IJPSR (2012), Vol. 3, Issue 02 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 October, 2011; received in revised form 21 November, 2011; accepted 28 January, 2012

EFFECT OF WATER STRESS BY POLYETHYLENE GLYCOL 6000 AND SODIUM CHLORIDE ON SEED GERMINATION AND SEEDLING GROWTH OF *CASSIA ANGUSTIFOLIA*

S. M. Shitole and K. N. Dhumal

Department of Botany, University of Pune, Pune- 411007, Maharashtra, India

Keywords:
Senna,
NaCl,
PEG-6000,
seed germination,
seedling growth

Correspondence to Author:

Shilpa Mohan Shitole

Ph. D., Scholar, Department of Botany, University of Pune, Pune- 411007, Maharashtra, India

ABSTRACT

The impact of salt stress and water stress on seed germination, root and shoot length, fresh and dry biomass, vigour index was investigated in Senna (*Cassia angustifolia*) at germination stage (7DAS). Salinity stress was induced by using NaCl and drought stress by using PEG-6000. Different concentration of NaCl (0.1 to 100 mM) and PEG-6000(-0.1 bars to -2.0 bars) were used for seed treatment. The objective of the present investigation was to study the comparatively impact of NaCl salinity stress and water stress on seed germination, seedling growth, fresh and dry weight in Senna.

INTRODUCTION: Seed germination is one of the most important phases in the life cycle of plant and is highly responsive to existing environment [1]. The abiotic factors such as salt and drought stresses are the two of the most important abiotic stresses that limit seed germination and seedling growth [2, 3, 4, 5]. Abiotic stress conditions negatively influence the survival of plants, biomass accumulation and yield [6].

Osmotic adjustment plays an important role in sustaining growth under water deficit conditions. The response of plants differs significantly at various organizational levels, depending upon intensity and duration of stress as well as plant species and its stage of development [7].

Both drought and salt stress is harmful during early stages of seed germination and seedling growth. With increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, more and more people are interested in the use of plants and plant based drugs being revived through out the world. So exploitation of medicinal plants has become more and more popular. For the past several years, several scales of physiology have been applied to study responses to salt stress tolerance mechanisms and methods to overcome salt stress in field crops [8]. It seems necessary to do research related to the correlation between medicinal plants and salt stress for the increasing need of medicinal plants. In order to meet the ever increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil salinity and other forms of pollutions pose serious threats to plant production [9]. So it seems valuable, to test the important medicinal plants for their salt tolerance capacity.

Cassia angustifolia (Family: Caesalpiniaceae), popularly known as Senna, is a valuable plant drug in Ayurvedic and Modern System of Medicine. The primary medicinal components of Senna is sennoside A and B, are the two anthroquinone glycosides that are responsible for purgative action of Senna [10] and is used to cure a large number of intestinal diseases.

Therefore, the objective of this research was to examine the influence of different concentrations of NaCl and PEG 6000 on the seed germination, root and shoot length, fresh and dry weight of *Cassia angustifolia*.

MATERIALS AND METHODS: Authentic seeds of *Cassia angustifolia* Vahl were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat. Seed germination bioassay was carried out during the month of July- August (2008-2009). The seeds were surface sterilized with 0.1 % $HgCl_2$ for five minutes with intermittent shaking; and thoroughly rinsed with distilled water. These seeds were put in 9 cm diameter sterilized petri plate containing single layer of seed germination paper. Salinity stress was induced by using NaCl and drought stress by using PEG-6000. Different concentration of NaCl (5 to 100 mM) and PEG-6000 (-0.25 bars to -2.0 bars) were used for seed treatment. The solution of different concentrations of Polyethylene Glycol (PEG 6000) was prepared by the method of Michel and Kaufmann [11].

Five ml solution of NaCl (5, 10, 25, 50 and 100 mM) and PEG 6000 (-0.25, -0.50, -1, -1.5, -2) bar were added in different petri plates. The control contained five ml of sterile distilled water. Each treatment was maintained in triplicate along with control. About 20 seeds were kept in each petri plate for germination. The germination studies were carried out in seed germination chamber at 30 ± 2^{o}C temperature, 94 ± 2 relative humidity and uniform light conditions. The germination percentage, the root and shoot length (cm), fresh and dry weight (gm) were measured after 7th day of germination in all the germinated seedlings. The seedling dry weight was determined after drying at 80^{o}C for 24 h. The average values were recorded. For seed germination experiment the control is always DW. Vigour Index was calculated by using formula,

Vigor Index (VI) = Germination percentage × (Root length + Shoot Length)

Statistical Analysis: The data was presented as arithmetic means of three replicates ±standard deviation. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2003 were used to carry out statistical analyses and graphical data presentations.

RESULTS AND DISCUSSION:

Effect of NaCl and PEG 6000 on seed germination and seedling growth: Decreasing osmotic potential (increasing NaCl and PEG 6000 concentration) caused reduction in seed germination percentage (%). The reduction in seed germination was proportional to the increasing concentration of NaCl and PEG 6000. Maximum retardation was noted at highest NaCl concentration (100mM) and PEG 6000 (-2 bars). There was absolute (100%) inhibition of germination above these concentrations. Similar results were observed by [12] Woodell [13] (1985); Gupta [14] Ungar [15] (1993), Safarnejad, [16] (2008).

This was attributed to the effect that seeds seemingly develop an osmotically enforced inhibition by NaCl and drought stress. This may be an adaptive strategy of seeds to prevent germination under stressful environment for ensuring proper establishment of the seedlings. Not only the seed germination but seedling growth was also obstructed by chloride and water stress. These results indicated that the radicle and plumule growth was adversely affected. The shoot and root length of senna was negatively influenced by NaCl and PEG. This ultimately affected the fresh and dry weight of the seedlings. Similar results were observed by Pratap and Sharma [6] (2010), Kaydan and Yagmur, [5] (2008).

It was claimed that decrease in the water potential gradient between seeds and their surrounding media adversely affect the seed germination and subsequent events in seedling growth and development. Water uptake leads to the activation of the metabolic processes as the dormancy of seed is broken [17]. High concentration of NaCl and PEG might have hampered the process of water uptake by seeds and thereby inhibited the process of seed germination [18]. Water and salinity stress induced by PEG and NaCl caused reduction in seed germination and also delayed germination time [5]. Similar results for decreased seed germination and seedling growth due to NaCl and PEG [19, 20].

PEG and NaCl decreased germination in pea, but effect of PEG on germination is more than NaCl [21]. Seed germination and seedling growth in legumes decreased under drought (PEG) treatment [22]. Drought stress cause reduction of seedlings growth in beet [23]. Germination percent, radical and plumule length, fresh and dry weight of pea seedling decreased by increase of drought stress (PEG) [21]. Drought stress caused reduction of germination rate and seedlings growth in okra plant [24].

Root length is an important trait against drought stress in plant varieties; in general, variety with longer root growth has resistant ability for drought [25, 26]. Seed germination, shoot and root length was decrease due to PEG in lentil [27].

NaCl stress decreased root and shoot length, dry matter production of *Glycine max* seedlings [28]. Shoot and root length, fresh and dry weight of the seedlings were also reduced in both salinity as well as drought stress which may be due to the metabolic disorders induced by stress and generation of Reactive Oxygen Species (ROS). There is a significant reduction in early seedling growth at higher concentrations of NaCl and PEG-6000 due to their toxic effect at high concentration on the seeds (**table 1**).

TABLE 1: EFFECT OF NaCl SALINITY STRESS ON SEED GERMINATION AND SEEDLING GROWTH OF SENNA

Treatments NaCl (mM)	Germination %	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigor Index
Control	74.32±3.05a	5.90±0.36a	2.50±0.08a	0.14	0.02	624.288
5	74.02±2.16b	5.86±0.32	2.37±0.09	0.14	0.02	609.1846
10	73.12±2.08	5.08±0.27	2.08±0.07	0.13	0.02	523.5392
25	74.71±3.81a	4.60±0.28b	1.90±0.10b	0.12	0.01	485.615
50	60.32±4.27b	2.90±0.12c	1.60±0.05c	0.10	0.01	271.44
100	53.39±1.69c	0.90±0.06d	0.50±0.03d	0.05	0.01	74.746
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Both seed germination and seedling growth was reduced due to NaCl and PEG induced osmotic stress. However, PEG induced osmotic stress caused more growth inhibition as compared to NaCl induced osmotic stress [18, 29, 30]. Drought stress induced by using PEG-6000 was most harmful to plant as compared to NaCl stress. Water deficient conditions created by PEG decreased seed germination and seedling growth with higher negative effects. The explanation of the higher inhibitory effects of PEG than NaCl lies in ion or solute entry into the seed [20]. Especially, the accumulation of Na^+ by the imbibing seed embryo functions to promote a water potential gradient between the embryo and substrate, and maintain water uptake during seed germination [19, 31] (**table 2**).

TABLE 2: EFFECT OF PEG-6000 ON SEED GERMINATION AND SEEDLING GROWTH OF SENNA

Treatments PEG6000 (- bars)	Germination %	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigor Index
Control	74.32±3.05a	5.90±0.36a	2.50±0.08a	0.14	0.029	624.288
-0.25	73.06±2.16b	5.85±0.30b	2.34±0.08b	0.13	0.024	598.3614
-0.5	71.12±2.08b	5.01±0.17c	2.03±0.05c	0.12	0.021	500.6848
-1	60.32±4.27c	4.53±0.22bd	1.81±0.05d	0.11	0.017	382.4288
-1.5	53.39±1.69d	2.76±0.10ce	1.52±0.04e	0.08	0.013	228.5092
-2	41.42 ±1.05e	0.86±0.06f	0.43±0.03f	0.05	0.007	53.4318
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

REFERENCES:

1. Besma B D and Mounir D: Salt stress induced changes in germination, sugars, starch and enzyme of carbohydrate metabolism in *Abelmoschus esculentus* (L) Moench seeds. African Journal of Agricultural Research 2010; **5**(6): 408-415.
2. Almansouri M, Kinet JM, Lutts S: Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf). Plant Soil 2001; 231: 243-254.
3. Kaya MD, Okcu G, Atak M, Cýkýlý Y, Kolsarýcý O: Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L). Eur J Agron 2006; 24: 291-295.
4. Atak M, Kaya MD, Kaya G, Cýkýlý Y, Ciftçi CY: Effects of NaCl on the germination, seedling growth and water uptake of triticale. Turk J Agric Fore 2006; 30: 39-47.
5. Kaydan D and Yagmur M: Germination, seedling growth and relative water content of shoot in different seed sizes of

triticale under osmotic stress of water and NaCl. African Journal of Biotechnology 2008; 7(16): 2862-2868.
6. Pratap V and Sharma Y K: Impact of osmotic stress on seed germination and seedling growth in black germ (*Phaseolus mungo*) Journal of Environmental Biology 2010; 31: (5) 721-726.
7. Chaves M M, J P Maroco and J Pereira: Understanding plant responses to drought from genes to the whole plant. Funct Plant Biol 2003; 30,239-264.
8. Jaleel, CA, Gopi, R, Manivannan, P, Panneerselvam, R: Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents. EurAsian J BioSci 2008; 2: 18–25.
9. Jaleel, CA, *et al*. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L) GDon plants under salinity stress. Turk J Bot 2007; 312; 45-251.
10. Aktar MW, Rajlakshmi, P, Anjan B: Status of sennosides content in various Indian herbal formulations: Method standardization by HPTLC. Bangladesh J Pharmacol 2008; 3: 64–68.
11. Michel BE and Kaufmann M R: The osmotic potential of polyethylene glycol 6000 Plant physiology 1973; 51 (914-916).
12. Harradine AR: Effect of salinity on germination and growth of *Pennisetum macrourum* in southern Tasmania. J of Applied Ecology 1982; 19: 273-282.
13. Woodell, SR: Salinity and seed germination patterns in coastal halophytes Vegetation 1985; 61, 223-229.
14. Gupta A K, J Singh, N Kaur and R Singh: Effect of polyethylene glycol induced water stress on germination and reserve carbohydrates metabolism in chickpea cultivars differing in tolerance to water deficit Plant Physiol Biochem 1993; 31: 369-378.
15. Ungar, IA: Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). Ame J Bot, 1996; 83, 604-607.
16. Safarnejad A: Morphological and biochemical response to osmotic stress in Alfalfa (*Medicago sativa* L) .Pak J Bot 2008; 40: (2) 735-746.
17. Katembe, WJ, JA Ungar and JP Mitchell: Effects of salinity on germination and seedling growth of two *Atriplex* spp (Chenopodiaceae). Annals of Botany 1998; 82:165-175.
18. Afzali SF, MH Hajabbasi, H Shariatmadari, K Razmjoo and AH Khoshgoftarmanesh: Comparative adverse effects of PEG- or NaCl- induced osmotic stress on germination and early seedling growth of a potential medicinal plant *Matricaria chamomilla* Pak J Bot 2006; 38:(5) 1709-1714.
19. Dodd GL, Donovan LA: Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. Am J Bot 1999; 86: 1146-1153.
20. Alam MZ, Stuchbury T, and Naylor REL: Effect of NaCl and PEG induced osmotic potentials on germination and early seedling growth of rice cultivars differing in salt tolerance. Pak J Biol Sci 2002; 5 (11): 1207-1210.
21. Gamze O, Mehmet DK, Mehmet A: Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum L)*. Turk J Agric 2005; 29: 237-242.
22. Murillo A, Lopez BR, Aguilar C, Kaya T, Larrinaga A, Flores H: Comparative effects of NaCl and Polyetylene glycol on germination, emergence and seedling growth of cowpea J Agron Crop Sci 2002; 188: 235-247.
23. Sadeghian SY, Yavari N: Effect of water –deficit stress on germination and early seedling growth in sugar beet. J Agron Crop Sci, 2004; 190(2), 138-144.
24. Amin Baghizadeh, Mahmood Hajmohammadrezaei: Effect of drought stress and its interaction with ascorbate and salicylic acid on okra (*Hibiscus esculents* l) Germination and seedling growth Journal of stress physiology & biochemistry 2011; 7:1 55-65.
25. Leishman MR, Westoby M: The role of seed size in seedling establishment in dry soil conditions -experimental evidence from semi-arid species. J Ecol 1994; 82(2): 249-258.
26. Leishman MR, Westoby M: The role of seed size in seedling establishment in dry soil conditions -experimental evidence from semi-arid species. J Ecol 1994; 82(2): 249-258.
27. Haq A, R Vamil and RK Agnihotri: Effect of osmotic stress (PEG) on germination and seedling survival of Lentil (*Lens culinaris* MEDIK). Research Journal of Agri Sciences, 2010; 1(3): 201-204.
28. Panneerselvam R, M Mutthukumarsamy and SN Rajan: Amelioration of NaCl stress by triadimefon in soyabean seedlings. Biologia Plantarum 1998; 41(1): 133-137.
29. Hampson,CR and GM Simpson: Effect of temperature, salt and water stress on early growth of wheat (*Triticum aestivum* L) germination. Can J Bot 1990; 68,524-528.
30. Gulzar S and Khan A: Seed germination of a halophytic grass *Aeluropus lagopoidales.* Annals of Botany 2001; 87: 319-324.
31. Eddleman LE, Romo JT: Sodium relations in seeds and seedlings of *Sarcobatus vermiculatus.* Soil Sci 1987; 143: 120-123.

****