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ARTICLE

EFFECT OF SALICYLIC ACID PRETREATMENT ON SEED GERMINATION OF 16 DIFFERENT ORNAMENTAL LANDSCAPE ANNUALS UNDER DROUGHT STRESS

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ABSTRACT

Water deficit is one of the most important problems in arid and semi- arid regions. In drought condition, using some plant growth regulators such as salicylic acid might be an effective strategy to prevent destructive effect of drought and provide plant compatibility. In this investigation, seeds of 16 ornamental annuals (Zinnia elegans 'Lilliput rose', Ageratum houstonianum 'Blue Danube', Catharanthus roseus' Bright Eye, Catharanthus roseus 'Carmine', Petunia hybrida Red, Petunia hybrid,Celosia plumose,Gomphrena gnome 'Pink',Gomphrena gnome' Purple' (Buddy), Gomphrena gnome 'White', Callistephuschinensis'Milady Mix',Tagetspatula'Hero Harmony',Tagetserecta 'Discovery Yellow',Calendula officinalis,SanvitaliaprocumbensandPennisetumglaucum 'Purple Majesty') were chosen for study. The experimental was conducted as a factorial using a completely randomized design (CRD) with 3 replications. Drought stress at 2 levels (-0.33 and -3.75 bar) was imposed using polyethylene glycol (PEG 6000) and SA at (0 and 1 mM) was also prepared. Germination percentage, germination rate, root and shoot length, shoot fresh and dry weight were measured at the experiment.There were significant differences between germination ability both in percent and rate among 16 species and stress levels (p<0.01). SA showed no noticeable effect as not considered. Except for shoot fresh and dry weight interaction effects of species×stress for all studied traits were significant (p<0.01). Germination percent reduction was most recognized in Callistephuschinensis 'Milady Mix', Gomphrena gnome 'White', Catharanthus roseus 'Bright Eye with 78.75% , 72.38% and 48.27% reduction respectively compared to control. Overall it was obvious that germination abilities in this experiment can be pointed to species characteristics itself and secondly to drought stress.

KEY WORDS
Landscape features, environmental stress, water limitation, aesthetic quality

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INTRODUCTION

Drought is one of the major physical parameter of an environment, which determines the success or failure of plants establishment [1]. Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world [2]. Stages of seed germination and seedling emergence are critical stages for plant establishment in crops grown in arid and semi-arid regions. It is at these critical stages that crop stand density and final yield are determined [3]. It has been reported that water stress can reduce or delay germination or completely prevent germination [4]. Drought inhibited germination and seedling growth in okra (Hibiscus esculents L.) [5]. Drought decrease germination and seedling growth, and these are one important case to produce crops [1]. The studies that have been conducted show that the factor of origin is also an important factor that determines the tolerance for drought stress in addition to the species [6]. Drought tolerance screening related to Polyethylene (PEG) induced water stress has successfully been performed in many agricultural plant species such as *Matricariachamomilla*[7], Soybean [8], *Helianthusannuus*[9], *Triticumaestivum*[10]. Plants produce proteins to react biotic and abiotic stresses. These proteins were induced by some phytohormones such as salicylic acid (SA) and Ascorbic acid (As) [11]. These compounds can decrease drought effects in plants under stress. SA has been found to play a key role in the regulation of plant growth, development and in responses to environmental stresses [12]. Further, its role is evident in ion uptake and transport photosynthetic rate, stomatal conductance and transpiration [13]. Several methods of SA application (seeds soaking prior to sowing, adding to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress by inducing a wide range of processes involved in stress tolerance mechanisms [14]. At sowing time, water shortage lead to irregular seed germination and unsuccessful seedling growth, which have negative effects on the yield [15,16] Seed germination is the most sensitive stage to abiotic stress [17,1819]. It has been reported that water stress can reduce or delay germination or completely prevent germination [4]. Effects of drought stress reported that emergence was the most and germination was the least affected. Because

of water limitations and the importance of using hardy plants for near future, planning a research for such plants seems to be necessary.
The aim of this study were to identify the impact of drought stress and salicylic acid pretreatment on germination response of 16 ornamental annuals common in landscape .

MATERIALS AND METHODS

Experimental conditions and treatments

This study was conducted in the laboratory of the Department of horticultural Science and Landscape, Ferdowsi University of Mashhad, Iran during 2014. In this investigation, seeds of 16 ornamental annuals (Zinnia elegans 'Lilliput rose',Ageratum houstonianum 'Blue Danube', Catharanthus roseus'BrightEye, Catharanthus roseus'Carmine', Petunia hybrida Red, Petunia hybrid,Celosia plumose,Gomphrena gnome 'Pink',Gomphrena gnome' Purple' (Buddy), Gomphrena gnome 'White', Callistephuschinensis'Milady Mix',Tagetspatula'Hero Harmony', Tagetserecta 'Discovery Yellow',Calendula officinalis,SanvitaliaprocumbensandPennisetumglaucum 'Purple Majesty') were chosen for study. The experimen was conducted as a 2x2 factorial based on a completely randomized design (CRD) with 3 replications. Drought stress at 2 levels (-0.33 and -3.75 bar) was imposed using polyethylene glycol (PEG 6000) and SA at (0 and 1 mM) was also treated as alleviator. Seeds were surface sterilized using 3% sodium hypochlorite solution for 60 second and then rinsed three times with sterile distilled water. The first group of the seeds was soaked in distilled water while the second group was soaked in 1Mm salicylic acid for 12 hours. After soaking period the seeds were air dried. Germination test was conducted by three replications of 25 seeds from every treatment in 9 cm petri dishes.

The water potential of the germination substrates (-0.33 and -3.75 bars) was determined using PEG-6000 solution, prepared as described by Michel and Kaufmann (1993) the following formula was used:

$$\psi_s = -(1/018\times10^{-2})C - (1/18\times10^{-4})C^2 + (2/67\times10^{-4})CT + (8/39\times10^{-7})C^2T$$

where ψ_s is osmotic pressure in terms of bar, C is the concentration of PEG- 6000 in g/kg H_2O and T is the temperature in degrees celsius.

The experiment was conducted in a germination chamber at 25±3°C and daily observations were recorded for 10 days. Germination percentage (GP), germination rate (GR) (seeds day^{-1}), root and shoot length (cm), shoot fresh and dry weight (mg plant^{-1}) were finally measured at the end of the experiment. Seeds were considered to have germinated when radical had emerged and elongated by at least 2 mm [20].
Dry weights were measured after drying shoots at 80° C for 48 hours in an air oven.

Germination percent and Germination rate

The germinating seeds were counted at regular intervals. The germination percentage was calculated as follows:
Germination Percentage = n/N×100
Where n is the number of germinated seeds, and N is the total number of seeds [21].
Rate of germination (seeds day^{-1}) was estimated using Maguire's equation [22].

$$GR = \Sigma\ Si/Di$$

GR= germination rate (The number of germination seeds per day), Si is the number of germinated seeds at each counting and Di is the number of days to nth count.
Data were analyzed as factorial ANOVA using JMP8. Where significant ($p\leq0.05$), data means were separated by the LSD test.

RESULTS

Results showed with increasing in drought stress, there was significant difference between treatments in all evaluated traits germination rate, germination percent, root and shoot length. ($p<0.01$) [Table 1]. Results showed that, there are significant differences between germination ability both in percent and rate among 16 species. Stress also reduced the germination in some species markedly. Analysis of variance and mean comparison of this experiment shows that using salicylic acid could not increase plant tolerance to drought stress.

Table 1: Analysis of variance among studied traits of 16 ornamental annualsns, and ** not significant and significant at 0.01 percent levels of probability, respectively

SV	Df	Germination percentage	Germination rate	Root length	Shoot length	Shoot fresh Weight	Shoot dry weight
Species	15	5046.38**	262.20**	122.84**	23.676**	0.08ns	0.0000233ns
Stress	1	13197.02**	634.73**	28.61**	87.68**	0.10ns	0.0000370 ns
SA	1	33.67 ns	0.57 ns	0.96 ns	0.28 ns	0.07 ns	0.0000108 ns
Species × Stress	15	1181.92**	51.05**	6.97**	2.53**	0.07 ns	0.0000140 ns
Species ×SA	15	171.29 ns	7.39 ns	1.08 ns	0.24ns	0.07 ns	0.0000102 ns
Stress ×SA	1	12.81 ns	9.73 ns	0.31 ns	0.01 ns	0.07 ns	0.0000262 ns
Species×Stress×SA	15	142.26 ns	4.85 ns	0.48 ns	0.34 ns	0.06 ns	0.0000076 ns
Error	128	156.49	7.27	0.63	0.27	0.07	0.000026
Total	191	678.96	33.88	10.95	2.78	0.071	0.00000001
C.V		39.65	48.72	91.78	63.19	698.02	168.69

Germination percent

According to the results of this experiment, drought stress significantly affected germination percent (p≤0.01). These results were also observed for different species [table 1]. Under drought stress treatment, germination percentage of *Callistephuschinensis*'Milady Mix', *Gomphrena gnome* 'White', *Catharanthus roseus*'Bright Eye',*Catharanthus roseus*'Carmine' and *Celosia plumose* decreased 78.75% , 72.38%, 48.27%, 29.24% and 27.87% compared to control respectively. Although drought stress had no significant effect on germination percent of *Zinnia elegans* 'Lilliput rose', *Calendula officinalis,Gomphrena gnome* 'Pink',*Tagetspatula*'Hero Harmony',*Tagetserecta* 'Discovery Yellow', *Ageratum houstonianum* 'Blue Danube',*Sanvitaliaprocumbens,Pennisetumglaucum* 'Purple Majesty', *Petunia hybrida* Red, *Petunia hybrid, Gomphrena gnome*' Purple' (Buddy) butit decreased 0.89%, 3.9%, 6.96%, 10.20%, 13.92%, 14.04%, 15%, 15.60%, 17.57%, 17.61%, and 27.10%, respectively [Table 2].

The highest germination percent (95%) was observed in *Ageratum houstonianum* 'Blue Danube'followed by *Zinnia elegans* 'Lilliput rose'(93.33%) and *Pennisetumglaucum* 'Purple Majesty (90.83%) all in 100% FC treatment and the lowest value (14.16%) was observed in *Callistephuschinensis*'Milady Mix'and *Sanvitaliaprocumbens*(18.4%) in 50% FC [Table 2].

Table 2: Mean comparison of different germination indexes for 16 ornamental annualsMeans within columns for each treatment with the same letters are not significantly different at 5% level

Treatment		Germination percentage (%)	Germination rate (day-1)	Root length (cm)	Shoot length (cm)
Seed	Stress (%FC)				
Zinnia elegans 'Lilliput rose'	100	93.33a	19.15ab	5.91ef	3.63d
	50	92.5ab	19.14ab	6.58de	2.56fgh
Ageratum houstonianum 'Blue Danube'	100	95a	19.08ab	4.8gh	4.52c
	50	81.66a-f	15.49cde	5.33fg	2.45ghi
Catharanthus roseus 'Bright Eye'	100	72.5fgh	15.06cde	1.99nop	2.05hij
	50	37.5kl	5.44j	1.42opq	1.36mno
Catharanthus roseus 'Carmine'	100	88.33a-d	16.31bc	2.74k-n	3.33de
	50	62.5hi	10.65hi	2.01nop	1.42k-o
Petunia hybrida 'Red'	100	75.83d-h	13.14d-h	2.29mno	3.03efg
	50	62.5hi	10.26hi	2.28mno	1.29nop
Petunia hybrid	100	80.83a-g	14.51f-i	3.96hi	2.98efg
	50	66.66ghi	11.58c-f	3.32i-l	2.26hi
Celosia plumose	100	86.66a-f	15.84cde	3.39ijk	2.94efg
	50	62.5hi	10.41hi	2.58k-n	1.97h-l
Gomphrena gnome 'Pink'	100	84.16a-f	15.84cde	3.84ij	3.23de
	50	78.33b-g	14.08c-g	2.97j-m	1.96i-m
Gomphrena gnome' Purple' (Buddy)	100	40jk	6.41j	0.76grs	1.51j-n
	50	29.16klm	4.95jk	0.49rs	0.75p
Gomphrena gnome 'White'	100	87.5a-e	16.13bcd	1.16pqr	2.1hij
	50	24.16 lmn	3.12jk	0.81qrs	1.14nop
Callistephuschinensis 'Milady Mix'	100	66.66ghi	10.69hi	5.48fg	5.16b
	50	14.16n	1.51k	2.99j-m	3.19de
Tagetspatula	100	81.66a-f	14.66c-f	0.45rs	1.4l-o
	50	73.33e-h	12.86e-h	0.33s	0.9op
Tagetserecta	100	62.5hi	11.05ghi	1.03grs	1.13nop
	50	53.8ig	9.51i	0.89grs	0.98nop

Calendula officinalis	100	77.5c-g	13.58c-g	8.47c	7.01a
	50	74.5d-g	12.97c-g	7.08d	3.13def
Sanvitaliaprocumbens	100	21.66mn	2.59jk	2.42lmn	2.01h-k
	50	18.4mn	1.64k	2.06nop	1.30nop
Pennisetumglaucum 'Purple Majesty'	100	90.83abc	19.65a	15.62a	6.96a
	50	76.66c-h	15.44cde	9.92b	4.70bc

Germination rate

According to the results of this experiment, drought stress and species significantly affected germination rate ($p \leq 0.01$). [Table 1].

Under drought stress treatment, there was nearly no decrease in germination rate of *Zinnia elegans*'Lilliput rose', but it significantly decresed for *Callistephuschinensis* 'Milady Mix', *Gomphrena gnome* 'White', *Catharanthus roseus* 'Bright Eye', *Catharanthus roseus* 'Carmine', *Celosia plumose*, *Pennisetumglaucum* 'Purple Majesty' and *Ageratum houstonianum* 'Blue Danube' 85.96%, 80,65%, 63.87%, 34.7%, 34.27%, 21.42% and 18.81% respectively.[Table 2]. The highest germination rate (19.65 day^{-1}) was observed in *Pennisetumglaucum* 'Purple Majesty' followed by *Zinnia elegans*'Lilliput rose'(19.15 day^{-1}) and *Ageratum houstonianum* 'Blue Danube'(19.08 day^{-1}) all in 100% FC treatment and the lowest value (1.51 day^{-1}) was observed in *Callistephuschinensis*'Milady Mix'followed by *Sanvitaliaprocumbens*(1.64 day^{-1}) both in 50% FC [Table 2].

Root length

According to the results of this experiment, drought stress and species significantly affected root length ($p \leq 0.01$). [Table 1]. Drought stress showed different results for root length ondifferent species. The decrese were 45.44%, 36.49% and 16.41% for*Callistephuschinensis*'Milady Mix', *Pennisetumglaucum* 'Purple Majesty' and *Calendula officinalis* , respectively [Table 2].

The root length of *Zinnia elegans* 'Lilliput rose' and *Ageratum houstonianum* 'Blue Danube' were longer than the control when the seeds were treated with drought stress and this increase were, 11.33% and 11.04% respectively.

The highest and the lowest root length (15.62 and 0.33 cm) were observed in *Pennisetumglaucum* 'Purple Majesty' and *Tagetspatula*'Hero Harmony'in 100% and 50% FC treatment, respectively. [Table 2].

Shoot length

According to the results of this experiment, drought stress and species significantly affected shoot length ($p \leq 0.01$). [Table 1]. Drought stress treatment, decreased the shoot lengthof all cultivars compared to their relative control,but it was not significant for *Gomphrena gnome* 'Pink', *Tagetspatula* 'Hero Harmony' and *Tagetserecta*'Discovery Yellow'.
The highest shoot length (7.01 cm) was observed in *Calendula officinalis* in 100% FC treatment and the lowest value (0.75 cm) was observed in *Gomphrena gnome*' Purple' (Buddy) in 50 % FC [Table 2].

Shoot and root fresh and dry weight

The results indicated that shoot and root fresh and dry weight of plants were not significantly affected by drought stress and species. but($p \leq 0.01$) [Table 1].

DISCUSSION

These results indicated that drought stress treatment markedly decreased the germination percentage of all plants compared to their relative controls. Seed germination is negatively affected by drought stress [23]. If water absorption disturbed or delayed, it could delay in seed metabolic processes. Drought stress had the least effect on germination percent of Zinnia elegans 'Lilliput rose' and Calendula officinalis, whereas the germination percent of Callistephuschinensis 'Milady Mix' and Gomphrena gnome 'White' declined clearly under drought stress compared to their relative controls. Seeds for germination process must absorb enough water. Soluble material in the medium such as polyethylene glycol reduces water absorption and subsequent seed germination is delayed or stopped [24]. Drought stress treatment, had nearly no decrease in germination rate of Zinnia elegans 'Lilliput rose' but it decreased in other plants compared to their relative controls. The germination rate under drought stress can show the germination ability of drought resistance of various species [25]. It is resulted that time for radicle outing increased and therefore, germination rate is decreased [26]. This suggested that Zinnia elegans 'Lilliput rose' germinated more quickly than other plants, showing a stronger drought resistance of Zinnia elegans 'Lilliput rose'. Due to osmotic pressure loss under drought stress, water absorption process is disrupted and the alpha-amylase enzyme activity is inhibited [27]. Root is an important absorption organ of plants, developed level of roots is one of the main indexes of drought resistance [25].The ability to develop extensive root systems contributes to differences among cultivars for drought tolerance and root length is considered an important trait in selection of drought resistant cultivars [28,29]. In our study, root length decreased under drought stress treatment, more in Callistephuschinensis 'Milady Mix' than in the other plants compared to their relative controls, indicating a higher sensitivity to drought stress in this cultivar (species). The root length reduction in Callistephuschinensis 'Milady Mix' under drought stress may be associated to a reduced cellular division and elongation during germination [30]. Reduction of root length with increasing in water potential has been reported by Takel (2000).This results indicated the roots that growth under drought stress were longer but they were very thin and delicate. The root length of Zinnia elegans 'Lilliput rose' and Ageratum houstonianum 'Blue Danube' were longer than their control when the seeds were treated with drought stress suggesting that Zinnia elegans 'Lilliput rose' and Ageratum houstonianum 'Blue Danube' developed powerful root system to fully use of water under drought condition, thus showing stronger drought resistance. The results indicated that drought stress treatment, decreased the shoot length of all cultivars compared to their relative controls. Generally, germinated seeds in environments under stress conditions have shorter shoot and root [31]. With increasing drought stress, root and shoot length decreased [32]. Reducing in water absorption by seeds reduces secretion of the stress hormone and enzyme activity and it has negatively effect on seedling growth [33].

CONCLUSION

In general, drought stress imposed different reactions on ornamental plant seeds examined in this study. Results showed that, there are significant differences between germination ability both in percent and rate among 16 species. Stress also reduced the germination in some species markedly, but not in all. SA showed no noticeable effect. It seems, it is significantly related to the soak time and dosage of application of salicylic acid.

Overall it was obvious that germination abilities in this experiment can be pointed to species characteristics itself and secondly to drought stress. Therefore resistant and hardy species are suggested to be used for dry and low rainfall states. *Zinnia elegans* 'Lilliput rose' and *Ageratum houstonianum* 'Blue Danube' in this experiment, were most resistant to drought stress during seed germination and *Callistephuschinensis* 'Milady Mix' showed the least resistance.

CONFLICT OF INTEREST
There is no conflict of interest

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None

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