

## Seedling growth of primed seed under drought stress in sorghum

Mohammad Omar Shihab<sup>1,2</sup>  , Jalal Hameed Hamza<sup>\*2</sup>  , William Serson<sup>3</sup>  , Andre Amakobo Diatta<sup>4</sup>  , Martin Battaglia<sup>5</sup>  , Maythem Al-Amery<sup>6</sup>  , Hail Z Rihan<sup>7</sup>  

<sup>1</sup>Department of AEZ Maps, Planning & Follow-up Office, Ministry of Agriculture, Baghdad, Iraq.

<sup>2</sup>Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

<sup>3</sup>Pennsylvania State University, 2809 Saucon Valley Rd, Center Valley, PA 18034, United States.

<sup>4</sup>Department of Plant Production and Agronomy, UFR of Agricultural Sciences, Aquaculture and Food Technologies, (S2ATA), Université Gaston Berger, 234, Saint Louis, Senegal.

<sup>5</sup>Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA 24061, United States.

<sup>6</sup>Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

<sup>7</sup>School of Biological and Marine Sciences, Faculty of Science and Engineering, University of Plymouth, United Kingdom.

\*Corresponding Author.

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### Abstract

Drought stress is considered a limiting factor during the early growth stages of sorghum. An experiment was conducted under field conditions during the fall seasons of 2017 and 2018. The objective was to improve the growth of sorghum seedlings and their chemical properties to tolerate drought stress. Three variables were investigated: first factor cultivars (Inqath, Rabeh, and Buhoth70), second factor seed priming (primed and unprimed seed). Seeds were primed by soaking for 12 hours in a solution containing 300 mg L<sup>-1</sup> + 70 mg L<sup>-1</sup> of gibberellic (GA<sub>3</sub>) and salicylic (SA) acids, respectively, and third factor drought stress represented by the irrigation intervals (irrigation every 2, 4, and 6 days) through calculated quantities to deliver moisture content to the field capacity (100%) at each irrigation. A randomized complete block design with four replications was used. The results showed that cultivars varied in their ability to tolerate drought stress, and seed priming improved their performance to tolerate drought stress compared to unprimed seed in both seasons by giving the highest values of plant height, leaf area, dry seedling weight, and content of chlorophyll, carbohydrate, and proline in leaves. Reducing irrigation intervals led to an increase in the values of the traits studied, except proline content in leaves, which increased as long as irrigation intervals increased. The interaction between priming treatments and irrigation intervals was significant for most of the studied traits. It can be concluded that priming has improved seed performance's ability to tolerate drought stress compared to unprimed seed.

**Keywords:** carbohydrate, chlorophyll, irrigation intervals, proline, water stress.

### Introduction

Drought stress on plants requires reconsideration to optimize water quantity and exploitation. Seed tolerance to water stress was evaluated, and there was a significant reduction in callus average fresh

and dry weights and soluble carbohydrates concentration as drought stress increased in cultured medium, whereas average proline concentration increased in wheat <sup>1,2</sup>. Free proline accumulation

started when leaf water stress decreased, and proline accumulation in the leaves was related to the ability of cultivar to restore plants to the natural status after water stress has been removed<sup>3,4</sup>. Water stress reduces leaf area, chlorophyll pigments, energy production from light reactions<sup>5</sup>. The concentration of protoplasm increases to a toxic level during water stress, and leading to protein denaturation, which damages the membranes and affects the stomatal, thereby CO<sub>2</sub> concentration to decrease, and effects photosynthesis and accumulation of sugars and amino acids, especially proline<sup>6,7</sup>.

The priming technique makes plants tolerate water shortages<sup>8</sup> and adverse conditions<sup>9</sup>. Seed priming allows the cell membrane to be reorganized to reduce the loss of electrolytes, then increases and accelerates germination, field emergence and improves seedling growth in a wide range of environmental conditions<sup>10-12</sup>. Seed priming improves vitality under drought stress through an escaping mechanism by reducing the need for moisture to complete germination, as seeds complete a part of the germination phase during the pre-treatment<sup>13</sup>.

Sorghum (*Sorghum bicolor* L. Moench) is grown as a summer fodder crop for animals as green fodder or silage. Planting sorghum seeds is accompanied by several problems, including poor field establishment, which is due to a significant decrease in the field germination rate under drought conditions due to a negative impact on the seed's ability to absorb water, and thus inconsistent germination, field emergence and low forage or grain yield. Drought stress inhibits germination entirely at high osmotic potential, and priming was a useful technique to improve seed performance under drought stress in sorghum<sup>14</sup>. Sorghum seeds that primed with several concentrations of bio-stimulator (appetizer) and soaking period were enhanced, and gave highest first and final count, radical and plumule length, seedling vigor index and field emergence ratio<sup>15</sup>. Soaking the seed pre-sowing in water or using plant growth regulators could improve water relations of sunflower plants, and increase moisture content in plants tissues especially during flowering and seed filling, when the water requirements increased and associated with high temperature<sup>16</sup>. Wheat seeds priming by licorice root extract, acadian and humic was investigated, and the results showed that the soaking wheat seeds with licorice root extract enhances the standard laboratory germination, root length, and seedling vigor index<sup>17</sup>. Seed soaking with

nutrition materials enhanced germination, field emergence, and growth<sup>18-19</sup> in maize. Sorghum seed soaking with several concentrations of gibberellin acid and ethephon reduced the contribution of main stems in the grain yield but increased it for the branches, which referred to possibility of increase grain yield via hormonal regulation of branching<sup>20</sup>. Wheat seed soaking with acadian (seaweed extract) led to enhance first and final count, radicle and plumule length, seedling dry weight and seedling vigor index under salt stress<sup>21</sup>. Sorghum seed priming with growth regulators (gibberellic, salicylic, cytokinin, ascorbic) to enhance growth and grain yield, and results gave the highest germination speed, germination ratio, radical and plumule length, seedling dry weight and vigor<sup>22</sup>. Alfalfa seeds priming with colocynth fruit extract showed significant response and gave the highest both fresh and dry weight of callus<sup>23</sup>. Sorghum cultivars were investigated to tolerate salt stress according to inherent genetic and seed priming to improve cultivars' performance in germination, field emergence, seedling growth and chemical properties<sup>24,25</sup>. Seed stimulation technology contributes to improve seed germination and tolerance to adverse growing conditions, germination and its characteristics under NaCl stress was enhanced by seed stimulating or priming technique in wheat<sup>26</sup>, in broad bean<sup>27</sup>, and in sorghum<sup>28</sup>.

Salicylic acid was used to reduce the adverse effects of many types of stress, whether biological or non-biological. Salicylic acid played a role in improving growth indicators associated with tolerating the drought<sup>4</sup>. There is an important role played by GA<sub>3</sub>, SA and seeds soaking duration led to improve seed germination and seedling growth<sup>29</sup>. Germination and field emergence are delayed and prolonged due to low seed vitality and vigor<sup>30,31</sup>, which affects the subsequent stages of crop growth and yield<sup>32</sup>. Seed vigor was related to energy potential of seed, which plays an important role to complete metabolic processes and growth compared to the deteriorated seed<sup>33,34</sup>. Seed vitality, vigor, and its enzymatic system correlated to metabolic processes affect seed germination and emergence<sup>35</sup>. Many studies investigated gibberellin acid (GA<sub>3</sub>) as pre-treatment to enhance seed performance, germination and seedling growth of deteriorated seed in oat<sup>36</sup>, germination and traits of seed viability and vigor in sorghum<sup>37</sup>, emergence properties and seedling

growth in corn<sup>38</sup>, field emergence and its characteristics that relate to seedlings in oat<sup>39</sup>, and seeds that were treated with gibberellic acid have fewer requirements for emergence and seedling growth under elevated salt condition in sorghum<sup>40</sup>. Priming of sorghum seeds with different agents led to increase its characteristics under salt stress<sup>41</sup>. Improvement of cereals seeds with pre-germinating agents resulted in a significant increase in plant dry

weight and leaf content of chlorophyll, compared to the control treatment<sup>42</sup>.

Iraq suffers from fluctuating rainfall and a low water level, coinciding with the misuse of water sources in agriculture, so this study was conducted to determine the effect of seed priming by growth regulators to tolerate the drought stress by measuring some seedling growth traits and chemical properties.

## Materials and Methods

An experiment conducted at the College of Agricultural Engineering Sciences, University of Baghdad during the fall seasons of 2017 and 2018 examined the effects of three factors: first factor cultivars (Inqath, Rabeh, Buhoth70), second factor seed priming (primed and unprimed seeds), and a third drought stress represented by the irrigation intervals. The seeds were primed by soaking for 12 hours in a solution containing 300 mg L<sup>-1</sup> of gibberellin (GA<sub>3</sub>) + 70 mg L<sup>-1</sup> salicylic acid (SA), and third factor drought stress represented by irrigation intervals (irrigation every 2, 4, 6 days) through calculated quantities to deliver moisture content to the field capacity (100%) at each irrigation. At the beginning of the experiment; irrigation was done using tap water with electrical conductivity of 1.26 ds m<sup>-1</sup> to deliver two irrigations for germination without stress, then irrigation process was carried out according to the irrigation intervals using the gravimetric method by the following equations (1, 2, 3)<sup>43</sup>.

$$P_w = \frac{\text{dry soil weight in oven} - \text{wet soil weight}}{\text{dry soil weight in oven}} \times 100 \dots 1$$
$$\frac{w}{x} = \frac{Fc - PW}{100} \dots 2$$

Where:

W: The amount of water to reach the field capacity.

X: Weight of dry soil in the oven.

Fc: Gravimetric moisture content at field capacity.

PW: Moisture content based on mass.

Total weight at field capacity = weight of dry soil in the oven + weight of standard water (The weight of water at each irrigation period is calculated before each irrigation period, supplemented by weight, and recorded) + empty pot weight + filter weight + seeds

weight + fertilizer weight + plant weight according to the growth stages ... 3

The seeds were soaked with the prepared solution of GA<sub>3</sub>+SA (the used concentrations were approved based on the results of the preliminary laboratory experiment conducted previously<sup>29</sup>), then washed thoroughly with water, then planted in the last third of July in plastic pots sized 24×22×30 cm and containing 11 kg of sifted soil through 4 mm sieve with a filter made of very fine gravel and coarse sand and a filter paper was placed in pot bottom. 20 seeds per pot were sown in a distance of 3 cm between each one in a depth of 3 cm for a period of three weeks from the date of planting. DAP fertilizer (46:18%) was added at 436 kg ha<sup>-1</sup> by mixing with soil before planting. Seedling growth and some chemical properties were studied after 21 days of planting as follows:

1. Seedling height (cm). The height seedling was measured starting from the soil surface to the top.
2. Leaf area (cm<sup>2</sup>) was calculated by measuring leaf length × maximum leaf width × 0.75 for five seedlings<sup>44</sup>.
3. Dry seedling weight (mg) was calculated by placing three seedlings in a perforated paper bag and dried in an electric oven at 70°C until the weight was fixed.
4. Leaf content of total chlorophyll (μg gm<sup>-1</sup>) was estimated by the acetone method<sup>45</sup>, by taking 0.2 grams of the weight of the sample, and mashing well, using 20 ml of acetone at a concentration of 80% for the extracting. The liquid was taken and placed in a spectrophotometer, and readings were made at the wavelengths (660 and 645 nm) for chlorophyll a and b, sequentially. The two readings were summed for total chlorophyll.
5. Total soluble carbohydrate (mg g<sup>-1</sup>) was estimated<sup>46</sup> by using 5% phenol reagent and

sulfuric acid; 0.2 grams of the weight of the sample were taken and mashed well after adding 10 ml of distilled water until the mixture was homogeneous. The mixture was placed in a centrifuge at a speed of 1500 rpm<sup>-1</sup> for 10 minutes to obtain the filtrate and complete its volume by adding 10 ml of distilled water. 1 ml of the filtrate was taken and 1 ml of 5% phenol reagent and 5 ml of concentrated sulfuric acid were added, mixed well and placed in a water bath at a temperature of 25-35°C for half an hour. Soluble carbohydrates were determined using a spectrophotometer at a wavelength of 488 nm.

6. Leaf content of amino acid proline ( $\mu\text{mol g}^{-1}$ ) was estimated<sup>47</sup>. The reading was taken using a spectrophotometer device at a wavelength of 520 nm.

Data were analyzed statistically according to the variance analysis of randomized complete block design RCBD with four replicates. Means were compared using the least significant difference test at a probability level of 0.05 (LSD 5%)<sup>48</sup>. GenStat program for statistical analyses was used.

## Results and Discussion

### Seedling height (cm)

The results of the fall season of 2017 showed that Buhoth70 cv. was superior significantly by giving the highest mean of seedling height. The lowest mean was for Rabeh cv. which was different insignificantly from Inqath cv. Table 1. The primed

seed surpassed the unprimed seed significantly. The highest mean of seedling height was related to the irrigation every 2 days without differing significantly from the irrigation every 4 days, while the irrigation every 6 days gave the lowest mean.

**Table 1. Seedling height (cm) that affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming× Cultivars	Fall season 2018			Seeds priming× Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	26.0	25.6	23.7	25.1	32.5	30.1	19.7	27.5
	Inqath	26.2	25.3	19.6	23.7	28.2	28.6	23.9	26.9
	Rabeh	25.2	25.1	20.9	23.7	30.1	28.9	23.9	27.6
Unprimed seed	Buhoth70	23.5	22.5	22.7	22.9	30.6	27.5	13.6	23.9
	Inqath	23.4	22.8	14.9	20.3	26.8	23.7	20.0	23.5
	Rabeh	21.5	20.2	18.6	20.1	24.9	23.0	20.3	22.8
LSD 5%		NS			NS			NS	
Drought stress (irrigation interval)	Primed seeds	25.8	25.3	21.4	24.2	30.3	29.2	22.5	27.3
	Unprimed seed	22.8	21.8	18.8	21.1	27.4	24.7	18.0	23.4
	LSD 5%	NS			1.1	NS			1.0
Drought stress (irrigation interval)	Buhoth70	24.7	24.0	23.2	24.0	31.6	28.8	16.7	25.7
	Inqath	24.8	24.1	17.3	22.0	27.5	26.2	21.9	25.2
	Rabeh	23.3	22.7	19.8	21.9	27.5	26.0	22.1	25.2
LSD 5%		2.4			1.4				NS
Drought stress (irrigation interval)		24.3	23.6	20.1		28.8	27.0	20.2	
LSD 5%		1.4				1.2			

LSD 5%: least significant difference at a probability level of 5%; NS: Non-significant at  $P \leq 0.05$

Interaction between Inqath  $\times$  irrigation every 2 days was superior over the rest treatments significantly by giving the highest mean without difference significantly with interaction treatment between Buhoth70  $\times$  irrigation every 2 days, while the lowest mean of seedling height belonged to the interaction treatment between Inqath  $\times$  irrigation every 6 days Table 1.

The results of the fall season 2018 in Table 1 indicate that reducing the irrigation interval has led to a significant increase in the mean of seedling height, as irrigation every 2 days gave the highest mean, while irrigation every 6 days gave the lowest mean. The seed priming gave the highest mean with a significant difference from the unprimed seed. Interaction treatment Buhoth70  $\times$  irrigation every 2 days was superior significantly compare to the rest treatments by giving the highest mean, while the lowest mean of seedling height was for the treatment of Buhoth70  $\times$  irrigation every 6 days Table 1.

The variance in the seedling height between the cultivars may be due to the genetic variation among them, or may be due to treat the seeds with gibberellic acid because of its essential role in improving growth indirectly, especially in the early stage of germination when stimulating the metabolic processes, growth processes and division that occur during germination, this is consistent with another study<sup>49</sup>. Also, the variation in the seedling height that related to the drought stress, maybe due to the lack of moisture in the soil which led to a decrease in the carbon metabolism ratio, the rate of cell division and elongation, and therefore the seedling height decreased.

### **Leaf area (cm<sup>2</sup>)**

The results of the fall season of 2017 showed that seed priming was significantly different compared to the unprimed seed Table 2. Reducing the irrigation interval led to a significant increase in the leaf area, so the irrigation every 2 days was higher in leaf area in comparison to the irrigation every 6 days. Interaction of Rabeh  $\times$  primed seed was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was for the treatment of Rabeh  $\times$  unprimed seed. The treatment of Rabeh  $\times$  irrigation every 2 days was significantly

superior over the rest treatments by giving the highest mean, while the lowest mean was for the treatment of Inqath  $\times$  irrigation every 6 days. The treatment of primed seeds  $\times$  irrigation every 2 days was superior significantly by giving the highest mean, while the lowest mean was for the treatment of unprimed seed  $\times$  irrigation every 6 days. The triple interaction between Rabeh  $\times$  primed seeds  $\times$  irrigation every 2 days was superior significantly by giving the highest mean, while the lowest mean was for the treatment of Inqath  $\times$  unprimed seed  $\times$  irrigation every 6 days Table 2.

The results of the fall season 2018 Table 2 showed that Rabeh cv. was superior significantly over the cultivars and gave the highest mean, while the lowest mean was for Buhoth70 cv., which was different insignificantly with Inqath cv. Seeds priming gave the highest leaf area with a significant difference compare to unprimed seed. Reducing the irrigation intervals were led to a significant increase in the leaf area, where the irrigation every 2 days gave the highest mean, which didn't differ significantly with the irrigation every 4 days, while the irrigation every 6 days gave the lowest mean. Interaction between primed seeds  $\times$  irrigation every 4 days was superior significantly over the rest treatments by giving the highest mean, and differed insignificantly with the primed seeds  $\times$  irrigation every 2 days, while the lowest mean was for the unprimed seed  $\times$  irrigation every 6 days Table 2.

Variation in the leaf area between the cultivars may be due to genetic variation among them, or the seedling height can have a powerful effect on increasing the leaf area of seedling, perhaps by increasing the number of leaves or by the efficiency to compete for the growth requirements available to form a larger leaf area Table 2. The variability in leaf area may be due to soaking the seeds with gibberellic acid because of its essential role in the regulating germination process, growth, cell division and elongation compared to the unprimed seed. Moreover, the variation in the leaf area may also due to drought stress, as the leaf is the most affected organ by water stress, and the blade growth stops, then the leaf wraps and ages quickly. Also, water stress causes a decrease in the cells size of leaf by reducing cell's ability to divide, expand and differentiate during growth processes.

**Table 2. Leaf area (cm<sup>2</sup>) affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming× Cultivars	Fall season 2018			Seeds priming× Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	26.7	24.9	23.6	25.1	56.2	59.2	39.0	51.5
	Inqath	29.4	26.7	19.5	25.2	58.1	59.4	44.1	53.9
	Rabeh	36.3	28.4	18.5	27.7	63.5	64.6	49.5	59.2
Unprimed seed	Buhoth70	23.0	22.7	20.0	21.9	42.5	42.6	28.0	37.7
	Inqath	22.5	22.3	13.4	19.4	43.1	38.4	30.6	37.4
	Rabeh	21.0	16.7	14.5	17.4	50.3	49.3	34.0	44.6
LSD 5%		3.3			1.9	NS			NS
Drought stress (irrigation interval)	Primed seeds	30.8	26.6	20.5	26.0	59.3	61.1	44.2	54.9
	Unprimed seed	22.2	20.6	16.0	19.6	45.3	43.5	30.9	39.9
	LSD 5%	1.9			1.1	2.5			1.4
Drought stress (irrigation interval)	Buhoth70	24.9	23.8	21.8	23.5	49.3	50.9	33.5	44.6
	Inqath	26.0	24.5	16.4	22.3	50.6	48.9	37.4	45.6
	Rabeh	28.6	22.6	16.5	22.6	56.9	57.0	41.7	51.9
LSD 5%		2.3			NS	NS			1.7
Drought stress (irrigation interval)		26.5	23.6	18.2		52.3	52.3	37.5	
LSD 5%		1.3				1.7			

LSD 5%: least significant difference at a probability level of 5%; NS: Non-significant at  $P \leq 0.05$

### Dry seedling weight (g)

The results of the fall season 2017 showed superior of Rabeh cv. significantly that gave the highest mean, while Inqath cv. gave the lowest mean. Primed seed gave the highest mean of seedling dry weight with a significant difference compared to unprimed seed. The irrigation every 2 days gave the highest mean, while the irrigation every 6 days gave the lowest mean. Interaction between Rabeh × primed seeds surpassed significantly compared to other treatments by giving the highest mean without difference significantly with Buhoth70 × primed seeds, while the lowest mean was for Inqath × unprimed seed. The treatment of Rabeh × irrigation every 2 days was superior significantly over the rest treatments by giving the highest mean without difference significantly with Buhoth70 × irrigation every 2 days, while the lowest mean of dry seedling weight was for Inqath × irrigation every 6 days. The treatment of Rabeh × irrigation every 2 days was superior significantly over the rest treatments and

gave the highest mean without difference significantly with Buhoth70 × irrigation every 2 days, while the lowest mean of dry seedling weight was for Inqath × irrigation every 6 days. The treatment of primed seeds × irrigation every 2 days surpassed significantly and gave the highest mean, while the lowest mean was for unprimed seeds × irrigation every 6 days Table 3.

The results of the fall season 2018 Table 3 showed that Buhoth70 cv. was superior significantly compared to other cultivars and gave the highest mean, while Inqath cv. gave the lowest mean. Primed seeds gave the highest dry weight of seedlings with a significant difference in comparison with the unprimed seed. Reducing the irrigation interval led to increasing the dry weight of seedlings significantly, so the irrigation every 2 days gave the highest mean, while irrigation every 6 days gave the lowest mean. Interaction between Buhoth70 × primed seeds was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was for Inqath × unprimed seeds. The

treatment of Buhoth70 × irrigation every 2 days was superior significantly over the rest treatments and gave the highest mean, while the lowest mean was for Buhoth70 × irrigation every 6 days. Interaction treatment between Buhoth70 × primed seeds × irrigation every 2 days surpassed significantly and gave the highest mean, while the lowest mean was for Buhoth70 × primed seeds × irrigation every 6 days Table 3.

That probably confirms that the leaf area is the most affected by the dry seedling weight compared to its height. The variation in the dry weight of seedlings

may be due to the nature of the genotypes. Moreover, the variation in the dry weight of seedlings may be due to soaking the seeds with gibberellic acid because of its essential role in the early stages of regulating the germination process, growth, cell division, and elongation compared to the unprimed seed. This is consistent with another study<sup>50</sup>. This decrease is due to the effect of water stress in reducing the absorption of water and nutrients, which have an essential role in the growth process and dry matter accumulation. This is consistent with another study<sup>51</sup>.

**Table 3. Dry seedling weight (g) that affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming× Cultivars	Fall season 2018			Seeds priming× Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	1.866	1.665	0.828	1.453	4.913	4.419	2.415	3.915
	Inqath	1.766	1.735	0.831	1.444	4.575	3.290	2.721	3.529
	Rabeh	1.842	1.638	0.986	1.489	3.669	3.300	3.023	3.330
Unprimed seed	Buhoth70	1.537	1.363	0.615	1.172	4.021	3.509	1.413	2.981
	Inqath	1.348	1.120	0.557	1.008	2.614	2.170	1.812	2.199
	Rabeh	1.664	1.266	0.853	1.261	3.325	2.268	2.063	2.552
LSD 5%		NS			0.083	0.387			0.223
Drought stress (irrigation interval)	Primed seeds	1.825	1.679	0.882	1.462	4.385	3.669	2.720	3.591
	Unprimed seed	1.516	1.250	0.675	1.147	3.320	2.649	1.763	2.577
	LSD 5%	0.083			0.048	NS			0.129
Drought stress (irrigation interval)	Buhoth70	1.701	1.514	0.721	1.312	4.467	3.964	1.914	3.448
	Inqath	1.557	1.427	0.694	1.226	3.595	2.730	2.267	2.864
	Rabeh	1.753	1.452	0.919	1.375	3.497	2.784	2.543	2.941
LSD 5%	0.102			0.059	0.274			0.158	
Drought stress (irrigation interval)		1.670	1.464	0.778		3.853	3.159	2.241	
LSD 5%		0.059				0.158			

LSD 5%: least significant difference at a probability level of 5%; NS: Non-significant at P≤0.05

### Leaf content of total chlorophyll ( $\mu\text{g gm}^{-1}$ )

The results of the fall season 2017 Table 4 indicated that Buhoth70 cv. was superior significantly over the other cultivars by giving the highest mean, while the lowest mean was for Inqath cv. Primed seeds had the highest mean with a significant difference compared to unprimed seeds. Irrigation every 4 days gave the highest mean, while irrigation every 6 days gave the lowest mean. Interaction between Buhoth70x primed

seeds was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was for the treatment of Rabeh × unprimed seed. The treatment of Buhoth70 × irrigation every 4 days was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was for the treatment of Inqath × irrigation every 2 days. The treatment of primed seeds × irrigation every 4 days was superior significantly by giving the highest mean, while the

lowest mean was for the treatment of unprimed seed × irrigation every 6 days. Interaction treatment of Buhoth70 × primed seeds × irrigation every 4 days was superior significantly by giving the highest mean, while the lowest mean was for Rabeh × unprimed seed × irrigation every 4 days Table 4. The results of the fall season 2018 showed that Buhoth70 cv. was superior significantly over the other cultivars and gave the highest mean, while Inqath cv. gave the lowest mean. The primed seed had the highest mean with a significant difference in comparison to the unprimed seeds. Irrigation every 2 days gave the highest mean of total chlorophyll, while irrigation every 6 days gave the lowest mean. The variation in leaf content of total chlorophyll between the cultivars may be due to genetic variation among them and this is consistent with other studies<sup>3,7</sup>. And the variation in leaf content of total

chlorophyll may be due to soaking the seeds with gibberellic acid also, because of its essential role in the early stages of regulating the germination process to form a seedling capable of making the food itself through photosynthesis compared to the unprimed seed this is consistent with another study<sup>50</sup>. Moreover, the variation in leaf content of total chlorophyll may be due to the drought stress also, because it causes the increase of free radicals and then oxidation of photosynthetic dyes and lack of cell fullness and then closes the stomata, causing the lack of CO<sub>2</sub> releasing inside plant tissues, which coincides with the process of respiration and stop the activity of some enzymes due to increasing the leaf temperature and then reduce the production of plant dyes and thus reducing the photosynthesis. This is consistent with another study<sup>6</sup>.

**Table 4. Leaf content of total chlorophyll ( $\mu\text{g gm}^{-1}$ ) that affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming× Cultivars	Fall season 2018			Seeds priming× Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	2.169	2.528	1.805	2.167	2.234	2.000	1.611	1.948
	Inqath	1.480	2.072	1.798	1.783	2.061	1.894	1.396	1.784
	Rabeh	2.092	2.112	1.992	2.065	2.127	1.860	1.465	1.817
Unprimed seed	Buhoth70	2.295	2.349	1.450	2.031	1.499	1.270	0.966	1.245
	Inqath	1.559	1.947	1.518	1.674	1.361	1.216	0.819	1.132
	Rabeh	1.603	1.514	1.650	1.589	1.367	1.159	0.815	1.114
LSD 5%		0.151			0.087	NS			NS
Drought stress (irrigation interval)	Primed seeds	1.913	2.237	1.865	2.005	2.141	1.918	1.491	1.850
	Unprimed seed	1.819	1.937	1.539	1.765	1.409	1.215	0.867	1.163
	LSD 5%	0.087			0.050	NS			0.038
Drought stress (irrigation interval)	Buhoth70	2.232	2.439	1.627	2.099	1.866	1.635	1.288	1.596
	Inqath	1.519	2.009	1.658	1.729	1.711	1.555	1.108	1.458
	Rabeh	1.847	1.813	1.821	1.827	1.747	1.510	1.140	1.466
LSD 5%		0.107			0.061	NS			0.046
Drought stress (irrigation interval)		1.866	2.087	1.702		1.775	1.566	1.179	
LSD 5%		0.061				0.046			

LSD 5%: least significant difference at a probability level of 5%; NS: Non-significant at  $P \leq 0.05$

### Total soluble carbohydrate ( $\text{mg g}^{-1}$ )

The results of the fall season 2017 showed that primed seed had the highest mean with a significant

difference compared to unprimed seed Table 5. The irrigation every 4 days gave the highest mean, while the irrigation every 2 days gave the lowest mean without difference significantly with the irrigation

every 6 days. The interaction treatment between Buhoth70 × irrigation every 4 days was superior significantly over the rest of the treatments and gave the highest mean, while the lowest mean was for Buhoth70 × irrigation every 6 days Table 5.

The results of the fall season 2018 Table 5 indicated that primed seed had the highest mean with a significant difference compared to unprimed seed. The irrigation every 2 days gave the highest mean, while the irrigation every 6 days gave the lowest mean. The treatment of Rabeh × primed seeds was superior significantly over the rest of the treatments by giving the highest mean, while the lowest mean was for the treatment of Inqath × unprimed seed. The treatment of Buhoth70 × irrigation every 2 days was superior significantly and gave the highest mean, while the lowest mean was for the treatment of Buhoth70 × irrigation every 6 days Table 5. Moreover, Interaction between primed seeds × irrigation every 2 days was superior significantly over the rest treatments by giving the highest mean,

while the lowest mean was for unprimed seed × irrigation every 6 days Table 5. Interaction treatment between Rabeh × primed seeds × irrigation every 2 days was superior significantly by giving the highest mean, while the lowest mean was for Buhoth70 × unprimed seeds × irrigation every 6 days Table 5.

The variation in leaf content of carbohydrates may be caused by seeds priming in gibberellic acid which affected the biological reactions within the plant cell and led to increasing the rate of cell division and expansion, which led to producing a good shoot and root, thus increasing the metabolism of absorbed nutrients compared to unprimed seed. Moreover, the alteration in the leaf content of carbohydrates may be caused by the drought stress which reduced the number of leaves, leaf area, leaf content of chlorophyll Table 2,4 and decreased the photosynthesis due to reduction the receiving light and conversion to dry matter, in addition, the plant has little ability to absorb water and nutrients and move them to the shoot due to the root's growth lack.

**Table 5. Total soluble carbohydrate (mg g<sup>-1</sup>) that affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming × Cultivars	Fall season 2018			Seeds priming × Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	1.562	1.898	1.330	1.596	2.251	2.284	1.427	1.987
	Inqath	1.419	1.474	1.650	1.514	2.253	2.129	1.775	2.052
	Rabeh	1.494	1.544	1.755	1.597	2.346	2.188	1.839	2.124
	Buhoth70	1.190	1.446	1.121	1.252	2.167	1.980	1.326	1.824
Unprimed seed	Inqath	1.271	1.443	1.216	1.310	1.756	1.826	1.611	1.731
	Rabeh	1.066	1.320	1.155	1.180	1.967	1.750	1.541	1.752
	LSD 5%	NS			NS	0.097			0.056
Drought stress (irrigation interval)	Primed seeds	1.492	1.638	1.578	1.569	2.284	2.200	1.680	2.055
	Unprimed seed	1.176	1.403	1.164	1.248	1.964	1.852	1.492	1.769
	LSD 5%	NS			0.083	0.056			0.032
Drought stress (irrigation interval)	Buhoth70	1.376	1.672	1.225	1.424	2.209	2.132	1.377	1.906
	Inqath	1.345	1.459	1.433	1.412	2.005	1.977	1.693	1.892
	Rabeh	1.280	1.432	1.455	1.389	2.157	1.969	1.690	1.938
	LSD 5%	0.177			NS	0.068			NS
Drought stress (irrigation interval)		1.334	1.521	1.371		2.124	2.026	1.586	
	LSD 5%	0.102				0.039			

LSD 5%: least significant difference at a probability level of 5%; NS: Non-significant at P ≤ 0.05

### Leaf content of amino acid proline ( $\mu\text{mol g}^{-1}$ )

The findings from the fall season of 2017 (as indicated in Table 6) highlighted the significant superiority of Buhoth70 cultivar in terms of the highest mean, surpassing the other cultivars, with Inqath cultivar yielding the lowest mean. Furthermore, primed seeds exhibited a significantly higher mean when compared to unprimed seeds. When it came to irrigation frequency, the results revealed that irrigating every 6 days resulted in the highest mean, while irrigating every 2 days produced the lowest mean.

The interaction treatment between Buhoth70  $\times$  primed seeds surpassed significantly the rest treatments and gave the advanced mean, while the lowest was for Inqath  $\times$  unprimed seed Table 6. Interaction between Buhoth70  $\times$  irrigation every 6 days was superior significantly, while the lowest

value was for Inqath  $\times$  irrigation every 2 days. Moreover, the treatment of primed seeds  $\times$  irrigation every 2 days was superior significantly in comparison to other treatments, while the lowest mean was for unprimed seed  $\times$  irrigation every 2 days. Interaction treatment between Buhoth70  $\times$  primed seeds  $\times$  irrigation every 6 days was superior significantly for proline, while the lowest mean was for Inqath  $\times$  unprimed seeds  $\times$  irrigation every 2 days Table 6.

The findings from the fall season of 2018 showed that Buhoth70 cv. was superior significantly in comparison to other cultivars and gave the highest mean, while Inqath cv. was the lowest. Primed seed gave the highest mean and difference significantly compare to unprimed seed. The irrigation every 6 days gave the highest mean, while the irrigation every 2 days gave the lowest mean.

**Table 6. Leaf content of amino acid proline ( $\mu\text{mol g}^{-1}$ ) that affected by cultivars, priming and drought stress (irrigation interval) in sorghum seeds in fall seasons of 2017 and 2018**

Seeds priming	Cultivars	Fall season 2017			Seeds priming $\times$ Cultivars	Fall season 2018			Seeds priming $\times$ Cultivars
		Drought stress (irrigation interval) (day)				Drought stress (irrigation interval) (day)			
		2	4	6		2	4	6	
Primed seeds	Buhoth70	0.382	0.461	0.825	0.556	0.253	0.304	0.667	0.408
	Inqath	0.336	0.456	0.364	0.385	0.473	0.341	0.377	0.397
	Rabeh	0.362	0.447	0.329	0.379	0.331	0.245	0.315	0.297
Unprimed seed	Buhoth70	0.273	0.308	0.638	0.406	0.223	0.251	0.534	0.336
	Inqath	0.126	0.252	0.283	0.220	0.132	0.257	0.260	0.216
	Rabeh	0.255	0.312	0.419	0.328	0.317	0.441	0.417	0.392
LSD 5%		0.066			0.038	0.028			0.016
Drought stress (irrigation interval)	Primed seeds	0.360	0.455	0.506	0.440	0.353	0.297	0.453	0.367
	Unprimed seed	0.218	0.291	0.446	0.318	0.224	0.316	0.404	0.315
	LSD 5%	0.038			0.022	0.016			0.009
Drought stress (irrigation interval)	Buhoth70	0.327	0.385	0.731	0.481	0.238	0.277	0.600	0.372
	Inqath	0.231	0.354	0.323	0.303	0.303	0.299	0.318	0.307
	Rabeh	0.309	0.379	0.374	0.354	0.324	0.343	0.366	0.344
LSD 5%		0.047			0.027	0.020			0.011
Drought stress (irrigation interval)		0.289	0.373	0.476		0.288	0.306	0.428	
LSD 5%		0.027				0.011			

LSD 5%: least significant difference at a probability level of 5%

The interaction treatment of Buhoth70  $\times$  primed seed was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was

for the treatment of Inqath  $\times$  unprimed seed. Furthermore, the treatment of Buhoth70  $\times$  irrigation every 6 days surpassed others significantly and gave

the highest mean, while the lowest mean was for the treatment of Buhoth70 × irrigation every 2 days. Moreover, the interaction treatment of primed seeds × irrigation every 6 days was superior significantly over the rest treatments by giving the highest mean, while the lowest mean was for the treatment of unprimed seeds × irrigation every 2 days. The interaction treatment between Buhoth70 × primed seeds × irrigation every 6 days surpassed others significantly and gave the highest mean, while the lowest mean was for Inqath × unprimed seed × irrigation every 2 days Table 6.

The observed variation in leaf proline content among the different cultivars can likely be attributed to

## Conclusion

In conclusion, the observed variation among cultivars in their ability to tolerate drought stress underscores their inherent potential. Additionally, extending irrigation intervals during the seedling growth stage was found to be associated with a decrease in growth rate. Notably, seed priming emerged as an effective technique for enhancing seedling growth under conditions of drought stress. Therefore, it is advisable to consider the priming of sorghum

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## Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Tables in the manuscript are ours.
- No animal studies are present in the manuscript.

## Authors' Contribution Statement

M.O.S., conducted the experiment, collects and analyzed the data, and wrote the draft of the manuscript. J.H.H., supervised all the previous steps.

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genetic differences, reflecting the cultivars' varying abilities to synthesize and accumulate proline. Proline accumulation is recognized as a key physiological mechanism that enables plants to withstand and tolerate environmental stresses. This variance in proline content may be an important factor contributing to the cultivars' resilience in response to adverse environmental conditions. This is consistent with another studies<sup>3-7</sup>. Moreover, the variation in leaf content of proline may be due to the drought stress that leads to the accumulation of proline as a result of the inability of plant tissues to protein synthesis as well as its catabolism. This is consistent with other studies<sup>1,16</sup>.

seeds before planting, especially when dealing with water deficiency conditions. This practice has the potential to significantly improve the resilience and growth of sorghum seedlings in such challenging environments. Similar studies can be conducted on tolerance to biotic and abiotic stresses by using natural materials to increase seed vitality, which enhances the role of seed stimulation technology to boost sustainable agriculture.

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- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

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## نمو بادرات البذور المنشطة تحت إجهاد الجفاف في الذرة البيضاء

محمد عمر شهاب<sup>1,2</sup>، جلال حميد حمزة<sup>2</sup>، ويليام سيرسون<sup>3</sup>، اندريه اماكوبو دياتا<sup>4</sup>، مارتين باتاغليا<sup>5</sup>، ميثم العامري<sup>6</sup>، هائل زهير ریحان<sup>7</sup>

- <sup>1</sup>دائرة الدراسات والتخطيط، وزارة الزراعة، بغداد، العراق.
- <sup>2</sup>قسم المحاصيل الحقلية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق.
- <sup>3</sup>جامعة ولاية بنسلفانيا، 2809 شارع سوكون فالي، سنتر فالي، بنسلفانيا 18034، الولايات المتحدة.
- <sup>4</sup>قسم الإنتاج والنباتات والهندسة الزراعية، اتحاد العلوم والهندسة الزراعية وتربية الأحياء المائية والتكنولوجيات الغذائية (S2ATA)، جامعة غاستون بيرغر، 234، سانت لويس، السنغال.
- <sup>5</sup>قسم علوم المحاصيل والتربة البيئية، جامعة فرجينيا للتكنولوجيا، بلاكسبيرغ، فرجينيا 24061، الولايات المتحدة.
- <sup>6</sup>قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.
- <sup>7</sup>قسم العلوم البيولوجية والبحرية، كلية العلوم والهندسة، جامعة بليموث، المملكة المتحدة.

### الخلاصة

يعد إجهاد الجفاف عاملاً محدداً خلال مراحل النمو المبكرة للذرة البيضاء. أجريت التجربة تحت الظروف الحقلية خلال العروتين الخريفية 2017 و 2018 في كلية علوم الهندسة الزراعية جامعة بغداد. كان الهدف هو تحسين نمو شتلات الذرة البيضاء وخواصها الكيميائية لتحمل إجهاد الجفاف. تمت دراسة ثلاث متغيرات: العامل الأول هو الأصناف (انقاذ، رابح، وبحوث70). والعامل الثاني هو تنشيط البذور (بذور منشطة وغير منشطة). تم تنشيط البذور عن طريق النقع لمدة 12 ساعة في محلول يحتوي على 300 ملغم لتر<sup>-1</sup> من أحماض الجبريليك (GA<sub>3</sub>) + 70 ملغم لتر<sup>-1</sup> من الساليسيليك (SA). والعامل الثالث هو فترات الري (الري كل 2، 4، و 6 أيام) بكميات محسوبة لإبصال المحتوى الرطوبي إلى السعة الحقلية (100%) عند كل رية. تم استخدام تصميم القطاعات الكاملة المعشاة بأربعة مكررات. أظهرت النتائج تباين الأصناف في قدرتها على تحمل إجهاد الجفاف، كما أدى تنشيط البذور إلى تحسين أدائها لتحمل إجهاد الجفاف مقارنة بالبذور غير المنشطة في كلا العروتين وذلك بإعطاء أعلى قيم لارتفاع النبات والمساحة الورقية ووزن البادرات الجافة ومحتوى الكلوروفيل والكربوهيدرات والبرولين في الأوراق. ويزداد متوسط الصفات المدروسة مع تقليل فترات الري، باستثناء محتوى البرولين في الأوراق، والذي يزداد مع زيادة فترات الري. وكان التداخل بين معاملات التنشيط وفترات الري معنوياً في معظم الصفات المدروسة. يمكن أن نستنتج أن التنشيط أدى إلى تحسين أداء البذور في تحمل إجهاد الجفاف مقارنة بالبذور غير المنشطة.

**الكلمات المفتاحية:** الاجهاد المائي، البرولين، فواصل الري، الكاربوهيدرات، كلوروفيل.