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Studies on Nitrate uptake by *Sesuviumportulacastrum* (L.), an associate Halophyte

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Abstract:
Sesuviumportulacastrum (L.) L. (Aizoaceae) is a pioneer, psammophytic associate halophyte naturally growing in the subtropical, mediterranean regions. It is found localized in coastal and warmer zones of the world. The plant is utilized as a vegetable by the local people as well as forage for domestic animals in the coastal area. Environmentally, it is utilized for the bioreclamation of saline soil in the arid and semiarid regions and an alternative culture to problematic soils.
Studies indicate that Nitrogen play varied in role in plant life. Most plants entirely depend on fixed nitrogen, which is absorbed from the soil mainly as nitrate and also as ammonium.
Inpresent investigation,*Sesuviumportulacastrum* (L.) is used as a model system for evaluating the effect of environmental conditions on uptake of Nitrate and its effect on morphological and eco-physiological adaptations.

Keywords: Associate halophyte, Nitrogen metabolism

Introduction:
Associate halophytes grow in the fringe area of mangrove swamps, get inundated 1-5 times per fortnight during spring tide and are also found growing in mesophytic habitat.
Sesuviumportulacastrum L. (Seapurslane) is one such fast growing, herbaceous, dichotomous, perennial, pioneer, psammophytic halophyte naturally growing in the subtropical, mediterranean, coastal and warmer zones of the world.
*Sesuviumportulacastrum*is found occurring on the coastlines of five continents and widely distributed as a pioneer strand species on tropical and subtropical shores (Lonard and Judd loc.cit.). It grows naturally in the subtropical, mediterranean, coastal and warmer areas around the world (Balasubramanian*et al*., 2006).

*Sesuvium*frequently grows in the backshore topographic zone on sandy beaches as the initial pioneer species just above the high tide line on barrier islands.It is also a common species on the margins of hurricane washover channels, disturbed roadsides, and tidal flats (Lonard and Judd, 1997). In the tropics, the species occurs on estuarine mudflats adjacent to mangrove swamps (Joshi and Bhosale, 1982), in salt marshes and on calcareous shorelines, on the margins of lagoons, on coral sand and rubble shorelines (Lonard and Judd loc.cit.). It is also found along coasts and river mouths and in lower mountains (Hammer, 2001).

In India, it grows among the coastal sides of eastern and western regions as inland or seashore species including areas where mangrove plants are found. This includes coastal regions of Gujarat, Maharashtra, Goa, Kerala, Tamilnadu, Andhra Pradesh and Orissa

Objectives:
The following study on *Sesuviumportulacastrum* L. was done keeping following objectives in mind.
To evaluating the effect of environmental conditions on uptake of Nitrate and its effect on morphological and eco-physiological adaptations.
To get an insight in the world of associate halophyte helping in natural conservation of mangrove locations.

Materials and Methods

The plants of *Sesuviumportulacastrum* were collected from the natural habitat along with surrounding soil and brought to the laboratory in a polythene bags. The roots were

washed carefully to remove all adhering soil and were treated according to the procedure of Jackson *et al*. (1973). This consisted of choosing plants of the same height and almost same age group bearing almost same number of leaves with clean healthy roots. The roots were then washed again with de-ionized water and transferred to conical flasks containing Tris-HCl buffer (pH 7.0). The plants were left overnight and then transferred to the treatment solution. All the experiments were carried out according to the methods of Rao and Rains (1976) with necessary modifications.

Experimental Procedure:

All the experiments on different parameters of nitrate uptake were carried out in a dark chamber. About 5-6 plants were required for each experiment. Each assay was performed three times. The constitution of treatment solution was changed according to the parameter under investigation. After a fixed time period, 1ml samples from the treatment solution were taken in three different test tubes.

Determination of the rate of nitrate uptake

Absorption rate for nitrate uptake were determined by the disappearance of nitrate from the external solution. This represented the net flux of nitrates into the roots. This flux was expressed as μM KNO_3 absorbed /g root on a fresh weight basis. Net uptake of nitrate was determined by method of Johanson and Ulrich (1950). This method is also known as Phenol disulphonic acid method. One ml of test solution was pipetted into an evaporating dish.To this 4 drops of 1N NaOH were added and the mixture was dried over a sand bath. After cooling, 1ml of phenol disulphonic acid was added and mixed thoroughly with the dried material in the evaporating dish. This was followed by the addition of 10 ml of distilled water and 5 ml of liquor ammonia. A yellow colour developed. The entire solution was then transferred to a test tube. Absorbance of yellow coloured solution was read at 440 nm on EQIP-Tronics digital spectrophotometer model no EQ 820.

Assay for nitrate uptake:

(i) Time course study and the effect of pH:

The plants were grown in Hoagland's solution(Hoagland and Arnon, 1948) containing 0.6 mM $KNO_3$with varying pH from 4.0 to 8.0, for 24 hour and the solution was drawn out at an interval of 1hour, 3hour, 6hour and 24hour and was analyzed for KNO_3 content as described earlier.

(ii) Effect of Temperature variation:

The plants were grown in Hoagland's solution(Hoagland and Arnon,loc.cit.)containing 0.6 mM KNO_3 and subjected to different growth temperature *viz.*10°C, 20°C, 27°C and 37°C and after an acclimatization period of 1 hour, the amount of nitrate absorbed was measured from the samples of the external solution and it was compared with that of control at 0 hour.

(iii) Effect of Nitrate variation:

The young plants of *Sesuviumportulacastrum* were grown in Hoagland's solution(Hoagland and Arnon,loc.cit.)containing different concentration of KNO_3 varying

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between 0.2 mM and 2 mM and the solution from each was analyzed for amount of KNO_3 remained after 1hour.

Results and Discussion:

(i) Time course study and effect of pH on nitrate uptake:

Figure 3.3 depicts the results of time course study of nitrate uptake at various pH(4 to 8) by *Sesuviumportulacastrum*plants. From the graph, it is evident that at the end of 1 hour, maximum uptake was seen at pH 6.5, which was retained even at the end of 24 hour. The uptake appears almost nil from pH upto pH 5.5 at the end of 1hour and 3 hours, whereas during the same time period it slightly improved above pH 7.0 but drastically dropped above 7.5. The same pattern was evident till 24 hrs. Lycklama (1963) has reported that external pH has marked influence on the process of uptake, and the nitrate uptake decreases at pH values close to 7 and above.

(ii) Effect of temperature variation on the rate of nitrate uptake:

The effect of different temperature on the rate of nitrate uptake is represented in Figure 3.4. From the data obtained, it was observed that the nitrate uptake by*Sesuviumportulacastrum* is maximum at 27°C (0.06 ΔOD/g root/hr). At lower temperature, nitrate uptake is poor.

Hallmark and Huffaker (1978) recorded higher rates of nitrate uptake and nitrate reduction with higher temperatures. Cantliffe (1972 b) has shown accelerated nitrate uptake in *Spinaceaoleracea*in low temperature, whereas Sarangdhar (1986) has shown that high temperatures favoured nitrate uptake in *Aeluropuslagopoides*under salinity stress. Bhagwat (1981) showed that nitrate uptake in *Kalanchoepinnata* changed the K_m values with changes in temperature.

(iii) Effect of nitrate concentration:

The effect of varying concentrations of KNO_3 on the rate of nitrate uptake was evaluated at assay pH 6.5 and temperature 27°C. The results obtained are represented in figure 3.5.

When the results were analysed by the Michaelis-Menten equation (1913), the curve obtained was a typical rectangular hyperbolic curve. The rate of nitrate uptake showed an increase with increase in KNO_3 concentration, maximum at concentration close to1.0mM. The V_{max}at1.0mMwas 0.17ΔOD with a corresponding apparent K_m of 0.48mM KNO_3.K_m and V_{max}values given here are representative values after considering nitrate uptake as a total activity, regulated by number of enzymes. The kinetic parameters are indicative of good nitrate uptake by *Sesuviumportulacastrum*.

According to Fowden (1977), nitrate uptake is mechanically linked with nitrate reduction. Hinginbotham*et al*., (1967) that the accumulation of nitrate occurs against electrochemical gradient and this could be due to active inward transport of these ions towards the tissues.

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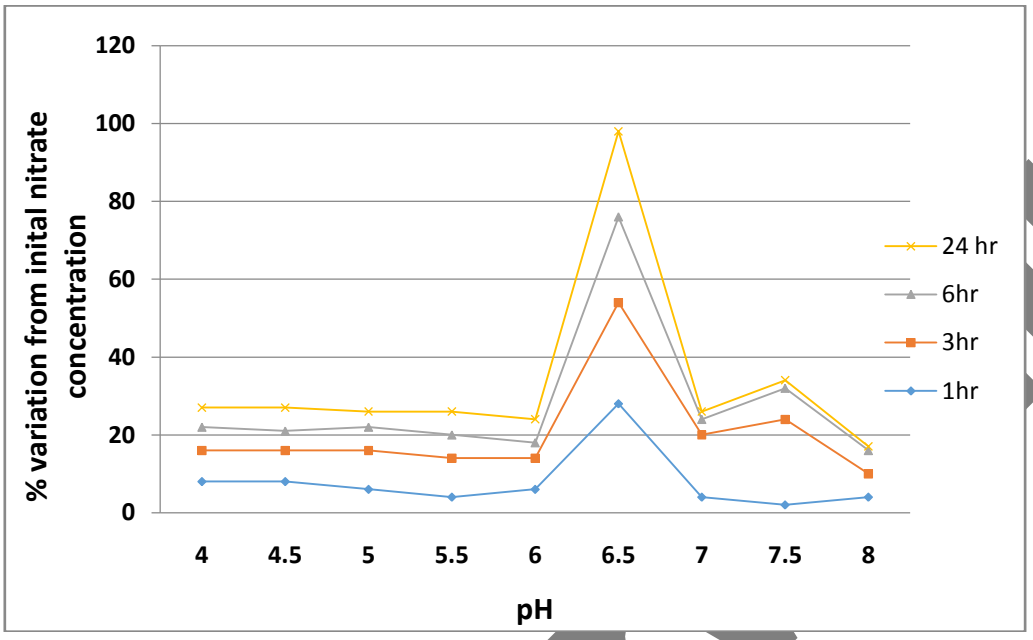
Fig. 3.3 Effect of pH and time variation on nitrate uptake in *Sesuviumportulacastrum*



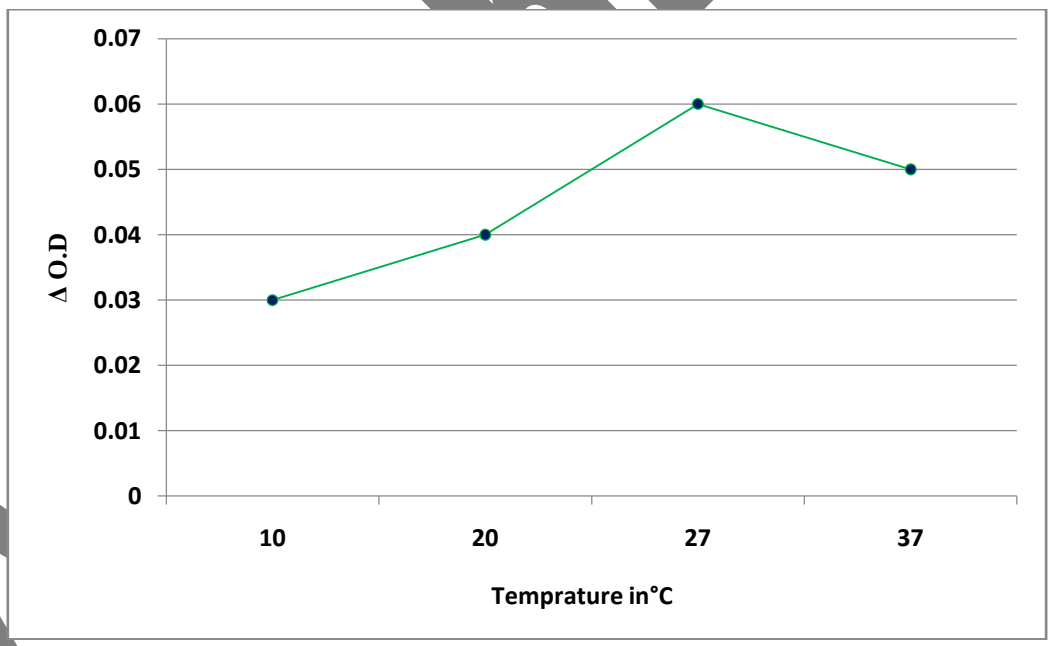


Fig. 3.4 Effect of temperature variation on nitrate uptake in *Sesuvium portulacastrum*

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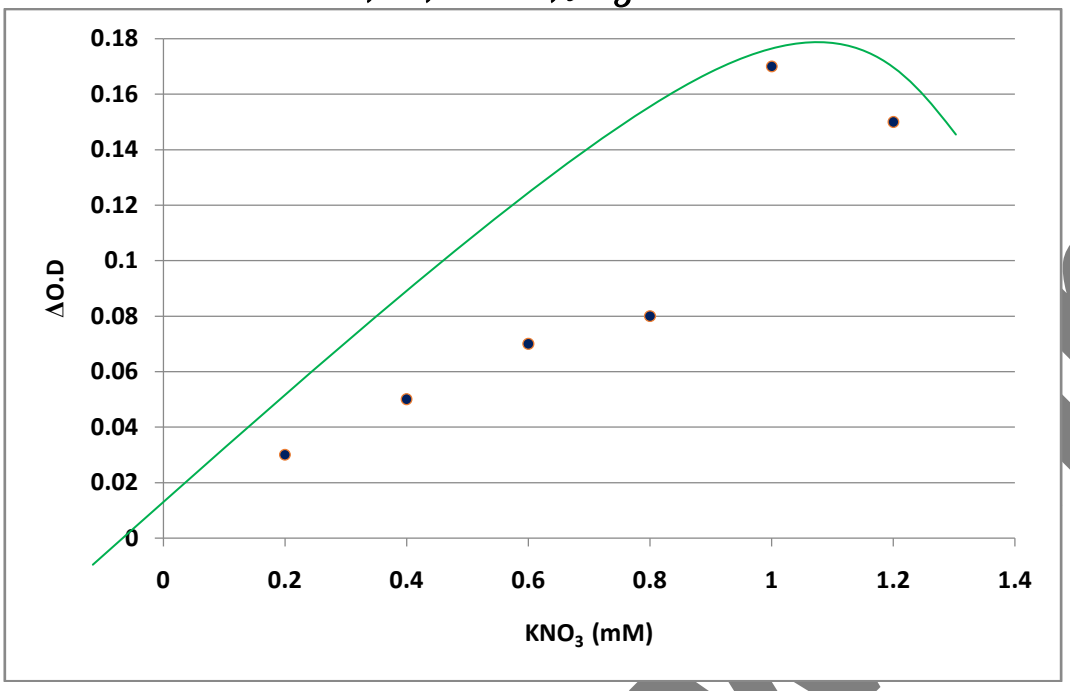

Fig. 3.5 Effect of substrate variation on nitrate uptake in *Sesuviumportulacastrum*

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