Journal of Horticultural Science & Ornamental Plants 7 (2): 56-65, 2015
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2015.7.2.1156

Effect of Organic Osmolytes on Pea Seed Yield under Drought Stress Conditions Imposed at Different Growth Stages

H.G. Abd El-Gawad

Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Abstract: Drought is one of the major abiotic stresses which adversely affect crop growth and production worldwide as water is vital for every aspect of plant growth and development. The present experiment was carried out during the two growing seasons of 2012/2013 and 2013/2014, at The Experimental Farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effecting of using foliar application of organic osmolytes on vegetative growth, seed yield and quality of pea (*Pisum sativum* L) under drought stress conditions imposed at different growth stages. The plants were exposed to drought stress at different growth stages; vegetative growth stage, flowering stage, all growth stages and control plants (no stress imposed) and foliar application of organic osmolytes; glycine betaine (GB) was used at 2 mM/L and proline was used at 2 mM/L. Results showed that untreated plants (no stress imposed) and the both foliar GB or proline had the highest significant value of vegetative growth. No significant differences observed in the first season in abortion, while in the second season GB had the highest value but didn't differ significantly with proline, control and its combination had the lowest value. The foliar application of GB produced the highest pod yield per plant and feddan compared with the other treatments. While, foliar application of GB or proline produced the highest hundred-seed weight compared with control treatment. The interaction among studied factors indicated that combination of GB or proline foliar application without drought stress had a little highest value followed by drought in vegetative and flowering stages.

Key words: Drought stress · Glycine betaine · Organic osmolytes · Pea · Proline · Seed yield

INTRODUCTION

Up to 45% of the world agricultural lands are subjected to continuous or frequent drought [1]. In legumes, the reproductive stage is the most sensitive stage to drought stress, whether it takes place during flower formation, full flowering, pod formation and grain filling. This is because the water deficit causes falling or abortion of reproductive structures [2]. In shortage water conditions, water had been to use at critical growth stages. Thus, it is important to recognize these critical growth stages for any crops. In addition, one of the main goals in breeding program is selection of genotypes that had been high yield in drought stress conditions [3].

Pea (*Pisum sativum* L.) yield depends strongly on environmental conditions such as drought, particularly at vegetative, flowering and pod filling [4]. In this respect, Baigorri *et al.* [5] reported that dry matter (DM) production was always smaller in peas until the beginning of drought treatment. During drought, shoot DM production decreased significantly. Flower production was also greater, although they exhibited about 24% flower abortion under well-watered conditions. Under drought, abortion percentage increased to 47%. Under drought produced significant decreases in pod and seed dry matter in peas, during drought seed size decreased. Drought caused significant decreases in most yield components.

Water stress causes accumulation of compatible solutes such glycine betaine (GB) and proline. Proline and GB may act as non-toxic osmotic solutes and as enzyme protectants, stabilizing the structures of macromolecules and oraganelles [6]. under stress conditions, the lowering of the cellular water potential and the ABA accumulation give rise to a recognition of the cellular metabolism, accumulation of osmolytes, such as proline, glycine, betaine can be one of the most molecular responses to water stress [7]. Recently, a number of drought stress

Corresponding Author: H.G. Abd El-Gawad, Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

induced genes at transcriptional level have been identified in several plants species including legumes [8]. Baroowa and Gogoi [9] mentioned that application of drought at flowering stage was found to be more detrimental to seed quality than at vegetative and pod filling stages. Also, Rezaei *et al*. [10] reported that seed crops are sensitive to soil water deficits during the reproductive growth stage of pea.

One of the common stress responses in plants is over production of different types of compatible organic solutes. Compatible solutes are low molecular weight, highly soluble compounds that are usually nontoxic at cellular concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity and stabilization of enzymes / proteins. These solutes also protect cellular components from dehydradation injury; they are commonly referred to as osmoprotectants like proline, sucrose, polyols, trehalose and quaternary ammonium compounds such as glycine betaine [1]. The GB a member of quaternary ammonium compounds is an osmolyte that is pre-dominated in higher plants subjected to drought conditions [11].

Glycine betaine and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stress such as drought, salinity, extreme temperature, UV radiation and heavy. GB is known to accumulate in response to stress in many crop plants, including sugar beet, spinach, barely, wheat and sorghum. In these species tolerant genotypes normally accumulate more GB than sensitive genotypes in response to stress. Externally applied GB and proline can rapidely penetrate through leaves and be transported to other organs [1]. The affective and efficiency doses of GB may vary with plant species and the results may vary significantly depending on development stage. Foliar application of GB is an effective approach for imparting tolerance among plants against heavy metals and other abiotic stresses, it scavenges reactive oxygen species (ROS), osmolytes [12]. Glycine betaine is environmentally safe, non-toxic and water-soluble and is found in animal, microbe and plant cells. Most halophytes, when grown under stress, synthesis GB in their chloroplasts and accumulate it as an osmoprotectant [13, 14]. In addition, the GB are well-known as compatible solutes that play a pivotal role in the process of osmotic adjustment in different organisms including higher plants, salt stress up-regulates the enzymes involved in proline and betaine biosynthesis in several plant species [15]. Proline is a compatible osmolyte with an ability to scavenge free-radicals. In addition to its role as an osmolyte, proline can also protect enzymes and increase membrane stability under various conditions. Proline may also assist in the non-enzymic detoxification of free radicals [16]. The GB play as an osmoregulation, a compatible solutes, small organic metabolite soluble in water and nontoxic at high concentrations, It is a compound that can potentially play a role in effective protection against salt, drought and extreme temperature stress [17]. Also, there have been indications that GB treatment induces grain yield increase, up to 25% in maize and up to 11% in sorghum when plants are suffering drought [18].

Drought stress is one of the major abiotic stresses that are a threat to crop production worldwide. Drought stress impairs the plants growth and yield. Therefore, this research aims to reduce the caused hazards resulting from water fluctuations on seed yield and quality of pea plants grown in flood irrigated clay loam Egyptian Delta lands through enhancing the plant tolerance to irrigation water fluctuations by spraying with some organic osmolytes.

MATERIAL AND METHODS

The field experiment was carried out during the two growing seasons of 2012/2013 and 2013/2014, at The Experimental Farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effecting of using foliar application of organic osmolytes on vegetative growth, seed yield and quality of pea (*Pisum sativum* L) under drought stress conditions imposed at different growth stages.

Seeds of pea "Master-B" cultivar were sown on the 1^{st} of September 2012 and 2013 seasons. The area of the experimental plot was 14 m^2 consisted of five rows; each row was 4 m length and 0.7 m width. The plant distance was 15 cm apart on one side, an alley (1.6 m wide) was left as boarder between drought stress treatments.

Calcium super-phosphate (15% P_2O_5) at 250 kg / fed. was banded on rows at two times, the first (150kg) was added during the soil preparation and the second one (100kg) was carried out in the flowering period. Ammonium nitrate (33% N), at 200kg / fed., was applied as soil application in two portions, the first addition was done after two weeks from sowing at the first irrigation and the second one was carried out after one month from the first addition. Potassium sulphate (48% K_2O) was applied at a rate of 100 kg K_2O fed^{-1} at two times. The first portion took place two weeks after sowing at the first

Table 1: *Field capacity, wilting point and available soil water of the experimental soil in the two seasons of 2012 and 2013

	2012			2013		
Depth of soil (cm)	FC%	Wilting percentage	Available water%	FC%	Wilting percentage	Available water%
5-15	24.77	13.81	10.96	25.14	13.58	11.56
15-30	21.90	13.45	8.45	22.65	12.51	10.14
Average	23.34	13.63	9.71	23.90	13.05	10.85

*Soil department, Fac. Agric., Ain Shams University, Cairo, Egypt

irrigation, whereas, the second part was added one month later. Cultural management, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture. Harvesting was carried out for each sowing date when seeds were matured (start yellowing and drying of leaves, i.e., after 125:140 days from planting).

Soil samples from experimental site were taken at random at depths of 5-15 and 15-30 cm from soil surface before soil preparation for determination of field capacity, wilting point and available soil water during the two seasons (Table 1). The experimental soil was clay loam.

The Experimental Design and Treatments

The Plants Were Exposed to Drought Stress at Different Growth Stages:

- Control plants (no stress imposed) were irrigated continuously at the optimum moisture regime (irrigation was conducted after the depletion of 60% of available soil water).
- Vegetative growth stage (after full germination (18 day-old) until start flowering): for imposing drought, water was withheld until the plants showed symptoms of wilting and leaf rolling (irrigation was conducted after the depletion of 90% of available soil water).
- Flowering stage (after start flowering (40 day-old) until the last seed harvesting): irrigation was conducted after the depletion of 90% of available soil water
- All growth stages (after full germination until the last seed harvesting): irrigation was conducted after the depletion of 90% of available soil water

Foliar Application of Organic Osmolytes: Glycine betaine (MW 117.18) of Sigma Aldrich was used at 2 mM/L, proline (MW 115.13) was used at 2 mM/L and distilled water (control) applied after 30, 50 and 70 days from seed sowing.

Samples of soil in the 0-30 cm layer were taken regularly at two-day intervals between irrigations to measure the soil moisture content to determine the timing of irrigation treatments. Soil water content was calculated as (fresh weight-dry weight)/fresh weight of the soil samples. Soil dry weight was obtained after drying at 105°C for three hours or until the stability of weight). The experiment was laid out in a split plot design with three replicates. Drought stress treatments were assigned in the main plots but foliar applications of organic osmolytes were distributed in the sub-plots.

Studied Characteristics: Vegetative characteristics: plants were chosen at random from three replications (from the inner rows) at 75 days from sowing to study the following parameters: leaf number, shoots fresh and dry weight [19]. Leaf chlorophyll reading (SPAD) was determined using the recently full expanded mature upper leaf of 6 plants in the middle row per plot. A digital chlorophyll meter, Minolta SPAD-502, (Minolta Company, Japan) was used.

Fruit set: three plants from each plot were labeled at random before flowering stage. Fruit set percentage was calculated as follows:

$$\text{Fruit set\%} = \frac{\text{Number of setting pods/plant}}{\text{Total number of flowers/plant}} \text{ X } 100$$

% abortion: Floral bud number was counted at 90 days from sowing and three plants from each plot were considered as the sum of initiated floral buds, fully opened flowers and pods according to Baigorri *et al.* [5]. Open flower number was counted as the sum of open flowers and pods and the percentage of flower abortion was calculated as:

$$\text{Abortion\%} = [1- (\text{Open flower number} / \text{Floral bud number})] \times 100$$

Seed yield and its component: one hundred-seed weight and seed yield per plant and feddan were calculated. Seed total carbohydrate was measured colorimetrically according to A.O.A.C. [19]. Total protein percentage on seed was determined according to the method of Bradford [20]. Seed calcium concentration was determined colorimetrically using a Specol spectophotometer according to the procedure described by Piper [21].

Statistical Analysis: Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.). The comparison among means of the different treatments was determined, as illustrated by Snedecor and Cochran [22].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics: Data in Table (2) showed that drought stress at different growth stages gave the lowest values for all measured vegetative growth traits as compared with control plants (no stress imposed) in the two seasons. Untreated control plants increased leaf number, fresh weight, dry weight and chlorophyll reading significantly in the two seasons. The impact of exposed to drought stress at flowering and all growth stages more visible in vegetative growth compared with exposed to drought stress at vegetative growth stage. This result may be attributed that water deficit treatments induced a reduction in the relative water content (RWC) of leaves, reduction in RWC indicates a loss of turgor that results in limited water availability for cell expansion [7]. This results agreed with Sorensen and Edelenbos [23] who observed that drought stress during flowering and pod filling of pea didn't significantly increase the dry matter concentration, dry matter was unaffected by drought stress. In this respect, Kirnak *et al.* [24] observed that water stress resulted in reduced vegetative growth and leaf chlorophyll content of soybean. Also, Pervez *et al.* [25] found that number of per plant showed significant results toward drought stress signifying drought effects on growth of tomato.

The vegetative growth was increased to reach a significant level by the application of organic osmolytes. On the other hand, chlorophyll readings were not significantly affected by application of all treatments. This effect was most obvious with GB followed by proline. This may be due to this compatible organic solutes protect cellular components from dehydration injury. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency

Table 2: Effect of two organic osmolytes foliar application on some vegetative growth characters of pea plant under drought stress at different growth stages in the two seasons (2012/2013 and 2013/2014)

	1st season				2nd season			
Treatments	Control	GB	Proline	Mean	Control	GB	Proline	Mean
Leaf number								
Cont.	19.22 c	22.73 a	21.11 b	21.02 A	17.15 cd	19.20 a	18.72 ab	18.36 A
D. Veg	17.03 d-f	18.45 cd	18.05 c-e	17.84 B	14.67 e	17.30 b-d	16.77 cd	16.25 C
D. Flow	15.98 fg	18.05 c-e	16.68 ef	16.90 C	16.20 d	18.31 a-c	17.35 b-d	17.29 B
D. All	13.14 h	15.94 fg	15.07 g	14.72 D	11.18 f	14.17 e	13.24 e	12.86 D
Mean	16.34 B	18.79 A	17.73 A		14.80 B	17.24 A	16.52 A	
Fresh weight (g)								
Cont.	86.48 b	98.35 a	94.18 a	93.00 A	77.86 cd	86.02 a	85.05 a	82.98 A
D. Veg	70.47 de	79.85 c	75.59 cd	75.30 B	65.01f	75.52 d	74.78 d	71.77 C
D. Flow	71.59 de	87.17 b	79.73 c	79.50 B	69.52 e	80.26 bc	82.83 ab	77.54 B
D. All	56.16 f	70.36 de	66.88 e	64.46 C	52.51 h	63.02 fg	60.08 g	58.53 D
Mean	71.17 C	83.93 A	79.10 B		66.22 B	76.20 A	75.68 A	
Dry weight (g)								
Cont.	8.45 b	9.22 a	9.16 a	8.94 A	7.54 bc	8.31 a	8.32 a	8.06 A
D. Veg	6.77 d	7.62 c	7.46 c	7.28 C	6.63 d	7.43 c	7.46 bc	7.18 B
D. Flow	6.75 d	8.55 b	7.83 c	7.71 B	6.84 d	7.80 a-c	7.97 ab	7.54 AB
D. All	5.67 e	6.95 d	6.71 d	6.45 D	5.07 f	5.98 e	5.83 e	5.63 C
Mean	6.91B	8.09 A	7.79 A		6.52 B	7.38 A	7.40 A	
Chlorophyll readings (SPAD)								
Cont.	41.10 b-d	41.22 bc	42.53 ab	41.62 A	39.20 b-d	40.22 a-c	41.32 ab	40.25 A
D. Veg	39.41 cd	42.15 ab	41.79 a-c	41.12 A	39.24 b-d	40.27 a-c	40.82 ab	40.11 A
D. Flow	40.93 b-d	43.91 a	40.62 b-d	41.82 A	39.67 b-d	42.37 a	40.56 ab	40.87 A
D. All	38.60 d	39.42 cd	38.52 d	38.85 B	38.15 cd	38.19 cd	37.59 d	37.98 B
Mean	40.01 A	41.68 A	40.87 A		39.06 A	40.26 A	40.07 A	

Means followed by different letters are significantly different at P≤ 0.5 level; Duncan's multiple range test.
GB = Glycine betaine, D. Veg = Drought at vegetative growth stage, D. Flow = Drought at flowering stage,
D. All = Drought at all growth stages

Table 3: Effect of two organic osmolytes foliar application on fruit set and abortion percentage of pea plant under drought stress at different growth stages in the two seasons (2012/2013 and 2013/2014)

	1st season				2nd season			
Treatments	Control	GB	Proline	Mean	Control	GB	Proline	Mean
Fruit set (%)								
Cont.	19.22 b	22.73 a	21.11 a	21.02 A	17.15 cd	19.20 a	18.72 ab	18.36 A
D. Veg	17.03 bc	18.45 b	18.05 b	17.84 B	14.67 ef	17.30 bc	16.77 bc	16.25 B
D. Flow	15.98 ef	18.05 de	16.68 de	16.90 C	16.20 g	18.31 e-g	17.35 de	17.29 C
D. All	13.14 f	15.94 cd	15.07 f	14.72 C	11.18 h	14.17 fg	13.24 g	12.86 D
Mean	16.34 B	18.79 A	17.73 AB		14.80 B	17.24 A	16.52 A	
Abortion (%)								
Cont.	4.8 f	5.4 ef	1.3 g	3.8 C	6.4 e	7.1 de	5.2 e	6.2 C
D. Veg	7.0 c-e	7.8 cd	9.0 c	8.0 B	7.6 de	10.7 c	10.9 c	9.7 B
D. Flow	6.6 d-f	9.0 c	9.0 c	8.2 B	9.3 cd	11.5 c	11.6 c	10.8 B
D. All	12.1 b	15.0 a	12.6 a	13.2 A	12.0 bc	16.3 a	14.8 ab	14.3 A
Mean	7.6 A	9.3 A	8.0 A		8.8 B	11.4 A	10.6 AB	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test.

GB = Glycine betaine, D. Veg = Drought at vegetative growth stage, D. Flow = Drought at flowering stage,

D. All = Drought at all growth stages

[11]. Proline accumulation under stress conditions related to increase content of precursor for protein biosynthesis, including glutamic acid, ornithine and arginine [1]. In this respect, foliar application of GB improves the growth vigor by increasing the shoot/ root length and fresh/dry masses under drought in wheat. Additionally, Rezaei *et al*. [26] GB was observed by the leaves and remained stable there, indicating a long term protective capability. GB also improves the growth, survival and tolerance of a wide variety of accumulator/ non-accumulator plants under various stress conditions. On the other hand, Abdelhamid *et al.* [16] found that exogenous spray applications of proline significantly improved all growth parameters in medium or high salt-stressed common bean plants. In this respect, Shahid *et al.* [27] reported that the promotion of growth in response to exogenous application of proline could have been due to the decline in the rate of cell death under stressed conditions in *Pisum sativum*.

Concerning the interaction between organic osmolytes foliar application and drought stress at different growth stages, untreated control plants (no stress imposed) and foliar GB or proline had the highest significant value. Meanwhile, in chlorophyll readings, drought applied during flowering with spraying GB had the highest significant value and most of interaction treatment didn't differ significantly from each other, also the fully drought treatment had the lowest value. These results are in harmony with Gadallah [6] who observed that proline and GB reduced membrane injury, improved K^+ uptake and growth. Also both solutes increased chlorophyll content under salt stress. GB and proline treated plants had more chlorophyll and produced more biomass, this probably due to the ability of these solutes to effect of activity of some enzymes and protein synthesis, or these solutes as reservoirs of C and N could enhance growth, also Cha-um *et al*. [11] investigated the role of GB applied exogenously in alleviating water deficit induced stress in rice, also found that exogenous GB increased proline concentration in the leaf tissues, the author discussed the significant decline in photosynthetic pigments of water stressed plant without GB application is likely to directly affect the light harvesting and electron transport system in photosystem II, which is indicated by chlorophyll fluorescence parameters. Both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plant grown under stress conditions [1].

Fruit Set and Abortion: Concerning fruit set and abortion, the data in Table (3) indicated that control treatment increased fruit set and abortion in the two seasons followed by drought during vegetative stage then flowering and the least value was drought in all stages. In this regards, Mwanamwenge *et al*. [28] noted that pod set was reducing by water deficit, particularly during the reproductive phase, due to the abortion of flowers and small pods. Also, the author concluded that when stress imposed earlier in the growth at foliar initiation and flowering, the plants had more opportunity to recover from loss of dry matter production during the stress period than at pod filling.

Concerning the effect of organic osmolytes, the same data in Table 3 showed that foliar application of GB or proline gave the highest fruit set percentage. However, foliar application of GB or proline tended to reduce the

Table 4: Effect of two organic osmolytes foliar application on yield components of pea plant under drought stress at different growth stages in the two seasons (2012/2013 and 2013/2014).

	1st season				2nd season			
Treatments	Control	GB	Proline	Mean	Control	GB	Proline	Mean
Yield (g / plant)								
Cont.	18.7 ab	19.8 a	18.3 a-c	18.9 A	21.0 ab	22.4 a	20.3 ab	21.2 A
D. Veg	16.0 c-e	17.3 b-d	15.6 d-f	16.3 B	19.9 b	19.7 b	19.4 b	19.6 AB
D. Flow	14.7 e-g	15.9 c-e	15.5 d-f	15.4 B	19.0 b	19.9 b	15.8 c	18.2 B
D. All	11.8 h	12.7 gh	13.3 f-h	12.6 C	13.6 d	14.8 cd	15.9 c	14.8 C
Mean	15.3 B	16.4 A	15.7 B		18.3 B	19.2 A	17.8 C	
Yield (kg / feddan)								
Cont.	675.3 b	715.6 a	662.7 b	684.5 A	758.5 b	810.7 a	733.1 c	767.4 A
D. Veg	580.7 d	626.6 c	563.1 de	590.1 B	718.5 cd	712.8 de	700.4 ef	710.6 B
D. Flow	532.2 f	577.1 de	562.1 e	557.1 C	686.7 f	718.8 cd	572.8 g	659.4 C
D. All	426.2 i	458.9 h	479.7 g	455.0 D	49.6 i	534.5 h	576.6 g	533.9 D
Mean	553.6 B	594.6 A	566.9 B		663.5 B	694.2 A	645.7 C	
hundred-seed weight (g)								
Cont.	20.99 b-d	22.98 ab	23.44 a	22.47 A	21.61 b-d	23.54 ab	23.80 a	22.98 A
D. Veg	21.02 b-d	22.55 a-c	23.69 a	22.42 A	21.10 c-e	22.84 a-c	22.95 a-c	22.30 AB
D. Flow	18.31 f	20.87 b-d	21.90 a-d	20.36 B	20.47 de	22.10 a-d	22.06 a-d	21.54 B
D. All	18.46 ef	20.28 d-f	20.50 c-e	19.75 B	19.14 e	21.31 cd	21.33 cd	20.59 C
Mean	19.70 B	21.67 AB	22.38 A		20.58 B	22.45 A	22.53 A	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test.
GB = Glycinebetaine, D. Veg = Drought at vegetative growth stage, D. Flow = Drought at flowering stage,
D. All = Drought at all growth stages

abortion in the second season, while, in the first season insignificantly affected. This result agrees with Abou El- Yazied [12] who found that foliar application of GB on common bean exhibited the highest fruit percentage.

Combination treatment with the both foliar application without drought treatment had the highest value of fruit set and abortion. In contrast drought stress at all growth stages without foliar application of organic osmolytes had the lowest value. As the studied interactions, in general plants with full irrigated combined with foliar application of GB and proline increased fruit set percentage in both tested seasons. Respecting abortion the highest significant value reported by fully drought followed by drought at flowering and vegetative stages which didn't differ from each other in the two seasons. No significant differences observed in the first season in abortion, while the second season GB had the highest value but didn't differ significantly with proline, control and its combination had the lowest value. This result may be attributed that exogenous glycine betaine application at reproductive development stage is more effective for accumulation of organic osmolytes than that of vegetative stage of water deficit stressed sunflower [29]. Also, Abou El-Yazied [12] reported that the effect of water stress and foliar application of GB were more pronounced when applied at flowering stage than at the budding stage. Moreover, exogenous GB application was only advantageous under water stress conditions. In addition, Baigorri *et al*. [5] reported that pea plant increased partitioning of total plant DM to leaves, roots and flowers, but decreased strongly percentage allocation to pods and seeds, this may cause increase flowering abortion and revealed its results because pea is an intermediate plant, vegetative development continuous during formation of reproductive structures. the presence of two sinks may results in a greater competition for assimilates especially at the beginning of pod filling and may have a crucial importance under drought, this competition may cause the abortion of flowers and pods because seed filling has priority over other sinks.

Yield and its Component: Data presented in Table (4) revealed that the drought at all growth stages gave the lowest pod yield per plant as well as pod yield per feddan and hundred-seed weight compared with other treatments in the two seasons. In this respect, the decreased in seed yield under water deficit conditions is largely due to the reduction in fruit set of pea [5]. On pea, Sorensen and Edelenbos [23] found that severe drought stress imposed during flowering reduced the yield more than an equal stress imposed during pod filling. Also, Masoumi *et al*. [7] mentioned that limited irrigation can significantly decrease seed yields especially during two growth periods flowering and filling stage on soybean. In this ward, when drought stress imposed during the pod filling stage, the yield of both cultivars of green pea under study was reduced as compared to that of non-stressed plants, revealing that the effect of drought stress on yield was more severe during flowering than during the pod filling [23]. On the contrast, Martin and Jamieson [30] concluded that the time of drought stress has no effect on pea yield. On faba bean, Siddiqui *et al.* [31] reported that a decrease in growth parameters may be due to the impairment of cell division, cell enlargement caused by loss of turgor and inhibition of various growth metabolisms.

Concerning the effect of organic osmolytes, data indicated that foliar application of GB produced the highest pod yield per plant and feddan compared with the other treatments. While, foliar application of GB or proline produced the highest hundred-seed weight compared with control treatment. In this respect, foliar application of GB increase its content in soybean plants leading to an improvement in photosynthetic activity, nitrogen fixation, leaf area development and seed yield of both well-irrigated and drought- stressed soybean plants [32]. Opposite trend found by Tahir *et al*. [33] who found that, exogenous application of GB either in the form of foliar spray or seed treatment in sunflower had no effect on seed weight and reported that application of soybean is time- cultivar and dose- dependent.

With regard to the effect of the interaction between organic osmolytes foliar application and drought stress at different growth stages (Table 4), the same data indicated that GB or proline foliar application combined with drought stress at different growth stages increased pod yield per plant and feddan as well as hundred-seed weight compared with control treatment. The previously obtained results on yield (Table 4) could be attributed to the stimulation effect of organic osmolytes foliar application and drought stress at different growth stages on plant growth (Table 2) and fruit set and abortion (Table 3). In respect of interaction foliar application with GB or proline were less affective with treatment of drought during two seasons. Agboma *et al*. [34] reported that the application of GB at the 50% irrigation regime on soybean reduced weights of seeds also, in the 75% and 100% irrigation levels showed increases in weight of total and large seeds. Iqbal *et al*. [18] mentioned that foliar application of GB at vegetative stage is more beneficial in alleviating the effects of water stress and improving the 100 achene weight and this improving the 100 achene weight consequently increasing achene yield of sunflower and the explanation of yield increase of stressed –plants after application of GB has been proposed to be partly located on the increased net photosynthesis, decreased rate of photorespiration, stomatal conductance, induced more efficient gas exchange [13] and thus better availability of carbon for photosynthetic processes and ability to avoid possibly photoinhibtion [32], water use efficiency chloroplast ultra-structure, chloroplast volume and increased chlorophyll in plants. Proline accumulation in water stress leaves serving as a source of respiratory energy to the recovering plant [7].

Seed Chemical Constituents: Data in Table (5) showed that fully irrigation increased total carbohydrates, protein and calcium generally followed by both droughts in vegetative and flowering stages which didn't differ significantly from each other and from fully drought however it has the lowest value. In this respect, Kirnak *et al*. [24] cleared that water stress during the different reproductive stages significantly affected protein content. The lowest protein value was obtained in beginning of flowering and full bloom, while the highest values were obtained from beginning of pod. The no differences existed between different stages of drought may be due to the concentration of sucrose and other sugars have been reported to increase in leaves of many plant species exposed to drought stress, this is probably caused by osmotic adjustment, which is the active accumulation of solutes within the plant tissue in response to a lowering of soil water potential [23]. Also it may be a genetic property, Masoumi *et al.* [7] indicated that RWC reflects water status of plants, while, ion leakage implicates the injury of plasma lemma, higher RWC and water ion leakage in cultivars under drought stress indicating that, those cultivars had an increased drought resistance. The protein damage (dityrosine content) in leaves increased markedly to a higher extent in soybean at moderate and extreme water deficit by high lipid and protein oxidation [7]. Also, Shinde and Thakur [35] found that the increase in the water stress causes a decrease in the amount of protein in the seeds of pea.

GB and proline increased total carbohydrate and protein and didn't differ significantly from control except in protein in first season. In this respect, Ashraf and Foolad [1] observed that amino acid proline play as an osmolyte for osmotic adjustment, proline contribute to stabilizing sub-cellular structures (e.g. membrane and protein), scavenging free radicals and buffering cellular redox potential under stress conditions. In addition, Hossain and Fujita [36] suggested that GB and proline roles as osmoprotectants, proline and GB also act as protectants of membranes, enzymes and other

Table 5: Effect of two organic osmolytes foliar application on some chemical constituents of pea seed under drought stress at different growth stages in the two seasons (2012/2013 and 2013/2014).

	1st season				2nd season			
Treatments	Control	GB	Proline	Mean	Control	GB	Proline	Mean
Total carbohydrates (mg / g f.w.)								
Cont.	43.66 b-d	46.27 a	45.56 ab	45.16 A	45.08 bc	48.02 a	48.75 a	47.28 A
D. Veg	41.54 d-f	44.40 a-c	43.63 b-d	43.19 B	43.02 c-e	46.67 ab	44.93 bc	44.87 B
D. Flow	42.26 c-e	44.03 bc	41.25 ef	42.51 B	42.40 d-f	44.79 bc	43.84 cd	43.68 B
D. All	37.94 g	39.43 fg	39.56 fg	38.98 C	40.96 ef	42.27 d-f	40.10 f	41.11 C
Mean	41.35 B	43.53 A	42.50 AB		42.87 B	45.44 A	44.41 AB	
Protein (mg / g f.w.)								
Cont.	22.13 c-e	28.04 a	26.01 ab	25.39 A	22.36 bc	26.41 a	23.74 b	24.17 A
D. Veg	20.93 e-g	24.24 bc	21.78 c-f	22.32 B	21.69 bc	20.96 cd	21.61 bc	21.42 B
D. Flow	19.58 fg	24.02 b-d	22.00 c-f	21.86 B	19.19 de	21.42 c	18.69 e	19.77 C
D. All	19.02 g	22.89 c-e	21.65 d-f	21.19 B	14.81 f	18.23 e	18.59 e	17.21 D
Mean	20.41 B	24.80 A	22.86 A		19.51 B	21.76 A	20.66 AB	
calcium (mg / g f.w.)								
Cont.	2.68 cd	2.92 ab	3.02 a	2.87 A	2.80 ab	2.88 a	2.82 ab	2.83 A
D. Veg	2.51 d-f	2.76 bc	2.64 cd	2.63 B	2.71 a-e	2.75 a-d	2.64 b-f	2.70 AB
D. Flow	2.61 c-e	2.66 cd	2.65 cd	2.64 B	2.50 ef	2.79 a-c	2.65 b-f	2.65 AB
D. All	2.40 f	2.43 ef	2.57 c-f	2.46 B	2.54 d-f	2.58 c-f	2.49 f	2.54 B
Mean	2.55 B	2.69 AB	2.72 A		2.64 A	2.75 A	2.65 A	

Means followed by different letters are significantly different at P≤ 0.5 level; Duncan's multiple range test.
GB = Glycinebetaine, D. Veg = Drought at vegetative growth stage, D. Flow = Drought at flowering stage,
D. All = Drought at all growth stages

proteins. GB is dipolar in nature, but electrically natural molecule and highly soluble in water, due to these characteristics GB protects or stabilizes 3D structures of enzymes and membranal proteins by interacting with both hydrophilic and hydrophobic domains and accumulates in cytosol and chloroplast [14].

The interaction among studied factors indicated that combination of GB or proline foliar application without drought stress had a little highest value followed by drought in vegetative and flowering stages. In this respect, Gadallah [6] reported that stressed plants generally had low content of soluble proteins and higher concentration of amino acids. In addition, Iqbal *et al*. [18] pointed out that GB protects stabilizes and activates the proteins of photosynthetic reactions. Also, Masoumi *et al.* [7] showed that the principle role of proline probably is not to reduce the osmotic potential, but to protect enzymes against dehydradation and salt accumulation.

In conclusion, it was, therefore, concluded that vegetative growth, dry seed yield and its chemical constituents of pea were responded positively to foliar application of organic osmolytes under drought stress in clay loam soil in the Qaliobia region of Egypt.

REFERENCES

1. Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaiene and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59: 206-216.
2. Saucedo, M.C., L.C. Tellez, V.A. Hernandez, A.D. Alvarado, A.S. Varela and G.G. Santos, 2009. Physiological performance, yield and quality of dry bean seeds under drought stress. Interciencia, 34: 748-754.
3. Seghatoleslami, M.J., M. Kafi and E. Majidi, 2008. Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* l.) genotypes, Pak. J. Bot., 40(4): 1427-1432.
4. Martin, I., J.L. Tenorio and L. Ayerbe, 1994. Yield, growth and water use of conventional and semi-leafless peas in semi-arid environments. Crop Sci., 34: 1576-1583.
5. Baigorri, H., M.C. Antolin and M.S. Diaz, 1999. Productive response of two morphologically different pea cultivars to drought. European Journal of Agronomy, 10: 119-128.

6. Gadallah, M.A.A., 1999. Effects of proline and glycinebetaiene on *Vicia faba* responses to salt stress. Biological Plantarum, 42(2): 249-257.
7. Masoumi, H., F. Darvish, J. Daneshian, G. Mohammed and D. Habibi, 2011. Chemical and biochemical responses of soybean (*Glycine max* L.) cultivars to water deficit stress. Australian Journal of Crop Science, 5(5): 544-553.
8. Jovanovic, Z., N. Stanisavijevic, A. Mikic, S. Radovic and V. Maksimovic, 2013. The expression of drought responsive element binding protein (DREB2A) related gene from pea (*Pisum sativum* L.) as affected by water stress. Australian Journal of Crop Science, 7(10): 1590-1596.
9. Baroowa, B. and N. Gogoi, 2015. Changes in plant water status, biochemical attributes and seed quality of black gram and green gram genotypes under drought. International Letters of Natural Sciences, 42: 1-12.
10. Rezaei, A., M. Yazdanpanah, Z. Khosrotaj, Z. Bahreman, S. Poureidi, R. Rashnavadi and B. Khosravi, 2014. Effect of drought stress on yield and yield components of pea hybrids. Technical Journal of Engineering and Applied Sciences, 4(4): 324-327.
11. Cha-um, S.T., L. Samphumphuang and C. Kirdmanee, 2013. Glycinebetaiene alleviates water deficit stress in indica rice using proline accumulation, photosynthetic efficiencies, growth performances and yield attributes. Australian Journal of Crop Science, 7(2): 213-218.
12. Abou El-Yazied, A., 2011. Foliar application of glycine betaine and chelated calcium improves seed production and quality of common bean (*Phaseolus vulgaris* L.) under water stress conditions. Research Journal of Agriculture and Biological Sciences, 7(4): 357-370.
13. Makela, P., K. Jokinen, M. Kontturi, P. Peltonen-Sainio, E. Pehu and S. Somersalo, 1998. Foliar application of glycine betaine a novel product from sugar beet—as an approach to increase tomato yield. Industrial Crops and Products, 7: 139-148.
14. Bhatti, K.H., S. Anwar, K. Nawaz, K. Hussain, E.H. Siddiqi and D. Aneelakhalid, 2013. Effect of exogenous application of glycinebetaiene on wheat (*Triticum aestivum* L.) under heavy metal stress. Middle Journal of Scientific Research, 4(1): 130-137.
15. Hoque, M.A., M.N.A. Banu, E. Okuma, K. Amako, Y. Nakamura, Y. Shimoishi and Y. Murata, 2007. Exogenous proline and glycine betaine increase NaCl-induced ascorbateglutathione cycle enzyme activities and proline improves salt tolerance more than glycine betaine in tobacco Bright Yellow-2 suspension-cultured cells, J. Plant Physiol., 164: 1457-1468.
16. Abdelhamid, M.T., M.M. Rady, A.S. Osman and M.A. Abdalla, 2013. Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. Plants Journal of Horticultural Science & Biotechnology, 88(4): 439-446.
17. Cha-um, S. and C. Kirdmanee, 2010. Effect of glycine betaine on proline, water use and photosynthetic efficiencies and growth of rice seedlings under salt stress, Turk. J. Agric. Forest., 34: 517-527.
18. Iqbal, N., M.Y. Ashraf and M. Ashraf, 2005. Influence of water stress and exogenous glycine betaine on sunflower achene weight and oil percentage. Int. J. Environ. Sci. Technol., 2: 155-160.
19. A.O.A.C., 2005. Official methods of analysis. 15^{th} ed. Association of Official Agricultural Chemists. 12^{th} Ed., Washengton, D.C., USA.
20. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. Analytical Biochemistry, 72: 248-254.
21. Piper, G.S., 1947. Soil and plant analysis. Inter Science Publishers, Inc. New York pp: 368.
22. Snedecor, G.W. and W.G. Cochran, 1982. Statistical methods. 7^{th} ed. Iowa State Unv., press, lowa, U.S.A.
23. Sorensen, J.N. and M. Edelenbos, 2003. Drought effect on green pea texture and related physical-chemical properties at comparable maturity. J. Amer. Soc. Hort. Sci., 128(1): 128-135.
24. Kirnak, H., E. Dogan, M. Alpaslan, S. Celik, E. Boydak and O. Copur, 2008. Drought stress imposed at different reproductive stages influences growth, yield and seed composition of soybean. The Principle Agricultural Scientist, 91(3): 261-268.
25. Pervez, M.A., C.M. Ayub, H.A. Khan, M.A. Shahid and I. Ashraf, 2009. Effect of drought stress on growth, yield and seed quality of tomato (*Lycopersicon esculentum* L.). Pak. J. Agri. Sci., 46(3): 174-178.

26. Rezaei, M.A., B. Kaviani and A.K. Masouleh, 2012. The effect of exogenous glycine betaine on yield of soybean [*Glycine max* (L.) Merr.] in two contrasting cultivars Pershing and DPX under soil salinity stress. Plant Omics Journal, 5(2): 87-93.
27. Shahid, M.A., R.M. Balal, M.A. Pervez, T. Abbas, M.A. Aqeel, M.M. Javaid and F. Garcia-Sanchez, 2014. Exogenous proline and proline-enriched *Lolium perenne* leaf extract protects against phytotoxic effects of nickel and salinity in *Pisum sativum* by altering polyamine metabolism in leaves. Turkish Journal of Botany, 38: 914-926.
28. Mwanamwenge, J., S.P. Loss, K.H. Siddique and P.S. Cocks, 1999. Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L.). European Journal of Agronomy, 11: 1-11.
29. Iqbal, N., M. Ashraf and M. Ashraf, 2008. Glycine betaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.): water relations and yield. South African Journal of Botany, 74: 274-281.
30. Martin, R.J. and P.D. Jamieson, 1996. The effect of timing and intensity of drought on the growth and yield of field peas. New Zealand Journal of Crop and Horticultural Science, 24: 167-174.
31. Siddiqui, M.H., M.Y. Al-Khaishany, M.A. Al-Qutami, M.H. Al-Whaibi, A. Grover, H.M. Ali, M.S. Al-Wahibi and N.A. Bukhari. 2015. Response of different genotypes of faba bean plant to drought stress, Int. J. Mol. Sci., 16: 10214-10227.
32. Makela, P., P. Peltonen-Sainio, K. Jokinenb, E. Pehu, Harri Setd, R. Hinkkanen and S. Somersalo, 1999. Uptake and translocation of foliar applied glycine betaine in crop plants. Plant Science, 121: 221-230.
33. Tahir, M., M. Asraf and M. Shahbaz, 2009. Dose exogenous application of glycine betaine as a pre-sowing seed treatment improve growth and regulate some key physiological attribute in wheat plants grown under water deficit conditions? Pak. J. Bot., 41(3): 1291-1302.
34. Agboma, P.C., P.S. Sainio, R. Hinkkanen and E. Pehu, 1997. Effect of foliar application of glycine betaine on yield of drought- stressed tobacco plant. Exp. Agric., 33: 345-352.
35. Shinde, B.P. and J. Thakur, 2015. Influence of Arbuscular mycorrhizal fungi on chlorophyll, proteins, proline and total carbohydrates content of the pea plant under water stress condition. Int. J. Curr. Microbiol. App.Sci., 4(1): 809-821.
36. Hossain, M.A. and M. Fujita, 2010. Evidence of a role of exogenous glycinebetaiene and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedling under salt stress. Physiol. Mol. Biol. Plants, 16(1): 19-29.