Correlation of fertilizer application, growth and nutrient transporter gene expression in Thai cassava

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Abstract

Cassava is an economically important crop of Thailand which can resistance to drought condition or poor soil quality. Macronutrients including nitrogen, phosphorus, potassium are important for cassava growth. This research is used molecular tools to investigate how plant response to nutrient application at different stages of growth and development. To study responses of nutrient transporter genes to fertilizer, the three transporter genes namely *AMT2*, *PHT1* and *HAK5* that encode for protein transport N, P, and K respectively were examined in cassava applied with fertilizer for every 4 week and 8 week. In addition, other related growth parameters were also measured. Then the semi-quantitative PCR was used to determine gene expression. The results showed that at week 4, only *PHT1* genes was upregulated significantly between with and without fertilizer treatment. However, the expression level of all transporter genes was highly response to fertilizer treatment at week 8. This concluded that cassava responses differently to nutrient application during different stages of growth and development.

Keywords: cassava, nutrient transporters, fertilizer, gene expression, essential elements

Introduction

Cassava is one of the major economic crops in Thailand. Production of cassava was used in various industries such as food industry, animal feeds and also used as raw materials for some industrial products (FAO and IFAD 2000). Cassava is an easy growing plant and well adapted in poor soils and drought stress. Due to its adaptability and relatively high productivity, it has become a popular crop for farmers. The planted areas found in many areas of the country, especially in the Northeast (OAE 2000).To increase high yield cassava production, there are many factors involved such as, cultivars, environmental factors and also good crop management. Even though cassava can usually grow in poor soil areas, but continuous growing plant can lead to soil nutrients depletion and also affect to cassava growth and yield production. To overcome this problem, good crop management is important. The application of fertilizer causes significant increases in yields of storage roots as well as starch content (Howeler2002). Potassium promotes the formation of starch while nitrogen and phosphorus are essential for growth. Not only fertilizer was used for plant to produce high root yield, but also used for maintain fertilizer status in soils. Previous study about nutrient requirements in cassava showed that cassava require large amount of macronutrient in order to grow. Nitrogen is the highest amount of nutrients taking by cassava and followed by potassium and phosphorus (Howeler 1985a). Generally, fertilizer 50 – 100 kg per rai of 15-15-15 will be applied at 2 stages of cassava. The first fertilizer will be applied one month after planting and another at three months after planting (DOA 2009). However, the formula of fertilizer is not designed specifically to support the actual need of cassava and can lead to unnecessary loss fertilizer. Besides, soil erosion is one of problem that can lead to

loss of fertilizer (Howeler2001). The general practice to improve fertilizer efficiency in cassava is to add excessive amount of fertilizer and this can lead to high cost of cassava production. To reduce costs for excessive fertilizer and also protect unnecessary loss fertilizer**,** suitable management for fertilizer in the field is under investigation. Among the fertilization application studies in cassava, there are only few reports about the molecular response of cassava to fertilizer and also preferences of essential elements during different growth and developmental stages. Study at molecular levels about uptake, transport, storage as well as utilization of nutrients in cassava may provide more understanding about response of cassava to fertilizer application. Uptake and transportation of nutrients in plant cell requires several membrane proteins which are nutrient transporters including ammonium, phosphate and potassium transporter (Lodish et al. 2000). Plant ammonium transporter (AMT) play an essential role in NH_4^+ uptake which is a preferred source of nitrogen for plants growth and development (Sohlenkamp et al. 2000). Whereas, phosphate transporter (PHT) are functionally involved in uptake of inorganic phosphate (Pi) from the soil and its translocation within the plant (Nussaume et al. 2011). Additionally, potassium transporters play an essential in taking up K^+ which is the most abundant inorganic cation in soil (Glass, 1976). The expression of these transporters can influence overall nutrient status as well as the growth and development of the plants. Investigation of these transporter gene expressions might provide more insight into cassava nutrient absorption. In this study, expression patterns of transporter genes related to macronutrient uptake and utilization in cassava under fertilizer application were studied. The results may provide important information that can be used to improve suitable formula of fertilizer to improve yield of cassava.

Methodology

Plant material and growth conditions

The cassava cultivar Kasetsart50 (KU50) was planted in pots with low-nutrient commercial soil mix. In fertilizer experiment, the cassava plants were applied with macronutrients at 3 and 7 weeks after planting and unfertilized use as a control. Roots tissues were collected at 4 and 8 weeks after planting and subjected to RNA extraction. All experiments were done in three replicates.

Plant morphology and growth analysis

To study effect of fertilizer on plant growth, cassavas were grown in pots filled with low-nutrient commercial soil mix and divided into two treatments. The first group was added with 15-15-15 fertilizer and compared to non-fertilized group (control). Plants at 4 and 8 weeks were measured for plant length, and leaf dry weight. To measure leaf dry weight, plant fresh tissues were harvested and dried in oven at 65^{o}C and weighed.

Expression analysis

Total RNAs from roots were extracted using RNA extraction method (Stiekema et al. 1988). The concentrations of RNAs were quantified using Nanodrop^{TM} (Thermo Scientific) and checked for integrity by gel electroporesis. To eliminate contamination of genomic DNA, RNA were treated with DNase I treatment. DNase-treated RNA samples were quantified and converted to cDNA using SuperScript III kit (Invitrogen). To study expression pattern semi-quantitative PCR was employed and expressed in term of relative expression with internal control 18SRNA. PCR reactions were carried out with gene-specific primers were: AMT2 forward, 5'- CCTGGAT GGCTTTTGTACCT-3'; AMT2 reverse, 5'-GCAGCGAAGACTTTTTGTGA-3';PHT1 forward, 5'- ATACAGGTTGGTGGGGAACA-3'; PHT1 reverse, 5'- GCAAACACAGCAGCGATA AA-3' ; HAK5 forward, 5'- CAT

TAGCGAAGGAGGAGACG-3'; HAK5 reverse, 5'-GAA CCTGATGAAACCCGAGA-3'. Additional reaction components are composed of 10 mM polymerase buffer, 1 mM dNTPs, 0.1 U *Taq polymerase*, 50 mM $MgCl_2$, DEPC-treated water, and 1 µM specific primer. The conditions for the PCR : 5 min at 94°C, 30 sat 94°C, 30 sat 55°C, and 1 min at 72°C for 35 cycles and 72°C for 10 min. The concentration of templates is 100 ng per 50 mL PCR reaction. Amplified products were visualized on a 1% TAE agarose gel containing ethidium bromide. Bands were photographed using the Quantity One 4.5.1 ChemidocEQ™ Software System (Bio-Rad, CA, USA).

Results

Determination of cassava growth

To demonstrate the importance of fertilizer application for cassava growth, fertilizer was applied to cassava and compared with control plants. It was found that cassavas showed different growth in both week 4 and 8. In week 4, cassava applied with fertilizer had higher leaf dry weight when compared to non-fertilized treatment. In week 8, plant with no fertilizer showed a reduction in dry weight significantly compared to fertilizer treatment (Figure 1). The average plant height showed only small difference in week 4, however, significantly higher in week 8 in plant applied with fertilizer compared to control.

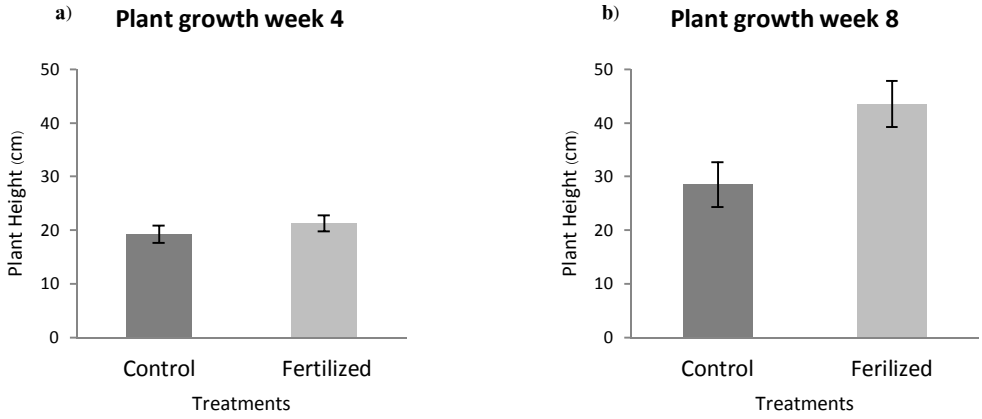

Figure 1 Cassava height in two different treatments at week 4 (a) and week 8 (b)

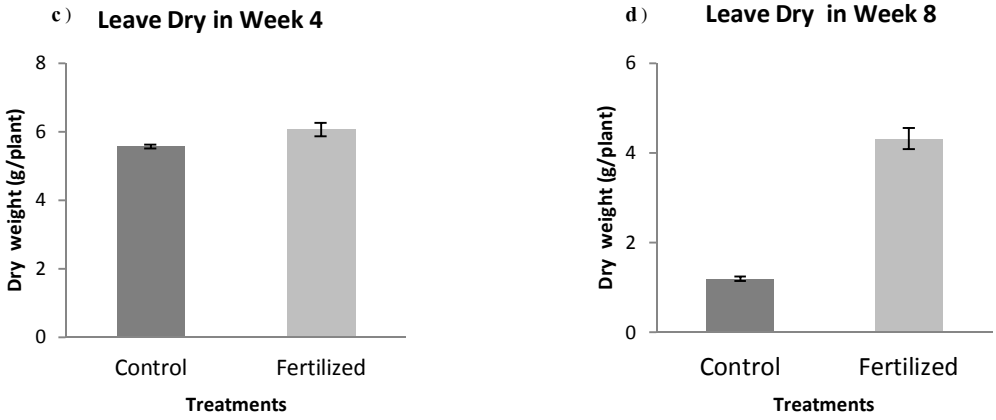

Figure 2 Leave dry weight of cassava in two different treatments at week 4 (c) and week 8 (d)

Expression analysis

To study expression of nutrient transporter genes that might response to fertilizer application, *AMT2, PHT* and *HAK5* genes that encode transport proteins for taking up N, P and K respectively were studies and 18S rRNA was used as an internal control. Expression analysis was conducted by using semi-quantitative PCR. The results showed that all transporter genes expressed in all tissues in both week 4 and week 8 experiments (figure 3 and 4). In week 4, expression of *AMT2* and *HAK5* were found similar in both with fertilizer and without fertilizer treatments. Contrastly, *PHT1* gene showed higher expression in fertilizer treatment compared than control (figure 3) However, it was found that all transporter genes in week 8 had higher expression in fertilizer treatment compared to control (figure 4). These results indicated the expression of *AMT2, PHT* and *HAK5* genes are regulated by nutrient application, however, differently responded between week 4 and 8. These expression patterns might relate to different utilization of nutrients during growth and development stages

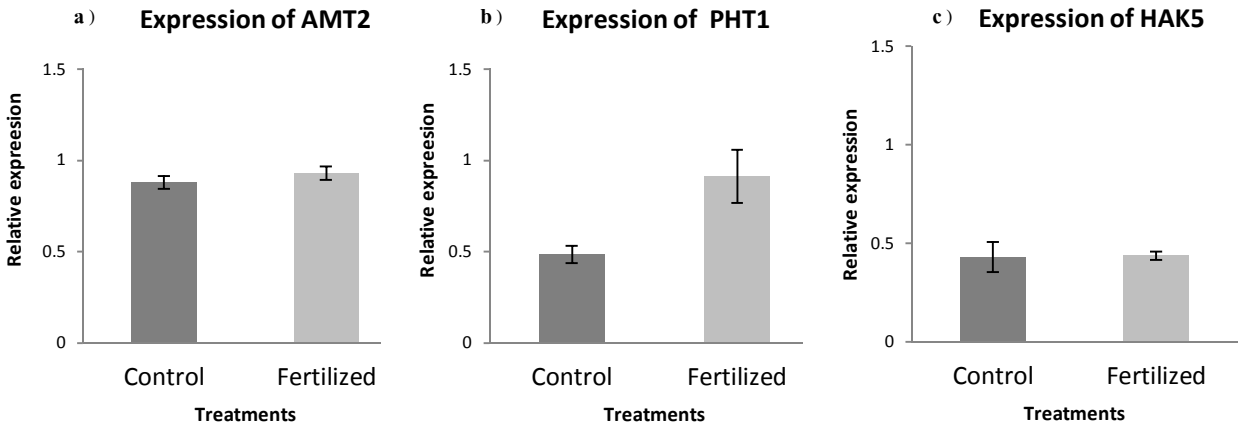

Figure 3 Relative gene expression of transporter genes from root tissues with and without fertilizer at week 4. Semi-quantitative PCR at 30 cycles were used. (**a**) *AMT1*; High Affinity $NH4^+$ transporter (**b**) *PHT1*; Phosphate transporter (**c**) *HAK5*; Potassium transporter.

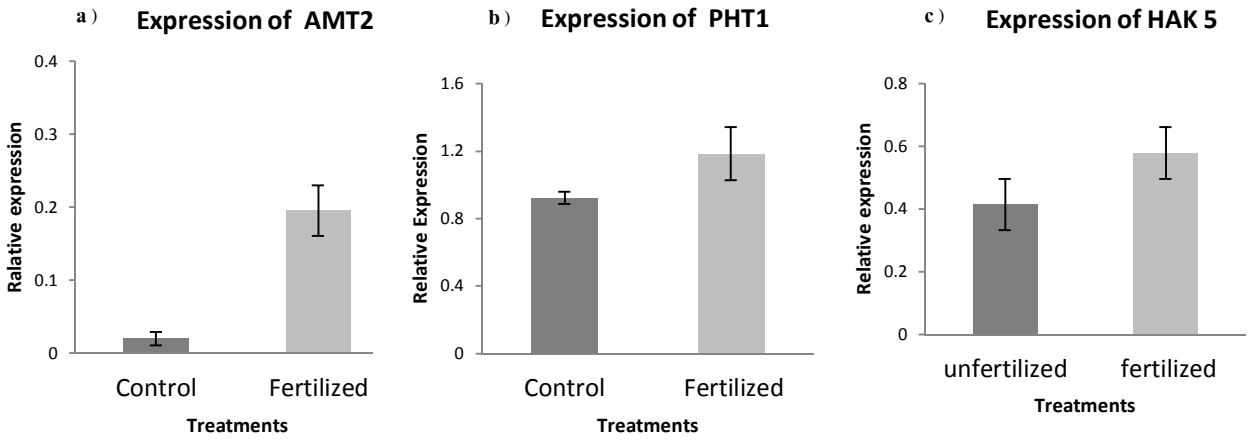

Figure 4 Relative gene expression of transporter genes from root tissues with and without fertilizer at week 8. Semi-quantitative PCR at 30 cycles were used. (**a**) *AMT1*; High Affinity $NH4^+$ transporter (**b**) *PHT1*; Phosphate transporter (**c**) *HAK5*; Potassium transporter.

Discussion

After applied N-P-K fertilizer (15-15-15) 50 kg/rai to cassava, it was found that fertilizer could increase dry weight of fertilized plants significantly. According to these results, the application of fertilizer can increase substantial growth in cassava. The positive effects of fertilizer demonstrated in these experiments are in agreement with previous studies (Howeler 2002). Study on expression of N, P and K transporter genes in these experiments found expression of all transporter genes in roots of cassava especially in fertilizer treatment.In week 4, it was showed that only *PHT1,* but not *AMT2* and *HAK5,* that was upregulated in response to fertilizer treatment. PHT1 transporter is the protein that involved in taking up phosphorus from soil (Koyama et al. 2004). Phosphorus is important role to promote early root formation and growth (Penas 1987). This might be the reason of *PHT1* regulation in fertilizer treatments compared to control at initial stage plant development. Meanwhile, the expression of *AMT2* transporter showed no difference between control and fertilizer treatment in week 4 but higher in fertilizer treatment in week 8. These results demonstrated the difference of nutrient requirement at different stage of plant growth and development. Generally, there are two forms of nitrogen sources in soils which are nitrate and ammonium and *AMT2* transporter is responsible of taking up nitrogen in form of ammonium (Sohlenkamp et al. 2000). Although, plant requires large amount of nitrogen for growth, difference forms of nitrogen transporter genes might play role during each stage of development. Regarding this, no different expression of *AMT2* between control and fertilizer treatment at week 4 might suggest the presence of another transporters that play a role to uptake nitrogen. Likewise, *HAK5* was showed no different in both treatments at initial stage. However, the expression of *HAK5* was higher only when plants received fertilizer. This might be because the regulation of *HAK5* is Potassium-dependent (Wang et al. 2002). Previously, it was reported that nutrient uptake processes and their molecular components are often regulated in response to two major parameters, nutrient availability and plant nutritional status (Buchner et al. 2001,2004; Glass et al. 2002). Fertilizer usage is closely associated with the growth phases of cassava. The response of cassava to fertilizer at initial stage in this study suggested that cassava might uptake phosphorus more than other nutrients to promote early root and leave formation (Penas 1987). However, cassava response differently to fertilizer applied at the later stage, especially to nitrogen. Previous study has been shown application of nitrogen will be promote branch production, leaf area and plant height (Fox et al. 1975). The optimal levels of fertilizer should be established specifically for producing high-quality plant as well as maintaining low cost of fertilizer. The further study will help us understand more about regulation of these transporter genes.

Conclusion

Fertilizer application promoted cassava growth and induced expression of nutrients transporter genes differently between treatments and time. It suggested that nutrient uptake in cassava might be regulated both by fertilizer availability and developmental stages. The expression of nutrient transporters suggest that PHT gene play important role in cassava growth at initial stage, while HAK and AMT genes play a role in the earlier stage. Information gained from these studies could benefit cassava fertilizer formulation and routine of application for maximum nutrient use efficiency and lower cost of production.

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