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THE DYNAMIC OF NITRATE REDUCTASE IN TEA LEAVES

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ABSTRACT

Crop productivity depends on adequate nitrogen supply. Nitrate is one form of nitrogen in the soil which is absorbed by plant root and the process of nitrate reduction in plant cells was catalyzed by nitrate reductase, therefore NRA might be able to be used as biochemical tools to estimate tea leaves productivity and the dynamics of NRA can be used to check the nitrogen availability in the soil.

To study the dynamic of nitrate reductase in respond to fertilizer application, an experiment which includes two factors i.e. plant materials and nitrogen fertilizer application was conducted during rainy season at experimental field of Pagilaran tea plantation. Factorial treatment design with plant materials i.e. 2 vegetative origin of TRI-2024 and TRI-2025 and 1 seed origin were used as first factor, while 2 dosages of nitrogen fertilization (0 and 250 kg/ha urea + 60 kg/ha SP36 + 60 kg/ha KCl) used as the second factor with 3 replications. The activity of nitrate reductase was then measured every week continuously started 8 weeks before fertilizer application (WBF) and ended 8 weeks after fertilizer application (WAF).

There result showed that the dynamics of NRA was depend on soil moisture and nitrate availability in the soil. When urea was used as nitrogen source, urea will be hydrolyzed first to produce ammonium and then ammonium will be nitrified to nitrate. This process could be studied from NRA in tea leaves, though NRA seemed could not be used as biochemical tool to estimate tea leaves productivity.

Keyword : Nitrat reductase, tea, production

INTRODUCTION

Plant growth which affects the crop product quantity and quality is depend on an adequate nitrogen supply. Nitrogen is generally supplied through urea application. Excessive nitrogen fertilizer application nevertheless may contribute to ground and surface water solution through leaching and soil erosion (Sisson *et al*., 1991). In most well-drainage agricultural soil such as in

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tea plantation, nitrate typically accumulates, regardless of the form of nitrogen inputs and most plants take up nitrogen in the form of nitrate (Wickremasinghe *et al*., 1980). Nitrate is absorbed by plant root and is available for translocation to the shoot, storage in the vacuole and assimilation into reduced nitrogen products. The processes of nitrate uptake, translocation, and assimilation are interdependent and closely regulated in higher plants (Sivasankar and Oak, 1996). Leaf tissue accumulates nitrate when the rate of nitrate delivery exceeds the nitrate accumulation capacity. Enzyme nitrate reductase (NR) catalyzes the reduction of nitrate to nitrite and is considered to be a limiting factor in the growth and development of plants (Campbell, 1997).

An increase in the amount and activity of nitrate reductase leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for general amino acid synthesis, protein synthesis or total nitrogen assimilation (Vincentz *et al.*, 1993). Nitrate availability, product of nitrate assimilation regulates nitrate assimilation (Lillo, 1994) and nitrate assimilation is also determined by genotype (Venkatesan, 1995).

Nitrate availability in the soil can vary by several orders of magnitude, both seasonally, from place to place, even over short distance, time, and dosage of nitrogen fertilizer application. NRA which is affected by nitrate availability in the soil was recommended as a biochemical tool for the prediction leaf tea yield, therefore in this opportunity, the nitrogen availability in the soil will be elucidated by using nitrate reductase dynamic in the leaves.

MATERIALS AND METHODS

To study the dynamic of nitrate reductase in respond to fertilizer application, an experiment which includes two factors i.e. plant materials and nitrogen fertilizer application was conducted during rainy season at experimental field of Pagilaran tea plantation. Factorial treatment design with plant materials i.e. 2 vegetative origin of TRI-2024 and TRI-2025 and 1 seed

origin were used as first factor, while 2 dosages of nitrogen fertilization (0 and 250 kg/ha urea + 60 kg/ha SP36 + 60 kg/ha KCl) used as the second factor with 3 replications. The nitrate reductase activity (NRA) was then measured based on the protocol developed by Johnsen *et al.* (1991) every week continuously started 8 weeks before fertilizer application (WBF) and stopped 8 weeks after fertilizer application (WAF). The collected data then were evaluated by analysis of variance with 5% significant level. When there were significant different at every source of variation, further analyses based on mean comparison and graph will be executed (Gomez and Gomez, 1984).

RESULTS AND DISCUSSIONS

Tea is one of the most popular beverages in the world (Su *et al*., 2007) and requires high soil nutrient such as N, P, K, Ca, and Mg (Bore, 1996), however nitrogen and kalium are the major nutrient of tea without which it would not be feasible to achieve the commercial production level (Venkatesan *et al*., 2005). Nitrogen is the single most important growth limiting factor and when supllied in the form of urea, has proved to be the most instrumental among all major elements in boosting the yield of numerous plants (Ashraft *et al*., 2006). In the soil, urea which is synthesized from ammonia, by Nitrozomonas and Nitrobacter bacteria will be nitrified to produce nitrate. In most plants, it has been shown that both NO_3^- and NH_4^+ forms are taken up and metabolized by plants. The preferential source has been shown to be independent on plant species, cultivar, and environment (Taghavi *et al*., 2004). Most species grow best if they have access to both nitrogen forms (Errebhi and Wilcox, 1980). When NO_3^- was dominantly used as nitrogen source of plants, nitrate reductase is the most important enzyme responsible for nitrate assimilation in plants. Nitrate reductase activity (NRA) is than considered to be limiting factor for growth, development, and protein synthesis (Solomonson and Barber, 1990) and was reported to be highly correlated with plant growth (Shen *et al*., 1993).

Table 1. Analysis of Variance of NRA in Tea Leaves

Source of variation	Degrees of freedom	Mean square	Probability
Planting materials	2	0.8181	0.0728
Fertilizer applications	1	1.7879	0.0201
Planting materials* fertilizer applications	2	0.3266	0.3054
Error (a)	12	0.2490	
Observation times	10	11.5903	0.0001
Planting materials*obsevation times	20	0.3518	0.0837
Fertilizer*observation times	10	0.8139	0.0004
Planting materials*fertilizer applications*observation times	20	0.1799	0.7306
Error (b)	120	0.2301	

There was a very high significant interaction effect between fertilizer and time of NRA measurement (table 1). High significant effect were also detected in observation times (P: 0.0001) and fertilizer application (P: 0.0201). Fertilizer tended to give higher nitrate reductase 6 weeks after fertilizer applications (table 2). Similar result has been reported in tea by Suryani (1999). NRA was affected by nitrogen sources (Taghavi *et al*., 2004; Villora *et al*., 2002). NRA was induced by NO_3^- (Johnsen *et al*., 1991) and can be altered by the changing of NO_3^- concentration (Crawford, 1995). In soils, nitrogen is available as dissolved organic nitrogen, NH_4^+, NO_3^-, or any combination of these and will be absorbed by plants (Glass and Siddiqi, 1995). Normally, the relative amount of nitrogen forms depended upon a number of biotic and abiotic factors which determine the rate of mineralization, nitrification, denitrification, leaching, relative uptake by organims (Stark and Hart, 1997). With the soil applied urea as nitrogen source, the nitrogen from urea is recycled as NH_4^+ following the action of urease in anaerobic condition (Goodwin and Mercer, 1983; Wickermasinge *et al*., 2006). The hydrolysis of urea in tea soil is influenced by soil pH and

incubation period (Sedaghathoor *et al*., 2009). There was a strong positive correlation between urease activity and organic matter content in the soil. The urease activity reached maximum within minimum 2 weeks after fertilizer application. As the amount of NH_4^+ in the soil increase, characterically nitrification rate, and soil NO_3^- concentration increase subtantially (Prescott, 1997). As soon as NO_3^- was produced, it will be absorbed. As a result, the NRA then increase started 3 weeks after fertilizer application. The maximum NRA was then achieved 8 weeks after fertlizer application.

Table 2. The Effect of Fertilizer Applications on NRA at Different Observation Times Started 2 Weeks Before and 8 Weeks After Fertilizer Applications

Observation times (weeks before and after fertilizer applications)	*NRA (μmol NO_2/g/h)*	
	With fertilizer application	*Without fertilizer application*
-8	1.343 de	1.035 e
-6	1.128 e	1.198 de
0	0.492 f	0.309 f
1	0.533 f	0.466 f
2	0.604 f	0.996 e
3	1.676 cd	1.532 d
4	1.514 cde	1.414 de
5	1.697 cd	1.534 cb
6	1.926 c	1.960 cb
7	3.434 a	2.103 b
8	2.951 b	2.659 a

Remarks: Means followed by similar letters were statistically no different by DMRT at 5% significant level.

It is very difficult to discuss about the dynamic of NRA in the tea plant without fertilizer application. Theoretically, the NRA must be very low, unfortunately in this experiment the pattern of NRA dynamic in such environment was similar to area with fertilizer application. The nitrogen at the tea plant without fertilizer application might come from the organic matter decomposition (Dogo, 2001). The rate of total biomass production of plucked tea plantation was ranged from 9.43–11.40 ton/ha/year (De Costa *et al*., 2007) and it was actually equal to 350 kg N/ha (Ranganathan, 1973). Pruning

as an essential agronomic practice in tea leaves production will be cycled every 4 years (Rarichandran, 2004) and contribute to a flow of nutrient in tea ecosystem. Such huge total amount of organic matter was never removed from the field, so that it could increase the total organic matter in the tea field. During rainny season, organic mater degradation might be more active, as a result the availability of nitrogen to plant will increase. Though the nitrogen availability to plant increase in area without fertilizer application, but the amount was considered lower compared to area with fertilizer application. This phenomenon can be seen 7 weeks after fertilizer application where there was a significant different of NRA from both tea plant and the different was quiete high. The significant different respond of NRA 7 weeks after fertilizer application might be caused by the dynamic portion of NH_4^+ and NO_3^-. NO_3^- concentration in the soil might decline faster in the area without fertilizer application. In both area, there was a trend that RNA will increase linearly after fertilizer application. Wisnubroto *et al*. (2002) reported that in Pagilaran tea plantation where the experiment was conducted, dry season normally happened from June to September. In this experiment, fertilizer was applied at the beginning of September when it was sometimes rain. Due to this experiment started at the beginning of rainy season, it was suspected that soil moisture also determine the nitrogen availability to tea plant (Figure 1).

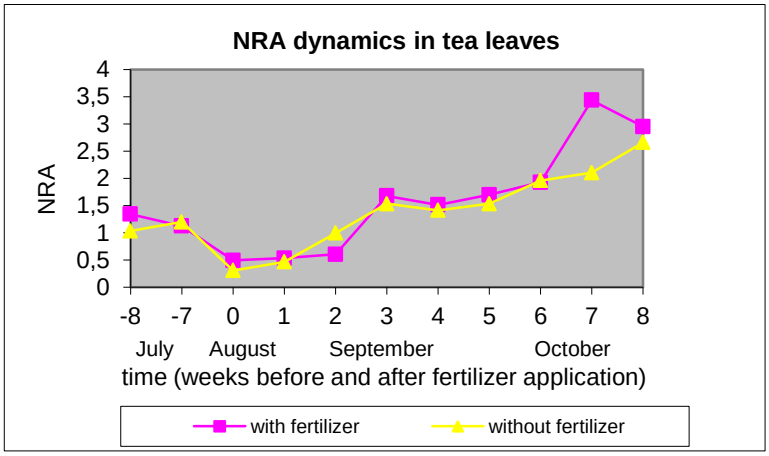

Figure 1. NRA Dynamics in Tea Leaves

The effect of soil moisture to nitrogen availability especially NO_3^- actually could be studied on the NRA before fertilizer application when it was dry season. From 8 weeks before fertilizer application, the NRA significantly decrease in both area. Grundmann *et al*. (1985) mentioned that soil moisture affect NO_3^- availability through nitrification process. Water availability surely influenced the NRA at the beginning of the experiment, because Foyer *et al*. (1998) reported that water stress decrease NRA. Although there was no data, watering a week after fertilizer application and rain which occurred 2 weeks after fertilizer application significantly influenced NRA. The positive respond of NRA to water availability can be observed as fast as 2 weeks after watering in area without fertilizer application and 3 weeks in area with fertilizer application.

CONCLUSION

In this experiment, it could be concluded that the dynamics of NRA was depend on soil moisture, nitrogen availability in the soil especially nitrate. When urea was used as nitrogen source, urea will be hydrolyzed first to produce ammonium and then ammonium will be nitrified to nitrate. This process could be studied from NRA in tea leaves, though NRA seemed could not be used as a biochemical tool to predict tea leaves productivity.

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