

NITRATE REDUCTASE STUDY FROM HEAT SUSCEPTIBLE AND HEAT TOLERANT CULTIVAR OF WHEAT (*TRITICUM AESTIVUM* L.)

MOHAN CHAVAN¹, ARTI KAROSIYA², SUMA TC³, POORNIMA R⁴, UMASHANKAR KUMAR N⁵ &
PRIKSHAYAT SINGH⁶

^{1,2,4,5,6} Assistant Professor of Biochemistry, University of Agricultural Sciences, Bangalore,

Agricultural Biotechnology Block, Agricultural College, Hassan, Karnataka, India

³ Assistant Professor, Crop Physiology, College of Agriculture, Bheemarayanagudi,

University of Agricultural Sciences, Raichur, Karnataka, India

ABSTRACT

The present work leads to the study of Nitrate reductase enzyme particularly from heat tolerant and heat susceptible cultivars (*Triticum aestivum* L) plants were continuously subjected to different kinds of stress at different levels and of different intensities. In the present study different cultivars of wheat (*Triticum aestivum* L) identified by plant breeders of IARI as heat tolerant or heat susceptible were used to study the process of nitrate reduction in leaves. The enzyme nitrate reductase (EC: 1.6.6.1) was studied under *in vitro* conditions. The extraction medium for the enzyme was made up of phosphate buffer (pH 7.5), EDTA and Cystein HCl. The leaf extract was centrifuged at 10000 x g, 15 min. and supernatant was used as the source of enzyme. Nitrate reductase activity from C-4 (maize) was slightly higher as compare to both heat tolerant and susceptible cultivars of wheat. Enzyme was fairly stable up to 24 hours when stored frozen (-20°C). The loss of activity was only 15-20% after 48 hours. The two kinds of cultivars though have near similar Michealis Menton constant (Km) values for co-enzyme (NADH), but km for nitrate (substrate) was significantly different. The km value with heat tolerant cultivar was 9.52X10⁻³M and for heat susceptible it was 33.3X10⁻³M. Nitrate reductase activity decreased after the exposure of seedling to temperature of 40°C in light, and major decrease in activity was during the first 30 min. Sucrose imparted protection to the enzyme in heat tolerant cultivar, when excised leaf blades were exposed to 45°C for 30 min. Nitrate reductase (*in vitro*) from both the cultivars showed unusually higher activity against the control when Mg²⁺ ions were present in the assay medium, while EDTA showed marginal decrease in the enzyme activity. The Wheat enzyme seems to have unique property of activation by divalent cat ion (Mg²⁺).

KEYWORDS: Wheat, Nitrate Reductase, NADH, Mg²⁺, EDTA, Km, Line Weaver-Burk