Seedling Parameters as affected by Soaking in Humic Acid, Salinity Stress and Grain Sorghum Genotypes

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Abstract—To study the effect soaking in humic acid and salinity stress on sorghum seedling parameters, a laboratory experiment accompanied in Seed Science Laboratory Faculty of Agriculture Mansoura University from June 2017 to July 2017. This exploration intended to study performance of seedling parameters of five grain sorghum cultivars viz. Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 to soaking in humic acid under salinity concentrations of 3, 6, 9, 12 and 15 dSm⁻¹ beside the control, and humic acid soaking. The results showed that seed soaking in humic acid recorded the tallest shoot and root, weight of fresh shoot and root, weight of dry shoot and the lowest percentages of relative dry weight and highest salinity tolerance index. Seed soaking in humic acid exceeded shoot and root length (cm), weight of fresh shoot and root, weight of dry shoot and tolerance index by 14.3, 92.6, 8.7, 4.5, 4.7 and 40.8%, respectively compared without soaking in humic acid. The tallest shoot and the fresh shoot weight were produced from germinating Giza 15 cultivar. In addition, the tallest roots and highest values of stress tolerance index were recorded from sown Mecca hybrid and Giza 15 cultivars without significant differences. It could be stated that sown Mecca hybrid surpassed H-305 cultivar in root length by 36.3 %. Moreover, Giza 15 cultivar surpassed H-305 in shoot length, shoot fresh weight and stress tolerance index by 30.6, 10.1 and 29.1 %, respectively. The results clearly revealed that accumulative salinity concentrations from 3, 6, 9, 12 and 15 dSm⁻¹ significantly produced the shortest shoot, root, weight of fresh shoot, root, weight of dry shoot, and root, highest percentages of seedling height reduction, and stress tolerance index, except the percentage of relative dry weight increased with salinity levels increased. The shortest shoot and root (cm), were recorded from the highest salinity concentrations of 15 dSm⁻¹. The gradually increases in salinity till of 15 dSm⁻¹ significantly diminished the length of shoot and root, the fresh weight of shoot and root, the dry weight of shoot and root, seedling height reduction percentages, and stress tolerance index by 51.7, 17.7, 4.7, 59.5, 38.7 and 57.4, respectively compared the control treatment. Accordingly, sown Mecca hybrid or Giza 15 cultivar with soaking in humic acid under salinity of 6 dSm⁻¹ maximized seedling parameters and could recommended to cultivated in saline new reclaimed soils.

Keywords—Sorghum varieties, humic acid, salinity levels.

I. INTRODUCTION

Salinity is harmfully influenced crop yield of various crops extents of Egypt and the world. Irrigation water sources in Egypt was limit. There is a shortage in cereal production with annually increases of population. To overwhelmed the lack of cereal productivity, it could be achieved by growing grain sorghum cultivars tolerant of salinity to get economical yields from saline reclaimed soils, particularly in early seedling stages is very important. Soil salinization is a great determinate factor of crop productivity, especially in dried area (Ahmed, 2009). Many physiological variations induced in plants affecting their growth, development and seedling parameters in arid and semi-arid districts (Saroj and Soumanu, 2014).

Humic acid activate many processes accompanied emergence of primary root and shoot emergence. Soaking in potassium humate at concentration of 100 mL⁻¹ for 24 hours the length of root and shoot and the weight of dry shoot were increased with (Ali and Elbordiny, 2009). The root and shoot length increased in the seedlings which preserved with potassium humate over control (Patil, 2010). The seeds primed at level of 750 mg 1 for 12 h recorded the greatest seedling growth parameters. Increasing concentration up to 500 and 750 mgL⁻¹ had highest influence of seedling growth parameters (Asgharipour and Rafiee et al., 2011). Germination percentage and tallest root produced from seed of wheat priming with humic acid compared with those primed in water (Ali et al., 2014). Seedling growth
inhibiting by salt and varying due top retreatment of humic acid (Cavusoğlu and Ergin, 2015).

The recommended cultivar for new reclaimed saline soils were Soavecultivar (Almodares et al., 2007). Root length affected by salt stress more than shoot length. 235461 and 69239 genotypes recorded the more salt tolerant during seedling growth (Geressu and Gezahagne, 2008). The most salt tolerance genotypes recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. Nevertheless, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 (Asfaw, 2011). Sorghum genotypes differed from response to salinity, the medium tolerant recorded by Hegariand JS-263 medium cultivars, while Noor cultivar produced the medium sensitive and the sensitive produced from FJ-115 and PSV-4 cultivars (Kausar et al., 2012). The STI, GMP and MP genotypes were better to cultivate under saline and non-saline conditions. In contrast, cluster of the highest tolerance index and stress sensitivity index is sensitive to saline or non-saline conditions (Hefny et al., 2013). The highest seedling growth characters recorded from Meko, Gambella-1107, ICSV-111 and Melkmv genotypes and were more salt tolerant cultivars. However, germinating ESH-2 and Goybe genotypes were salt sensitive throughout growth stage. (Tigabo et al., 2013). The most salt tolerant cultivars produced from germinated Shallu, Desert Maize, and 1790E genotypes, while the least salt tolerance genotypes produced from Schrock and RTx430 cultivars (Sun et al., 2014). Wad Ahmed cultivar recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive (El Naim et al., 2012). The seedling characters decreased as the salinity concentrations increased in all studied cultivars. Salinity stress reduced root and shoot length and weight of dry seedling of all genotypes (Chauhan et al., 2012). The shortest shoot androot, the lowest weight of dry root and chlorophyll contents were produced from increasing of salinity levels of all studied cultivars (Movafegh et al., 2012). The control and salinity level of 5 dS.m⁻¹ produced the tallest root and shoot. As salinity increased, the shoots and root length decreased. The highest shoot length recorded by KFS2 cultivar, so this genotype was salt tolerant cultivar (Tabatabaei and Anagholi, 2012). Seedling growth of sorghum decreased due to salinity. Seedling dry weight decreased due to increasing in salinity concentration (Bezadnejad and Tohidinejad, 2014). Radical and shoot length and weight decreased with increasing salinity concentration (Dadar et al., 2014). The plumule and radical fresh and dry weight were decreased as the NaCl concentrations increased (Sam et al., 2014). Plumule and radical length reduced as NaCl level was amplified. The sensitive cultivar to salinity produced from Barbarei cultivars compared to Tabat and Wad-Ahmed cultivars. Tabat cultivar recorded the more-salt tolerant cultivars (Siddig and Idris, 2015). Therefore, the goals of this study aimed to study the behavior of seedling parameters of some grain sorghum cultivars soaking in humic acid under salinity stresses.

II. MATERIALS AND METHODS

2.1. Treatments and Experimental Design:

A laboratory experiment carried out in the Faculty of Agriculture, Mansoura University from June 2017 to July 2017 in Agronomy Department, Seed Science Laboratory. The aimed of the investigation conducted to screening forfive (Sorghum bicolor L. (Moench) cultivars i.e. Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 under salinity stress, sodium chloride (Na Cl) at the levels of 0 (as control), 3, 6, 9, 12 and 15 dS/m⁻¹ soaked or non-soaked in humic acid. RCBD design in factorial experiment in four replications was used. The first factor include two treatments with and without soaking in humic acid. The second one includes the five-grain sorghum cultivars i.e. Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305. Six different concentrations of NaCl include 0, 3, 6, 9, 12 and 15 dS/m⁻¹ were allocated as third factor. The experiment included 240 Petri dishes, then, the Petri dishes take placed in a growth chamber for 14 days at 28 ± C ° for germination according to ISTA, 2016 roles.

2.2. Studied characters:

Eight seedling trails were studied as follows:

1-The length of shoot (cm): It measured as average of five shoot length in centimeters (cm).
2-The length of root (cm): It was calculated as means of five root length in centimeters (cm).
3-Weight of fresh shoot (g): Means of five shoots were weighted in gram (g).
4-Weight of fresh root (g): Means of five seedlings roots was weighed in gram (g).
5-Weight of dry shoot (g): Mean of five seedlings shoots was weighted after oven drying at 75 °C for 48 h.
6-Weight of dry root (g): Mean of five seedlings roots was weighted after oven drying at 75 °C for 48 h.

Physiological Parameters:

7-The percentage of seedling height reduction: It calculated as described by the following equation (Islam and Karim, 2010).

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The percentage of relative dry weight: It calculated according (Islam and Karim, 2010) equation:

\[
SRH(\%) = \left( \frac{\text{Plant height at control} - \text{Plant height at saline condition}}{\text{Plant height at control condition}} \right) \times 100
\]

To calculate the germination stress tolerance index (GSI), promptness index (PI) estimated using following (Ashraf et al., 2006) formula:

\[
P\text{romptness index (PI)} = \text{nd1 (1.00)} + \text{nd2 (0.75)} + \text{nd3 (0.50)} + \text{nd4 (0.25)}
\]

Where nd1, nd2, nd3 and nd4 = Number of seeds germinated on the 1st, 2nd, 3rd and 4th day, respectively.

The germination stress tolerance index (GSTI) calculated in terms of percentage as follows:

\[
G\text{STI} = \left( \frac{\text{PI of stress seeds}}{\text{PI of control seeds}} \right) \times 100
\]

2.3. Statistical Analysis:

Rendering to the system of variance (ANOVA) was used for the factorial in RCBD as published by Gomez and Gomez (1991). The subject data was statistically analyzed. LSD method was used as defined by Snedecor and Cochran (1980). The data as (Russel, 1986). method was statistically analyzed using RCBD design by MSTAT-C computer package.

III. RESULTS AND DISCUSSIONS

3.1. Humic acid soaking effect:

The results presented in Tables (1 and 2) clearly showed that soaking in humic acid significantly affected shoot and root length (cm), weight of fresh shoot and root, weight of dry shoot, relative dry weight and germination stress tolerance index, however insignificantly affected root dry weight and percentage of seedling height reduction. The results showed that seed soaking in humic acid recorded the tallest shoot (5.75 cm) and radical (8.67 cm), the highest weight of fresh shoot (0.39 g) and root (0.36 g), the dry shoot weight (0.34 g) and lowest percentages of relative dry weight (79.95) and the highest salinity tolerance index values (80.59). It could noticed that soaking seed in humic acid exceeded the length of shoot and root (cm), weight of fresh shoot and root, weight of dry shoot and tolerance index by 14.3, 92.6, 8.7, 4.5, 4.7 and 408 %, respectively compared without humic acid soaking. Root and shoot length increased in the seedlings treated with potassium humate over control (Patil, 2010). Seeds primed at level of 750 mg L-1 recorded the greatest seedling growth parameters. Increasing concentration up to 500 and 750 mgL-1 had highest influenced of seedling growth parameters (Asgharipour and Rafiei et al., 2011). Germination percentage and tallest root produced from seed of wheat priming with humic acid compared with those primed in water (Ali et al., 2014). Seedling growth inhibiting by salt and varying due to pretreatment of humic acid (Çavuşoğlu and Ergin, 2015).
3.2. Cultivars performance:
The outcomes of accessible results in Tables (1 and 2) clearly revealed that studied sorghum cultivars significantly influenced the length of shoot and root (cm), the weight of fresh shoot, seedling height reduction and relative dry weight percentages as well as stress tolerance index, however insignificantly affected root fresh and dry weight and shoot dry weight. The results clearly indicated that the tallest shoot (6.37 cm) and shoot fresh weight (0.39 g) were produced from sown Giza 15 cultivar. In addition, the tallest roots (10.47, 7.81 cm) and highest values of stress tolerance index (69.47, 70.83) were recorded from sown Mecca hybrid and Giza 15 cultivars without significant differences. The maximum percentages of seedling height reduction (44.18%) were found from germinating H-306 cultivar and highest relative dry weight from sown Dora cultivar. The shortest roots (4.42 cm) and shoot (6.66 cm) the lowest values of shoot fresh weight (0.35 g), relative dry weight (79.44%) and stress tolerance index (50.17) were obtained from sown H-305. Genotypically, it could be stated that sown Mecca hybrid surpassed H-305 cultivar in root length by 36.3%. Moreover, Giza 15 cultivar surpassed H-305 in shoot length, shoot fresh weight and stress tolerance index by 30.6, 10.1 and 29.1%, respectively. The recommended cultivar for new reclaimed saline soils were Soave cultivar (Almodares et al., 2007). Root length affected by salt stress more than shoot length. 235461 and 69239 genotypes recorded the more salt tolerant during seedling growth (Geressu and Gezahagne, 2008). NM-92 cultivar was more salinity tolerant (Ahmed, 2009). The more-salt tolerance recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. Nevertheless, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 (Asfaw, 2011). Wad Ahmed cultivar was recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive (El Naim et al., 2012). Shoot and root growth, weight of fresh and dry shoot and root were clearly demonstrated varietal differences (Khan et al., 2014).

3.3. Salinity level effects:
The outcomes of accessible results in Tables (1 and 2) clearly showed that studied sorghum cultivars significantly influenced all studied trials. Increment in salinity levels from 3, 6, 9, 12 and 15 dSm⁻¹ significantly abridged the length of shoot and root (cm), weight of fresh shoot and root (g), weight of dry shoot and root, percentages of seedling height reduction and stress tolerance index, except, the relative dry weight increased with increasing salinity levels. The highest values of shoot (7.42 cm) and root length (8.72 cm), shoot (0.38 g) and root (0.36 g) fresh weight (g), shoot (0.34 g) and root (0.32 g) dry weight (mg), and stress tolerance index(100.0) were recorded from without salinity. The

| Treatment | Shoot length | Root length | Weight fresh Shoot | Weight fresh root |
|-----------|--------------|-------------|--------------------|-------------------|
| Without   | 4.93         | 7.64        | 0.35               | 0.33              |
| Soaking   | 5.75         | 8.67        | 0.39               | 0.36              |
| F-test    | *            | *           | *                  | *                 |

| B. Sorghum cultivars |
|----------------------|
| Dora                 |
| H.306                |
| Giza 15              |
| Mecca hybrid         |
| H-305                |

| C. Salinity Level |
|-------------------|
| 0 dsm⁻¹            |
| 3 dsm⁻¹            |
| 6 dsm⁻¹            |
| 9 dsm⁻¹            |
| 12 dsm⁻¹           |
| 15 dsm⁻¹           |

| F-test | L.S.D at 5% |
|--------|-------------|
| *      | 0.42        |
| *      | 0.71        |
| *      | 0.02        |
The interaction m cultivars and salinity

The results showed that the salinity concentrations increased in all studied cultivars. Salinity stress reduced root and shoot length and weight of dry seedling of all genotypes (Chauhan et al., 2012). The shortest shoot and root, the lowest weight of dry root and chlorophyll contents were produced from increasing of salinity levels of all studied cultivars (Movafegh et al., 2012). The STI, GMP and MP genotypes were suitable for cultivation under salinity and without salinity. In contrast, cluster of the highest tolerance index and stress sensitivity index is sensitive to under salinity and without salinity (Hefny et al., 2013). Salt stress harmfully disturbs plants and crops at all stages (Hussain et al., 2013). Similar conclusions were described by Almodares et al. (2014), Behzadnejad and Tohidinejad (2014), Dadar et al. (2014) and Sam et al. (2014).

Table 2: Averages of root fresh weight and root dry weight as affected by humic acid soaking, sorghum cultivars and salinity levels.

| Treatments          | Weight of dry shoot | Weight of dry root | Seedling height reduction | Relative dry weight | Stress tolerance index |
|---------------------|---------------------|--------------------|---------------------------|---------------------|------------------------|
| A. Humic acid       |                     |                    |                           |                     |                        |
| Without             | 0.32                | 0.32               | 39.49                     | 84.41               | 47.67                  |
| Soaking             | 0.34                | 0.33               | 41.45                     | 79.95               | 80.59                  |
| F-test              | *                   | NS                 | N.S.                      | *                   | *                      |
| B. Sorghum cultivars:|                     |                    |                           |                     |                        |
| Dora                | 0.34                | 0.33               | 42.81                     | 86.81               | 65.12                  |
| H.306               | 0.34                | 0.33               | 44.18                     | 80.03               | 65.06                  |
| Giza 15             | 0.34                | 0.33               | 36.76                     | 82.11               | 70.83                  |
| Mecca hybrid        | 0.31                | 0.32               | 36.42                     | 82.51               | 69.47                  |
| H-305               | 0.32                | 0.31               | 42.12                     | 79.44               | 50.17                  |
| F-test              | NS                  | NS                 | *                         | *                   | *                      |
| L.S.D at 0.05       | -                   | -                  | 5.34                      | 2.30                | 7.50                   |
| C. Salinity Level   |                     |                    |                           |                     |                        |
| 0 dsm⁻¹             | 0.34                | 0.32               | 100.00                    | 100.00              | 100.00                 |
| 3 dsm⁻¹             | 0.33                | 0.32               | 13.58                     | 92.16               | 71.51                  |
| 6 dsm⁻¹             | 0.33                | 0.32               | 22.51                     | 87.02               | 66.53                  |
| 9 dsm⁻¹             | 0.32                | 0.31               | 31.14                     | 79.85               | 53.58                  |
| 12 dsm⁻¹            | 0.32                | 0.30               | 35.13                     | 72.79               | 50.56                  |
| 15 dsm⁻¹            | 0.31                | 0.29               | 40.49                     | 61.28               | 42.60                  |
| F-test              | *                   | *                  | *                         | *                   | *                      |
| L.S.D 0.05          | 0.02                | 0.02               | 5.86                      | 2.52                | 8.21                   |

3.4. Interaction effects:
3.4.1. Interaction between humic acid soaking and studied sorghum cultivars effects:
The results graphically demonstrated in Fig. 1, 2, 3, 4 and 7 showed the interaction effect between humic acid soaking and studied sorghum cultivars on shoot and root length, shoot and root fresh weight and stress tolerance index, respectively. This interaction significantly influenced shoot and root length, shoot and root fresh weight and stress tolerance index. The results showed that the tallest shoot (7.61 cm) and root (11.4 cm), shoot (0.49 g) and root fresh weight (0.37 g) and highest values of stress tolerance index (90.05) were recorded from sown Giza 15 cultivar with soaking in humic acid. However, the lowest values of shoot and root length and stress tolerance index were obtained from sown H-305 cultivar without soaking in humic acid. In addition, the interaction between humic acid soaking and studied sorghum
cultivars significantly seedling height reduction affected as graphically demonstrated in Fig.5, the highest seedling height reduction (45.22 %) was obtained from sown H-306 cultivar with soaking in humic acid and the lowest values (36.14 %) produced from sown H-305 cultivar with soaking in humic acid. Meanwhile, the interaction between humic acid soaking and studied sorghum cultivars significantly influenced relative dry weight as graphically demonstrated in Fig.6, the results rev that the highest relative dry weight (89.72 %) was recorded from sown Dora cultivar without soaking in humic acid. While, the lowest percentages of the relative dry weight was recorded from sown H-305 cultivar with soaking in humic acid. Cultivars resistant to levels of salinity due to the interaction between salt and its levels as well as the ways of seed soaking (Wei and Qing-Xiang, 2011).

Fig. 1. Averages of shoot length as influenced by humic acid soaking and studied sorghum cultivars.

Fig. 2. Averages of root length as affected by humic acid soaking and studied sorghum cultivars.

Fig. 3. Averages of shoot fresh weight as influenced by humic acid soaking and studied sorghum cultivars.

Fig. 4. Averages of root fresh weight as affected by humic acid soaking and studied sorghum cultivars.
3.4.2. Interaction between studied sorghum cultivars and salinity levels effects:
The outcomes graphically demonstrated in Fig.8 revealed that the interaction between studied sorghum cultivars and salinity levels significantly affected root length. The result showed that the tallest root (11.02 cm) was produced from sown Mecca hybrid at without salinity. The weight of fresh shoot as graphically demonstrated in Fig.9 as influenced by the interaction between studied sorghum cultivars and salinity levels. The highest fresh shoot weight (0.41 g) was produced from sown Giza 15 cultivar at without salinity. The results graphically demonstrated in Fig.10 showed the effect of the interaction between studied sorghum cultivars and salinity levels on weight of fresh root. The maximum fresh root weight (0.37 g) was recorded from sown H-306 cultivar at the control treatment. The results graphically established in Fig.11 and 12 showed the effect of the interaction between studied sorghum cultivars and salinity levels on weight of dry shoot and root, respectively. The results showed that the highest weight of dry shoot (0.36 g) and weight of dry root (0.36 g) obtained from sown Dora cultivar and without salinity, respectively. The results graphically illustrated in Fig.13 indicated that the interaction effect between studied sorghum cultivars and salinity levels on the percentage of seedling height reduction. The results showed that the less percentages of seedling height reduction (8.41 %) was produced from sown H-306 cultivar at salinity level of 3 dSm⁻¹. The results graphically confirmed in Fig.14 revealed the interaction effect between studied sorghum cultivars and salinity levels on the percentage of relative dry weight. The results showed that the great percentages of relative dry weight (95.41 %) was produced from sown Dora cultivar at salinity level of 3 dSm⁻¹. The results graphically illustrated in Fig.15 showed the interaction effect between studied sorghum cultivars and salinity levels on stress tolerance index. The highest stress tolerance index (100 80.13 and 79.5 %) was produced from sown Mecca hybrid at without salinity and at
salinity level of 3 dSm⁻¹ without significant difference as well as between Giza 15 cultivar at salinity level of 6 dSm⁻¹, respectively. The more-salt tolerance recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. However, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 (Asfaw, 2011). Sorghum genotypes differed from response to salinity, the medium tolerant recorded by Hegari and JS-263 medium cultivars, while Noor cultivar produced the medium sensitive and the sensitive produced from FJ-115 and PSV-4 cultivars (Kausar et al., 2012). The highest seedling growth characters recorded from Meko, Gambella-1107, ICSV-111 and Melkam genotypes and were more salt tolerant cultivars. However, germinating ESH-2 and Gobye genotypes were salt sensitive throughout growth stage (Tigabo et al., 2013). Inter cultivars genetic variation and concentration x cultivars interaction showed significant differences for all the parameters studied (Khan et al., 2014). The shoot length, shoot fresh and dry weight and radical decreased as the NaCl concentrations increased (Sam et al., 2014). Similarly, many investigators such as Dadar et al. (2014), and Siddig and Idris (2015) reported results.
3.4.3. Interaction between humic acid soaking and salinity levels effects:
The results revealed that the interaction between humic acid soaking and salinity levels insignificantly influenced the length of shoot and root, weight of fresh shoot and root, the percentages of seedling height reduction and relative dry weight besides stress tolerance index.

3.4.4. Interaction between humic acid soaking x cultivars x salinity levels effects:
The results revealed that the interaction among humic acid soaking studied sorghum cultivars and salinity levels insignificantly influenced the length of shoot and root, weight of fresh shoot and root, the percentages of seedling height reduction and relative dry weight besides stress tolerance index.
height reduction and relative dry weight besides stress tolerance index.

IV. CONCLUSION

Accordingly, sown Mecca hybrid or Giza 15 cultivar with soaking in humic acid under salinity of 6 dSm⁻¹ maximized seedling parameters and could recommended to cultivated in saline new reclaimed soils.

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