seed yield/plant, seed yield/fed, straw yield, 1000 seeds weight when compared with the untreated controls. Data also show that, foliar spray of salicylic acid increased oil% and oil yield (kg/fed) as compared with controls. Also, qualitative and quantitative changes in fatty acid compositions were obtained in response to SA under drought stress. In conclusion, cultivation of two linseed varieties under drought stress in newly reclaimed sandy soil were more effective with foliar spraying with 75 mg/l of SA plant.

Key words: linseed varieties; salicylic acid (SA); drought stress; photosynthetic pigments; lipid peroxidation.

Introduction

Flax plant (Linum usitatissimum L.) has been grown in many different countries as linen flax, linseed and double purpose plant (fibers and seed) in the same time. It is grown in Egypt as dual purpose crop. Its seeds containing about 36 to 40 % of oil, have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints and/or polymers. Recently, flax plant became an important industrial crop however, all biomass can be used as a raw material for many purposes. There has been a growing interest in the probiotic properties of flax and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Simopoulos, 2002). Flax oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food. Moreover, Flax oil is a rich source of the following unsaturated fatty acids: oleic, linoleic and linolenic acid (El-Beltagi et al., 2007).

Drought is one of the most serious world-wide problems for agriculture. Four-tenths of the world's agricultural land lies in arid and/or semi-arid regions. Egyptian new reclaimed land is characterized as arid and semi-arid regions with poor soil nutrients and unfavorable environmental conditions (drought or high temperature). Water stress is one of the most important environmental stresses that can regulate plant growth and development, limit in production. Water stress is known to increase the amount of secondary metabolites in plants. Accumulation of secondary metabolites is known as a defense mechanism of plants and plants can respond and adapt the water stress by altering their cellular metabolism to invoke various defense mechanisms (El-Tayeb, 2006). It involves the synthesis and accumulation of small compatible solutes (osmolytes), such as proline, glycine betaine, sugars and some inorganic ions (Chaves et al., 2003). These compounds help the cells to maintain their dehydrated state and the structural integrity of the membranes and this in turn provide resistance against drought and cellular dehydration (Ramanjulu and Bartels, 2002). In addition, drought stress often leads to the accumulation of reactive oxygen species (ROS). Excessive ROS production can cause oxidative stress to the photosynthetic apparatus seriously impair the normal function of cells (Niyogi, 1999).
The alleviation of oxidative damage and increase resistance to environmental stresses, at critical growth stages of plant, are often correlated with an efficient antioxidative system. Such systems may be induced or enhanced by the application of chemicals such as salicylic acid SA (He et al., 2005). Salicylic acid (SA) acts as a potential non-enzymatic antioxidant as well as an endogenous plant growth regulator of phenolic nature, naturally occurs in plants in very low amounts, which plays an important role in regulating a number of plant physiological processes such as photosynthesis, stomatal closure, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Arfan et al., 2007). The effect of salicylic acid on the physiological processes is variable, promoting some process and inhibiting others depending on its concentration, plant species, developmental stages and environmental conditions (El-Mergawi and Abdel-Wahed, 2004). Exogenous application of SA helps in the activation of a range of plant defense genes; increase resistance caused by exposure to environmental stresses such as it improves drought tolerance in plants (Sreenivasulu, et al., 2000). SA has a direct physiological effect through the alteration of antioxidant enzyme activities. SA plays an essential role in preventing oxidative damage in plants by detoxifying super oxide radicals, produced as a result of stress (Munns & Tester, 2008).

The objective of this study was to examine the effects of foliar application of SA on growth, some physiological responses, yield and yield components, oil contents and fatty acid composition of the yielded seed of two flax varieties grown under drought stress in newly reclaimed sandy soil.

Materials and Methods

A field experiment was carried out at the experimental Station of National Research Centre, Nubaria district El-Behrea Governorate – Egypt, during two successive winter seasons of 2010/2011 and 2011/2012. The soil of both experimental sites were Newly Reclaimed sandy soil where mechanical and chemical analysis is reported in Table 1 according to Chapman and Pratt (1978). The aim of this work was to investigate drought stress mitigation by foliar application of salicylic acid (SA) on linseed (Linum usitatissimum L.) varieties (Olin and Amon, from oil purpose types) grown under newly reclaimed sandy soils.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 cm depth</td>
<td>60 cm depth</td>
</tr>
<tr>
<td>Mechanical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>91.20</td>
<td>93.70</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>3.70</td>
<td>3.90</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>5.10</td>
<td>3.40</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Sandy</td>
<td>Sandy</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.80</td>
</tr>
<tr>
<td>E.C. m mohs/cm2</td>
<td>0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>CaCO3 (%)</td>
<td>1.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>0.30</td>
<td>0.21</td>
</tr>
<tr>
<td>Soluble N ppm</td>
<td>8.10</td>
<td>9.20</td>
</tr>
<tr>
<td>Available P ppm</td>
<td>3.20</td>
<td>3.60</td>
</tr>
<tr>
<td>Exchangeable K ppm</td>
<td>20.00</td>
<td>23.50</td>
</tr>
</tbody>
</table>

The experimental design was split plot design with four replication, which linseed varieties occupy the main plots and Salicylic acid treatments were allocated at random in sub-plots. Seeds of linseed varieties (Linum usitatissimum L.) cvs. (however, Olin is Romanians while Amon is eruption origin and Amon, oil seed types) were sown on the 15th November in both season in rows 3.5 meters long, and the distance between rows was 20 cm apart, Plot area was 10.5 m² (3.0 m in width and 3.5 m in Length). The recommended agricultural practices of growing linseed were applied and the seeding rate was 2000 seeds/m²). Pre-sowing, 150 kg/fed. of calcium super-phosphate (15.5 % P₂O₅ ) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 75 Kg/fed. was applied at five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52 % K₂O) was added at two equal doses of 50 kg/fed, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Linseed plants were foliar sprayed with salicylic acid at the rate of (0, 25, 50 and 75 mg/ L). In both seasons, foliar application of salicylic acid was carried out twice; where plants were sprayed after 45 and 60 days from sowing. Skipping the irrigation at 45 and 60 days after sowing after sprayed (SA). Plant samples were taken after 75 days from sowing for measurements growth characters were measured in terms of, plant height shoots (cm) fresh and dry weight g/plant, roots length(cm), root fresh and dry weight(g). Plant samples were dried in an electric oven with drift fan at 70°C for 48 hr. till constant dry weight. Plant samples were taken for chemical analysis after 75 days from sowing for chemical analysis of total soluble sugars, polysaccharides, total carbohydrates, total IAA, total phenol content, proline, free amino acids contents and lipid peroxidation.
Flax plants were pulled when signs of full maturity were appeared, then left on ground to suitable complete drying. Capsules were removed carefully. At harvest the following characters were recorded on random samplers of 10 guarded plants in each plot to estimate the following characters: Straw yield and its related characters (Plant height (cm), technical stem length (cm), straw yield/ plant (g), biological yield (ton/fed), Straw yield (ton/fed). Seed yield and its related Characters (number of fruiting branches/ plant, number of capsules/ plant, fruiting zone length, seed yield/ plant (g), 1000-seed weight (g), seed yield (ton/fed), Oil yield (kg/fed) was calculated by seed yield (kg/ fed) * Seed oil percentage and fatty acid profile.

Chemical analysis:

Photosynthetic Pigments:

Total chlorophyll a and b and carotenoids contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophyll a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/100g FW.

Total soluble sugars (TSS):

Total soluble carbohydrates (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme et al. 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H2SO4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol SpectrocolorimeterVEB Carl Zeiss (Yemm and Willis, 1954).

Total carbohydrate:

Determination of total carbohydrates was carried out according to Herbert et al., (1971). A known mass (0.2-0.5 g) of dried tissue was placed in a test tube, and then 10 ml of sulphuric acid (1N) was added. The tube was sealed and placed overnight in an oven at 100ºC. The solution was then filtered into a measuring flask (100ml) and completed to the mark with distilled water. The total sugars were determined Colorimetrically according to the method of Smith et al., (1956) as follows: An aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed in a water bath at 23-30ºC for 20 minutes. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Indole acetic acid content:

A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0ºC. The combined extracts were collected and made up to a known volume with cold methanol. Then take 1ml of the methanolic extract and 4ml of PDAB reagent (para-dimethylamino benzoic acid 1 g dissolve in 50 ml HCl, 50 ml of ethanol 95%) and left for 60 min in 30-40ºC. The developing colour was spectrophotometrically measured at wave length of 530 nm. As described by Larsen et al., (1962).

Total phenol content:

The extract was extracted as IAA extraction, and then 0.5 ml of the extraction was added to 0.5 ml Folin, shaked and allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by Danil and George (1972).

Proline:

Proline was assayed according to the method described by Bates et al. (1973). 2.0ml of proline extract, 2.0ml of acid ninhydrin and 2.0ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath
followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocolourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

**Free amino acids:**

Free amino acid content was extracted according to the method described by Vartanain *et al.* (1992). Free amino acid was determined with the ninhydrin reagent method (Yemm & Cocking 1955). 1.0ml acetate buffer (pH 5.4) and 1.0 ml chromogenic agent were added to 1.0ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocolourimeter VEB Carl Zeiss.

**Lipid Peroxidation:**

The level of lipid peroxidation was measured by determining the malondialdehyde (MDA) contents. Malondialdehyde is the product of Lipid peroxidation and that assayed by thiobarbaturic acid reactive substance (TBARS) contents (Stewart & Bewley, 1980).

**Oil determination:**

The oil of flax seeds were extracted according to Kates and Eberhardt (1957), the powdered seeds is shaken overnight with isopropanol: chloroform (1:1). The solvent were evaporated under reduced pressure of CO₂ atmosphere. The lipid residue is taken up in a chloroform: methanol (2:1 v/v) and given a folch wash, the dissolved total oils were purified by washing with 1% aqueous saline solution. The aqueous phases were washed with chloroform that was combined with the pure oil solution. Chloroform was evaporated and the total pure oil was weighed.

**Fatty acid determination:**

To oil sample 20 ml methanol, 10 ml benzene and 1ml concentrated sulphoric acid were added and refluxed for 90 minutes, the methyl esters obtained were extracted with petroleum ether (b.p. 40-60 °C). The petroleum ether was then evaporated; the residue was dissolved in chloroform (Harbone, 1984). The methylated samples were subjected to analysis by GLC in GVC pye Unicam gas-liquid chromatograph equipped with dual flame ionization detector and dual channel recorder.

**Statistical analysis:**

The data were statistically analyzed on complete randomized design system according to Snedecor & Cochran (1980). Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

**Results and Discussion**

**Growth parameters:**

Drought is an important factor that could influence growth and physiological characteristics of plants (Xiangwen, *et al.*, 2009). It is well known that the response of plants to drought stress depends on the species and genotype, the time of water severity deficit, plant age and stage of development and other related characters could be affected on flax growth parameters. The results reported in Table (2) showed that there was an increase in all growth parameters due to salicylic acid (SA) application. Plant height, fresh and dry weight of shoot (g/plant), RWC of shoot system and root length, fresh and dry weight of root plant of the two flax varieties under test compared with untreated control plants. This promoting effect reached maximum at 75 mg/l SA for all growth characters except RWC of Amon variety which was at 50 mg/l. Data showed also that, salicylic acid treatment were more effective on Olin variety than Amon in general response. These results are in good agreement with those reported by others investigators, AbdEl–Wahed *et al.*, (2006) on maize, Yildirim *et al.* (2008) on cucumber and El Tayeb & Ahmed (2010) on wheat. Singh and Usha (2003) suggested that the promotive effect of SA on linen plant under drought stress may be related to the induction of antioxidant responses which protect plant from damage. Also, the promotive effect of salicylic acid could be attributed to its bioregulator effects on physiological processes in plants such as ion uptake, cell elongation, cell division, cell differentiation, morphogenesis, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as reported by (El-Tayeb, 2005). Salicylic acid as anti-stress substance may enhance the
plant tolerance to drought stress (Sreenivasulu et al., 2000). Increases in RWC of linen varieties treated with SA were also reported for other crops grown under drought stress including barley (El-Tayeb, 2005). This phenomenon may be attributed to the fact that foliar SA treatment application can increase the leaf diffusive resistance and lower transpiration rates and these conclusions confirmed our findings.

Table 2: Effect of salicylic acid on morphological criteria of flax varieties grown under drought stress in newly reclaimed sandy soil (combined analysis of two growing seasons 2009/010 and 2010/011).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Olin</th>
<th>Amon</th>
<th>LSD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid (mg/l)</td>
<td>0</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Shoot Length (cm)</td>
<td>42.67</td>
<td>47.00</td>
<td>56.33</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>2.20</td>
<td>3.00</td>
<td>4.35</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>0.35</td>
<td>0.40</td>
<td>0.58</td>
</tr>
<tr>
<td>RWC</td>
<td>84.17</td>
<td>86.0</td>
<td>86.75</td>
</tr>
<tr>
<td>Root Length (cm)</td>
<td>11.67</td>
<td>12.33</td>
<td>13.00</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>0.25</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Photosynthetic pigments:

Regarding to photosynthetic pigments of flax plant grown under drought stress in newly reclaimed sandy soil is represented in Fig. 1. Data show that SA applications with different concentrations (25, 50 and 75 mg/l) caused significant increases in chlorophyll a, chlorophyll b, carotenoids and total pigments of the two tested varieties (Olin and Amon). The highest promotive effect was obtained from 75 mg/l of SA application in both varieties. SA at 75 mg/l caused significant increases (67.33% & 65.18%) for chlorophyll a, (87.01% & 64.18%) for chlorophyll b, (76.44% & 74.79%) for carotenoids (72.74% & 66.83%) and for total pigments in Olin and Amon varieties respectively. Data clearly showed that the response of Olin variety is more apparent than Amon variety this means that, Olin is more tolerant than Amon variety. The increase in photosynthetic pigments content owing to SA confirmed the reported by Daneshmand, et al., (2009) for Solanum bulbocasanum plant and Idrees, et al., (2010) for periwinkle plant. This positive effect of SA could be attributed to an increased in CO₂ assimilation and photosynthetic rate which increased mineral uptake by the plant (Szepesi et al., 2005). Also, salicylic acid is one of antioxidant substances concentrated in the chloroplast and protect the photosynthetic apparatus when a plant is subjected to drought stress, by scavenging the excessively reactive oxygen species known as free radicals (Kramner, et al., 2002). The results obtained by these of other investigators are in good agreement with our findings and these in turn confirmed our results.

It is worthy to mention that, carotenoids content was significantly higher in both varieties under SA treatment as compared to control. Carotenoids might play a role as a free radical scavenger. Therefore, increasing of carotenoids in both varieties treated with SA could enhance their capacity to reduce the damage caused by ROS, which in turn increased chlorophyll content of such plants. The same findings were reported by Eraslan et al. (2007).
Fig. 1: Effect of salicylic acid on photosynthetic pigments contents (as mg/100g fresh wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar =Olin variety horizontal bar = Amon variety).

Total soluble sugar, polysaccharides and total carbohydrate:

The effect of foliar application of SA on the total soluble sugar, polysaccharides and total carbohydrate of the two tested varieties are shown in Fig. 2. Data clearly showed that, SA treatment caused significant decreases in TSS in Olin variety, while significant increase in Amon variety as compared with the corresponding control plants. In the meantime, it caused significant increases in polysaccharides and total carbohydrate of the two varieties Olin and Amon as compared with the corresponding control plants. SA at 75mg/l was the most effective treatment in both varieties, it caused significant increase (35.48% & 26.44%) for polysaccharides (22.59% & 75.28%), for total carbohydrates in Olin and Amon varieties, respectively. The significant increases in total carbohydrates, in shoots of flax cultivars concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to salicylic acid treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of flax plants. Similar results to our obtained results were obtained by El-Tayeb and Ahmed (2010). The reduction in TSS was suggested that SA application might activate the metabolic consumption of soluble sugars to form new cell constituents as a mechanism to stimulate the growth of flax plants reported in this study. SA treatment might also be assumed to inhibit polysaccharide-hydrolyzing enzyme system on one hand and/or accelerate the incorporation of soluble sugars into polysaccharides. Our assumption could be supported by the result that SA increased polysaccharide level on the sake of soluble sugars (Sharma and Lakhvir, 1988). While, in Amon variety the total soluble sugar increased as a result of SA treatment this means that the osmotic adjustment was associated with an increase in total soluble sugars. In this connection, Benbella, (1999) reported that, the increasing of all soluble carbohydrate in the shoot during drought stress is effective on the balance against osmotic pressure. The plant cell in order to escaping from plasmolysis performance and creation during drought stress conditions should be changed and analyzed from macro to micro molecules.
Fig. 2: Effect of salicylic acid on TSS, Polysaccharides and total carbohydrates contents (as mg/100g dry wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).

Proline and free amino acids:

Data presented in Fig.3 shows that SA increased significantly proline and free amino acids content of both varieties (Olin and Amon) compared to the corresponding control plant, SA at 75 mg/l was effective treatment in both varieties in most parameters. These results are in agreement with those of El Tayeb and Ahmed (2010), Delavari et al. (2010). Many functions have been postulated for proline accumulation in plant tissues, proline and free amino acids could be involved in the osmotic adjustment of plants (Gzik, 1999) and could also be a protective agent of enzymes and membranes (Bandurska, 1993). When plant subjected to drought stress, plants maintain their water content by accumulation of compatible organic solutes act as osmoprotectants, such as proline, in their cytoplasm (Harinasut et al., 2000). Proline also functions as hydroxyl radical scavenger (Hoque et al., 2007). proline accumulation under drought stress has been reported and suggested to be a biochemical marker for increased stress tolerance in plant species under stress conditions.

Indole acetic acid content:

Fig.4 shows that SA increased IAA content significantly of both varieties (Olin and Amon) compared to the corresponding control plant SA at 75 mg/l was the most effective treatment in both varieties, this increase in IAA concurrent with the increase in growth rate as shown in Table (2). It could be concluded that this increase may be due to the role of endogenous hormone in stimulating cell division and/or the cell enlargement and this in turn improve plant growth. In this connection, Shakirova, et al., (2003), reported that, SA promoted plant growth via increase in IAA and cytokinins under no stress conditions. Moreover, Sakhabutdinova, et al., (2004) found that, the SA treatment caused accumulation of both ABA and IAA in wheat seedlings.
**Phenol content:**

In the present study, total phenol content increased of both varieties (Olin and Amon) compared to the corresponding control plant, SA at 75 mg/l was the most effective treatment in both varieties as show in Fig.4. Increase in phenol content in different tissues under osmotic stress has been reported in many plants (Pokorny, 2001). This increase may be one aspect of the role played by SA in alleviating the suppressive effects of drought; phenols possess ideal structural chemistry for free radical scavenging activity. This increase may be due to total phenols role that play a significant mechanism in regulation of plant metabolic processes and consequently overall plant growth (Lewis and Yamamoto 1990). Moreover, phenols act as a substrate for many antioxidants enzymes, so, it mitigates the salinity stress injuries (Khattab, 2007). Another mechanism underlying the antioxidative properties of phenolic compounds is the ability of phenols to decrease membrane fluidity (Gaballah *et al.*, 2006).

![Figure 4: Effect of salicylic acid on IAA and phenol (as mg/100g fresh wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).](image)

**LSD at 5%: 4.49**

**LSD at 5%: 20.06**

**Fig. 4:** Effect of salicylic acid on IAA and phenol (as mg/100g fresh wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).

**Lipid peroxidation:**

Drought stress induced membrane injury which may therefore due to changes in the membrane lipids or protein or both (Scandalios, 1993). Lipid peroxidation is the symptom most easily ascribed to oxidative damage (Zhang and Kirkham, 1996). As shown in Fig.5 SA treatment reduced the amount of MDA can assess the tolerance capacity of both varieties to membrane damage induced by drought stress. There were many reports about the role of salicylic acid in removing the stress of peroxidative, salicylic affecting the antioxidant enzymes and lipids peroxidation, would protect the Arabidopsis plant against warm and drought stress (Yildirim *et al.*, 2008).

![Figure 5: Effect of salicylic acid on MDA (as µg/100g fresh wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).](image)

**LSD at 5%: 1.64**

**Fig. 5:** Effect of salicylic acid on MDA (as µg/100g fresh wt) of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).
Yield and yield attributes:

Data in Table (3) show that yield and its components such as plant height (cm), technical stem length (cm), fruiting zone length (cm), number of fruiting branches/plant, number of capsules/plant, biological yield/plant (g), seed yield /plant (g), 1000 seed weight(g), seed yield (kg/fed), straw yield (ton/fed), oil % and oil yield (kg/fed) were significantly enhanced with foliar application of salicylic acid compared with control plants. It is clear from the results that, the enhancement effect of SA increased with the increase in SA concentration up to 75 mg/l in both cultivars, Olin and Amon. These results are confirmed by the results of Abd el-Wahed et al., (2006), Daneshmand et al., (2009), Khan et al., (2010) on different plant species. These increases in yield and yield components might be due to SA stimulation of physiological processes that were reflected on improving vegetative growth (Table 2) that followed by active translocation of the photosynthetic products from source to sink in flax varieties.

Regarding with the increment in oil% and oil yield (kg/fed) with foliar treatments of SA these increases may be due to the increase in vegetative growth, nutrients uptake. Similar observations were noted by Noreen and Ashraf (2010). It is worthy to mention that, in Amon variety the increase in oil yield (kg/fed) by application of SA due to the increase in oil%, the most pronounced increase was at 75 mg/l SA it caused an increase by 21% as compared with control plant.

Table 3: Effect of salicylic acid on yield and yield components of flax varieties grown under drought stress in newly reclaimed sandy soil.

<table>
<thead>
<tr>
<th>varieties</th>
<th>Salicylic acid mg/l</th>
<th>LSD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olin</td>
<td>Amon</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>73.67 78.00 85.33 69.00</td>
<td>84.00 87.67 75.33</td>
</tr>
<tr>
<td>Technical length (cm)</td>
<td>61.00 62.00 67.00 62.33</td>
<td>55.00 63.33 62.67 46.33</td>
</tr>
<tr>
<td>Fruiting zone length (cm)</td>
<td>12.67 16.00 18.33 20.00</td>
<td>14.00 20.67 25.00 29.00</td>
</tr>
<tr>
<td>No. of fruiting branches/plant</td>
<td>3.67 4.00 4.67 5.67</td>
<td>3.67 4.67 5.00 7.00</td>
</tr>
<tr>
<td>No. of capsules/plant</td>
<td>19.67 26.00 24.00 15.50</td>
<td>34.00 40.00 41.00 2.13</td>
</tr>
<tr>
<td>Bio. yield/plant (g)</td>
<td>1.35 2.01 2.28 1.31</td>
<td>1.45 1.73 2.51 0.18</td>
</tr>
<tr>
<td>seed yield/plant (g)</td>
<td>0.30 0.45 0.51 0.60</td>
<td>0.29 0.32 0.38 0.56</td>
</tr>
<tr>
<td>1000 seed weight(g)</td>
<td>8.03 8.50 9.46 10.85</td>
<td>4.12 4.85 5.90 6.07</td>
</tr>
<tr>
<td>Seed yield (kg/fed)</td>
<td>504.00 688.80 672.00 380.16</td>
<td>651.04 656.88 697.20 840.00</td>
</tr>
<tr>
<td>Straw yield (ton/fed)</td>
<td>2.35 2.94 2.98 3.11</td>
<td>2.02 2.81 2.98 3.36</td>
</tr>
<tr>
<td>Oil%</td>
<td>37.14 37.26 38.03 38.67</td>
<td>35.31 36.80 40.05 0.22</td>
</tr>
<tr>
<td>Oil yield (kg/fed)</td>
<td>187.19 256.65 255.56 332.62</td>
<td>182.72 231.94 269.12 336.42</td>
</tr>
</tbody>
</table>

Fatty acid composition:

The results of gas chromatographic analysis of the methyl esters of fatty acids of yielded flax seeds revealed that Palmitic acid was the most predominant saturated fatty acid. While Oleic acid and Linoleic acid were the major unsaturated fatty acid in the two varieties (Olin and Amon). Olin variety have more Linoleic acid (44.064%) than Oleic acid (37%). In the meantime, Amon variety have Oleic acid (68.6041%) more than Linoleic acid (10.9621%) Fig..6&7. The exposure of flax plant to different concentrations of SA induced marked increases in the levels of unsaturated fatty acids particularly Oleic and Linoleic acids. On the other hand, the saturated fatty acids markedly decreased. The magnitude of such increase was much more pronounced by applying 75 mg/l SA, it caused 5.95% and 5.63% in the total unsaturated fatty acids in Olin and Amon varieties respectively. These results are quite similar to those obtained by Noreen and Ashraf (2010). Abdel Rahim et al., (2000) reported that the percentage of unsaturated fatty acids proved the efficiency of desaturation in oil. Data also show that the effects of SA treatments on individual fatty acids varied between varieties) In addition, Noreen and Ashraf (2010) mentioned that high doses of salicylic acid caused a marked increase in sunflower achene oil content as well as some key fatty acids and significant decrease in stearic acid.

The increase in unsaturated fatty acid with decreasing of saturated fatty acids and consequently, increasing in TUS/TS (Table 4). Thus the yielded oil becomes safer for human consumption.
Fig. 6: Effect of salicylic acid on saturated fatty acids of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil.

Fig. 7: Effect of salicylic acid on unsaturated fatty acids of two flax varieties (Olin and Amon) under drought stress in newly reclaimed sandy soil (clear bar = Olin variety horizontal bar = Amon variety).

Table 4: Effect of salicylic acid on total saturated, unsaturated fatty acids and their ratio of two flax varieties under drought stress in newly reclaimed sandy soil.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Salicylic acid mg/L</th>
<th>Olin</th>
<th>Amon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Total unsaturated</td>
<td>86.248</td>
<td>89.689</td>
<td>90.555</td>
</tr>
</tbody>
</table>

References


