

## Response of Lime Seedlings (*Citrus aurantifolia* L.) to Exogenous Spermidine Treatments under Drought Stress

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**Abstract:** Drought is one of the most important problems that restrict cultivation of crops in arid and semi-arid regions; Lime (*Citrus aurantifolia* L.) is one of the most important trees that grown in these restricts. In this greenhouse study, the effects of exogenous spermidine treatments and drought stress on growth of Lime evaluated. A factorial experiment in completely randomized design in 2008 in greenhouse were carried. For periodic irrigation 7, 15 and 21 respectively, each 7, 15 and 21 days one time and weight were given to the FC. 4 repeat for each treatment were selected, total dry period lasted 180 days. Concentrations of spermidine treatments 0.1 and 2  $\mu$ M were used. The results show that polyamine spermidine treatment improved growth of root, shoot and total plant biomass in Lime seedlings. This treatments increased the proline concentration, and reduce cell membrane ion leakage. The results of this study show that increased periods of drought, reducing the rate of growth and total plant biomass is associated with. This research has shown that use of different degrees of exogenous polyamine can reduce the effects of stress on growth of Lime seedlings.

**Key words:** drought stress, lime seedling, poly amine spermidine, proline

### INTRODUCTION

Polyamines (PAs) are organic polycations found in all living organisms. In higher plants putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant PAs and are implicated in various developmental processes (Tonon *et al.*, 2004). Because the levels of PAs increase during the adaptation to stresses in a variety of plants, it is thought that they are also involved in these processes. However, the physiological role of stress-induced PA accumulation remains unknown. Polyamines titers altered in different manners dependent upon several factors, such as plant species, tolerance or sensitivity to stress, and duration of stress. The exogenous addition of polyamines to stress-treated cells or tissues could lead to injury alleviation and growth promotion in most cases, although the effects varied between polyamines and among plant species (Shen *et al.*, 2000; Bouchereau *et al.*, 1999; Capell *et al.*, 2004; Kasukabe *et al.*, 2004).

They have been proposed as a new category of plant growth regulators that are purported to be involved in a large spectrum of physiological processes, such as embryogenesis, cell division, morphogenesis, and development (Bais and Ravishankar 2002; Liu *et al.*, 2006). In addition, they have been shown to be an integral part of plant stress response (Bouchereau *et al.*, 1999; Alcazar *et al.*, 2006). Though the physiological significance of polyamines in stress is not thoroughly understood, much progress has been made. Since an increase in Put due to potassium deficiency was reported decades ago, changes in polyamines have been extensively investigated when plants are exposed to single or combined stresses (Scalet *et al.*, 1995; Shen *et al.*, 2000; Urano *et al.*, 2003; Liu *et al.*, 2006). In many cases, stress led to an accumulation of free or conjugated polyamines, indicating that polyamine biosynthesis might serve as an integral component of plant response to stress (Bouchereau *et al.*, 1999). An increase in polyamine under stress may be caused by de novo synthesis or reduced degradation, although the exact mechanism remains a matter of debate. The activation of polyamine biosynthetic enzymes in response to stress might argue in favor of the former notion (Liu *et al.*, 2006).

Several lines of research have demonstrated that changes in polyamines are divergent under stress treatment for different periods. In recent years, attention has been focused on the role of PAs in plant defence against abiotic and biotic stresses (Liu *et al.*, 2006). A general phenomenon observed is that PAs can alter their titres in response to various types of environmental stresses such as low and high temperatures (Imai *et al.*, 2004),

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salinity (Roy *et al.*, 2002; Liu *et al.*, 2006), and water stress (Capell *et al.*, 2004; Kasukabe *et al.*, 2004). As compared with stressintolerant plants, stress-tolerant plants generally have a large capacity to enhance PA biosynthesis in responses to stress, resulting in a 2- to 3-fold increase of endogenous PA levels over those in unstressed plants (Kasukabe *et al.*, 2004). Treatment with a PA biosynthetic enzyme inhibitor reduces stress tolerance but the concomitant treatment with exogenous PAs restores it (Liu *et al.*, 2004). However, there is a report that Put and Spd levels decrease, rather than increase, in salt-stressed rice (*Oryza sativa* L.) plants, and Spm accumulation induced by treatment with cyclohexylamine shows no reduction in leaf injury associated with the stress. Increases in Spd and Spm in wheat (*Triticum aestivum*) plants under water stress are observed to be associated with a reduction in drought tolerance. So far, the physiological role of PAs in tolerance to environmental stress remains uncertain (Bais and Ravishankar, 2002; Capell *et al.*, 2004).

## MATERIALS AND METHODS

The experiment was carried out during during July 2008 until February 2009 in the greenhouse located in the department of horticulture of shiraz university.

Three drought levels of irrigation water (7, 15 and 21 days) were used. 72 hours after drought treatments foliar treatments of Spermidine (0, 1 and 2 mM) were used a non-ionic wetting agent (ricka0.5mL) was used in all treatments.

Lime seedlings were studied in this experiment. The seed of lime were obtained from a lime orchard located in Darab. The seeds were germinated and transplanted in a medium with 1:1 sand-soil.

Once the test period to measure total biomass, all plants, removed from the pot and two were divided into root and shoot. For measurement of leaf relative water content (RWC), of fully developed leaves were used. The relative amount of water content using the following relationship was calculated:

$$\% \text{ RWC} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$$

Shoot and root length and fresh weight was measured. Amine acid proline in the leaves, using Bytz and colleagues evaluated. Leakage using the following equation and the method by Bloom was modified, was calculated.

$$\text{EL} = (\text{Ci} / \text{Cs}) \times 100$$

The effects of treatments were evaluated using analysis of variance and the means compared by Duncan's Multiple Range Test (DMRT) at the 95% significance level, using SAS software (SAS Version 6, 4th Edn).

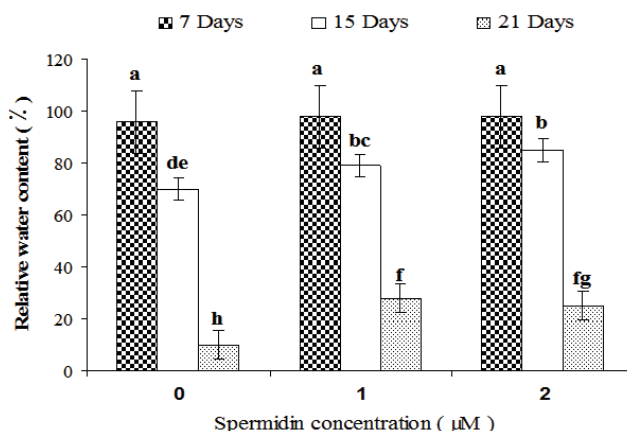
### **Results:**

#### ***Effects of Drought and Spermidine on Shoot and Root Growth:***

The growth of the seedlings was significantly reduced when the plants were subjected to long-term water stress (**Table 1**). In period 7 days with or without the use of spermidine we witnessed the highest shoot length. But with increasing irrigation seedlings shoot height was decreased with treatments poly amine spermidine some of this reduction was compensated shoot height. To zero concentration and shoot height were significantly increased (**Table 1**). In long-term water stress, unlike in shoot growth, root growth increased, so that the increase from 7 days to 21, roots increased significantly (**Table 1**). In 7 days with or without the use of spermidine we witnessed the lowest root length. But with increasing water stress, was added to the root length (**Table 1**). With the effect of root length polyamine Spermidine improve concentration was zero during the roots significantly increased (**Table 1**).

#### ***Effects of Drought and Spermidine on Relative Water Content of Leaves:***

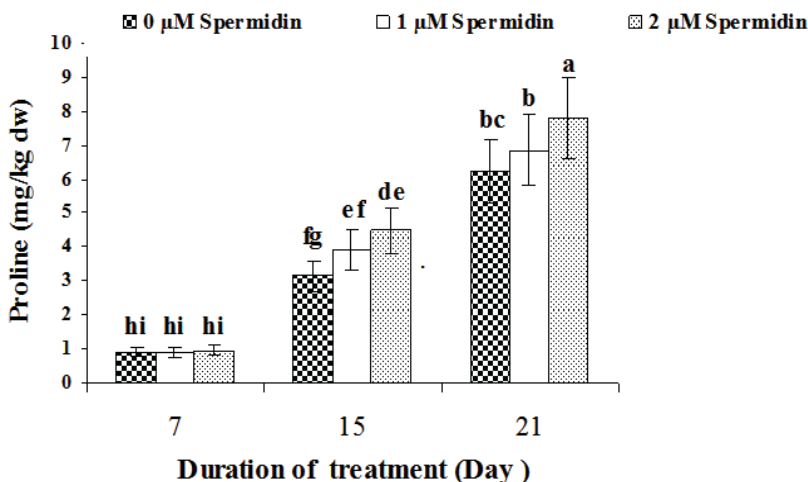
Results of statistical analysis for relative water content attribute, that represents the difference between concentrations of stress and spermidine this property was very significant. With long-term water stress, leaf relative water content also decreased. In 21 days, lowest in leaf relative water content was observed (**Fig. 1**). Polyamine spermidine use, the amount of leaf relative water content than control significantly increased. Leaf relative water content in 21 days + polyamine spermidine the same assembly without spermidine poly amine, significantly increased (**Fig. 1**). The results show that poly amine spermidine increasing rate relative leaf water content, can tolerate much drought stress the plant increases.



**Fig. 1:** Effects of drought and spermidine on relative water content (RWC) of lime leaves. For each date, means followed by the same letter are not significantly different at the 95% probability level (DMRT). Statistical analysis is shown only for the dates when significant differences were obtained.

**Effects of Drought and Spermidine on Amino Acid Proline in Leaves:**

Rate of increase in proline increased. Means to enter the plant with the amount of stress this amine acid was added to the seedlings (Fig. 2). Plants synthesize the amine acid tolerance threshold of the stress applied to the high range. But this increase in proline concentration to a limit is possible, and increasing plant stress tolerance can not stress conditions. Applying the polyamine spermidine rate of this increase was more proline. So that the plants were under treatments significantly in their amine acid proline levels increased (Fig. 2). Perhaps one of the reasons that polyamine increasing resistance to environmental stress in plants is, is the increase in proline concentration. With smoking in comparison with poly amine normal stress conditions increased proline concentration in the plant.

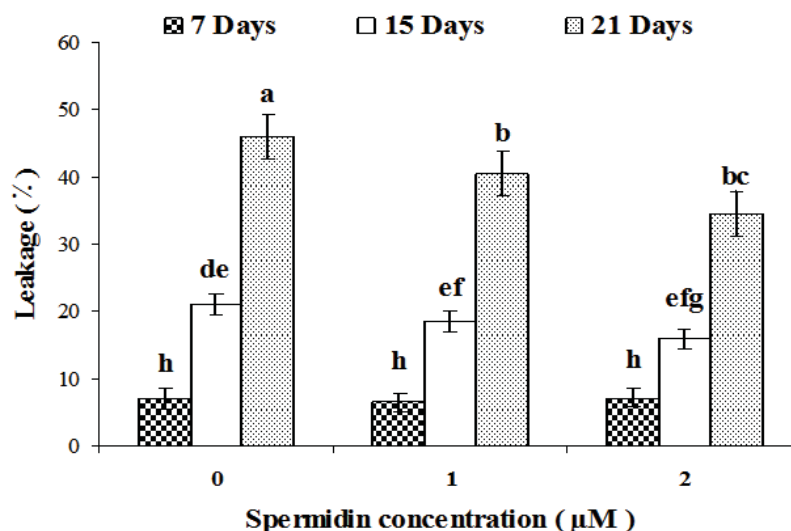


**Fig. 2:** Effects of drought and spermidine on proline accumulation in lime leaves. Accumulation of proline was enhanced by drought and spermidine in lime leaves. More proline concentration found in the leaves of lime leaves under drought and spermidine. For each date, means followed by the same letter are not significantly different at the 95% probability level (DMRT). Statistical analysis is shown only for the dates when significant differences were obtained.

**Effects of Drought and Spermidine on Leakage in Leaves:**

Percent damage to the cell membrane irrigation 7 days was very small, but with increasing irrigation rate of ion leakage and damage to the cell membrane also increased (Fig. 3). Spermidine using the improved status

and reduce cell membrane ion leakage compared with control cells decreased the stability and more resistance and cell membrane and the stress was increased the concentration of polyamine spermidine zero concentration of amount of damage to cell membranes significantly reduced, and the percentage of cell membrane stability significantly increased (Fig. 3).



**Fig. 3:** Effects of drought and spermidine on Percent damage to the cell membrane (leakage) of lime leaves. For each date, means followed by the same letter are not significantly different at the 95% probability level (DMRT). Statistical analysis is shown only for the dates when significant differences were obtained.

#### **Discussion:**

Drought causes adverse effects on plant growth and productivity (Das and Parida, 2005). Lime is semi-sensitive to drought. Drought stress resulted in the increase of membrane damage (Fig. 3) and RWC (Fig. 1) the suppression of growth of lime plants (Table 1). It has been suggested that the free radical scavenging of plants is an important component of the stress protective mechanism (Verma and Mishra, 2005), and higher antioxidant enzyme activity is closely correlated with drought tolerance (Das and Parida, 2005). Polyamines are involved in plant defense to environmental stresses (Bouchereau *et al.*, 1999). In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance polyamine biosynthesis in response to environmental stresses including drought. Suggested that the initiation of polyamine accumulation requires an osmotic signal (Imai *et al.*, 2004) also suggest that an osmotic, rather than ionic effect is the main signal triggering the polyamine response under drought. Long-term drought disturbed polyamine homeostasis in lime roots. It may act as a protective for the plasma membrane against stress damage by maintaining membrane integrity (Roy *et al.*, 2005), preventing superoxide-generating NADPH oxidases activation (Shen *et al.*, 2000) or inhibiting protease and RNase activity (Bais *et al.*, 2002). Recently, another mechanism of spermidine action has been proposed, suggesting that Spermidine may act as a signaling regulator in stress signaling pathways (Kasukabe *et al.*, 2004).

On the other hand, exogenous spermidine application alleviated growth inhibition of *Brassica juncea* (Verma and Mishra, 2005) and improved grain yield of rice plants under drought (Velikova *et al.*, 1998). These results indicate that the accumulation of free spermidine may be detrimental for growth and development of lime plants. These conjugated forms of polyamines could be a valuable source of free polyamines under conditions that demand their presence in active forms (Tonon *et al.*, 2007). Also, they have the potential to act as free radical scavengers (Bouchereau *et al.*, 1999). In the present study, among the enhanced polyamines, the insoluble bound spermidine content was most different between the lime under either drought conditions or spermidine application conditions. Therefore, it seems that the bound spermidine is most closely implicated in the drought tolerance of lime. These polyamines may serve as membrane surface stabilizers through interaction with phospholipids or other negatively charged groups of membrane (Wan *et al.*, 2006). In addition,

**Table 1:** Effect of water stress and spermidine treatment on shoot and root length [cm] and shoot and root fresh and dry masses [g] of lime seedlings.

Spermidine [mM]	Irrigation length (day)	Shoot Length (cm)	Shoot fresh mass(g)	Shoot dry mass (g)	Root length (cm)	Root fresh mass(g)	Root dry mass (g)
0	7	54.3 cd	19.5de	9cd	15.1fg	11.3de	4.1gh
	15	50.8 de	18ef	8.7de	17.8de	11.1de	4.2gh
	21	37.6 hi	10h	5.1fg	27.1b	10.5fg	5.1f
1	7	53.2 cd	21.3c	9.1cd	15.9fg	11.3cd	6.3de
	15	54.1 c	19.1d	9de	18.1d	12.1bc	6.4cd
	21	40.5 gh	13.1g	6.5f	28.1ab	11de	6de
2	7	58.6 ab	24.9ab	11.2ab	14.1fg	15.1a	7.1a
	15	60.4 a	26.8a	12.1a	21.2c	13.1ab	6.5bc
	21	48.1 ef	18.1ef	10.3bc	30.3a	11.1de	6.8ab

For each plant part, means in a column followed by the same letter are not significantly different at the 95% probability level (DMRT).

they have a function to protect against stress via their stabilizing protein structure and preventing the proteins from degradation by conjugation to proteins (Verma *et al.*, 2005; Waie *et al.*, 2003). Photosynthesis is one of the most important processes inhibited under drought. In recent years, there have been several reports establishing that polyamines, especially the thylakoid-bound polyamines, participate in the regulation of structure and function of photosynthetic apparatus under environmental conditions, like UV-B radiation (Wne *et al.*, 2006), low temperature (Urano *et al.*, 2003) and drought (Imai *et al.*, 2004). The proposed mechanism is based on polyamines inducing the reorganization of the photosynthetic apparatus and bounding to the photosynthetic complexes, like thylakoids, the light-harvesting complex and the PSII complex. However, Beauchemin *et al.* (2007) suggested that interaction of polyamines with proteins of PSII led to strong inhibition of photosynthetic oxygen evolution and PSII activity. Unfortunately, these results do not elucidate the possible roles of spermidine in increasing the drought tolerance of the photosynthetic apparatus in lime.

Oxidative damage in plants by acting as direct free radical scavengers or binding to antioxidant enzyme molecules to scavenge free radical (Bais *et al.*, 2002). Higher endogenous levels of polyamines, particularly spermidine are positively correlated with greater increase in antioxidant. Suggest that Spermidine accumulation is able to land through osmotic effects, metabolism and growth affect the plant.

As a result of osmotic effects, ions can be made by excessive accumulation of plant nutrition and balance are impaired. With increased irrigation and to long periods of drought, in addition to creating serious damage to plant, dry weight of plant tissue will decrease. That this subject suggests that the drought has reduced plant growth, and cause damage to plant tissue is. The accumulation of large amounts of some ions, the ions toxicity effects occur. The results of this study show that increased periods of drought, reducing the rate of growth and total plant biomass is associated with. This article shows that plant growth by water stress mainly to prevent photosynthetic activity is. The results show that stress leaves and stem growth more than root growth is affected.

Plants under drought stress conditions acting gathering compatible metabolism, such as proline are; seem to visitors of such substances as plant protection against stress must play. Studied in seedlings of this experiment, plants in response to drought stress action to proline accumulation.

Increasing irrigation intervals increased damage to the cell membrane, the percent damage to the plants under drought conditions with increasing irrigation intervals significantly increased compared to control plants, which indicates cell strength is less drought conditions.

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