8.1 List of publications

1. Effect of salt stress on seedling growth and survival of *Oenothera biennis* L.

2. Impact of water deficit and salinity stress on seed germination and seedling growth of *Capsicum annuum* ‘Solan Bharpur’.

3. Studies on seed germination and seedling growth in kalmegh (*Andrographis paniculata* Wall ex Nees) under abiotic stress conditions.

Effect of Salt Stress on Seedling growth and Survival of *Oenothera biennis*L.

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Abstract

Effect of salinity on emergence, seedling growth and seed vigor of *Oenothera biennis* L., a medicinal plant was considered using different concentrations of NaCl (25 mM, 50 mM, 75 mM and 100 mM). Increasing stress regimes upto50 mM led to enhancement of seed germination. At higher salt concentrations a negative relationship between seed germination was obtained. Seedling survived and grew up to salinity of 100 mM NaCl and eventually this species is tolerant to seedling stage. Elongation of stem and root was decelerated by increasing salt stress. Though, this species has a tendency for rapid root penetration and roots are able to draw water from saline soil. Seed vigor index decreased with increasing concentration of stress.

Keywords: Stress, germination, seedling growth, seed vigor index, evening primrose.

Introduction

At present salinity is the world-wide problem and is increasing day by day due to excessive use of chemical fertilizers and saline water for irrigation, especially in arid and semi arid areas. Salinity adversely affects the germination and survival of most of glycophytes. It is also well documented in literature. So understanding the morphological responses of plants to salinity are utmost importance. Germination of seeds is the first critical and most sensitive stage in life cycle of plants and the seeds exposed to unfavourable environmental conditions like salts stress may have to compromise the seedling establishment. Salinity either completely restrains germination at higher levels or induces state of dormancy at low levels. However, plant species varies in their sensitivity or tolerance to salts. The decrease in growth under salinity is a result of many physiological responses which include alteration of water status, photosynthetic efficacy, carbon allocation and utilization.

Medicinal herbs have been extensively studied because chemical medicines have proved to have side effects and human tend to use natural products as much as possible. Moreover, medicinal plants play a monumental role in the provision of health care in many developing countries. The need is to study the medicinal plants for their halotolerance. *Oenothera biennis* L., an important medicinal plant, known as evening primrose is cultivated in Indian gardens. It is an exotic as it originated in North America. The plant is recognized as oil seed crop contains approximately 7-10 percent G-linolenic acid (GLA), an essential fatty acid. GLA has been identified as a useful treatment for a variety of ailments including blood pressure, cardiovascular disease, skin disorder and diabetic neuropathy.

The purpose of present study is to analyze the *Oenothera biennis* L. in terms of germination and seedling growth to salinity stress.

Material and Methods

**Seed Source:** Seeds of *Oenothera biennis* L., collected from Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) were used in this study. The experiment was conducted from April to May, 2013 under laboratory conditions at Shoolini University of Biotechnology and Management Sciences, Solan. The detailed schedule of study was as:

**Seedling germination:** Seeds of *Oenothera biennis* L., selected on the basis of size and colour, were surface sterilized with 0.1 percent HgCl₂ solution for 5 minutes followed by thorough washing with distilled water. Then, the seeds were soaked in distilled water (control) or in solutions of stated concentrations of effector namely 25, 50, 75, 100 mM NaCl solution for 24 hours. Afterwards, the seeds were shifted to petriplates lined with three layers of filter papers moistened either only by distilled water (control) or by effect of different NaCl concentrations of same volume. The treatments were replicated six times and each petriplate contained thirty seeds. The seeds were then allowed to germinate in an incubator at 25 ± 2°C under continuous illumination provided by fluorescent white light. Emergence of 2-5 mm radicle was taken as seed germination. The seed germination was recorded at periodic intervals for few days until the final count.

**Seedling growth and seed vigor:** After 31 days of incubation, seedling growth was measured in terms of root length, shoot length, seedling fresh weight and seed-vigor index (SVI). The seed-vigor index (SVI) was determined with the help of Seghatoleslami method. Germination percentage × means of seedling length (cm)/100. Each experiment was performed in triplicate; each replicate comprised thirty seeds. At the end of experiment, data was subjected to analysis of variance.
Results and Discussion

Effect of salinity on seedling emergence: Seedling started to emerge 3 days after sowing and 72% seed germination was obtained over period of 31 days under control as presented in figure-1. Seedling emergence was noticed on the 3rd, 5th, 7th and 9th days after sowing in 25, 50, 75 and 100 mM NaCl, respectively and seed germination percentage was 76%, 73%, 60.9% and 48% respectively. Seedling did not emerge from soils with further increase in salinity. There was significant reduction in germination of seed with increasing salt stress. As a result it has been observed that this plant species is salt tolerant at seed germination phase up to certain concentration. However, salt concentrations exceeding 50 mM NaCl were detrimental to seed germination. The diminishing germination due to raising salinity can be correlated to the nature of salinity to restrict imbibitions of water due to lowered osmotic potentials of the medium and causes changes in metabolic activity. Different studies showed that different salinity stress levels affect germination in variety of plants such as *Satureja hortensis* and *Elymus junceus*. High level of soil salinity can significantly suppress seed germination in glycophytes and halophyte plants. This suppression is because of potential osmotic effects and ionic toxicity.

Effect of salinity stress on seedling growth: Shoot and root growth inhibition is a common response to salinity. However, shoots are usually sensitive to cation interference than roots and there are huge diversity among plant species in the capacity to avoid or tolerate the excess salt concentration. Shoot length of seedling given in figure-2(a) lessened with the increase of salt treatments. Salt stress inhibits the efficacy of the translocation and assimilation of photosynthetic products and might have caused decrease in shoot growth. In the present study, increasing NaCl given in figure-2(b) resulted in decrease in root length at higher concentrations but at lower concentration of 25 mM NaCl root length increased in comparison to control. NaCl had a stimulatory effect on the growth of root up to a certain concentration. High concentration of salt results in slow down or stop root elongation and causes reduction in root production. Similarly in *Catharanthus roseus; Withania somnifera* and *Salvadora persica*, root length was reduced in comparison to untreated plants with increase in salinity, which is in consensus with the present study. However, in *Plantago ovata*, initially root first enhanced then significantly decreased with increasing concentrations of NaCl.

Seedling fresh weight in *O. biennis* L. presented in figure-2(c) also decreased in concentration dependent manner. It seems that the decreased seedling fresh weight may be because of decreased water absorption due to osmotic stress induced by NaCl, which in turn causes a reduction in the amount of water in plant tissue. Massai et al. observed that salinity decreased plant growth due to reduction in photosynthesis, closing of stomata and reduction of water entrance into the plant. In *Artemisia annua* L. seedling fresh weight decreased with increase in salinity. In *O. biennis* L. seed vigor index declines as shown in figure-3 with increasing NaCl concentration. The most seed vigor index was related to control treatment. Generally, seed vigor index is related to special influence of ions and reduction of environmental water potential in the presence of salinity. If salinity raises there is reduction of environmental osmotic potential and seed vigor index show negative trends. Liu and co-workers opined that this might be due to suppression of cell division induced by chromosomal aberrations.
Effect of NaCl on seedling shoot length (A), root length (B) and seedling fresh weight (C) of *O. biennis* L. Values are mean±SE; n=6. Analyzed by One-way ANOVA followed by Tukey’s multiple comparison test. \(^a\)p<0.05 Vs control, \(^b\)p<0.05 Vs 25 mM NaCl, \(^c\)p<0.05 Vs 50 mM NaCl, \(^d\)p<0.05 Vs 75 mM NaCl, \(^e\)p<0.05 Vs 100 mM.
Conclusion

Plants have inbuilt ability to adjust to seasonal environmental variables. Apart from the environmental variables, there may be certain other rapid and predictable environmental disturbances resulting in stressful conditions. However, plants differ in ability to tolerate such stressful situations. This study shows that salinity stress restricts the seedling growth and seed germination of *O. biennis* L., decreasing shoot length, root length, fresh weight at higher stress regimes. However, plant can grow well under mild stress conditions. The finding presented demonstrate that, in terms of the parameters being investigated and in comparison of previous reports available in the literature, *O. biennis* L. would be classified as a species tolerant to salinity during seedling growth at lower level of salinity.

References

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**Figure-3**

Seed vigor index (SVI) of *Oenothera biennis* L. under NaCl stress conditions. Values are mean ± SE; n=6. *a* vs control, *b* vs 25 mM NaCl, *c* vs 50 mM NaCl, *d* vs 75 mM NaCl, *e* vs 100 mM NaCl, analysed by One-way ANOVA followed by Tukey’s multiple comparison test.

Seed vigour (SVI) of *Oenothera biennis* L. under NaCl stress conditions. Values are mean ± SE; n=6. *a* vs control, *b* vs 25 mM NaCl, *c* vs 50 mM NaCl, *d* vs 75 mM NaCl, *e* vs 100 mM NaCl, analysed by One-way ANOVA followed by Tukey’s multiple comparison test.


Impact of Water-deficit and Salinity stress on Seed Germination and Seedling Growth of Capsicum annuum ‘Solan Bharpur’

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Abstract

In both natural and agricultural conditions plants are frequently exposed to environmental stresses. The aim of present study is to determine the adverse effects of abiotic stresses viz. water and salinity stress on the growth of Capsicum annuum ‘Solan Bharpur’ at the germination and seedling growth stage using polyethylene glycol 6000 (5% PEG and 10% PEG) and NaCl (50 mM NaCl and 100 mM NaCl). Proline, MDA and chlorophyll content were also assessed. Seed germination and seedling growth reduced with increasing concentrations of PEG or NaCl. However, PEG induced water stress caused more growth inhibition compared to NaCl induced salinity stress. Water and salinity stress caused increase in the level of proline and MDA of both shoots and roots. The total chlorophyll content decreased with an increase in water or salinity stress.

Key words: Stress, Polyethylene glycol, NaCl, Proline, MDA, Chlorophyll content.

Introduction

The term stress is often used with various meanings. The definition and appropriate term for stress are referenced as responses to different situations. The flexibility of normal metabolism allows the development of responses to environmental changes; those fluctuate regularly and predictably over daily and seasonal cycles. Abiotic stresses including drought and salinity stress are currently the major factors which reduce crop productivity worldwide. Excessive amount of salts in soil severely reduced the seed germination and further seedling growth. Water deficit is defined as the absence of adequate moisture necessary for a plant to grow normally. When a plant is exposed to high salinity and water stress, its major processes such as lipid peroxidation, photosynthesis and protein synthesis are affected.

India is the second largest producer of vegetables in the world but production of vegetables is much less than the daily requirement of increasing population of India. Efforts are to increase the production of vegetables at national level. It is noticed in a report of Indian Council of Agricultural Research that the present production of 90.8 million tonnes needs to be raised to 250 million tonnes by 2024-2025. Nutritionally, vegetables are important as they are rich source of vitamins, minerals and dietary fibers. From many years growing vegetables has been the mainstay of rural economy and has emerged as an indispensable component of agriculture. Of the various vegetables capsicum’s nutritive value is high as it contains 1.29 mg protein, 11 mg calcium, 870 I.U. vitamin A, 17.5 mg ascorbic acid, 0.6 mg thiamin, 0.003 mg riboflavin and 0.55 mg niacin per 100 g of edible fruit. It is also a good source of ascorbic acid, tocopherols, provitamin A, carotenoids including lutein, β-carotene, capsanthin and violaxanthin which contribute to antioxidative activity. The major objective of present investigation is to study effects of water deficit and salinity stress on germination and growth of seedling, free proline, MDA level and chlorophyll contents in C. annuum ‘Solan Bharpur’ which is selection from cross between california wonder and chilli.

Material and Methods

Mature seeds of Capsicum annuum ‘Solan Bharpur’ were collected from Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. India. Seeds of C. annuum ‘Solan Bharpur’ selected for uniformity were surface sterilized, washed thoroughly under tap water and soaked in water (control), 5% PEG, 10% PEG, 50 mM NaCl and 100 mM NaCl solution for 24 hours. Thereafter, the seeds were transferred to petriplates lined with three layers of filter papers moistened either only by distilled water (control) or by effect of PEG or NaCl concentrations of same volume. The seeds were then allowed to germinate in BOD incubator at 25 ± 2°C under continuous illumination provided by fluorescent white light. Emergence of 2.5 mm radical was taken as seed germination. After 25 days of germination, seedling growth was measured in terms of root length; shoot length and seedling fresh weight. Seedlings, 15 days old were shifted to hydroponic culture containing Hoagland nutrient solution and allowed to grow in BOD incubator at 25±2°C for further growth. Nutrient medium was replaced by fresh one at regular intervals. After 14 days of shifting to BOD the plants were treated with 5% PEG, 10% PEG, 50 mM NaCl and 100 mM NaCl through appropriate addition to the nutrient medium. After 21 days of treatment, determination of proline, lipid peroxidation and chlorophyll contents were done.
Determination of free proline content: Free proline was estimated spectrophotometrically following the method of Bates et al. The leaves / roots weighing 200/150 mg were homogenized with 3 % sulphosalicylic acid (SSA). The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. Supernatant (2ml) was reacted with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 Molar orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling water bath at 100°C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the calibration curve.

Lipid peroxidation: Lipid peroxidation was estimated from accumulated malondialdehyde (MDA) following the method given by Dhindsa et al. Leaves or roots were used as test material. The leaf / root weighing 200/150 mg were homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. Supernatant (2ml) was reacted with 4 ml of 0.5 % thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and kept at 95 °C in a water bath for 30 minutes. The reaction was terminated by cooling the reaction mixture in ice for 5 minutes. Absorbance was read at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600nm. MDA content was determined using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Chlorophyll estimation: Leaf tissue weighing 100 mg was homogenized with 80% acetone. The homogenate was centrifuged at 5000 rpm for 3 min. Final volume was made approximately 5 ml with 80% acetone. The absorbance of extract was read at 663 and 645 nm. Amount of total chlorophyll, chlorophyll a (chl a) and chlorophyll b (chl b) was estimated by using following equations given by Harborne:

\[
\text{Chl a (mg/g)} = \frac{12.3 A_{663} - 0.86 A_{645}}{ax1000 \times w} \times v
\]

\[
\text{Chl b (mg/g)} = \frac{19.3 A_{645} - 3.6 A_{663}}{ax1000 \times w} \times v
\]

In these equations; v corresponds to volume (ml), a to length of path of light i.e. 1 cm and w to fresh weight of tissue (g).

Statistical analysis: The experiments had completely randomized design and each experiment was repeated at least thrice. Data are analyzed using T- test at p=0.05 for significance. The standard error is plotted in all graphics.

Results and Discussion: In the present study the effects of water deficit and salinity stress were monitored on seed germination and seedling growth. Seed germination and early seedling growth are the most vulnerable stages of the plant life cycle where plants are frequently subjected to water-deficit during the dry season or grown in salty lands. Salt and drought stresses are two most important abiotic stresses that limit the number of seedlings and seedling growth. C. annuum ‘Solan Bharpur’ seeds were non-dormant and responded differentially to PEG (water deficit) and NaCl concentrations (figures 1-2). Seeds germination in control started on 6th day of incubation and thereafter progressed gradually. Seed germination rate decreased at 5% PEG, 10% PEG and 100 mM NaCl. However, at 50 mM NaCl the germination increased. PEG treatments proved to be inhibitor of germination. But NaCl pre-treatments of seeds increased the germination of seeds at lower concentration. Interestingly, the seed germination initially imposed by NaCl got diminished with lapse of time. Since NaCl was applied only once, it is likely that NaCl concentration got diluted due to the growth of embryo and eventually seedlings.

The seedling growth of C. annuum ‘Solan Bharpur’ was measured in terms of seedling root length, shoot length and fresh weight as shown in figure 3. Root length of seedling increased at 50 mM NaCl concentration but it decreased at 5% PEG, 10% PEG and 100 mM NaCl. Seedling shoot length and seedling fresh weight decreased in all treatments (5% PEG, 10% PEG, 50 mM NaCl and 100 mM NaCl) compared to control. The root length of C. annuum ‘Solan Bharpur’ in PEG treatments decreased with an increase in concentration. This suggest that C. annuum ‘Solan Bharpur’ can tolerate drought up to a certain degree as root length is an important trait against drought stress in several plant species. But in case of NaCl root length increased at lower concentration (50 mM) but decreased at higher concentration (100 mM). An increased root growth might be due to mild salinity stress. Reduction in root length at higher concentration in response to salinity may be due to Na⁺ and Cl⁻ ions in growing media. The Na⁺ and Cl⁻ affects root permeability due to displacement of Ca⁺ ions from the plasma lemma, which inhibits roots growth and root length. Shoot length and seedlings fresh weight decreased with PEG and NaCl treatments which is similar to several earlier reports.

In another experiment, physiological / metabolic responses of C. annuum ‘Solan Bharpur’ under water and salinity stress were assessed in hydroponic system. The free proline content was measured in leaves and roots of C. annuum ‘Solan Bharpur’ after treating the plants with PEG and NaCl treatments in hydroponic culture for twenty one days (Figure 4). The proline content in leaves and roots increased significantly with an increase in PEG and NaCl concentration. The free proline content in leaves was 41.62 %, 44.97 %, 38.0 % and 44.1% greater than control at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl treatments, respectively. Likewise, the roots free...
proline content was 22.7 %, 29.16 %, 14.14 % and 22.7 % higher than control at the same treatments. It was observed that the increase in free proline content induced by the PEG treatments was much more than induced by NaCl treatments. It is evident from figure 4 that increase in proline content in leaves was much more as compared to roots at both PEG and NaCl treatments than control. The most prominent function of proline might be to act as an osmoregulator and thus keeping cells turgid. Free proline accumulation in water stressed plants has also been suggested to serve as an index of drought hardness; higher proline accumulation being a characteristic of resistant cultivars. Free proline has also been demonstrated to act as an antioxidant.

The MDA content in leaves and roots was measured as an index of lipid per oxidation. The quantitative estimation of MDA content was done in leaves and roots in figure 5. MDA content increased with an increase in concentration of NaCl. It also increased in 10 % PEG treatment more than 5 % PEG treatment. MDA content present in leaves was always higher than control and was 68.8, 83.8, 66.0 and 75.5 n mol g⁻¹ FW at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl treatments, respectively. In roots also the MDA content was higher than control and was 9.3, 11.0, 9.0 and 10.0 n mol g⁻¹ FW at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl treatments, respectively. But increase in MDA content was more in leaves as compared to roots. Generally, abiotic stresses caused an extensive lipid per oxidation which has been often used as an indicator of stress induced oxidative damage of membrane. The increase in MDA contents might be due to the increased antioxidative enzymes which have scavenged various reactive oxygen species (ROS) produced due to abiotic stresses.

The content of chlorophyll a in leaves decreased with an increase in PEG concentration. Likewise, the content of chlorophyll b in leaves decreased with an increase in PEG concentration. It is evident from figure 6 that chlorophyll a content was higher in leaves as compared to chlorophyll b. The chlorophyll a: b ratio altered marginally with the PEG (5 % or 10 %) and NaCl (50 or 100 mM) treatments. The total chlorophyll content of leaves decreased with an increase in PEG and NaCl concentrations. The magnitude of reduction was more in PEG treated plants than in NaCl treatments (Figure 6). The chlorophyll content was suppressed by both stresses; magnitude of reduction being greater in PEG treatments. Our results of decrease in chlorophyll content corroborated with the findings of, who also found a decrease in chlorophyll content with NaCl stress in five widely cultivated rice (Oryza sativa L.). The loss in chlorophyll content lead to disruption of photosynthetic machinery. Moreover, the high activity of chlorophylls enzyme which activated by salinity lead to chlorophyll reduction.

**Conclusion**

Plants have inbuilt ability to adjust to seasonal environmental variables. Apart from the environmental variables, there may be certain other rapid and predictable disturbances in environment resulting in stressful conditions. Plants are exposed to many stress conditions such as low temperature, salt, drought, flooding and heavy metal toxicity. The capacity for a plant species to adjust these stresses is usually limited and varies from plant to plant. The findings from present study reveal the nature and magnitude of the responses of C.annuum ‘Solan Bharpur’ important vegetable of mid hill of Himachal Pradesh to water deficit and salinity stress. Stress effects are evident from germination to plant growth. Water deficit and salinity stress seem to involved in observed growth suppression where as proline and MDA accumulation might help plants to stressful conditions to some extent.

![Germination Graph](image1)

**Figure 1**

Time–course of germination of C. annuum ‘Solan Bharpur’ seeds as affected by 5 % PEG (A) and 10 % PEG (B) treatments. The values are mean ± S.E.
Time-course of germination of *C. annuum* ‘Solan Bharpur’ seeds as affected by 50 mM NaCl (A) and 100 mM NaCl (B) treatments. The values are mean ± S.E.

Figure 2

Effect of PEG (%) and NaCl (mM) treatments on root length (A); shoot length (B) and seedling fresh weight (C) Growth measured after 25 days of incubation. Values are mean ± S.E. (n=15)

Figure 3
Figure 4
Effect of PEG-6000 and NaCl on free proline content in leaves and roots *C. annuum* ‘Solan Bharpur’. Seedlings (15 days old) were grown hydroponically for 14 days and subsequently exposed to stress for 21 days. Values are mean ± S.E.

Figure 5
Effect of PEG-6000 and NaCl on MDA contents in leaves and roots of *C. annuum* ‘Solan Bharpur’. Seedlings (15 days old) were grown hydroponically for 14 days and subsequently exposed to stress for 21 days. Values are mean ± S.E.
Effect of PEG and NaCl on chlorophyll a and b (A), chlorophyll a: b ratio (B) and total chlorophyll content (C) of C. annuum ‘Solan Bharpur’. Seedlings (15 days old) were grown hydroponically for 14 days and subsequently exposed to stress for 21 days. Values are mean ± S.E

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References


STUDIES ON SEED GERMINATION AND SEEDLING GROWTH IN KALMEGH (ANDROGRAPHIS PANICULATA WALL. EX NEES) UNDER ABIOTIC STRESS CONDITIONS

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Abstract: The changing environmental conditions and increasingly heavy metal concentration in soil has been a major concern for growth of medicinal plants widely used in pharmaceutical and ayurvedic formulations. The need is to develop effective means to regenerate and grow medicinal plants under changing environmental conditions. The realization of this strategy requires an understanding of agronomic requirements of the medicinal plant species including the responses to variations in growth conditions on account of abiotic stresses. Present study is an attempt to determine the influence of abiotic stress viz., water-deficit (PEG), salinity (NaCl) and heavy metal (Cd), germination, seedling growth and seed vigour index (SVI) of Kalmegh (Andrographis paniculata Wall. ex Nees), an important indigenous medicinal plant of Acanthaceae family. Seed germination was enhanced by all the three factors tested i.e. PEG, NaCl and Cd; the degree of enhancement was found to be maximum in case of PEG. Seedling growth and SVI was suppressed by PEG, NaCl and Cd. Cadmium stress was most inhibitory.

Keywords. Kalmegh, Medicinal plant, Stress, Germination, SVI.

1. INTRODUCTION

Kalmegh (Andrographis paniculata Wall. Ex Nees) belonging to the family Acanthaceae has wide range of medicinal and pharmacological application. It is also known as ‘King of bitters’, Maha-tita or bhui-neem because of its similarity in appearance and bitter taste as that of neem (Azadirachta indica A. Juss) though it is much smaller in size (Niranjan et al. 2010). Kalmegh is one of the nineteen species of the genus Andrographis, which is indigenous to India. It is an active constituent in majority of Ayurvedic preparations and is official in the Ayurvedic Pharmacopoeia (Rammohan et al. 2011). It is distributed in tropical Asian countries having hot and humid climatic conditions but it can be cultivated in subtropical regions during the monsoon season (Niranjan et al. 2010; Kumar, 2011). The herb is having a preventive effect from many diseases, due to its powerful immune strengthening benefits
(Chauhan, 2009). The therapeutic activities of this plant are attributed to andrographolide and related diterpens i.e., deoxyandrographolide, 14- deoxy-11, 12- didehydro-andrographolide and neo-androrapholide (Bahn, 2006). The demand of Kalmegh is increasing day by day (Chauhan, 2009). The propagation of kalmegh generally occurs through seeds, inspite of several germination problems. The production of any crop heavily relies on quality of planting seeds and for producing good quality seeds it would be desirable to have information regarding germination and associated germination parameters like germination energy, germination period etc.(Kumar, 2011).

During their life-cycle, plants experience a variety of abiotic stresses. Among the major abiotic stresses that affect the plant growth and yield are water-logging, drought, high or low temperature, excess soil salinity, heavy metals, inadequate mineral nutrients in the soil and too little or too much light. They cause considerable (upto 80%) economic losses in agriculture. Water-deficit, salinity and heavy metal stress affect the water relations of a plant on the cellular as well as whole plant level causing damages and reduction in growth rate and development. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by abiotic stresses. In general, the seeds and seedlings may be less stress tolerant than adult plants. The plants respond in a species-specific manner to different stresses. Keeping this in view, the present study was an attempt to standardize germination parameters under varying abiotic conditions.

1. Materials and methods

2.1. Seed Source
The seeds of *Andrographis paniculata* Wall. ex Nees were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. The experiment was conducted during Aril-May in laboratory conditions of Shoolini University of Biotechnology and Management Sciences, Solan. The methodology adopted is described below.

2.2. Germination test
Seeds of *Andrographis paniculata* selected for uniformity (on the basis of colour and size), were surface sterilized with 0.1% HgCl₂ for 5 min. followed by thorough washing under tap water. Then, the seeds were soaked in distilled water (control) or in solutions of stated concentrations of the effector substances namely, i) PEG (5%, 10%), ii) NaCl (25, 50 mM)
and, iii) CdSO\_4 (50, 100µM) for 24 h at 25±2°C. Thereafter, the seeds were transferred to petriplates lined with two layers of filter paper moistened with distilled water (control), PEG, NaCl and CdSO\_4 solutions of same concentrations. The treatments were replicated six times and each petriplate contained 30 seeds. The seeds were allowed to germinate in an incubator at 25±2°C under continuous illumination provided by fluorescent white light (PAR: 40 µmol m\(^{-2}\) s\(^{-1}\)). Emergence of 2-5 mm radicle was taken as seed germination (ISTA, 1966). Germination was recorded regularly until the final count.

2.3. Seedling growth & vigour: Seedling growth was measured after 30 d of incubation in terms of root and shoot length, seedling fresh weight and seed-vigour index (SVI). Seed-vigour Index (SVI): Germination percentage × Seedling length (cm). Experiments were performed in triplicate; each replicate comprised 15 seeds.

At the end of experiment, data was subjected to analysis of variance (ANOVA) and mean separation. The statistical analysis was done using GraphPad Prism\textsuperscript® 5.2. The least significance difference (LSD) at 5% level was used to compare the means of different test parameters under different stress conditions.

1. Results and discussion

The seeds of *Andrographis paniculata* were subjected to the described germination conditions in order to determine the status of germination and the effects of different effectors namely, PEG, NaCl and Cd there on. The seeds of *A. paniculata* were non-dormant and started germinating within 4 d of incubation. Seeds responded differently to water-deficit (PEG), salinity (NaCl) and cadmium (Cd) treatments (Fig. 1). The percent germination and days to germination varied with stress conditions. The first emergence of seedling was observed on 4\(^{th}\) day in control, 5\(^{th}\) PEG & 25mM NaCl, 6\(^{th}\) day in 10\(^{th}\) PEG & 50mM NaCl, 8\(^{th}\) day in 50µM Cd and 14\(^{th}\) day in 100µM Cd. During the initial period (until 12 d in case of 50 and 14 d in case of 100µM) Cd treatment led to the suppression of seed germination but after that it was increased. The degree of increase was maximum in case of 50µM CdSO\_4. Seedling growth was measured after 30 d of seed incubation for germination in terms of root, shoot length and seedling fresh weight. All stresses applied (PEG, NaCl, Cd) adversely affected the seedling growth (Fig. 2). In general, the root length was affected more than the shoot length.
The salt and osmotic stress (PEG) effects have been extensively studied in a wide range of plant species (Hampson and Simpson, 1990; Falleri, 1994; Huang and Redmann, 1995; Katembe et al., 1998; Raza et al., 2006; 2007). The osmotic conditions improved seed germination of soybean (Guidice et al., 1998), neem (Vanangamudi et al., 2000) and asparagus (Bittencourt et al., 2004). Under the dry climatic conditions, salt might accumulate in soil because of high evaporation demand and insufficient leaching of ions due to low precipitation. Cadmium is a non-essential element that affects plant growth and development (Sanita di Toppi and Gabrjelli, 1999). The seeds treated with Cd showed decreased seed germination at higher concentration. The root length of *A. paniculata* in PEG treatment increased with an increase in concentration.
This suggests that *A. paniculata* tolerate drought even at high concentration. It is known that species with longer root length are drought resistant (Leishman and Westoby, 1994). Roots play an important role in plant survival during periods of drought (Hoogenboom et al., 1987) and also drought resistance is characterized by an extensive root growth and small reduction in shoot growth in drought stressed conditions (Guoxiong et al., 2002). A detailed analysis of root-to-shoot ratio under stressful conditions would be useful. At 25 and 50mM NaCl concentrations, the reduction in shoot length was greater than in the root length. This effect has been shown earlier (Khan, 2004; Alam and Sheikh, 2007). In response to the cadmium stress, both root and shoot length and the seedling fresh weight & SVI of *A. paniculata* seedlings were inhibited in a concentration-dependent manner. In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants (Das et al., 1997). Cd treatment induced a greater inhibition of root length than that of shoot length and seedling fresh weight. Despite the different mobility of metal ions in plants, the metal content is generally greater in roots than in the above-ground tissues (Ramos et al., 2002). In most environmental conditions, Cd enters the roots first, and consequently they are likely to experience Cd damage first (Sanita di Toppi and Gabrielli, 1999). In conclusion, the findings from present study reveal the differential sensitivity of seed germination and seedling growth of *A. paniculata* to the tested abiotic stresses namely, water-
deficit, salinity and heavy metal stress. All the three stress factors tested increased the seed germination of *A. paniculata*. Unlike seed germination, seedling growth, measured in terms of root and shoot length, seedling fresh weight and seed vigor, was inhibited by the stress factors tested.

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Impact of water-deficit stress on the seed germination and growth of *Lycopersicon esculentum* ‘Solan Sindhur’

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ABSTRACT

Among the environmental stresses, water-deficit stress is one of the most adverse factors to plant growth and development. The present study was undertaken to evaluate the morphological, physiological and biochemical responses of *Lycopersicon esculentum* ‘Solan Sindhur’ to water-deficit stress under laboratory conditions. Osmotic stress was generated using PEG-6000 (Polyethylene glycol 6000) and the seed germination, seedling growth, proline, lipid and chlorophyll (a, b and total) content were evaluated. The increase in osmotic potential showed significant decrease in germination percentage. Seedling growth parameters increased with an increase in PEG concentration, except the root length which decreased at 10% and 15% PEG concentration. The content of proline and malondialdehyde (MDA) increased according to severity of water deficit stress in hydroponically grown plants. Proline content in roots was more as compared to leaf proline; whereas leaf MDA content was high to root. Chlorophyll (a, b and total) content decreased as PEG concentration increased except at 1% PEG.

KEYWORDS: Water-deficit, polyethylene glycol 6000, osmotic potential, hydroponically grown plants, proline.

Plants are subjected to various abiotic stresses due to unfavorable environmental conditions that affect their growth, metabolism and yield. Extensive studies have been conducted for understanding the plant tolerance in response to water-deficit. Water-deficit stress induces morphological, physiological and biochemical responses which in turn inhibit growth of plant (Shao et al., 2008). Osmotic solution such as PEG (Polyethylene glycol) has been used to impose water stress in different experimental approaches (Kulkarni and Deshpande, 2007). This neutral polymer could be used to modify the osmotic potential of nutrient solution culture (Langerwerff et al., 1961). Responses of plants to water deficit result in alternation of morphology, chlorophyll content, free proline and lipid content. Plants have evolved several mechanisms that allow perceiving the stresses and rapidly regulating their physiology and metabolism to cope them. Under drought stress condition, plant accumulates osmolytes such as proline and act as osmoprotectant (Kumar et al., 2011).

Tomato is one of the important vegetable crops grown throughout the world and ranks next to potato in terms of the area but ranks first as a processing crop. In India, it occupies an area of 571.70 million hectare with a production of 10054 million tonnes (Gore and Sreenivasa, 2011). Himachal Pradesh, a small mountainous state of India, is situated in Western Himalayan region. Tomato is the main cash crop grown in mid-hills of Himachal Pradesh during summer season (Thakur et al., 2010). The total area under tomato in Himachal Pradesh is 7,035 hectares with production of 243,950 metric tons (Anonymous, 2003). The Solan district of Himachal Pradesh produces a bulk of tomatoes and is often called the ‘tomato bowl’ of the hill state.

The present study was to evaluate the effect of PEG induced water stress on morphological, physiological and biochemical activities in tomato. Since a little attention has been given to improve the locally cultivated tomato in this area; the present study would help to understand the responses under water-
deficit stress condition and its further improvement of present cultivar.

**Materials and Methods**

Seeds of *L. esculentum* ‘Solan Sindhur’ selected on the basis of uniformity (ISTA, 1996) were surface sterilized. Washed under distilled water and soaked in control (distilled water), 1%, 5%, 10% and 15% PEG solution for 24 hours. Thereafter, the seeds were transferred to petriplates lined with three layers of filter papers moistened either only by distilled water (control) or by different concentrations of PEG. The seeds were then allowed to germinate in an incubator at 25±2°C under continuous illumination provided by fluorescent white light. After 24 days of germination, seedling growth was measured. Seedling (15 days old) was shifted to hydroponic culture containing Hoagland nutrient solution (Hoagland and Broyer, 1936) and allowed to grow in BOD incubator at 25±2°C. Nutrient medium was replaced by fresh one at regular intervals. After 14 days of shifting to BOD the plants were treated with 1%, 5%, 10% and 15% PEG through appropriate addition to the nutrient medium. After 21 days of treatment, leaf chlorophyll was estimated following the method of Harborne (1973). Lipid peroxidation and proline of leaf and root tissue was estimated by following the method of Dhindsa *et al* (1981) and Bates *et al* (1973), respectively. The data was analysed statistically using t-test.

**Results**

**Germination**

The seeds of *L. esculentum* ‘Solan Sindhur’ were non-dormant and responded differently to PEG concentration. PEG at high concentration 5%, 10% and 15% decreased the seed germination rate whereas at 1% PEG concentration it increased as compared to control (Fig. 1. A, B, C, D).

**Growth**

The growth was measured in terms of seedling root length, shoot length and fresh weight. Root length of seedling increased at 1% and 5% PEG concentration but as the concentration of PEG increased (10%, 15%) root length decreased gradually. Both seedling shoot length and fresh weight increased at all PEG concentrations (1%, 5%, 10% and 15%) (Fig. 2. A, B, C).

![Figure 1](image1.png)

**Figure 1.** Time-course of germination of *Lycopersicon esculentum* ‘Solan Sindhur’ seedling affected by (A) 1%, (B) 5%, (C) 10% and (D) 15% PEG treatment. Values are mean ± S.E.
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PEG Concentration

Figure 2. Effect of PEG on (A) Root length, (B) Shoot length and (C) Seedling fresh weight of Lycopersicon esculentum ‘Solan Sindhur’. Growth measured after 24 days of incubation. Values are mean±S.E.

Chlorophyll Content
PEG induced drought stress imposed to plants, significantly decreased chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll content at all the stress levels except at 1% PEG concentration. Unlike chlorophyll ‘a’, it is clear that a progressive stress adversely affect chlorophyll ‘b’ content (Figure 3. A, B).

Free Proline Content
Free proline content increased both in leaves and roots under different PEG concentration but the increase in proline content was more in roots as compared to leaves of the plant. Proline content present in leaves was 55.90%, 95.90%, 155.90% and 221.01% higher than the control at 1% PEG, 5%PEG, 10% PEG and 15% PEG treatments, respectively. Likewise the root free proline content was 63.74%, 102.92%, 183.04% and 264.33% higher than control at 1%, 5%, 10% and 15% PEG treatments, respectively (Figure 4.).

MDA Content
Lipid peroxidation, a measure of free radical generation, was determined in terms of malondialdehyde (MDA) content. Leaf and root MDA content increased with increase in PEG concentration. But increase in MDA content in leaf was much more as compared to root. MDA content present in leaf was 27.46%, 40.45%, 46.61% and 52.83% higher than the control at 1% PEG, 5%PEG, 10% PEG and 15% PEG treatments, respectively. Likewise the root MDA content was 34.41%, 46.82%, 58.52% and 75.41% higher than control at 1%, 5%, 10% and 15% PEG treatments, respectively (Figure 5.).
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**Discussion**

In the present investigation, tomato responses were studied to progressively induce different level of osmotic stress by using PEG-6000 in the medium. Many earlier studies, including present one, have conclusively proved that water stress is an important factor that limits germination of seeds (Vallejo et al., 2010). However, in the present study at lower concentration of PEG (1%) there is an increase in germination percentage. Roundy et al. (1985) and Wenfan et al. (2010) attributed increase in germination due to PEG.

In the present studies root length increased at lower concentration of PEG (1%, 5%) but decreased at higher PEG concentration. This suggests that *L. esculentum* ‘Solan Sindhur’ might tolerate drought up to certain degree only. An increased root growth due to water stress was reported in sunflower (Tahir et al., 2002) and *Catharansus roseus* (Jaleel et al., 2008). In the present study increase in shoot length and seedling fresh weight of plant was observed under different PEG concentrations. According to Bloor and Grubb, (2003) the increase in shoot length and seedling fresh weight could be due to high-light treatment. Chlorophyll content was also affected during the present investigation which shows that long progressive stress may affect photosynthetic ability of the plant. The decrease in chlorophyll content as affected by water deficit is because of production of reactive oxygen species (ROS), such as $\text{O}_2$ and $\text{H}_2\text{O}_2$, which lead to lipid peroxidation and consequently, chlorophyll destruction (Kumar et al., 2011). At 1% PEG concentration chlorophyll content increased as compared to control and this result was in agreement to finding of Garacia-Valenzuela et al. (2005), which stated that chlorophyll concentration of graminaceous chlorophillic cells (‘TADH-XO’) increased when exposed to different concentration of PEG. A variety of organic solute accumulates in osmotic ally stressed plants in which proline appears to be widely distributed osmolytes under stress condition (Yoshiba et al., 1997).
investigation, it was observed that proline content in leaf and root increased with increase in PEG concentration. Increased level of proline in PEG induced water stressed plants may be an adaptation to overcome the stress conditions. Proline accumulates under stress condition supplies energy for survival and growth and thereby helps the plant to tolerate stress (Kumar et al., 2011).

The MDA content in leaves and roots was measured as index of the rate of lipid peroxidation. Generally, various stresses cause an extensive lipid peroxidation which has been often used as an indicator of stress induced oxidative damage of membrane. Under water stress, plants produced large quantities of MDA content. In the present study the MDA content in root and leaves of L. esculentum ‘Solan Sindhur’ increased under drought stress. The observed increase of MDA under drought stress condition results from in production of reactive oxygen species (ROS) which lead to lipid peroxidation (Kumar et al., 2011). The earlier studies reported that MDA content increased under PEG stress (Gengsheng et al., 2001). Similar result was observed by Radic and Pevalek-Kozlina (2010).

Conclusion

Our present results indicate that progressive water stress induced by PEG 6000 cause significant morphological, physiological and biochemical changes in tomato. Drought stress affects the growth of the plant. Enhanced proline accumulation during stress indicates that proline play a cardinal role as an osmo-regulatory solute in plants. MDA content also increase due to reactive oxygen species. In addition to other factors, changes in photosynthetic pigments are of paramount importance to drought tolerance. Finally, the present findings revealed that, Lycopersicon esculentum ‘Solan Sindhur’ is susceptible to water-deficit stress. There is a need to select resistant genotype in order to improve the cultivar for growing under drought conditions.

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