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Anatomical Characterization and Protein Profiles in Adventitious and Storage Roots of Two Commercial Thai Cassava Varieties

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ABSTRACT

Anatomical changes and protein expression patterns were examined in adventitious and storage roots of two commercial varieties of cassava grown in Thailand, namely Rayong 1 (R1) and Kasetsart 50 (KU50). Both storage and adventitious roots were harvested consecutively every 7 days after planting for a period of 9 weeks. Adventitious and storage roots were collected separately, cross-sectioned to observe anatomical changes under light microscopy. Iodine-stain, indicating starch accumulation revealed that starch accumulation started 35 days after planting in both varieties. Total protein in adventitious and storage roots were extracted in ice-cold saline buffer and analyzed for protein profile using SDS-PAGE. Similar to the anatomical changes, SDS-PAGE revealed unique protein bands in the cassava roots from 35 days after planting onward. Further analysis will be carried out to investigate and differentiate the unique protein patterns between adventitious roots and storage roots using 2-dimensional gel electrophoresis technique.

Key words: cassava, adventitious root, storage root, protein, SDS-PAGE

INTRODUCTION

Storage root of cassava is the most important source of starch used in the industry and as a source of food in some tropical countries. In Thailand, cassava is a major commercial crop with a rapid increase in area coverage and production over a short period. Currently, the area coverage of cassava in Thailand is approximately 1.08 million hectare (Office of Agricultural Economics, 2003). At the same time, the total production of cassava in the country was estimated about 16-18 million tons of root annually (Santisopasri *et al*., 2001), and about half of the total yield was converted to starch and exported for the rests (Sriroth *et al*., 1996). Thailand has long been the world's leading exporter of cassava products over 90 % of world export 6-7 million tons shipped annually. From 1999, the world demand for cassava production increased dramatically so the goal of cassava breeding program in Thailand was to improve starch yield and quality to serve the global need (http://www.foodmarketexchange.com).

Moreover, other applications of cassava starch include adhesive, textile, binding agent and

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paper (Santisopasri *et al*., 2001), as well as in the pharmaceutical and cosmetic industries. However, high variation in starch quality makes the utilization restricted (Santisopasri *et al*., 2001). Thai cassava researchers have put their effort in conventional breeding programs for nearly three decades to develop cassava varieties to improve economic return. Rayong 1 (R1) was developed in 1975 from local varieties with the advantage of good germination and adaptability to all environmental conditions including low soil nutrient or prolonged dry periods. Later on Rayong 60 (R60) was released in 1987 with the advantage of shorter harvest time, while ensuring high yield (Sriroth *et al*., 2000). In the following decade, other varieties were developed for high starch content and better adaptability to poor environmental conditions. These varieties include Rayong 90 (R90), Kasetsart 50 (KU50) and Rayong 5 (R5) that were released in 1991, 1992 and 1994, respectively (Santisopasri *et al*., 2001).

With the focus of plant breeders to develop high-yielding varieties with high starch content, the storage roots became the main targets for improving these two important traits. Two different kinds of roots were identified in cassava plant; adventitious roots which sprouted from the base of the cutting within a week, and tuberous or storage roots which developed from an increase in diameter of the adventitious roots within eight weeks after planting (Ekanayake *et.al*., 1997). Storage root formation (tuberization) in cassava is an important trait related to starch accumulation and quality (Cabral *et al*., 2000), and gives rise to growth and differentiation of parenchyma cell of the secondary xylem to accumulate starch (Cabral *et al*., 2001).

Biotechnological tools have been used to identify the genes controlling the traits of interest, to facilitate breeding program. Salekdeh *et al*. (2002) used proteomic techniques to discover genes expression during rice response salt stress to find candidate stress-tolerance genes and utilize them in molecular breeding. Similarly, Dubey and Grover (2001) genetically engineered rice with improved flooding tolerance through proteomic maps of flood-tolerant and flood-sensitive proteins associated with O_2-deprivation stress and recover regimes.

Cassava biotechnology also has been used to unravel genes associated with initiation of cassava storage root. Cabral *et al*. (2000) reported two major proteins to be associated with early stages of development of the storage root, and two major proteins to be associated with adventitious root demonstrated by SDS-PAGE analysis.

However, information on the physiology, biochemistry and molecular biology of gene regulation and protein patterns during storage root development in cassava is limited. Therefore, with the advancement of biotechnology, there are numbers of ways to help elucidate the answers of the developmental process during tuberization and root tissue differentiation.

The objectives of this experiment were to observe anatomical changes and protein expression pattern of storage root of cassava during different stages of development. Further study was necessary to characterize unique proteins related to the storage root formation by using two-dimensional gel electrophoresis technique which provided basic information on how the storage roots are initiated. The integration of knowledge in physiology, biochemistry and molecular biology from this study would assist improving cassava breeding program of the country.

Therefore, the main objectives of this experiment were to compare protein expression and observe the anatomical changes between adventitious roots and storage roots at different stages of development in Thai Cassava.

MATERIALS AND METHODS

Plant materials

Two varieties of cassava, Rayong 1 (R1)

and Kasetsart 50 (KU50) were used in this experiment. R1 was developed from local varieties. It is well adapted to various environmental conditions. KU50 is the most popular variety (cultivated about 56% of the total cassava planting area). It contains high starch percentage. The 25 cm-long stem cuttings were planted vertically in 0.014 m^3 pitchers using sand and soil at the ratio of 3:1. The experiment was performed in 3 replications using completely randomized block design. Both storage and adventitious roots were harvested every 7 days after planting (DAP) for the period of nine weeks. Adventitious roots and storage root were collected separately then were cross-sectioned to observe anatomical changes of roots.

Anatomical characteristic of cassava roots

Tissues of root samples were cross-sectioned and stained in O-toluidine. Afterward observations were made on the pattern of anatomical change under light microscope (Olympus, research microscope system). Iodine stain was used for recognition of starch deposition.

Analysis of protein pattern of cassava roots

Total protein was extracted from both storage and adventitious roots using the method described by Cabral and Carvalho (2001). Two different kinds of roots, storage and adventitious roots, were collected from the outdoor pots 14, 21, 28, 35, 42, 49, 56 and 63 days after planting (DAP). Adventitious roots were washed, while storage roots were peeled, sliced and kept until extraction. Samples were ground in liquid nitrogen to fine powder. Ten grams of extracted powder were added with 3 volumes of ice-cold extraction buffer (25 mM Sodium phosphate pH 7.0, 250 mM NaCl, 10 mM EDTA, 10 mM thiourea, 10 mM DTT, 1 mM PMSF and 1.5% polyvinylpolypyrolydone) was added. The samples were incubated overnight at 4°C with constant agitation at 150 rpm in a rotary shaker. Extracts were filtered through four layers of cheesecloth and centrifuged at 10000 *xg* for 90 min twice to eliminate debris and starch. The supernatant was collected and proteins were precipitated by adding 2 volumes of cold ethanol. Samples were centrifuged at 10000 *xg*, for one hour and the pellets were dissolved in suspension buffer (9 M urea, 4% Triton X-100, 1% DTT, and 0.5% IPG buffer). Protein concentration was estimated by Bradford's method (Robertson *et al*., 1997).

RESULTS AND DISCUSSION

Morphology of cassava roots at different developmental stages

The morphology of cassava roots was studied on two commercial varieties of cassava, namely R1 and KU50 under field conditions. This result showed that both varieties started sprouting of cassava root within 14 DAP (Figures 1 and 2) when planted from December 2004 to February 2005 with the average day/night temperatures of 32/20°C. However, first week of cassava planting the average day/night temperatures of 31/15°C were observed. Comparing with other experiment that was conducted from February to April, 2004 with the average day/night temperatures of 35/24° C under same growing conditions. Both varieties started to the sprout within 10 DAP (Figures 3 and 4). The difference on time of sprouting might be due to weather variables such as temperature. Aleves (2002) found that cassava plant could grow in a wide range of climate, low soil fertility, with annual rainfall < 600 mm in the semiarid tropics to > 1500 mm in the sub-humid and humid tropics. The temperature affected on sprouting, leaf size, leaf formation and storage root formation of cassava. At low temperatures (16°C), sprouting of the stem cutting was delayed, however when the temperature reached more than 37°C, sprouting of cassava root was inhibited.

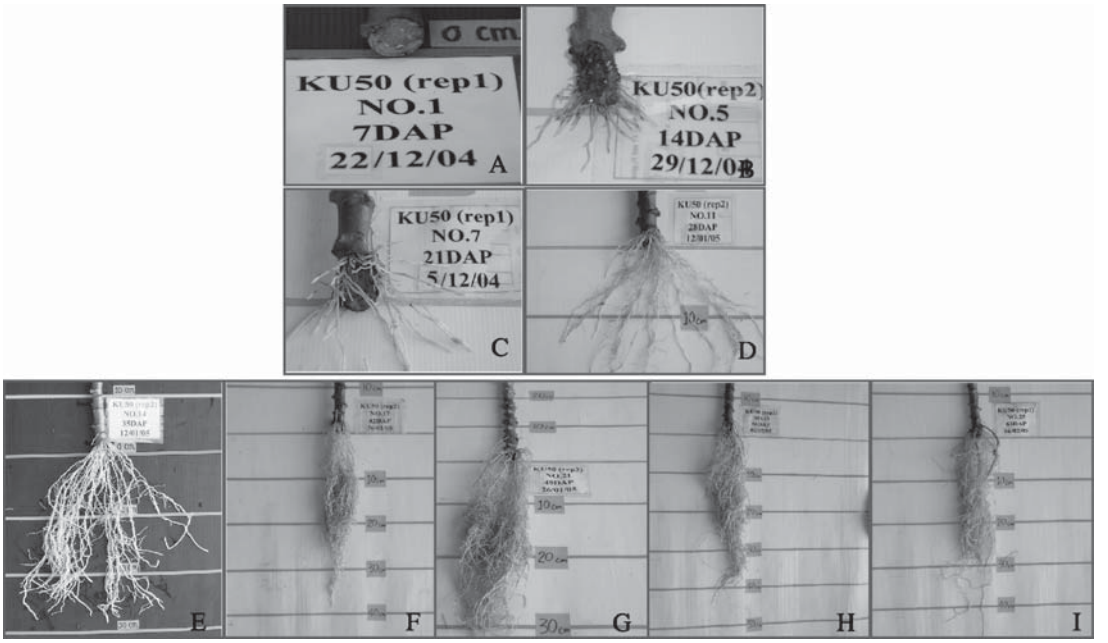

Figure 1 Sprouting of KU50 variety of cassava roots 14 DAP (B) from December 2004 to February 2005 (A-I: 7 to 63 DAP, at a 7-day interval).

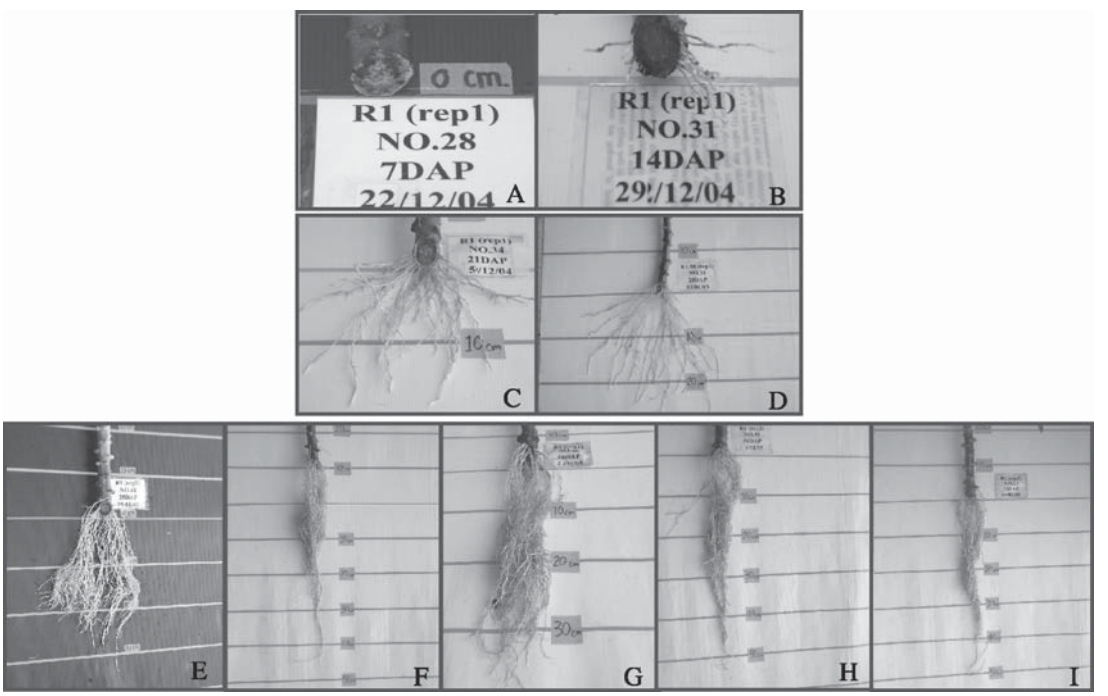

Figure 2 Sprouting of R1 variety of cassava roots 14 DAP (B) from December 2004 to February 2005 (A-I: 7 to 63 DAP, at a 7-day interval).

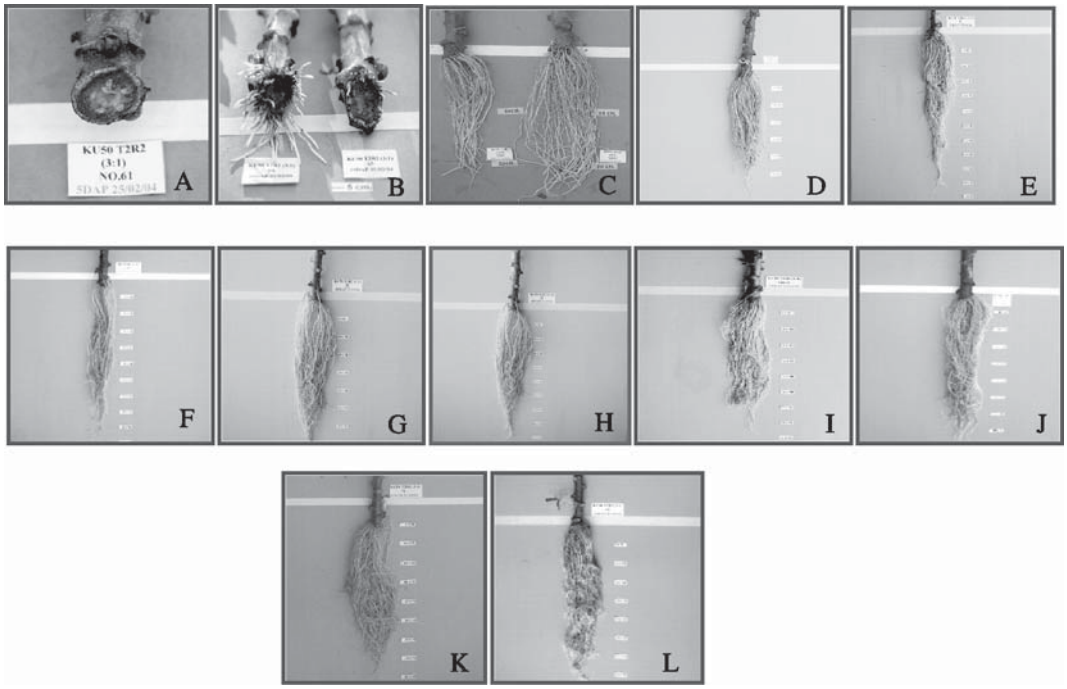

Figure 3 Sprouting of KU50 variety of cassava roots 10 DAP (B) from February to April 2004 (A-L: 5 to 60 DAP, at a 5-day interval).

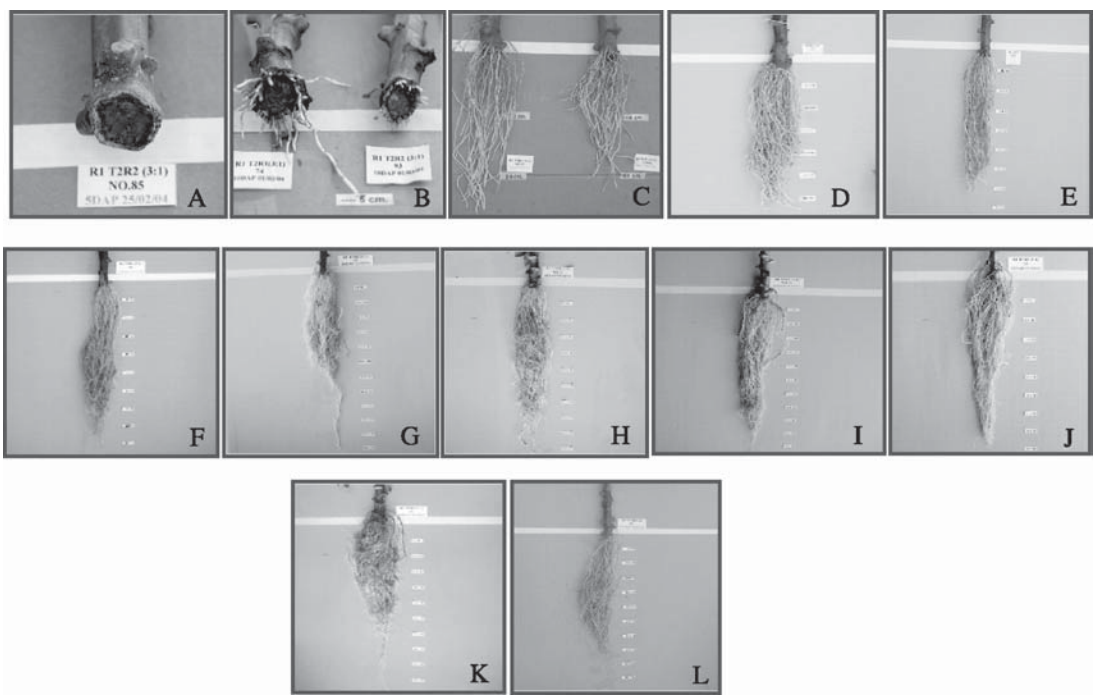

Figure 4 Sprouting of R1 variety of cassava roots 10 DAP (B) from February to April 2004 (A-L: 5 to 60 DAP, at a 5-day interval).

The storage root formation was observed 42 DAP when they were planted from December 2004 to February 2005 (Figure 5). When the same varieties were planted from February to April 2004, the storage root formation was observed 35 DAP (Figure 6). This finding was similar to that of Aleves (2002) which reported that low temperature directly affected the storage root formation. In the normal situation, i.e. at optimum temperature, storage root formation which distinguished from fibrous root, started about 52 to 76 DAP or 60 to 90 DAP (Cock *et al*, 1979; Cabral *et al*., 2000). Therefore, storage root formation might depend on the types of varieties and weather conditions during the growing season.

Anatomical characterization of cassava roots

During anatomical characterization of the cassava roots, more emphasis was given on the time and sites of starch accumulation on different tissues. A series of tissue cross-sections made in this experiment revealed that starch accumulation started 35 days after planting. The starch accumulation on these tissues was confirmed with blue or dark-blue color after iodine staining (Figure 7). The starch accumulation was mainly found on secondary xylem parenchyma and secondary phloem parenchyma (Figure 8) of cassava root tissue.

Analysis of protein pattern of cassava roots

Analysis of protein pattern using SDS-PAGE showed that clear and unique bands were obtained from roots collected after 35 DAP (Figure 9). This study, showed protein profile of cassava root extracted in the experiments from December 2004 to February 2005 only. Unique protein bands of interest were found in the ranges of 75-80 kDa and 55-60 kDa in both varieties. A further prove is needed to verify these unique proteins subunits that may be associated with the anatomical change and to compare these proteins with those reported by Cabral et.al. (2000) whose ranges were between 40-70 kDa.

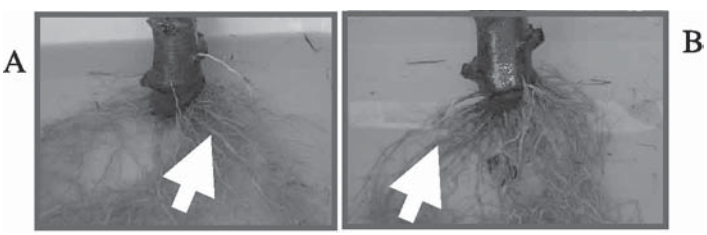

Figure 5 Storage root formation of two cassava varieties 42 DAP from December 2004 to February 2005 (arrows-formation of storage roots); (A- KU 50; B- R 1)

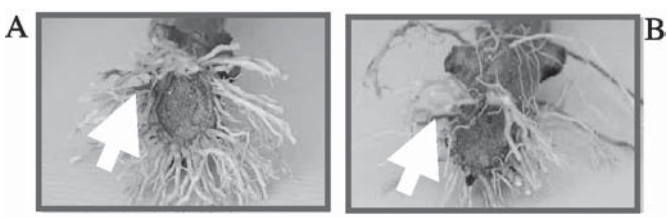

Figure 6 Storage root formation of two cassava varieties 35 DAP from February to April 2005 (arrows-formation of storage roots); (A- KU 50; B- R 1)

CONCLUSION

Sprouting of cassava root under field condition started within 14 DAP when planted from December 2004 to February 2005 and 10 DAP when planted from February to April, 2004. This difference might be a consequence of greater day/night temperature of 3/4 °C from February to April 2004 period when compared to a period from December 2004 to February 2005.

Anatomical study using cross-section and iodine staining suggested that starch accumulation started from 35 DAP. That could be assumed that storage root formation was initiated

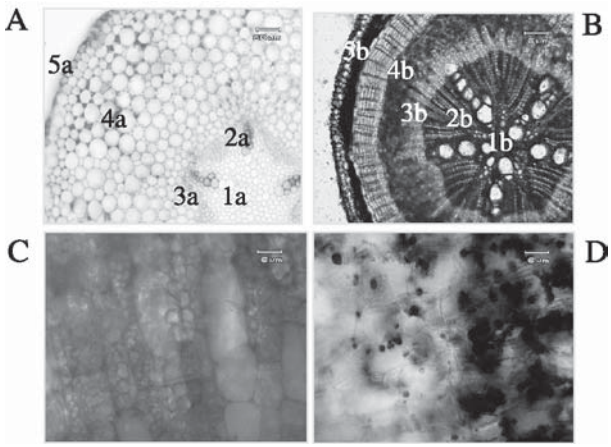

Figure 7 Cross-section of adventitious (A) and strorage root (B) and toluidine-and iodine-stained (C and D, respectively). In A, 1a; parenchymatous pith, 2a; primary xylem, 3a; primary phloem, 4a;cortex, 5a; periderm. In B, 1b; primary xylem, 2b; secondary xylem parenchyma, 3b; vascular cambium, 4b.secondary phloem parenchyma. In C and D, O-toluidine and iodine-stained (respectively) storage roots, showed distribution of starch accumulation around secondary phloem parenchyma.

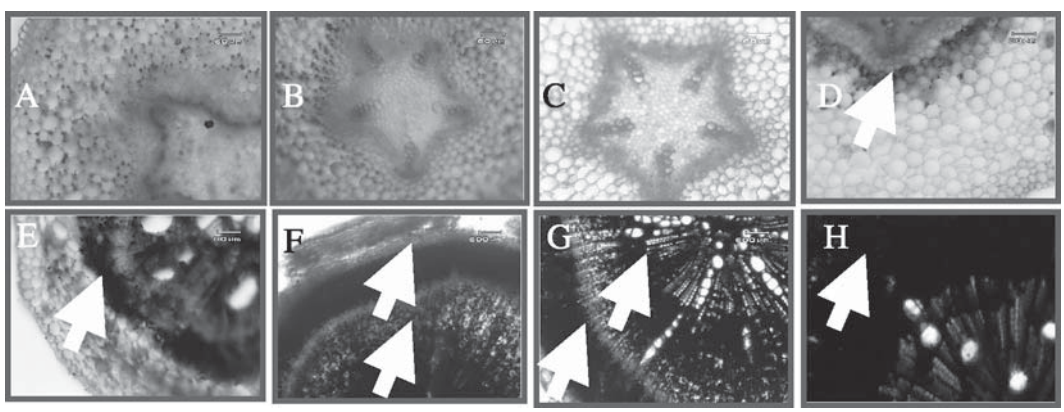

Figure 8 Starch accumulation of cassava roots at 35 DAP in December 2004 to February 2005 using iodine staining. A-H-cross-section (14,21,28,35,42,49,56 and 63 DAP respectively. Arrows: Sites of starch accumulation.

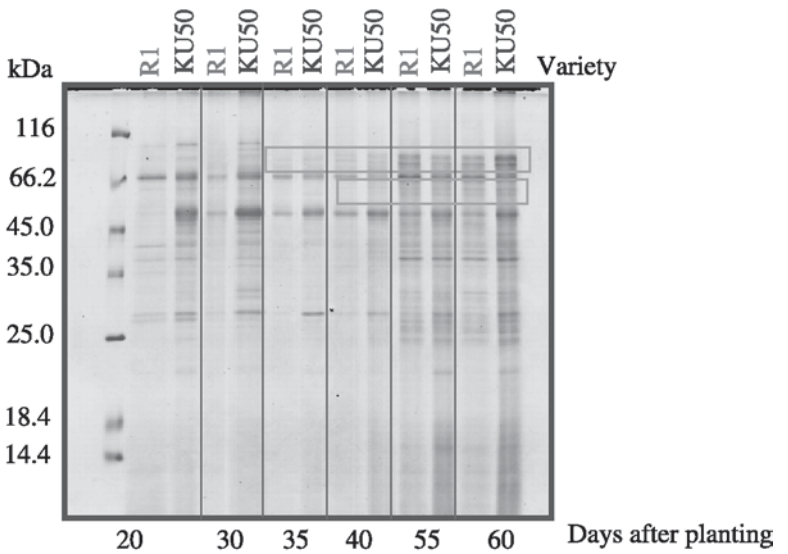

Figure 9 Protein expression during different developmental stages of cassava roots. The squares show unique patterns started 35 DAP in both varieties.

around the same period. Similarly, protein profile analysis showed unique protein band starting from 35 DAP. Molecular mass in the ranges of 75-90 kDa and 55-60 kDa were observed during this period. A further prove is needed to verify these unique proteins subunits.

Further analysis will be carried out to investigate and differentiate the unique protein patterns between adventitious roots and storage roots using two-dimensional gel electrophoresis.

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