



ORIGINAL ARTICLE

EFFECT OF MANNITOL AND PEG INDUCED WATER STRESS ON SEEDLINGS GROWTH OF BROAD BEAN (*VICIA FABA* L.)

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Abstract: Drought is one of the abiotic stress that affecting on plants growth and productivity. This study was conducted to examine the effects of water stress on seedlings growth of two varieties, luzdeotono (Spanish) and local variety of faba bean (*Vicia faba* L.) by using mannitol (0%, 10%, 20% and 30%) and PEG-4000 (0%, 10%, 20% and 30%). The results showed that root length, protein content decreased with increasing mannitol and PEG-4000 concentrations, whereas proline content, dry weight and enzymes activities of catalase (CAT), Superoxide dismutase (SOD), increased with increasing of mannitol and PEG-4000 concentrations compared to the control (distilled water), but the local variety had a higher in the studied traits and it was considered more tolerant than luzdeotono to drought stress.

Key words: Mannitol, PEG, seedlings, Faba bean.

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1. Introduction

Drought has been one of the most common factors that cause harmful for plants growth and development, and it has become a serious problem that threatens global food in many regions around the world. Water deficiency cause physiological and morphological changes in plants which in turn reduce the yield and production of the crops and inhibits photosynthesis due to stomatal closure and the reduction of internal water transport, respiration, translocation, carbohydrates formation [Duan *et al.* (2017)]. For plants, water limitations lead to the production of (ROS), such as hydrogen peroxide and superoxide anion radicals which results in growth inhibition decreases in photosynthetic functions (lipid peroxidation and the higher frequency of programmed cell death processes). Finally, Water Stress conditions inhibit seedlings growth, resulting in poor crops, reduced biomass and yield [Issa *et al.* (2019)]. Faba beans (*Vicia faba* L.) are one of the most important food crops in the world and the fresh and dry seeds are used as important food for humans

because they contain protein (35%) in dry seeds and are considered a good source of many nutrients, such as potassium, calcium, magnesium, iron and zinc. Faba bean seeds also, contain several other bioactive compounds, such as carotenoids, polyphenols [Salama *et al.* (2019), Turco *et al.* (2016)] and carbohydrates [Lafta and Habib (2021)]. Currently, a new technology has been used which is the use of chemical compounds that cause industrial water stress, such as mannitol, polyethylene glycol. Polyethylene glycol (PEG) and mannitol have been used to stimulate osmotic stress and these neutral polymers are being widely used to impose water stress in plants. Polyethylene glycol (PEG) of higher molecular weight, cannot enter into cell through plant cell wall [Radhi and Abdul-Hasan (2020)] is considered to cause blockage of the pathway of water movement, reducing water absorption and causing desiccation of plant. Mannitol is a white, solid of the chemical formula $C_6H_8(OH)_6$ which has a low molecular weight sufficient to enter into cells and cause toxicity. The aim of this experiments is to study the

effect of water stress that induced by PEG and mannitol on the seedlings growth of two varieties of faba bean and determine which the variety is more tolerant for water stress.

2. Materials and Methods

The Factorial Experimental was conducted according to the complete randomized design with three factors and three replications. The averages were compared using the lowest significant differences (LSD) at $P > 0.05\%$ to study the effect of water stress by using Mannitol (0%, 10%, 20% and 30%) and Polyethylene glycol (PEG-4000) (0%, 10%, 20% and 30%) on seedlings growth of two varieties (luzde otono (Spanish) and local variety) of faba bean (*Vicia faba* L.). The seeds washed with distilled water and were germinated in growth chamber at $25 \pm 1^\circ\text{C}$ and relative humidity 60-70%, then the seedlings of faba bean were treated with mannitol in concentration (0%, 10%, 20% and 30%) and poly ethylene glycol (PEG-4000) (0%, 10%, 20% and 30%) for 3 days, after the study ended, the following results were taken:

1. Plant height (cm): The Plant height was measured using tape measure.
2. Dry weight: The dry weight (DW) was obtained

after drying the plant for 48 hr at 75°C in oven advise.

3. Proline was estimated according to the method of [Bates *et al.* (1973)].
4. Protein was measured according to the method of [Bishop *et al.* (1985)].
5. Catalase (CAT) (EC 1.11.1.6) enzyme activity was measured according to the method of Aebi (1984).
6. Superoxide dismutase (SOD) (EC 1.15.1.1) enzyme activity was measured according to the method of [Giannopolitis and Ries (1977)].

3. Results

Table 1 showed a significant decrease in plant height of faba bean seedlings with increase concentrations of PEG and mannitol. PEG was found to be more effective than mannitol in decrease of plant height of faba bean seedlings. Also, Table 1 showed the superiority of the local variety compared to the Luzdeotono variety in plant height. The interaction between (Luzdeotono variety + mannitol 30% + PEG 30%) caused significant decrease in plant height at (31.58 cm) compared to control at (60.78 cm).

Table 1: Effect of Mannitol and PEG on plant height of faba bean seedlings (cm).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	60.78	58.89	50.78	41.78	53.06
	10	55.67	52.78	49.80	41.75	50.00
	20	52.96	49.63	44.67	39.02	46.57
	30	44.67	41.78	37.89	31.68	39.00
Luzdeotono variety	0	64.78	56.78	49.45	40.26	52.82
	10	53.65	52.67	43.67	39.45	47.36
	20	48.89	42.68	40.76	37.67	42.50
	30	39.67	37.67	32.67	31.58	35.45
PEG average		52.63	49.11	43.71	37.92	1.73
LSD 5%		2.57				
Variety * PEG						Variety average
local variety		53.52	50.77	45.79	38.56	47.17
Luzdeotono variety		51.75	47.45	41.64	37.29	44.53
LSD 5%		1.73				1.05
Mannitol * PEG						Mannitol average
0		62.78	57.84	50.12	41.02	52.94
10		54.66	52.74	46.74	40.60	48.69
20		50.93	46.16	42.72	38.35	44.54
30		42.17	39.73	35.28	31.73	37.23
LSD 5%		1.62				1.78

Table 2: Effect of Mannitol and PEG on dry weight of faba bean seedlings (gm).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	0.32	0.33	0.38	0.43	0.37
	10	0.33	0.35	0.41	0.47	0.39
	20	0.39	0.43	0.45	0.48	0.44
	30	0.44	0.51	0.49	0.54	0.50
Luzdeotono variety	0	0.32	0.29	0.35	0.38	0.34
	10	0.34	0.36	0.41	0.42	0.38
	20	0.39	0.40	0.43	0.44	0.42
	30	0.41	0.43	0.45	0.46	0.44
PEG average		0.37	0.39	0.42	0.45	0.07
LSD 5%		0.13				
Variety * PEG						Variety average
local variety		0.37	0.41	0.43	0.46	0.42
Luzdeotono variety		0.37	0.37	0.41	0.43	0.40
LSD 5%		0.07				n.s.
Mannitol * PEG						Mannitol average
0		0.32	0.31	0.37	0.41	0.35
10		0.34	0.35	0.41	0.45	0.39
20		0.39	0.41	0.45	0.46	0.43
30		0.43	0.47	0.47	0.50	0.47
LSD 5%		0.09				0.09

It is evident from Tables 2 and 3 that the dry weight and Proline content in leaves of faba bean seedlings increased with increasing water stress that induced by PEG and mannitol. PEG was increased dry weight and proline content in leaves more than mannitol and increased dry weight and proline content in leaves of local variety (0.42 and 24.66) seedlings was more than that the Luzdeotono variety (0.40 and 22.49). The interaction between the study factors showed a significant superiority of the treatment (local variety + mannitol 30% + PEG 30%) compared to the control in studied traits.

Table 4 showed that the protein in leaves decreased with increasing concentrations of PEG and mannitol and decreased with exogenous PEG more than mannitol. Also, increased in local variety more than Luzdeotono variety. The interaction between study factors (Luzdeotono Variety + Mannitol 30% + PEG 30%) showed a significant decreased in protein at (80.56%) compared to control at (145.23%).

It is evident from Tables 5,6 that the enzymes activities of CAT and SOD increased in leaves with increasing concentrations of PEG and mannitol. Also, increased in local variety (161.88 unit mg^{-1} protein min^{-1})

more than Luzdeotono variety (157.49 unit mg^{-1} protein min^{-1}). The interaction between study factors (Local variety + Mannitol 30% + PEG 30%) showed a significant increased in CTA and SOD at (234.56) compared to control at (130.45%).

4. Discussion

In this study, plant height of faba bean seedlings decreased with increasing PEG and mannitol concentrations. A decrease in plant height might be due to inhibition of cell enlargement and cell division. Also Table 1 indicates that superiority of local variety over the Spanish variety may be due to genetic factors and adaptation. Water stress caused a significant increase in dry weight of seedlings Table 2 might be due to that the water stress induced dry weight increase and attributed to the increased synthetic activity associated with a new material synthesis [Ashraf *et al.* (2011)]. Also, the Proline content Table 3, a significant increased with increase of PEG and mannitol concentrations due to protein breakdown. Proline regulates the oxidation of the plant tissues cells and reduces the ionic effect resulting from the water stress and contributes to the restriction of the toxic elements absorbed under stress conditions and the accumulation of proline in plant

Table 3: Effect of Mannitol and PEG on proline content of faba bean seedlings ($\mu\text{g}/\text{gm.D.W.}$).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	12.45	16.45	22.45	28.89	20.06
	10	17.67	19.23	25.35	30.23	23.12
	20	21.56	22.12	25.98	30.99	25.16
	30	27.89	28.78	30.78	33.67	30.28
Luzdeotono variety	0	11.34	12.89	15.67	19.45	14.84
	10	15.34	16.89	18.89	22.78	18.48
	20	20.67	23.78	25.65	28.76	24.72
	30	28.78	29.67	33.67	35.76	31.97
PEG average		19.46	21.23	24.81	28.82	3.17
LSD 5%		6.27				
Variety * PEG						Variety average
local variety		19.89	21.65	26.14	30.95	24.66
Luzdeotono variety		19.03	20.81	23.47	26.69	22.49
LSD 5%		3.17				1.13
Mannitol * PEG						Mannitol average
0		11.90	14.67	19.06	24.17	17.45
10		16.51	18.06	22.12	26.51	20.80
20		21.11	22.95	25.77	29.88	24.93
30		28.34	29.23	32.23	34.72	31.13
LSD 5%		2.17				2.17

Table 4: Effect of Mannitol and PEG on protein content of faba bean seedlings ($\text{mg}/\text{gm.F.W.}$).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	145.23	137.67	129.78	111.78	131.12
	10	123.56	121.23	118.45	112.98	119.06
	20	121.45	119.23	112.39	107.85	115.23
	30	109.38	104.34	100.43	95.34	102.37
Luzdeotono variety	0	144.34	138.39	116.56	101.78	125.27
	10	137.89	131.45	118.78	90.87	119.75
	20	120.34	119.98	91.78	83.67	103.94
	30	101.43	99.45	85.67	80.56	91.78
PEG average		125.45	121.47	109.23	98.10	6.43
LSD 5%		12.55				
Variety * PEG						Variety average
local variety		124.91	120.62	115.26	106.99	116.94
Luzdeotono variety		126.00	122.32	103.19	89.22	110.18
LSD 5%		6.43				2.13
Mannitol * PEG						Mannitol average
0		144.79	138.03	123.17	106.78	128.19
10		130.73	126.34	118.62	101.93	119.40
20		120.90	119.61	99.03	95.76	108.83
30		105.41	101.90	93.05	87.95	97.08
LSD 5%		4.07				4.07

Table 5: Effect of Mannitol and PEG on CAT activity of faba bean seedlings (unitmg⁻¹protein min⁻¹).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	130.45	137.67	149.78	164.89	145.70
	10	141.89	154.89	162.34	169.78	157.23
	20	159.65	178.90	185.89	189.99	178.61
	30	169.90	181.77	194.65	234.56	195.22
Luzdeotono variety	0	123.78	134.78	144.69	158.78	140.51
	10	135.78	143.80	154.61	169.89	151.02
	20	140.67	150.55	162.89	188.89	160.75
	30	159.45	162.72	178.89	223.78	181.23
PEG average		145.20	155.64	166.72	187.57	8.56
LSD 5%		10.56				
Variety *PEG						Variety average
local variety		121.22	163.31	173.17	189.81	161.88
Luzdeotono variety		136.36	147.97	160.27	185.34	157.49
LSD 5%		8.56				4.78
Mannitol *PEG						Mannitol average
0		127.12	136.23	147.24	161.84	143.12
10		138.84	149.35	158.48	169.84	154.13
20		150.16	164.74	174.39	189.44	169.69
30		164.68	172.25	186.77	229.17	188.22
LSD 5%		5.91				5.91

Table 6: Effect of Mannitol and PEG on SOD Activity of faba bean seedlings (units·mg⁻¹·protein·min⁻¹).

Variety	Mannitol (%)	PEG (%)				Variety * Mannitol
		0	10	20	30	
Local variety	0	8.97	10.65	16.78	24.46	11.45
	10	10.65	12.78	18.89	26.78	17.28
	20	19.43	23.78	25.78	29.98	24.74
	30	25.65	29.64	30.31	34.76	30.09
Luzdeotono variety	0	8.23	9.98	14.78	23.49	14.12
	10	11.89	12.72	17.39	24.89	16.72
	20	15.89	19.43	25.76	30.69	22.94
	30	21.89	26.65	28.67	32.61	27.46
PEG average		15.33	18.21	22.29	28.45	7.32
LSD 5%		10.54				
Variety *PEG						Variety average
local variety		16.18	19.21	22.94	28.98	21.83
Luzdeotono variety		14.48	17.21	21.65	27.92	20.32
LSD 5%		7.32				6.54
Mannitol *PEG						Mannitol average
0		8.60	10.34	15.78	23.98	14.67
10		11.27	12.75	18.14	25.84	16.99
20		17.66	21.61	25.77	30.34	23.84
30		23.77	28.15	29.49	33.69	28.77
LSD 5%		5.74				5.74

[Ashraf and Foolad (2007)]. Osmotic adjustment involves an active accumulation of cellular solutes such as proline and soluble proteins within the plant in response to lowering of the soil water potential and reducing the harmful effects of water deficit [Morgan (1984)]. Protein content (Table 4) decreased with increase concentrations of PEG and mannitol. The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may affect water stress tolerance [Jiang and Huang (2002)]. Plant growth under water deficit can also be affected by changes in gene expression, leading to the synthesis and activation of novel proteins under water deficit conditions activity of antioxidant enzymes (CAT and SOD) were significantly increased with increasing levels of drought stress (PEG, mannitol) in seedlings of faba bean (Tables 5 and 6). Abiotic stress such as water stress leads to the generation of reactive oxygen species (ROS) that may react with a large variety of biomolecules [Siddiqui *et al.* (2012)]. To overcome oxidative damage, plants develop an antioxidant system to scavenge ROS. In the present study, activity of antioxidant enzymes (CAT and SOD) in seedlings increased under drought stress.

5. Conclusion

This study was conducted to study the effect of drought stress on faba bean seedlings. Different concentrations of Mannitol and PEG were used to induce drought stress. PEG and mannitol induced drought stress efficiently, but PEG 30% were more severe. The results indicate that local variety more tolerance than Luzdeotono variety. Also, tolerant local variety had a better ability to reduce oxidative damage by increasing activity of CAT and SOD. These findings will be helpful to improve tolerance of Faba bean against drought stress. Proline content and dray weight increased with increased drought stress.

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