Responses of *Andrographis paniculata* to salinity for stress tolerance

Sujata Bhattacharya, Sunil Puri
Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan
Email: sujata0685@gmail.com

ABSTRACT

Response of plants to abiotic stresses during seed germination and at different growth stages is important for understanding the mechanisms of stress tolerance or sensitivity. The aim of present study is to know the response of *Andrographis paniculata* to salinity (NaCl) stress, with respect to its morphological, physiological, biochemical parameters and therapeutically active constituents. Germination percentage enhanced in lower NaCl concentration but decreased by 21% in plants treated with higher concentration as compare to control. However, germination got delayed due to salinity. There was an increase in enzymatic and non-enzymatic antioxidants indicating that this plant has the ability to scavenge or control the level of cellular ROS and can be grown successfully under stressful conditions. The study revealed for the first time, the tolerance ability of *A. paniculata* and has anti-oxidant defense mechanism to tolerate abiotic stress. The results of the present study can be used as a baseline for further study involving screening and selection of tolerant genotypes and using these traits in breeding programs.

INTRODUCTION

- Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches
- India has a rich history of using potent herbs and herbal components as traditional medicines for various ailments.
- *Andrographis paniculata* (Acanthaceae), occurring wild in India and is used both in Ayurevda and Unani system of medicine (CSIR, 1985)
- Its major secondary metabolites are andrographolide and related diterpens, i.e., dexoyandrographolide, 14-deoxy-11, 12-didehydroandrographolide and neo-andrographolide
- Abiotic stress (salinity, drought, heat/cold, light and other hostile conditions) leads to the overproduction of reactive oxygen species (ROS), which stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhances the medicinal value of the plant
- Salinity stress is detrimental to plant growth and productivity as it causes nutritional imbalances by altering the uptake of nutrients (nitrogen, phosphorus, potassium, calcium, etc.) and interferes with the cellular metabolism by causing ion toxicity and osmotic perturbations (Joshi *et al.*, 2011)
- It is clear that plant growth, productivity and production of secondary metabolites are adversely affected due to abiotic stress factors

AIMS AND OBJECTIVES

The aim of the present study is to evaluate the relative tolerance of *Andrographis paniculata* to salinity stress factors in terms of growth, morphological, physiological and biochemical characters, antioxidant defense potential and the concentration of active constituents i.e., andrographolide.

MATERIALS & METHODS

Materials

Seeds were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Methods

- **Seed germination** assays in solutions of NaCl (50 and 100 mM) Emergence of 2-5 mm radicle was taken as seed germination (ISTA, 1966)
- **Physiological aspects**
  - Total carbohydrates (Hedge *et al.*, 1962)
  - Protein (Lowry *et al.*, 1951
  - Proline (Bates *et al.*, 1973)
- **Plant antioxidants**
  - Superoxide Dismutase (Kakkar *et al.*,1984)
  - Catalase (Luck, 1974)
  - Peroxidase (POD) (Reddy *et al.*, 2005)
  - Ascorbic acid (AA) (Roe and Keuther, 1943)
  - Tocopherol (Rosenberg, 1992)
  - Lipid peroxidation (Dhindsa *et al.*, 1981)
- **Quantitative determination of Andrographolide**
  - HPLC system of Agilent technologies
  - Andrographolide (98% pure Chromadex)
  - Mobile phase combination of H_2O and methanol of HPLC grade in the ratio of 35:65 v/v with RP-HPLC at flow rate of 0.8 ml/min at wavelength 223nm
  - Retention time of 3.5456±0.0145 minutes
- **Statistical analysis**
  - The data was analyzed statistically using GraphPad Prism® 5.2

RESULTS

- **Seed germination assays**

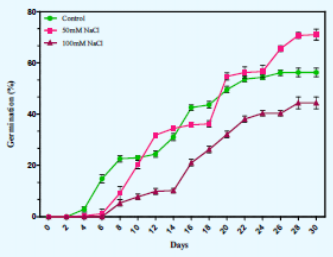

Table 1: Time-course of germination of *Andrographis paniculata* seeds as affected by NaCl. Values are mean ± S.E. n=6

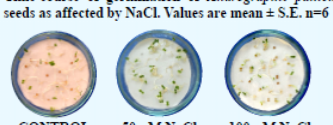

Figure 2: Germination of *Andrographis paniculata* seeds as affected by NaCl after 30 days of incubation

Table 2: Growth parameters of *Andrographis paniculata* after 30 days of incubation under NaCl; values are mean±S.E.; n=6

Sr. No	Parameters	Control	50mM NaCl	100mM NaCl
1	Stem length (cm)	5.71± 010	2.5±0.12	1.22±0.11
2	Root length (cm)	2.53±0.05	2.06±0.06	0.73±0.10
3	Swelling fresh weight (gm)	17.5±0.24	12.9±0.44	8.93±0.24
4	Seed vigor index (SVI)	464.7±9.8	304.6±7.3	86.6±8.34

- **Physiological aspects**

Sr. No.	Parameter	Treatment		
		Control	50mM NaCl	100mM NaCl
1	Total carbohydrate (mgg^{-1} FW)	4.80±0.14	4.96±0.08	5.89±0.10
2	Total protein (mgg^{-1} FW)	0.62±0.02	0.61±0.02	0.78±0.01
3	Proline (mgg^{-1} FW)	0.123±0.002	0.126±0.0033	0.221±0.0028

RESULTS

- **Plant antioxidants**

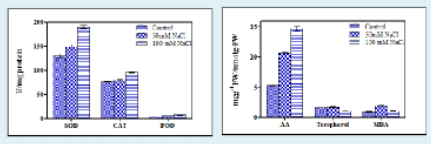

Figure 3: Quantitative estimation of antioxidants (SOD, CAT, POD, ascorbic acid, tocophrol and lipid peroxidation) in the leaves of *Andrographis paniculata* under different concentrations of NaCl; values are mean±S.E.; n=6.

- **Quantitative determination of Andrographolide**

Table 3: Retention time of the main peaks

Peaks	Retention time (min.)	Compound
A	1.8-1.9	Andrographolic acid
B	3.4-3.5	Andrographolide
C	6.6-7.1	14-deoxy-11,12-didehydroandrographolide

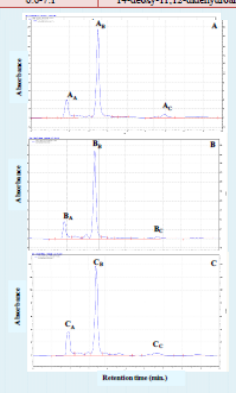

Figure 4: Andrographolide content in leaves of *Andrographis paniculata* under control (A), 50mM NaCl (B) and 100mM NaCl (C) after 120 days

Table 4: Andrographolide content in the leaves of *Andrographis paniculata* under salinity stress

Sr. No.	Treatments	Andrographolide content (mgg^{-1}DW)
1	Control	34.362
2	50mM NaCl	41.280
3	100mM NaCl	23.950

Conclusion

Seed germination in *Andrographis paniculata* was affected due to higher NaCl concentration. Plant growth was also affected due to salinity stress. There was an increase in antioxidants indicating that this plant has the ability to scavenge or control the level of cellular ROS and can be grown successfully under stressful conditions. The study revealed for the first time, the tolerance ability of *A. paniculata* and has a strong anti-oxidant defense mechanism to tolerate abiotic stress. Andrographolide content increased under salinity stress conditions. The results of the present study can be used as a baseline for further study involving screening and selection of tolerant genotypes and using these traits in breeding programs.

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