Role of Salicylic Acid in the Improvement of PEG – Induced Drought Stress in Maize (Zea mays L.) cv. Baghdad

Hussein K. Al-Kaaby* Luma H. Abdul-Qadir Wasan F. Abdul- Hussein
Department of Biology, College of Education for Pure Sciences, University of Basrah

Abstract

Laboratory experiment using hydroponic culture system was conducted at plant physiology laboratory, Department of Biology, College of Education for Pure Sciences, University of Basrah, in order to study the role of seeds soaking with salicylic acid (SA) and the effect of PEG8000 induced water stress on germination and early seedling growth stages of maize (*Zea mays* L.) cv. Baghdad. Seeds were soaked with three concentrations of SA (0.1,0.3 and 0.5 mM) in addition to control treatment with distilled water, while PEG8000 were used at concentrations of 0% (distilled water) 1%, 2%, 3%, 6%, 9% and 12%. The data was recorded on various parameters like seeds germination percentage, seedlings roots and shoot length and seedlings roots and shoot fresh and dry weight.The experiment was designed as completely randomized factorial with three replicates for each treatment. Results indicated that SA at 0.5 mM caused a significant increase in germination percentage roots and shoot length, roots and shoots fresh and dry weight while PEG8000 caused a significant decrease especially at its high concentrations (6% and 9%) in all the previous parameters. Interaction between SA at 0.5 mM and PEG had improved significantly germination percentage, shoot and roots length, shoot and roots fresh and dry weight so it had a positive significance effect on increasing the drought tolerance in maize.

Keywords: Maize, Salicylic acid, PEG8000, Hydroponic, Germination, Seedling growth.

1. Introduction

Maize (*Zea mays* L.) is an annual monocot grass belonging to the family poaceae, growing up to four meter tall. Maize is used in the preparation of food or drinks, high proportion of maize produced is used as stock feed and it is the major source of starch worldwide (McCutcheon, 2007). Corn starch can be fermented into alcohol, including fuel ethanol, while the paper industry is the biggest non-food user of maize starch. The oil and protein are often of commercial value as by-products of starch production and are used in food manufacturing (McCutcheon, 2007). In Iraq maize is cultivated in the spring and autumn in many areas especially in Kirkuk and Babel cities.

Drought stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production. Salinity and drought are the major abiotic stresses that reduce plant growth and crop productivity worldwide, scarcity resulting from global climate change is accompanied by more frequent and more severe summer droughts in many regions (Munns, 2005). Abiotic stresses resulting from excessive salinity or water deficit led to reduction in photosynthesis, transpiration and other biochemical processes associated with plant growth, development and crop productivity, furthermore, abiotic stress lead to oxidative stress in the plant cel resulting in a higher leakage of electrons towards O_2 during photosynthetic and respiratory processes which leading to enhancement of reactive oxygen species (ROS) generation (Asada, 2006).

Salicylic acid (SA) is a phenolic compound that had been recognized as a plant hormone (Hayat and Ahmad 2007), it plays an important role in photosynthetic rate, stomatal conductance and transpiration abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under adverse environmental conditions (Arfan *et al*., 2007). Salicylic acid appears as a signal molecule or chemical messenger and its role in defense mechanism has been well established in plants (Gunes *et al*.,2007).

So because of the economic importance of maize in the world and in our motherland, the present study was aimed to explore the role of seeds soaking with SA on germination percentage and seedling characters and to examine whether SA could improve the drought stress (induced with PEG8000) tolerance of maize at germination and early seedling growth.

2. Materials and Methods

2.1 Plant materials

Seeds of maize (*Zea mays* L. cv. Baghdad) were obtained from Directory of seeds Testing and Evaluation in Abo- Gharib, Baghdad. Seeds were surface sterilized with 70% of ethanol solution for 5 min then rinsed with commercial bleach contain 5% of sodium hypochlorite with frequent shaking for thirty minutes, and after that thoroughly washed many times with distilled water.

2.2 Hydroponic culture description

A small hydroponic culture system designed by Carolina Biological Supply-USA (Fig 1) was used. It consists from a plastic container with a board with six holes for fixing the net pots, each net pot filled with glass wool for seeds and plant establishment. The whole supplied with an electrical aeration system.

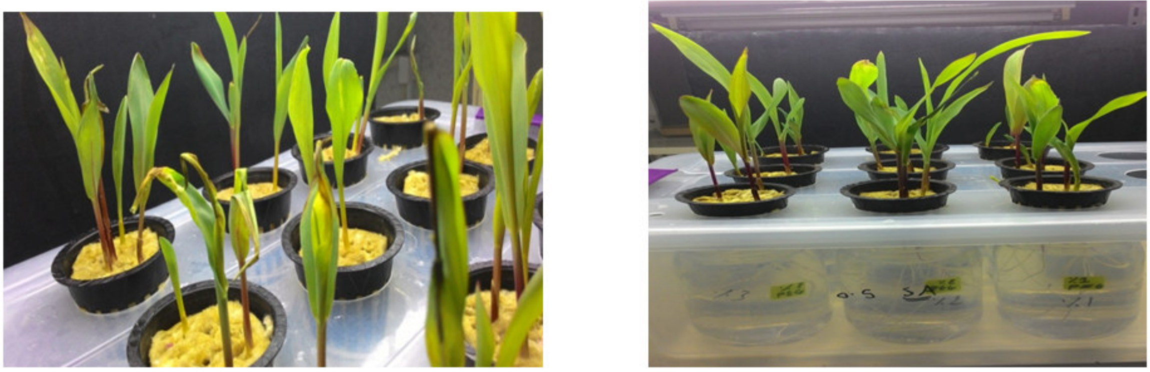

Fig 1. The hydroponic culture with corn (*Zea mays* L.) seedling.

2.3 Seeds germination percentage test:

One hundred corn seeds were spread equally on 4 plastic Petri dishes 10 cm in diameter lined with Whittman No. 1 filter paper (9cm diameter). Filter paper was wetted with 5 ml of distilled water and kept for 48 hours after which germination percentage was calculated according to the following equation:
% Germination= No. of germinated seeds / Total No. of seeds x 100%.

2.4 Seeds soaking treatments:

Seeds were soaked as follows:
1-with distilled water for six hours (control treatment).
2- with different concentration (0.1, 0.3 and 0.5 mM) of salicylic acid in order to choose the optimum soaking concentration for germination to be used in the experiment, for six hours also.

2.5 Water stress induction:

Water stress was induced by using PEG8000 at concentrations of 1%, 2%, 3%, 6%, 9% and 12%.

2.6 Seeds planting and irrigation

Four seeds from the previously soaked with distilled water or salicylic acid, were planted in each net pot on the glass wool which was moistened with the same solution used for irrigation and fixed in the plastic containers. The plastic containers were filled with the irrigation solution consist of Hoagland nutrient solution with the addition of PEG8000 at 1%, 2%, 3%, 6%, 9% and 12%, irrigation with distilled water was used as control with three replicates for each treatment. Seeds were kept in the hydroponic culture for three weeks after which the following parameters were estimated:
1- Germination percentage.
2- Shoot and roots length (cm.).
3- Fresh weight of shoot and roots (gm.).
4- Dry weight of shoot and roots (gm.) which estimated after oven drying at 65 °C for 48 hours.

2.7 Statistical analysis

Experiment was designed as a completely randomized factorial experiment with two factors (type of seeds soaking x PEG concentrations) with three replicates for each treatment. Data were subjected to two way analysis of variance (ANOVA) using Genstat program, means were compared using least significant difference test (LSD) at $P<0.05$.

3. Results and discussion

Effect of SA and PEG on:

3.1 Germination percentage

Results in table 1 indicated the gradual significant increase in seeds germination percentage with the increase in SA concentration, when compared with control treatment. Seeds soaked with 0.5 mM of SA have the highest germination percentage equal to 76% while it was 42.86%, 54,%, 69% at control, 0.1 mM and 0.3 mM of SA respectively, so we used 0.5 mM of SA in the rest experiments. Regarding the effect of PEG, table1 indicate that increasing PEG concentration in the irrigation water caused a significant decrease in germination percentage when compared with control, a severe reduction was noticed at concentration of 9% PEG (42%) comparing with control (80.5%), there was no germination at 12% PEG which was excluded. Interaction results showed that the highest germination percentage was at control and interaction between 0.5 SA and PEG at 1%.

Table (1) Effect of SA and PEG on % germination of maize seeds in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	65.00	60.00	55.00	50.00	40.00	30.00	42.86
0.1	75.00	70.00	66.00	60.00	40.00	38.00	54.00
0.3	85.00	80.00	75.00	70.00	55.33	45.00	69.05
0.5	97.00	95.00	90.00	85.00	65.00	55.00	76.00
Water stress (PEG) mean	80.50	76.25	71.50	66.25	50.08	55.00	
LSD: SA= 1.12 PEG= 1.48 SA x PEG= 2.96							

3.2 Shoot height

Table 2 showed that shoot length was increased significantly at 0.5 mM SA(21.71 cm) when compared with control (17.88 cm), while there was a gradual significant decrease in shoot length started at 2% of PEG and continued in the higher concentrations comparing with the control. Shoot length at control was equal to 25.88 cm while it was 15.17 cm at 9% of PEG. Interaction results in table 2 indicate that the highest shoot length was at control, 1%, 2% and 3% of PEG without a significant difference among these treatments.

Table (2) Effect of SA and PEG on shoot height of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	25.93	20.80	18.50	15.60	13.60	12.83	17.88
0.5	25.83	24.10	23.53	21.00	18.27	17.50	21.71
Water stress (PEG) mean	25.88	22.45	21.02	18.30	15.93	15.17	
LSD: SA= 2.29 PEG= 3.96 SA x PEG= 5.61							

3.3 Shoot fresh and dry weight

Results in table 3 showed that shoot fresh weight of the plants developed from seeds soaked with 0.5mM of SA was higher significantly comparing with plants developed from distilled water treated seeds, the increment in fresh weight reached 101.9%. The significant effect of PEG on shoot fresh weight started at 2% PEG and the lowest fresh weight was at 9% PEG (0.305 gm) compared with the control (0.691 gm).

Table (3) Effect of SA and PEG on shoot fresh weight (g) of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	0.587	0.446	0.278	0.305	0.204	0.044	0.311
0.5	0.794	0.757	0.654	0.496	0.502	0.567	0.628
Water stress (PEG) mean	0.691	0.602	0.466	0.400	0.353	0.305	
LSD: SA= 0.084 PEG= 0.146 SA x PEG= 0.207							

Effect of SA on shoot dry weight was similar to its effect on fresh weight (table 4) i.e. SA caused a significant increase in shoot dry weight compared with control. Table 4 indicated also that PEG caused a significant decrease in shoot dry weight at 3% while there was no significant effect on shoot dry weight at other concatenations. Interaction results showed that highest dry weight was at interaction between SA and 1% PEG (.091 gm) while the lowest dry weight was at interaction between control treatments and 2% and 3% PEG.

Table (4) effect of SA and PEG on shoot dry weight (g) of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	0.059	0.038	0.026	0.026	0.045	0.034	0.038
0.5	0.070	0.091	0.082	0.049	0.056	0.062	0.068
Water stress (PEG) mean	0.065	0.065	0.054	0.037	0.050	0.48	
LSD: SA= 0.010 PEG= 0.0.18 SA x PEG= 0.025							

3.4 Root length

Table 5 showed that SA caused a significant increase in root length when compared with control, root length was 23.06 cm and 16.64 respectively. PEG caused a significant decrease in root length at 2%, 3%,6% and 9% when compared with control. Root length in the interaction between SA, control and 1% PEG treatments, have the highest root length (35.47 and 32.47) when compared with other interaction results.

Table (5) Effect of SA and PEG on root length (cm) of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	20.17	20.80	18.60	17.00	13.33	9.93	16.64
0.5	35.47	32.47	27.67	15.00	12.50	15.27	23.06
Water stress (PEG) mean	27.82	26.63	23.13	16.00	12.92	12.60	
LSD: SA= 2.134 PEG= 3.697 SA x PEG= 5.228							

3.5 Root fresh and dry weight

Results in table 6 showed that there was no significant difference between roots fresh weight developed from seeds soaked with 0.5 mM SA and that developed from control. The significant decrease in root fresh weight started at 3% PEG and continued in the subsequent concentrations compared with control. Root fresh weight at the Interaction between SA and PEG at control, 1% and 2% was higher than other interaction values.

Table (6) Effect of SA and PEG on roots fresh weight of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	0.335	0.320	0.393	0.368	0.140	0.109	0.278
0.5	0.488	0.342	0.457	0.171	0.105	0.247	0.302
Water stress (PEG) mean	0.425	0.331	0.425	0.269	0.178	0.170	
LSD: SA= 0.089 PEG= 0.154 SA x PEG= 0.218							

Table 7 shows the effect of SA and PEG on roots dry weight and it cleared that SA caused significant increase in root dry weight and that PEG caused a significant decrease in root dry weight starting at 1% concentration and continued at the subsequent concentrations compared with control. The highest roots dry weight was recorded at control treatment (0.047 gm).

Table (7) effect of SA and PEG on roots dry weight (g) of maize growth in hydroponic culture

SA mM.l^{-1}	PEG %						SA mean
	0	**1**	**2**	**3**	**6**	**9**	
0	0.059	0.038	0.026	0.026	0.045	0.034	0.038
0.5	0.070	0.091	0.082	0.049	0.056	0.062	0.068
Water stress (PEG) mean	0.065	0.065	0.054	0.037	0.050	0.48	
LSD: SA= 0.010 PEG= 0.0.18 SA x PEG= 0.025							

4. Discussion

Germination is the most critical and sensitive stage in the life cycles of plants (Ahmad *et al*., 2009) and the seeds exposed to unfavorable environmental conditions such as drought may compromise the subsequent seedling establishment (Soleymani *et al*. 2012). PEG is considered as a superior chemical to induce water stress (Kaur *et al*, 1998). PEG molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress in crop plants (Landjeva *et al*, 2008). PEG especially at higher concentrations had reduced the germination percentage inhibited the lines and caused them in record low germination percentage. Dodd and Donavon, (1999) stated that PEG induced reduction in germination percentage.Water availability is usually the limiting factor for the germination of non-dormant seeds, affecting the percentage, speed, and uniformity of emergence. A threshold level of hydration is required for the synthesis of hydrolytic enzymes which are responsible for the hydrolysis of stored substrates.

Shoot length, fresh and dry weight was reduced significantly with the increase in PEG concentrations. The results clearly indicated reduction rate in shoot growth characters with high concentration of PEG. The decline in shoot length, fresh and dry weight traits in response to induced osmotic stress is a commonly observed phenomenon which is depends on the tolerance capacity of the plant. Decreasing in growth rate with increasing osmotic stress was reported in several studies (Abdel- Raheem *et al*, 2007; Aazami *et al*, 2010).

Roots length, fresh and dry weight were affected by increasing PEG concentrations. Roots are the primarily effected plant part under drought conditions than any other parts (Ghafoor, 2013). Root trait of all varieties provided useful information against different levels of PEG and this is very important attribute to study the drought stress. A gradual reduction in root length, fresh and dry weight with an increasing concentration of PEG was the common tendency observed in our experiment. It is well known fact that root architecture influences the yield and other agronomic traits, particularly under stress conditions (Dorlodot *et al*, 2007). Remarkable decrease in root length has been observed with increasing PEG concentrations, Kulkarni and Deshpande, (2007) reported that early and rapid elongation of roots is a key trait of drought tolerance.

Results in this study revealed that Seeds soaking with SA had improved significantly all growth parameters studied. Plant growth regulators play important roles in the regulation of plant developmental processes and signaling networks as they are involved either directly or indirectly in a wide range of biotic and abiotic stress responses and tolerance in plants (Asgher *et al*., 2015). SA is a phenolic compound involved in the regulation of growth and development of plants, and their responses to biotic and abiotic stress factors (Miura and Tada, 2014). SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, and proline metabolism, production of glycinebetain, antioxidant defense system, and plant- water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Khan *et al*., 2014). Apart from its involvement in the induction of defense- related genes and stress resistance in biotic stressed plants (Kumar, 2014), SA has been shown to improve plant tolerance to major abiotic stresses such as metal (Zhang et al., 2015), salinity (Nazar *et al*., 2015), osmotic (Alavi *et al*., 2014), drought (Fayez and Bazaid, 2014), and heat stress (Khan et al., 2013b). Exogenously sourced SA to stressed plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying was reported to induce major abiotic stress tolerance-mechanisms (Palma *et al*., 2013). SA influences plant functions in a dose dependent manner, where induced or inhibited plant functions can be possible with low and high SA concentrations, respectively. Recent molecular studies have established that SA can regulate many aspects in plants at gene level, and thereby can improve plant-abiotic stress tolerance. SA was reported to induce several genes responsible for encoding chaperone, heat shock proteins (HSPs), antioxidants and secondary metabolite (Jumali *et al*., 2011).

5. Conclusion

In order to develop sustainable agriculture as well as to improve overall plant performance in the conditions of the changing climate and the increased severity of abiotic stresses, it would be imperative to exploit the information available on the involvement of SA in abiotic stress tolerance in plants. In the current study the role of SA in plants exposed to abiotic stress conditions is discussed, and it's clear that SA had potential role in mechanisms controlling plant drought stress and the capability of using SA in agricultural operations.

References

Aazami, M.A., Torab, M. and Jalili, E. (2010). In vitro response of promising tomato genotypes for tolerance to osmotic stress. Afr. J. Biotechnol., 9: 4014-4017.

Abdel-Raheem, A.T., Ragab, A.R., Kasem, Z.A., Omar, F.D. and Samera, A.M. 2007. In vitro selection for tomato plants for drought tolerance via callus culture under polyethylene glycol (PEG) and mannitol treatments. Afr. Crop Sci. Soc., 8: 2027-2032.

Ahmad, P., Nabi, G., and Ashraf, M. (2011). Cadmium-induced oxidative damage in mustard [Brassica juncea (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. South Afr. J. Bot. 77, 36–44.

Alavi, S. M. N., Arvin, M. J., and Kalantari, K. M. (2014). Salicylic acid and nitric oxide alleviate osmotic stress in wheat (Triticum aestivum L.) seedlings. J. Plant Interac. 9, 683–688.

Arfan, M. (2009). Exogenous application of salicylic acid through rooting medium modulates ion accumulation and antioxidant activity in spring wheat under salt stress. Intl. J. Agri. Biol. 11, 437–442.

Asgher, M., Khan, M. I. R., Anjum, N. A., and Khan, N. A. (2015). Minimizing toxicity of cadmium in plants– role of plant growth regulators. Protoplasma 252, 399–413.

De Dorlodot, S., Forster, B., Page, L., Price, A., Tuberosa, R. and Draye, X. (2007). Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Pl. Sci., 12: 474-481.

Dodd, G.L. and Donovan, L.A. (1999). Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. Am. J. Bot., 86: 1146-1153

Fayez, K. A., and Bazaid, S. A. (2014). Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. J. Saudi Soc. Agri. Sci. 13, 45–55.

Ghafoor, A. (2013). Unveiling the mess of red pottage through gel electrophoresis: a robust and reliable method to identify Vicia sativa and Lens culinaris from a mixed lot of split “red dal”. Pak. J. Bot., 45: 915-919.

Gunes, A., A. Inal, M. Alpaslan, F. Eraslan, E.G. Bagci,and N. Cicek.(2007). Salicylic acid induced changeson some physiological parameters symptomatic foroxidative stress and mineral nutrition in maize (Zeamays L.) grown under salinity. J. Plant Physiol. 164:728–736.

Hayat S, Ahmad A. (2007). Salicylic acid a plant hormone.The Netherlands: Springer.

Jumali, S. S., Said, I. M., Ismail, I., and Zainal, Z. (2011). Genes induced by high concentration of salicylic acid in ‘Mitragyna speciosa’. Aust. J. Crop Sci. 5, 296–303.

Kaur, S. Gupta, A.K. and Kaur, N. (1998). Gibberellic acid and kinetin partially reverse the effect of water stress on germination and seedling growth in chickpea. Plant Growth Regul., 25: 29–33.

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S., and Khan, N. A. (2013). Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal. Behav. 8:26-37.

Khan, M. I. R., Asgher, M., and Khan, N. A. (2014). Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetain and ethylene in mungbean (*Vigna radiata* L.). Plant Physiol. Biochem. 80: 67–74.

Kulkarni, M. and Deshpande, U. 2007. In-vitro screening of tomato genotypes for drought resistance using polyethylene glycol. Afr. J. Biotech., 6: 691-696.

Kumar, D. (2014). Salicylic acid signaling in disease resistance. Plant Sci. 228:127–124.

Landjeva, S., Neumann, K., Lohwasser, U. and Borner, A. 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol. Plantarum, 52: 259-266.

McCutcheon, A. (2007). Victoria, Australia, Agriculture Notes - Bioethanol in Victoria. AG1314, 1-3 State of Victoria, Department of Primary Industries.

Miura, K., and Tada, Y. (2014). Regulation of water, salinity, and cold stress responses by salicylic acid. Front. Plant Sci. 5:4-9.

Munns, R. (2005). Genes and salt tolerance: bringing them together. New Phytol. 167: 645–663.

Nazar, R., Umar, S., and Khan, N. A. (2015). Exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate-glutathione metabolism and S assimilation in mustard under salt stress. Plant Signal. Behav. 10:100-110.

Siahsar BA, Ganjali S, Allahdoo M. (2010). Evaluation of drought tolerance indices and their relationship with grain yield of lentil lines in drought-stressed and irrigated environments. Aust. J. Basic Appl. Sci. 4:4336-4346. .

Soleymani A, Hesam M, Shahrajabian H. (2012). Study of cold stress on the germination and seedling stage and determination of recovery in rice varieties. Int J Biol.4:23-30.

Zhang, Y., Xu, S., Yang, S., and Chen, Y. (2015). Salicylic acid alleviates cadmium- induced inhibition of growth and photosynthesis through upregulating antioxidant defense system in two melon cultivars (*Cucumis melo* L.). Protoplasma 252: 911–924.