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Dehydrogenase Activity in the Rhizosphre Soils of Sugarbeet

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The field experiment was conducted at Agricultural Research Station, Mudhol, University of Agricultural Sciences, Dharward during the rabi season of 2011 and 2012 (two years) in order to investigate dehydrogenase activity in rhizosphere soils of sugarbeet. The dehydrogenase activity was investigated with sugarbeet genotypes and varied levels of nitrogen and potassium. Three genotypes were evaluated, among them Magnolia recorded significantly higher dehydrogenase activity than other genotypes and the treatment application 100 kg N and K_2O recorded significantly higher dehydrogenase activity among the fertilizer N and K_2O levels. The interaction effect was found significant. Increased levels of fertilizers decreased the dehydrogenase actitvity (DHA). The DHA was increased from 45 days to 90 days after sowing (DAS).

Keywords: Dehydrogenase actitvity, Rhizosphere soils of sugarbeet, levels of fertilizers and sugarbeet.

The enzyme Dehydrogenase plays a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2010). Many specific dehydrogenases transfer hydrogen to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Subhani *et al.*, 2001). Throughout mentioned co-enzymes hydrogen atoms are involved in the reductive processes of biosynthesis. Due to this fact, the overall dehydrogenase activity (DHA) of a soil depends on the activities of various dehydrogenases, which are fundamental part of the enzyme system of all living microorganisms, like enzymes of the respiratory metabolism, the citrate cycle and N metabolism (Subhani *et al.*, 2001). Thus, DHA serves as an indicator of the microbiological redox-systems and could be considered a good and adequate measure of microbial oxidative activities in soil.

Environmental factors such as soil type, temperature and moisture, application of organic and in-organic fertilizers play an important role in variation of microbial activity. Soil microorganisms participate in the processes that are crucial for long-term sustainability of agricultural systems (Nannipieri *et al*., 2003). In organic systems, plant production depends primarily on nutrient cycling in soils that are controlled by microbes and soil enzymes (Monokrousos *et al.* 2006; Karaca *et al.* 2011). As is stated by many researchers, different farming systems may change soil parameters especially soil microorganisms and enzymes (Monokrousos *et al*. 2006; Fliessbach *et al.* 2007; Karaca *et al.* 2011).

The continuous cultivation of soil without replenishing sufficient quantity of essential elements either through organic or in-organic sources may deteriorate the soil fertility

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status. Micro organisms play a very important role in nutrient recycling process. Analyzing DHA of a soil is an indirect indicator for checking the soil health. Thus, experiment was conducted with one of the objective to estimate the DHA. The experimental details and methodology followed are furnished under the sub heading material and method.

MATERIALS AND METHODS

The field experiment was conducted during *rabi* season of 2011-12 and 2012-13 at Agricultural Research Station, Mudhol, University of Agricultural Sciences, Dharwad. The soil of experimental site was clayey with minimum drainage. Prior to the experiment composite soil samples were drawn during both the years 2011 and 2012 from the experimental site and analyzed for the physical and chemical properties. The soil fertility status was 225 (low), 41.2 (medium) and 278 (medium) kg in available N, P_2O_5 and K_2O ha^{-1} respectively with an EC of 0.15 dSm^{-1}, pH of 8.4 and OC of 0.41% (low) during the season 1 (2011). Similarly, during the season 2 (2012) the soil contained 272 (low), 29.3 (medium) and 340 (high) kg available N, P_2O_5 and K_2O ha^{-1} respectively with an EC of 0.27 dSm^{-1}, pH of 7.3 and OC of 0.59 % (medium).

The experiment was laid-out in a split plot design with three replications. Main plot treatments consisted of three sugarbeet genotypes which were obtained from private organizations. Sub plot treatments included different N and K_2O levels with split application, 10% N and K_2O basal application, 30% N and K_2O at 30, 50, 90 DAS. 60 kg P_2O_5 ha^{-1} was common to all treatments and was applied as basal dose. The poultry manure was applied as an organic manure @ 3.5 t/ha fifteen days before sowing. The sugarbeet seeds were treated with *Trichoderma*, PSB and *Azospirillum* cultures. The experimental field was ploughed once with mould board plough and twice by tractor drawn cultivator. The ridges and furrows were formed with tractor drawn ridger. The crop was sown on 13th&12th respectively during August 2011&12 with the spacing of 75 cm x 10 cm. The seeds were dibbled on one side of the ridge on the top 3/4 portion from furrow. Fertilizer was applied as nitrogen through urea, phosphorus through complex fertilizer and potassium through muriate of potash as per treatment schedule. Hand weeding was carried out thrice during the season and need based irrigation was given to the crop. The plant protection was taken against pest and diseases as per the recommendation. The crop was harvested on 9th & 8th February, respectively during 2012&13. The statistical analysis was done by using MSTAT-C Software.

Dehydrogenase activity in rhizosphere soil samples was determined during 45 and 90 DAS by following the procedure as described by Casida *et al.* (1964). Ten grams of soil and 0.2 g of $CaCO_3$ were thoroughly mixed and dispensed in 250 ml conical flask. To each flask, 3 ml of 3% 2, 3, 5-triphenyl tetrazolium chloride (TTC), 1 ml of 1 per cent glucose solution and 8.0 ml of distilled water were added, which was sufficient to leave a thin film of water above the soil layer. The flasks were stoppered with rubber cork and incubated at 30°C for 24h. At the end of incubation, the contents of flasks were rinsed down into a small beaker and slurry was made by adding 10 ml methanol. The slurry was filtered through Whatman No. 50 filter paper. Repeated rinsing of soil with one ml of methanol was continued till the filtrate ran free of red colour. The filtrate was pooled and made upto 50 ml with methanol in a volumetric flask. The intensity of red colour was measured at 485 nm against a methanol blank using spectrophotometer. The concentration of formazan in soil samples were determined by reference to a standard curve prepared by using graded concentration of formazan. The results were expressed as µg of triphenyl formazan (TPF) formed g^{-1} soil day^{-1}.

EXPERIMENTAL RESULTS

The data was analyzed independently during both the years of study, the pooled data has been highlighted in the experimental results. The DHA was increased from 45 DAS to 90 DAS in general. The experimental results are furnished in the **Table 1**.

Significantly higher dehydrogenase activity (16.2 and 21.5 µg TPF g^{-1} soil day^{-1} at 45 DAS and 90 DAS, respectively) was recorded in rhizosphere soil where Magnolia was cultivated (Fig. 1). The lowest dehydrogenase activity was seen in SZ 35 (12.8 and 18.5 µg TPF g^{-1} soil day^{-1} at

Table 1. Soil dehydrogenase activity of sugarbeet at different growth stages as influenced by genotypes, nitrogen and potassium levels during 2011 and 2012

Treatment	Soil dehydrogenase activity (µg TPF g^{-1} day^{-1})					
	45 DAS			90 DAS		
	2011	2012	Pooled	2011	2012	Pooled
Genotype (V)						
SZ 35 (V_1)	11.60	15.10	12.8	14.00	21.90	18.5
PAC 60008 (V_2)	12.50	15.60	14.2	15.80	25.50	20.6
Magnolia (V_3)	12.60	13.80	16.2	19.70	29.20	21.5
S.Em±	0.20	0.51	0.14	0.30	0.26	0.33
CD at 5%	0.79	2.01	0.47	1.17	1.04	1.07
N and K_2O level (F)						
100:100 kg N: K_2O ha^{-1} (F_1)	17.90	20.20	17.7	17.60	31.20	25.7
120:120 kg N: K_2O ha^{-1} (F_2)	16.60	18.50	17.0	17.40	27.90	23.2
140:140 kg N: K_2O ha^{-1} (F_3)	11.60	15.70	14.4	17.20	26.10	20.9
160:160 kg N: K_2O ha^{-1} (F_4)	9.30	11.70	12.6	15.90	22.40	17.0
180:180 kg N: K_2O ha^{-1} (F_5)	5.70	8.30	10.1	14.50	20.10	14.2
S.Em±	0.55	0.76	0.53	0.83	0.73	0.57
CD at 5%	1.61	2.22	1.50	2.41	2.14	1.63
Interaction (VxF)						
V_1F_1	16.40	21.90	15.5	14.70	30.80	26.3
V_1F_2	15.60	21.30	15.0	14.50	22.10	21.7
V_1F_3	12.20	14.70	13.3	14.30	20.20	17.5
V_1F_4	9.50	10.70	11.4	13.20	19.60	15.2
V_1F_5	4.10	7.10	8.6	13.10	16.60	11.9
V_2F_1	18.40	18.50	17.9	17.20	29.10	23.8
V_2F_2	17.10	18.20	17.2	17.30	28.40	23.3
V_2F_3	11.50	17.10	14.2	17.00	28.60	22.9
V_2F_4	8.60	13.50	11.8	14.90	20.90	17.2
V_2F_5	7.00	10.90	9.9	12.70	20.40	15.7
V_3F_1	18.80	20.30	19.8	20.80	33.90	27.1
V_3F_2	17.10	15.90	18.8	20.40	33.20	24.6
V_3F_3	11.20	15.10	15.7	20.30	29.40	22.3
V_3F_4	9.80	10.90	14.7	19.50	26.50	18.7
V_3F_5	6.20	7.00	11.9	17.60	23.20	15.1
S.Em ±	0.96	1.32	0.91	1.43	1.27	0.99
CD at 5%	NS	NS	NS	4.18	NS	2.82

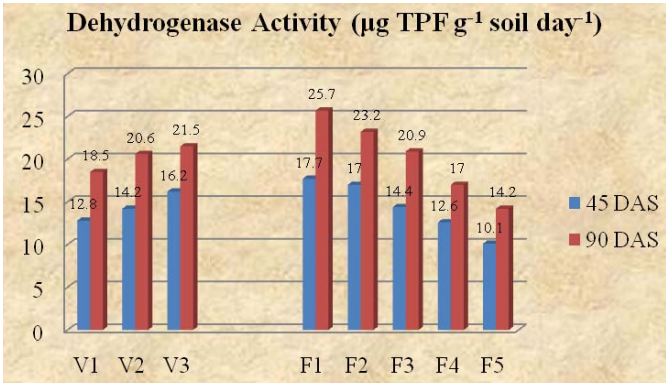

Fig. 1. Dehydrogenase activity as influenced by genotypes and Fertilizer N and K_2O levels (Pooled).

45 and 90 DAS, respectively). The dehydrogenase activity in rhizosphere soils of Magnolia was higher than the other genotypes. These variations in the microbial activity surrounding the rhizosphere soil might be due to differential root exudation and rhizo-deposition. This may vary from one genotype to another due to its substantial composition of the particular genotype.

With increase in fertilizer levels the enzyme activity in rhizosphere soil declined markedly. The highest soil dehydrogenase activity was recorded in application of N and K_2O @ 100 kg ha^{-1} (17.7 and 25.7 µg TPF g^{-1} soil day^{-1} at 45 and 90 DAS, respectively). The lowest enzyme activity was observed at 180 kg ha^{-1} (10.1 and 14.2 µg TPF g^{-1} soil day^{-1} at 45 and 90 DAS, respectively). Changes in soil organic matter due to different fertilization practices can be characterized by evaluating a long-lasting increase or decrease in total organic C content in top soils (Kubát and Lipavský, 2006).

Apart from the fertilization several environmental factors, including soil moisture, oxygen availability, oxidation reduction potential, pH, organic matter content, depth of the soil profile, temperature, season of the year, heavy metal contamination and soil fertilization or pesticide use may affect DHA significantly in the soil environment.

The interaction effect was found significant during 90 DAS. The highest dehydrogenase enzyme activity was observed in the soil where Magnolia was cultivated with N and K_2O @ 100 kg ha^{-1} (27.1 µg TPF g^{-1} soil day^{-1}) and was on par with Magnolia cultivated with N and K_2O @ 120 (24.6 µg TPF g^{-1} soil day^{-1}). The lowest enzyme activity was seen in SZ 35 with higher level (180 kg N and K_2O ha^{-1}) of fertilizers (11.9 µg TPF g^{-1} soil day^{-1}).

However, fertilization could affect on the population of soil microorganisms and consequently soil enzymatic activities. It is often assumed, that inorganic fertilizers had relatively less effect on soil enzymes activity than organic fertilizers (Chu *et al.*, 2007; Xie *et al.*, 2009; Romero *et al.*, 2010). Macci *et al.* (2012) reported that DHA usually reached higher level in the organic treatments. As was suggested by Chu *et al.* (2007) and Xie *et al.* (2009) long-term balanced fertilization greatly increased DHA level in the soil environment, rather than nutrient-deficiency fertilization.

CONCLUSION

Application of 100 kg N and K_2O to the Magnolia genotype has enhanced the dehydrogenase activity. The increase in the levels of N and K fertilizers to the sugarbeet has deteriorated the enzyme activity. However, long term balanced application of fertilizers may maintain the activity of microbes. It is recommended to grow along with application of organic manures which might be increasing the dehydrogenase activity in the rhizosphere soils.

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