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OPTIMUM CONDITION FOR *IN VIVO* ASSAY OF NITRATE REDUCTASE IN LEAVES OF BLACK PEPPER (*PIPER NIGRUM* L.)

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ABSTRACT

The study conducted to understand the optimum pH, temperature, nitrate concentration, and phosphate buffer concentration for the *in vivo* assay of Nitrate Reductase (NR) in leaves of *Piper nigrum* showed that neutral pH (7.0), temperature at 30°C, phosphate buffer concentration at 0.1 M and nitrate concentration at 0.1 M, were found as best conditions for maximisation of activity. The activity was maximum at 12 noon and 5 hours incubation period were required to get complete activity.

Key words : Black Pepper, Nitrate Reductase,Methodology.

The Nitrate Reductase (NR) enzyme is considered to be a limiting factor for growth and development. NR activity is controlled mainly by the substrate. Without nitrate in the medium, little or no enzyme activity can be detected (Cheng et al., 1986; Crawford and Davis, 1988). Wallace and Pate (1965) found that NR is most active in fully mature leaves. Optimum pH for in vivo NR activity was reported between 7-8 for soybean leaves (Jowerski, 1978) and 7.5-8.0 in *Eichornia* leaves (Wignaraja, 1990). It has been shown that the levels of this enzyme were severly affected by change in temperature (Shivashankar and Ramadasan, 1983). The present study was undertaken with a view to find out optimum pH, temperature, nitrate concentration, and phosphate buffer concentration for in vivo NR assay in leaves of *Piper nigrum*.

Rooted cuttings of Black pepper (*Piper nigrum* L. karimunda) were planted in 30 cm earthen pots containing soil, sand and cowdung in the ratio 1:1:1. Samplings were done when plants were 3 month old, having five to six leaves. To standardise the sampling of leaf from the tip to the base of the plant in the order of maturity was assayed for NR activity. The youngest mature leaf, which was the third leaf from the tip was found to be best for sampling. Raju and Rajagopal (1988) have also reported that the highest NR activity is in youngest mature leaf of Black Pepper. The standard assay medium of the enzyme contained 0.25 g leaf disc in a total volume of 6 ml containing potassium nitrate and phosphate buffer. The discs were infiltrated for 3 minutes in the medium and incubated at 30°C for one hour in the dark. After incubation the samples were kept in a boiling water bath for two minutes to arrest the reaction and the nitrate content was estimated according to the method of Evans and Nason (1953). The activity is expressed in n.mol nitrite formed/h/g fresh weight. The experiments were repeated twice and gave similar trends, but only data from a single experiment has been reported here.

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The data are presented in table 1 and 2. The enzyme assay characters were studied in the Pepper leaf tissue at pH 6.4 to 7.6 in combination with phosphate buffer concentration at 0 to 0.25 and nitrate concentration at 0 to 0.25 in the assay medium. Table 1(A) shows the NR activity at different pH with optimum concentration of nitrate (0.1 M) and phosphate concentration (0.1M) and it was found that pH 7.0 gives the highest value. The pH 7.0 to 8.0, were reported to be the optimum pH range for maximum activity in most plants (Joworski, 1978). In Table 1(B) the activity at different phosphate concentrations are depicted. It may be noted that the activity is maximum at 0.1 M phosphate concentration. Table 1(C) shows the NR activity at different phosphate concentrations are depicted. It may be noted that the activity is maximum at 0.1 M phosphate concentration. Table 1(C) shows the NR activity at different nitrate concentrations, estimated at pH 7.0, Pi. concentration at 0.1 M. The highest activity is obtained at 0.1 M nitrate concentration. The effect of different temperatures on NR activity studied at pH 7.0, Pi concentration at 0.1 M and nitrate concentration at 0.1 M gives optimum temperature for maximum activity at 30°C(Table 2A). The NR activity shows rapid increase upto 5 hours and thereafter it declined gradually (Table 2 B). The decline, after

Table 1. Effect of pH, Phosphate buffer concentration (Pi) and nitrate on NR activity in vivo (in.mol. NO_2/h/g FW). Values in the parentheses are S E of Mean.

A	B		C		
pH	Activity	Pi Con. (M)	Activity	NO_3 Con. (M)	Activity
6.4	408 (14)	0	167 (9)	0	29 (1)
6.6	487 (13)	0.05	194 (5)	0.05	538 (13)
6.8	587 (15)	0.10	407 (16)	0.10	706 (18)
7.0	635 (12)	0.15	147 (8)	0.15	609 (13)
7.2	616 (16)	0.20	127(7)	0.20	286 (12)
7.4	456 (12)	0.25	115(4)	0.25	244 (11)
7.6	541 (9)				

Table 2. Effect of temperature and incubation time on NR activity and diurnal variation of NR Activity (n.mol No_2/g FW). Values in the parentheses are S.E. of Mean.

A	B		C		
Temp. (°C)	Activity	Inc. Period (hour)	Activity	Time	Activity (hour)
20	319 (10)	2	453 (18)	6 AM	203 (7)
25	543 (21)	4	1260 (70)	8 AM	367 (8)
30	701 (25)	5	1872 (83)	10 AM	496 (10)
35	625 (15)	6	1735 (56)	12 NOON	684 (21)
40	250 (5)	8	856 (33)	2 PM	280 (8)
				4 PM	178 (8)
				6 PM	164 (5)

5 hours, may be due to either feed back inhibition, or by the removal of nitrite by substrate induced Nitrite Reductase activity. In the diurnal variation, the activity increased from at 6 A.M. and attained maximum of 685 n.mol NO_2/h/g F.W. at 12 noon (Table 2C). This might be due to the corresponding increase of light intensity. Light intensity induced NR activity had been reported by Beevers and Hageman (1969) in Radish cotyledons. Oaks *et al.* (1982) found an enhancement of NR protein in light.

In conclusion, the study indicates that NR activity in Black pepper leaf tissue was optimised at 30°C in the medium with 0.1 M nitrate at pH 7.0 with 0.1 M phosphate concentration.

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