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Path Analysis of Agronomic Traits of Thai Cassava for High Root Yield and Low Cyanogenic Glycoside

Kongsil, P.[1]*, Kittipadakul, P.[1], Phumichai, C.[1], Lertsuchatavanich, U.[2] and Petchpoung, K.[3]

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
[3]Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok 10900, Thailand

ABSTRACT

Most high-yielding cassava cultivars have high cyanogenic glycoside (CNglcs) content in their roots and the CNglcs content of < 50 ppm in fresh root is considered safe for consumption. The root yield and CNglcs content which are agronomic traits involving several genes and environmental interactions can be evaluated only during the harvest time. In this study, 83 breeding lines and parents were evaluated for the variation and correlation between root yield and CNglcs content with 17 agronomic traits: root weight, leaf weight, stem weight, starch content, harvest index, root number, plant type, plant height, the first branch height, cyanide-equivalent contents in root and in leaf, chlorophyll content, carotenoid contents in leaf and in root, and cassava bacterial blight, fibrous and tuberous root-knot symptom scorings that may affect root yield and CNglcs content. The multiple regression and path analysis indicated that: a) harvest index, leaf weight and stem weight, and b) stem weight, starch content, CNglcs content in leaf, the first branch height and leaf weight could produce root weight with predicted R^2 = 86.03 and 47.05%, respectively. Also, a) chlorophyll content, CNglcs content in leaf, and root-knot symptom scoring, and b) carotenoid content in leaf and CNglcs content in leaf could be used in screening for low CNglcs content in root with predicted R^2 = 52.20 and 55.06%, respectively. However, CNglcs content in leaf and root did not show any correlation with cassava bacterial blight and fibrous root-knot symptom scorings. Further evaluation and trial in other locations are required for the verification.

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E-mail addresses:
fagrpjk@ku.ac.th (Kongsil, P.),
kittipadakul@yahoo.com (Kittipadakul, P.),
agrcpp@ku.ac.th (Phumichai, C.),
agrusl@ku.ac.th (Lertsuchatavanich, U.),
rdikyp@ku.ac.th (Petchpoung, K.)
* Corresponding author

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is the fourth most important staple crops after rice, wheat and maize. It is one of the cyanogenic plants such as bamboo (*Bambusa* spp., Gramineae) and sorghum (*Sorghum bicolor* (L.) Moench, Gramineae) and some other cultivated plants, with cyanogenic glycoside (CNglcs) present in its leaves and roots. It is cultivated for tuber production in Central America, the Caribbean, Central, East and West Africa, India, Sri Lanka, Southeast Asia and most tropical regions in the equatorial belt between 30°N and 30°S latitudes. It is also used to a less extent for leaf consumption in West Africa, Brazil, Indonesia, Malaysia, the Philippines and Thailand (Tindall, 1983). Thailand ranks next to Nigeria and Brazil on cassava annual production, but it was the world largest exporter of dried cassava at 1.4 million tons in 1973-1974 to 6.7 million tons in 1983-1985 before it dropped to 3.9 million tons in 1988 (Ratanawaraha et al., 2001). Cassava export from Thailand was accounted at 77% of the total world export in 2005 and in 2012 the area harvested was 1,362,080 ha with 29,848,000 metric tons production (FAO, 2015).

Cassava originated in the northeastern region of Brazil, western and southern Mexico, and part of Guatemala. It was known to be taken by the Portuguese in the latter half of the sixteenth century to West Africa and from there, it spread to East Africa; taken from Brazil to Réunion and Madagascar in 1736; recorded in Zanzibar in 1799; introduced from Mauritius to Sri Lanka in 1786 and reached Calcutta in 1794; and probably taken at an earlier date to the Philippines from Mexico (Purseglove, 1981; Tindall, 1983). It was also reported that cassava was transported directly from Brazil to Java, Singapore and Malaysia in 1850.

Cassava is not native to Thailand and it is not known exactly when it was introduced for cultivation. Sarakarn et al. (2001) reported that cassava was imported mainly as germplasms nearly 40 years ago, and since cassava production in Thailand is mostly for starch-based industrial purpose, the key objective of the cassava breeding programme has been to increase root yield and starch content. According to Ratanawaraha et al. (2001), some 20 cassava cultivars were introduced from Malaysia, Indonesia and Mauritius before 1960, and more clones were introduced from Indonesia in 1963, the Virgin Islands in 1965, and the first introduction from the International Centre for Tropical Agriculture (Centro Internacional de Agricultura Tropical - CIAT), Cali, Colombia was in 1975. They were observed and evaluated for root yield, starch, dry matter and HCN contents, harvest index and other agronomic traits.

It was known that CNglcs content of the cassava cultivars commercially cultivated in Thailand is relatively high due to the traits in the parent plants used in the breeding programme. However, most cassava lines core collection and germplasms

at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, the International Centre for Tropical Agriculture (CIAT) in Cali, Colombia, and in Cameroon are known to have low CNglcs content (Bokanga, 1994). The known toxic principle found in all parts of the cassava plant is a CNglcs called linamarin. Bolhuis (1954) set the level of its toxicity at an intake of 50-60 mg daily for a European adult. Bhattacharya et al. (2009) reported that consumption of cassava with high CNglcs content, without proper processing, could cause acute or chronic intoxication due to the toxins and paralytic disease.

Mahunga (1994) reported that the CNglcs content in the root of cassava had a correlation with the root yield with a coefficient of 0.19 to 0.20. This correlation indicated low genetic linkage between the two traits leading to the effort in cassava breeding for high root yield with low CNglcs. However, Kizito et al. (2007) and Whankaew et al. (2011) reported two and five quantitative trait loci (QTLs), respectively associated with CNglcs content in cassava roots, indicated difficulty in selecting for roots with low CNglcs. Moreover, the environmental conditions can also affect the CNglcs content in roots. There were reports that nitrogen and fertiliser applications increased or reduced CNglcs content in cassava roots (Bokanga et al., 1994; Gleadow & Møller, 2014). Water deficit also has an effect on CNglcs content (Bokanga et al., 1994; Hular-Bograd et al., 2011; Vandegeer et al., 2013).

Therefore, an investigation into the agronomic traits associated with root yield and CNglcs would be of a benefit in selecting cassava with high root yield and low CNglcs content. However, there were studies showing difference on the correlation coefficients of these two traits. For instance, Moh (1976) found a positive correlation between CNglcs content in cassava leaf and root with a coefficient of 0.59, while Mahungu (1994) reviewed the relationships between CNglcs content of cassava and other agronomic traits. In addition, Cooke et al. (1978) and Mahungu et al. (1992) reported a positive correlation between CNglcs contents in cassava leaf and root with correlation coefficients ranging from 0.20 to 0.36.

The CNglcs content in the cassava plant may also be affected to some noticeable extent by the environmental variables such as an infestation by insect pests and infections by diseases such as bacterial leaf blight and root-knot nematodes. Bernays et al. (1977) demonstrated that yellow senescent leaves of cassava were readily eaten by the variegated grasshopper, *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) but green leaves were not, and this was apparently because the senescent leaves lacked CNglcs. Meanwhile, Bellotti and Arias (1993), Bellotti and Riis (1994) and Riis et al. (2003) also gave an account of cassava cyanogenic content and resistance to insect pests especially the burrowing bug, *Cyrtomenus bergi* Froschner (Hemiptera: Cydnidae), feeding on cassava roots.

Among the diseases, in Africa, Zinsou et al. (2005) reported that cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* and *X. campestris* pv. *manihotis* could reduce cassava yield up to 76%. In Thailand, Lertsuchatavanich et al. (2014) found a negative correlation between the HCN content in cassava leaves and the incidence of cassava bacterial blight caused by *X. axonopodis* pv. *Manihotis*, and the root-knot nematodes, *Meloidogyne* spp. (Tylenchida: Heteroderidae), which could cause up to 70% yield loss in cassava production (Lertsuchatavanich & Chinnasri, 2012). A preliminary screening of Kasetsart 50 with high CNglcs in the roots and resistant to the root-knot nematode is on-going (Udomsak Lertsuchatavanich, pers. comm.). Coyne et al. (2006) attempted to score the intensity of root-knot disease by using the root gall index at the harvesting period, which was found either positively or negatively correlated with the root yield depending on the cultivars. These reports suggested that high CNglcs content in the roots and leaves of cassava might have an implication in the pest and disease resistance mechanism.

The objective of this study was to find the variation of some agronomic traits for further stepwise multiple regression analysis for the correlations between the fresh root weight, CNglcs content in roots and other agronomic traits so as to choose the significant and highly correlated traits as the selection criteria for rapid screening of 83 cassava breeding lines. The 17 selected quantifiable and observable agronomic traits in this study were fresh root weight, fresh leaf weight, fresh stem weight, starch content in root, harvest index, root number, plant type, plant height, the first branch height, CNglcs contents in root and leaf, chlorophyll content in leaf, carotenoid contents in root and leaf, and cassava bacterial blight and fibrous and tuberous root-knot symptoms scorings. The data obtained were evaluated and analysed using the stepwise multiple regression analysis or the path analysis originally developed by Wright (1934). The ultimate objective was to verify the effects of the significantly correlated traits on the fresh root weight and CNglcs content in the root for verification and further investigation.

MATERIALS AND METHODS

Plant Materials and Field Experiment

Eighty-three cassava breeding lines were used for clonal selection trial carried out at Thai Tapioca Development Institute (TTDI) Experiment Station in Huai Bong, Dan Khun Thot, Nakhon Ratchasima (15º12.444′ N, 101º 42.911′ E). They were grown from stakes obtained from the cassava plants which were hybridised between the cultivars with high root yield and high CNglcs content in the root (Kasetsart 50, Huai Bong 60, Huai Bong 80 and Rayong 5), and the cultivars with low CNglcs content in the root but low root yield (Kolok, Rayong 2, and Ha Na Thi). They were planted along with the parent cultivars to serve as check every two rows. In each row, six plants were planted with the plant to plant distance of 1 m, while the row to row distance was 2 m.

The stakes were planted in May 2012 and the plants harvested in May 2013. Three plants in each row were selected and the agronomic traits measured were fresh root weight (RW), fresh leaf weight (LW), fresh stem weight (SW), starch content in root (SC), harvest index (HI), number of roots (RN), plant type (PT), plant height (PH), first branch height (FBH), and the cyanide-equivalent contents (HCN) in root and in leaf (R-HCN and L-HCN), chlorophyll content in leaf (CHLO), carotenoid contents in leaf (L-CA) and in root (R-CA) were measured in 28 selected lines having either white, cream, or yellow root flesh in appearance, whereas cassava bacterial blight (CBB) and cassava root-knot symptoms in fibrous and tuberous roots (FRK and TRK) with the scorings of 1 to 5, 1 to 4, and 1 to 5 respectively, were estimated in 60 breeding lines. The starch content in root (SC) was analyzed by Reimann Scale Balance (GENIX, Samutprakarn, Thailand) described in Prammanee et al. (2010). The harvest index (HI) was calculated from the fresh root weight (RW) divided by fresh stem weight (SW) of the whole plant. The plant types (PT) were scored from 1 to 5, ranging from highly branched plant type (1) to straight plant type (5), consecutively.

Cyanogenic Glycoside Analysis

The cyanide-equivalent contents in the root and in leaf were analysed following the modified methods described in Bradbury et al. (1999). Root parenchyma was collected using a 4 mm diameter cork borer to cut the largest section of root, using a portion with the final weight of 100 mg without cortex layer. The leaf tissue was cut from a fully expanded young leaf with a 1 cm^2 circular cork borer to obtain around 10 mg leaf. The fresh tissue was ground in a 15 ml tube containing 50 µl of phosphate buffer, pH = 8.0, and then sealed with the cap attached with picrate paper. The reaction was held at 30°C for 24 h before the picrate paper was soaked in 5 ml of water for 30 min for quantitating cyanide-equivalent content at 510 nm by spectrophotometer (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) using a standard curve of HCN generated from linamarin.

Chlorophyll and Carotenoid Analysis

The chlorophyll and carotenoid contents were analysed as follows. A leaf disc was cut from a fully expanded young leaf with a 1 cm^2 circular cork borer and put in a vial with 3 ml of *N, N*-dimethylformamide (DMF). The vial was held in the dark for 24 h before the solution absorbance was measured at 248, 647, and 664 nm, respectively, with a spectrophotometer (GE Healthcare Life Sciences, Little Chalfont, United Kingdom). The chlorophyll *a* and *b* contents per leaf in mg per cm^2 were estimated using the formulae described in Equations [1] to [3] (Porra et al., 1989).

$$\text{Chlorophyll } a = 12.00\ A_{664} - 3.11\ A_{647} \quad [1]$$

$$\text{Chlorophyll } b = 20.78\ A_{647} - 4.88\ A_{664} \quad [2]$$

Total chlorophyll
= Chlorophyll *a* + Chlorophyll *b* [3]

Meanwhile, the total carotenoid content per leaf in mg per cm^2 was estimated using the formula described in Equation [4] (Wellburn, 1994).

Carotenoid
= (1000 A_{480} – 1.12 Chlorophyll *a* – 34.07 Chlorophyll *b*) /245 [4]

The total carotenoid content in roots was extracted by 80% (v/v) acetone and estimated in micromoles per g, as described in Equation [5] (Edwards et al., 1998).

Carotenoid
= $\{(A_{480} + 0.114\ A_{663} – 0.638\ A_{645}) \times V \times 1000\} + 112.5\ W$ [5]

where, V is extraction volume, W is fresh root weight.

Cassava Bacterial Blight Scoring

The cassava bacterial blight disease symptoms caused by *X. axonopodis* pv. *manihotis* were estimated and scored from three plants per line and averaged from six leaves per plant, with two leaves selected from the top, middle and bottom portions of the stem. The severity was scaled into five levels (1 to 5), associated with 0, 5, 10, 15, or 20% leaf damage, consecutively as described in Teri (1978).

Cassava Root-knot Scorings

The root-knot symptoms caused by the nematodes *Meloidogynes* spp. were scored for fibrous root-knot (FRK) and tuberous root-knot (TRK) using three plants per line. The symptom scorings for the fibrous root-knot and tuberous rot-knot were somewhat different and the systems were adapted from Coyne et al. (2006), as follows.

Fibrous root-knot symptom scoring criteria:

Symptom Score	Severity
0	No root- knot symptom appeared
1	25% of fibrous roots have root knots
2	50% of fibrous roots have root knots
3	More than 50% of fibrous roots have small root knots
4	More than 50% of fibrous roots have large root knots

Tuberous root-knot symptom scoring criteria:

Symptom Score	Severity
0	No root knot appeared in fibrous roots and no effect on tuberous roots
1	Root knot appeared in fibrous roots but no effect on tuberous roots
2	Root knot appeared in fibrous roots and 25% of tuberous roots no longer developed
3	Root knot appeared in fibrous roots and 50% of tuberous roots no longer developed
4	Root knot appeared in fibrous roots and 75% of tuberous roots no longer developed
5	Root knot appeared in fibrous roots and 100% of tuberous roots no longer developed

Statistical and Path Analysis

Mean, standard deviation, coefficient of variation and range across all 83 breeding lines and parent lines were calculated

by using the values obtained from 17 agronomic traits of each line. They were then calculated for the phenotypic correlation coefficients. The effects of agronomic traits were formulated using the stepwise multiple regression analysis and the Pearson correlation coefficients (*r*) among the agronomic traits analysed. The multiple regression analyses were formulated using the stepwise method. The path analyses were calculated from correlation coefficients. The Statistic Tool for Agricultural Research STAR 2.0.1 software (IRRI, 2013) was used for all calculation and analysis.

RESULTS AND DISCUSSION

A. Variation Evaluation of Agronomic Traits

The mean ($\bar{x}$), standard deviation (SD), range and coefficient of variation (CV) of 17 agronomic traits, including the fresh root weight (RW), fresh leaf weight (LW), fresh stem weight (SW), starch content (SC), harvest index (HI), roots per plant (RN), plant type (PT), plant height (PH), the first branch height (FBH), cyanide-equivalent contents in root (R-HCN) and in leaf (L-HCN), chlorophyll content in leaf (CHLO), carotenoid contents in leaf (L-CA) and in root (R-HCN), as well as cassava bacterial blight (CBB) symptom scoring, and fibrous root-knot (FRK) and tuberous root-knot (TRK) symptoms scorings are summarised in Table 1.

Fresh Root Weight (RW), Fresh Leaf Weight (LW) and Fresh Stem Weight (SW)

The preliminary evaluation of agronomic traits revealed a high variation in fresh root weight (RW), fresh leaf weight (LW) and fresh stem weight (SW) with the CV values from 41.62 to 53.66%. The variation in dry matter content of cassava root was also reported in cassava in the sub-Saharan Africa by Kawaki et al. (2011). Among these traits, fresh leaf weight (LW) was found to have greater variation than fresh root weight (RW) and fresh stem weight (SW). It was also observed that many traits showed relatively high variation, which could be due to segregation of genes caused by the heterozygosity of cassava breeding parents used in this trial.

Harvest Index (HI) and Starch Content (SC)

However, the harvest index (HI) and starch content (SC) had lower percentages of CV, than the fresh root weight, being 22.07, 18.95 and 46.76%, respectively. The harvest index (HI) averaged 0.49±0.11, ranging from 0.22–0.75, indicating that the assimilate partitioning between the above-ground and underground parts of cassava lines used in this trial were mostly balanced. The starch content (SC) averaged 19.26±3.65%, ranging from 10.0–26.80% in this trial. The results obtained were somewhat lower than those obtained from 266 germplasm varieties evaluated by Yurasit (2007), with the range of 10.0–35.9%. The low coefficient variation of the starch content

(SC) of this trial might be limited by the genetic variation of selected parents compared to the germplasm evaluation.

Plant Type (PT), First Branch Height (FBH), Plant Height (PH) and Roots per Plant (RN)

The cassava lines in this clonal evaluation trial had different plant types (PT) from being straight to being branchy from 1 to 5. As a result, the variation in plant type (PT), averaging at 2.55±0.99, and the first branch height (FBH), averaging 81.99±29.56 cm and ranging from 0–240.00 cm, were relatively high compared with the plant height (PH) variation, averaging at 206.40±30.70 cm and ranging from 125.00–283.33 cm (Table 1). The number of roots per plant (RN) averaged 12.02±3.80 roots per plant, ranging from 4–38 roots per plant. The number of roots per plant (RN) which is a component of fresh root weight (RW) also had a high coefficient variation at 46.76%, averaging at 5.54±2.59 kg plant^{-1} and ranging from 1.00 – 15.60 kg plant^{-1}. However, since the size of cassava root is not uniform, the number of roots per plant (RN) may not be a suitable parameter to be used for selecting for high root yield, not unless they could be clearly classified or categorised into a number of large and/or small roots.

Cyanide-Equivalent Contents

This study revealed that the cyanide-equivalent contents in root (R-HCN) and leaf (L-HCN) averaged at 110.30±62.33 ppm, ranging from 12.52 – 271.72 ppm, and at 1,231.20±312.20 ppm, ranging from 512.90 – 2,248.10, respectively (Table 1). The result indicated that the cyanide-equivalent content in the leaves was higher than that in the roots. The higher cyanide-equivalent content in fresh leaves compared to in fresh roots was obvious because CNglcs were shown to be synthesised in the leaves and translocate to the root tissues through the phloem (Selmar, 1994). It was also obvious that the root had a higher coefficient variation of cyanide-equivalent content than the leaf (i.e., 56.51 vs. 25.35%). The cyanide-equivalent contents in root obtained in this trial had a range from the innocuous group (<50 mg kg^{-1} root fresh weight) to the toxic group (>100 mg kg^{-1} root fresh weight) as reported in Bolhuis (1954, 1966) and reviewed in Lukuya et al. (2014). Nevertheless, the coefficient of variation of cyanide-equivalent content in the roots was much higher than that in the leaves (i.e., 56.51% to 25.35%). Therefore, the variation of the cyanide-equivalent content in the roots may not only be due to the cyanide-equivalent in the leaves, but also the CNglcs transported from the leaves to roots through the phloem, which may have complex enzymatic mechanisms involving diglucosidase (Selma, 1994) and hydroxynitrile lyase (Siritunga & Sayre, 2004). It is also well known that CNglcs accumulated in all cassava lines and there has never been any report of cassava with zero CNglcs content, except for the transgenic cassava lines, with *CYP79DI/D2* knockout (Siritunga & Sayre, 2004).

Table 1
Mean ± SD, range, and coefficient of variation of 17 agronomic traits in cassava breeding lines in clonal evaluation trial

Agronomic traits	Mean ± SD ($\bar{x}$)	Range	CV (%)
RW (Fresh root weight, kg plant^{-1})	5.54 ± 2.59	1.00 - 15.6	46.76
LW (Fresh leaf weight, kg plant^{-1})	2.70 ± 1.45	0.10 - 8.75	53.66
SW (Fresh stem weight, kg plant^{-1})	2.86 ± 1.19	0.40 - 7.85	41.62
SC (Starch content, %)	19.26 ± 3.65	10.00 - 26.80	18.95
HI (Harvest index, ratio)	0.49 ± 0.11	0.22 - 0.75	22.07
RN (Root number, number/plant)	12.02 ± 3.80	4 - 38	31.57
PT (Plant type, 1-5)	2.55 ± 0.99	1 - 5	38.79
PH (Plant height, cm)	206.40 ± 30.70	125.00 - 283.33	14.87
FBH (First branch height, cm)	81.99 ± 29.56	0 - 240	36.05
R-HCN (Cyanide-equivalent in root, ppm)	110.30 ± 62.33	12.52 - 271.72	56.51
L-HCN (Cyanide- equivalent in leaf, ppm)	1,231.20 ± 312.20	512.90 - 2,248.10	25.35
CHLO (Chlorophyll content in leaf, mg cm^{-2})	9.37 ± 1.93	6.19 - 13.54	20.58
L-CA (Carotenoid content in leaf, mg cm^{-2})	2.15 ± 0.33	1.54 - 2.97	15.56
R-CA (Carotenoid content in root, µg g^{-1})	3.94 ± 1.76	2.00 - 7.77	44.68
CBB (Cassava bacterial blight symptom, 1-5)	1.27 ± 0.52	1 - 5	40.62
FRK (Fibrous root-knot symptom, 1-4)	2.60 ± 0.89	1 - 4	30.80
TRK (Tuberous root-knot symptom, 1-5)	2.90 ± 0.95	1 - 5	36.64

The amounts of cyanide-equivalent content in the leaves and roots could vary with the environmental condition, especially under high nitrogen fertilisation (Bokanga et al., 1994; Gleadow & Møller, 2014), and with water deficits (Bokanga et al., 1994; Hular-Bograd et al., 2011; Vandegeer et al., 2013). In this experiment, the harvest time was during the early monsoon season of May 2013; therefore, the drought effect should not have any significant impact on the CNglcs transportation or storage in cassava breeding lines under this trial.

Chlorophyll and Carotenoid Contents

For the chlorophyll content in leaf (CHLO), the average content was 9.37±1.93 mg cm^{-2}, ranging from 6.19–13.54 mg cm^{-2} and the lower level of variation was 20.58%. However, the pattern of variation of the carotenoid content in leaf and in root (L-CA and R-CA) was similar to those of the cyanide-equivalent contents, i.e., higher variation in the roots than in the leaves. The average carotenoid contents in root (R-CA) and in leaf (L-CA) were 3.94±1.76 µg^{-1}, ranging from 2.00–7.77µg^{-1} and 2.15±0.33 mg cm^{-2}, ranging from 1.54 – 2.97 mg cm^{-2}, respectively. The carotenoid content, as appeared in the roots, varied from the

white-coloured flesh, through the cream-colored flesh and to the yellow-coloured flesh.

Cassava Bacterial Blight Symptom Scoring

The incidence of cassava bacterial blight (CBB) disease caused by (*X. axonopodis* pv. *manihotis*) on cassava breeding lines in this trial, evaluated by using the symptom scoring of 1 to 5, was 1.27±0.52. This indicated a relatively low or rather negligible overall cassava bacterial blight incidence during the trial.

Root-Knot Symptom Scorings

Scorings of the root-knot nematodes (*Meloidogyne* spp.) infection causing fibrous root-knot (FRK) symptom and tuberous root-knot (TRK) symptom, from 1- 4 and 1- 5, averaged at 2.60±0.89 and 2.90±0.95, respectively. This indicated a moderate nematode incidence which caused both fibrous root-knot and tuberous root-knot symptoms.

B. Agronomic Traits and Path Analysis

Phenotypic Correlation Coefficients in Cassava Breeding Lines

The phenotypic correlation coefficients between 17 agronomic traits in cassava breeding lines in the clonal evaluation trial are summarised in Table 2. The stepwise multiple regression analysis on the effects of agronomic traits on fresh root weight (RW) and cyanide-equivalent content in the root (R-HCN) from the clonal evaluation trial is shown in Table 3.

The path analysis of the agronomic traits evaluated, in terms of the standardised partial regression coefficients (*b*) and the correlation coefficients (*r*), which showed the effects of the harvest index (HI), leaf weight (LW) and stem weight (SW); as well as the stem weight (SW), starch content (SC), cyanide-equivalent content in leaf (L-HCN), the first branch height (FBH) and leaf weight (LW) on the root weight (RW), are illustrated and presented in Figure 1 and Figure 2, respectively.

The standardised partial regression coefficients (*b*) and the correlation coefficients (*r*), which showed the effects of the chlorophyll content (CHLO), cyanide-equivalent content in leaf (L-HCN) and tuberous root-knot symptom scoring (TRK), as well as the carotenoid content in leaf (L-CA) and the cyanide-equivalent content in leaf (L-HCN) on the cyanide content in root (R-HCN), are illustrated in Figure 3 and Figure 4, respectively.

Effects on Root Weight

It is evident in Table 2 that the fresh root weight (RW) showed a positive correlation with fresh stem weight (SW) ($r = 0.51$) and harvest index (HI) ($r = 0.56$) (Figure 1). This is probably because stems and roots are both the carbohydrate storage tissues. The stepwise analysis showed a standardised linear regression that the harvest index (HI), fresh leaf weight (LW) and stem weight (SW) had contributions to the fresh root

Table 2
Phenotypic correlation coefficients between 17 agronomic traits in cassava breeding lines in clonal evaluation trial

Traits†	RW	LW	SW	SC	HI	RN	PT	PH	FBH	R-HCN	L-HCN	CHLO	L-CA	R-CA	CBB	FRK	TRK
RW	1.00	0.26**	0.51**	0.38**	0.56**	0.38**	-0.26**	0.23*	0.25**	0.29**	0.36**	0.11	0.12	0	0.19	0.11	- 0.09
LW		1.00	0.51**	-0.02	-0.49**	0.21*	-0.52**	0.40**	0.06	-0.15	-0.19	-0.04	-0.10	0.16	0.14	0.29*	0
SW			1.00	0.11	-0.24**	0.36**	-0.42**	0.53**	0.19*	0.07	0.22*	0.02	-0.01	0.01	0.22	0.25	-0.10
SC				1.00	0.35**	0.09	-0.38**	-0.14	0.03	0.20*	0.12	0.18	0.13	0.71	0.16	-0.05	-0.42**
HI					1.00	0.08	0.25**	-0.26**	0.22*	0.32**	0.33**	0.03	0.10	-0.02	-0.03	-0.15	-0.01
RN						1.00	-0.07	0.19*	0.23**	0.29**	0.24**	0.11	0.06	-0.40	-0.07	-0.06	-0.08
PT							1.00	0.01	0.18*	0.07	0.11	-0.07	-0.03	-0.63	-0.15	-0.08	0.21
PH								1.00	0.18*	0.04	0.12	0.18	0.15	-0.77*	0.11	0.30*	-0.09
FBH									1.00	0.17	0.06	0.15	0.14	0.28	0.09	0.15	0
R-HCN										1.00	0.48**	0.65**	0.67**	-0.43	0.17	0.04	-0.33*
L-HCN											1.00	0.19	0.25	-0.27	0.09	-0.02	-0.13
CHLO												1.00	0.95**	0.10	0.36	0.23	-0.45*
L-CA													1.00	0.45	0.26	0.21	-0.49*
R-CA														1.00	-0.39	0.45	0.07
CBB															1.00	0.08	-0.29*
FRK																1.00	0.04
TRK																	1.00

* significant at 95% confidence level, ** highly significant at 99% confidence level
†RW = Fresh root weight (kg plant^{-1}), LW = Fresh leaf weight (kg plant^{-1}), SW = Fresh stem weight (kg plant^{-1}), SC = Starch content in root (%), HI = Harvest Index (ratio), RN = Root number, PT = Plant type (1-5), PH = Plant height (cm), FBH = First branch height (cm), R-HCN = Cyanide-equivalent content in root (ppm), L-HCN = Cyanide-equivalent content in leaf (ppm), CHLO = Chlorophyll content in leaf (mg cm^{-2}), L-CA = Carotenoid content in leaf (mg cm^{-2}), R-CA = Carotenoid content in root (µg g^{-1}), CBB = Cassava bacterial blight symptom (1-5), FRK = Fibrous root-knot symptom (1-4), TRK = Tuberous root-knot symptom (1-5)

Table 3
Stepwise multiple regression analysis of effects of agronomic traits on (A) Fresh root weight and (B) Cyanide-equivalent content in root from clonal evaluation trial

Traits†	R^2 (%)	P-value
(A) Fresh Root Weight (RW)		
	86.03	0
Regression model: RW = 0.89 HI + 0.44 LW + 0.49 SW		
HI (Harvest index)	49.46	0
LW (Fresh leaf weight)	11.68	0
SW (Fresh stem weight	24.89	0
Residual	13.97	
Regression model: RW = 0.24 SW + 0.32 SC + 0.31 L-HCN + 0.23 FBH + 0.22 LW	47.05	0
SW (Fresh stem weight)	12.29	0.012
SC (Starch content)	12.05	0
L-HCN (Cyanide-equivalent in leaf)	11.16	0
FBH (First branch height)	5.75	0.010
LW (Fresh leaf weight)	5.80	0.025
Residual	52.95	
(B) Cyanide-equivalent in Root (R-HCN)		
Regression model: R-HCN = 0.53 CHLO + 0.37 L-HCN-0.04 TRK	52.20	0
CHLO (Chlorophyll content in leaf)	32.84	0.011
L-HCN (Cyanide-equivalent content in leaf)	17.94	0.033
TRK (Tuberous root-knot symptom scoring)	1.42	0.031
Residual	47.80	
Regression model: R-HCN = 0.59 L-CA + 0.33 L-HCN	55.06	0
L-CA (Carotenoid content in leaf)	39.21	0.001
L-HCN (Cyanide-equivalent in leaf)	15.85	0.038
Residual	44.94	

†RW = Fresh root weight (kg plant^{-1}), LW = Fresh leaf weight (kg plant^{-1}), SW = Fresh stem weight (kg plant^{-1}), SC = Starch content in root (%), HI = Harvest index, FBH = First branch height (cm), R-HCN = Cyanide- equivalent content in root (ppm), L-HCN = Cyanide- equivalent content in leaf (ppm), CHLO = Chlorophyll content in leaf (mg cm^{-2}), L-CA = Carotenoid content in leaf (mg cm^{-2}), TRK = Tuberous root-knot symptom scoring

weight (RW), with R^2 = 86.03%, as shown in Table 3. This model was, however, in contrast with the report by Boakyn et al. (2013) showing a non-significant correlation between the root weight (RW) and the so-called top weight consisting of leaf and stem weights. In this study, the harvest index (HI) had a direct effect on root weight (RW) with a standardised partial regression coefficient (b) = 0.89 (Figure 1), when calculated from the fresh root weight (RW), leaf weight (LW) and stem weight (SW). The path analysis in Figure 1 also showed various standardised partial regression coefficients with these parameters affecting the root weight (RW). Therefore, the stepwise multiple regressions were analysed without the harvest index (HI). It was also found that the leaf weight (LW), stem weight (SW), starch content (SC), the first branch height (FBH) and cyanide-equivalent content in leaf (L-HCN) contributed significantly to the fresh root weight (RW), with R^2 = 47.05%.

Ntawuruhunga and Dixon (2010) proposed a regression model to predict root yield using leaf area, petiole length, storage root number, root size, root girth, stem weight, and starch content. The agronomic traits used in the said model, which are common with our model, were the stem weight (SW), starch content (SC) and leaf weight (LW), assuming that the leaf area relates to the leaf weight. Our analysis showed that both stem weight (SW) and root weight (RW) were equally correlated with the leaf weight (LW) (r = 0.26) and the stem weight (SW) (r = 0.51 (Table 2 & Figure 2). Apparently, the stem weight (SW) was the only agronomic trait or parameter in the standardised regression

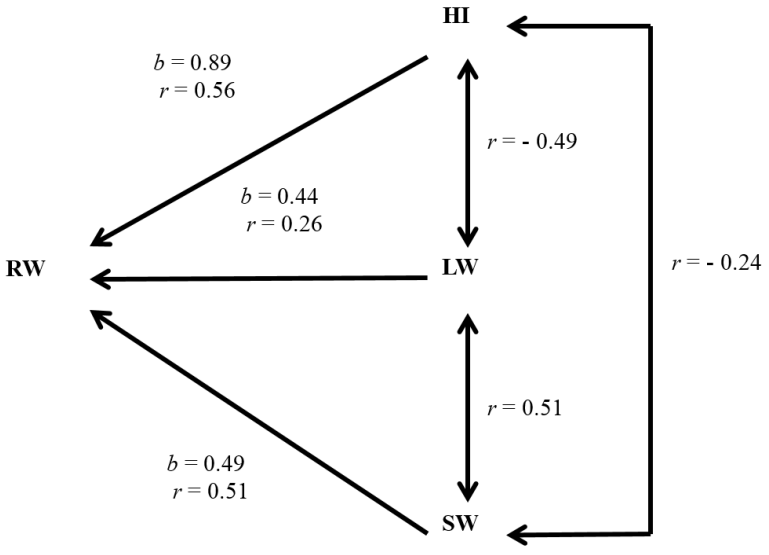

Figure 1. Path analysis effect of harvest index (HI) (ratio), fresh leaf weight (LW) (kg plant^{-1}), and fresh stem weight (SW) (kg plant^{-1}) on fresh root weight (RW) (kg plant^{-1}). b = standardized partial regression coefficient, r = correlation coefficient

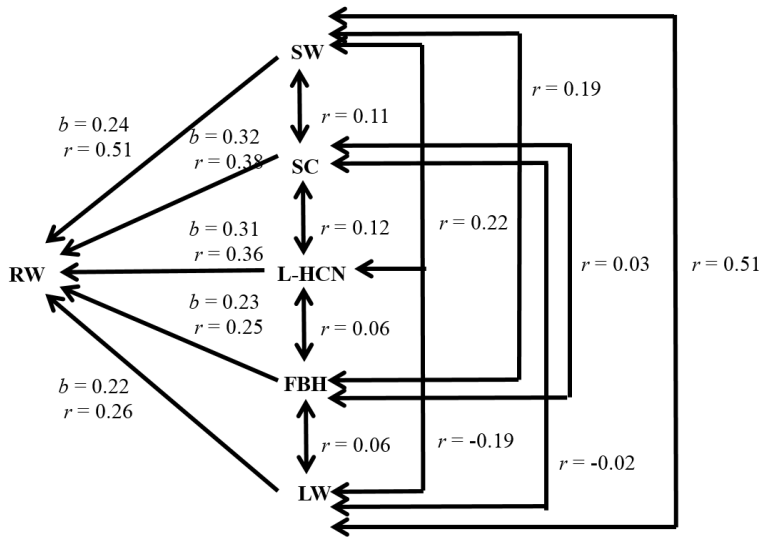

Figure 2. Path analysis effect of fresh stem weight (SW) (kg plant^{-1}), starch content in root (SC) (%), cyanide-equivalent content in leaf (L-HCN) (ppm), first branch height (FBH) (cm), and fresh leaf weight (LW) (kg plant^{-1}) on fresh root weight (RW) (kg plant^{-1}). *b* = standardized partial regression coefficient, *r* = correlation coefficient

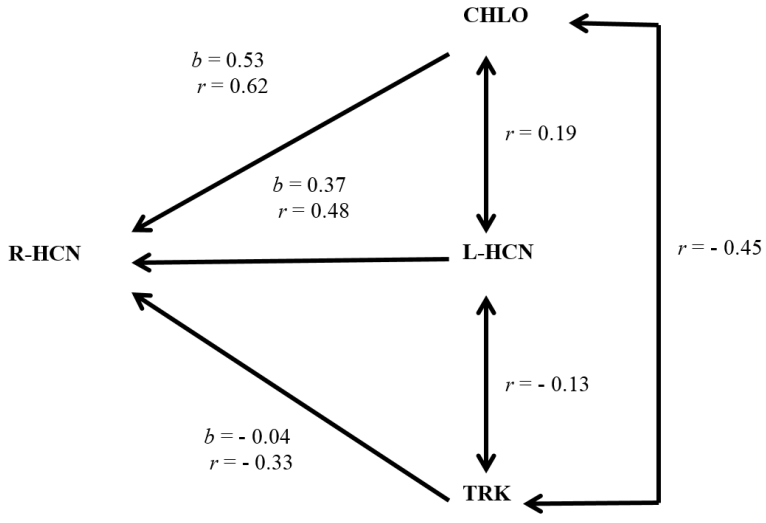

Figure 3. Path analysis effect of chlorophyll content in leaf (CHLO) (mg cm^{-2}), cyanide-equivalent content in leaf (L-HCN) (ppm), and tuberous root-knot symptom scoring on cyanide-equivalent content in root (R-HCN) (ppm). *b* = standardized partial regression coefficient, *r* = correlation coefficient

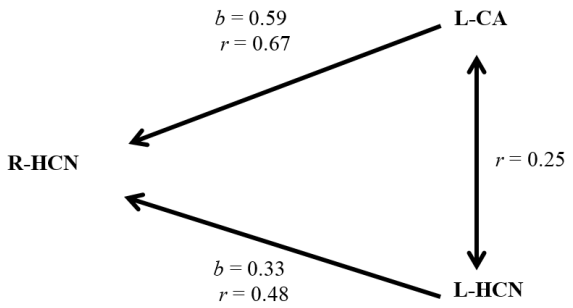

Figure 4. Path analysis effect of carotenoid content in leaf (L-CA) (mg cm^{-2}) and cyanide-equivalent content in leaf (L-HCN) (ppm) on cyanide equivalent content in root (R-HCN) (ppm). *b* = standardized partial regression coefficient, *r* = correlation coefficient

model which had a correlation coefficient (*r* = 0.51) higher than the standardised partial regression coefficient (*b* = 0.24) (Figure 2), while other four parameters [starch content (SC), cyanide-equivalent content in leaf (L-HCN), the first branch height (FBH), and leaf weight (LW)] showed no significant difference between the correlation coefficient and the standardised partial regression coefficient. Thus, the findings indicated that the stem weight (SW) had direct and indirect effects on the root weight (RW). The highest indirect effect of the stem weight (SW) on root weight (RW) was obviously through the leaf weight (LW) (Figure 2), while other traits tended to have a direct effect on the root weight (RW) rather than an indirect effect.

It was also observed that the cassava plants having branchy plant type and rather tall plant height tend to have a higher leaf weight (LW) and stem weight (SW) with significant correlations (*r* = - 0.52 and 0.53, respectively) (Table 2). However, the parameter which was included in the standardised regression analysis was the first branch height (FH) instead of the plant height (PH) or the plant type (PT). The first branch height (FBH) had non-significant positive correlation with the stem weight (SW) and the leaf weight (LW) with *r* = 0.19 and 0.06, respectively (Table 2).

The starch content (SC) was also correlated with the root weight (RW), implying that starch is the most common carbohydrate stored in the roots. The correlation between the starch content (SC) and the harvest index (HI) also indicated the importance of carbohydrate partitioning to root. The root number (RN) also had a high positive correlation with root weight (*r* = 0.38), leaf weight (*r* = 0.21) and stem weight (SW) (*r* = 0.36). In this study, however, the root number (RN) was not included in the standardised partial regression model analysed by the stepwise method. A significantly positive correlation (*r* = 0.64) between the root number (RN) and root weight (RW) was also reported by Boakyn et al. (2013). Ntawuruhunga and Dixon (2010) also proposed a regression model with the root number (RN) and other root characters

in the model. In this study, the roots were not characterised by size or girth. Therefore, the root number used in Ntawuruhunga and Dixon (2010) might be accounted for the root storage capacity which could be reasonably included in the model.

Effects on Cyanide-Equivalent Content in Root

In this study, the cyanide-equivalent content in the root (R-HCN) had a positive correlation (r = 0.29) with the fresh root weight (RW). It was consistent with previous reports that the cyanide-equivalent content in cassava roots was correlated with the root yield, with coefficients ranging from 0.19 to 0.20 (Muhungu, 1994). Therefore, these two traits were likely unlinked. However, when the data were analysed separately within the parent group and breeding line group, the parent group had a lower positive correlation (r = 0.22) between these two traits than the breeding line group (r = 0.35), which is in contrast with the hypothesis that the parent lines have a higher correlation between the two traits.

It was also found in the trial that the root cyanide-equivalent content (R-HCN) was correlated with the cyanide-equivalent content in leaf (L-HCN), chlorophyll content in leaf (CHLO) and carotenoid content in leaf (L-CA) (Table 2). Interestingly, the cyanide-equivalent content in root (R-HCN) showed a higher correlation with the carotenoid content in leaf (L-CA) than the chlorophyll content in leaf (CHLO). However, it was found that the chlorophyll content in leaf (CHLO) and the carotenoid content in leaf (L-CA) were highly correlated to each other, although the carotenoid content in leaf (L-CA) was calculated by subtracting the chlorophyll content in leaf (CHLO) in Equation [4].

Although it is known that the high nitrogen environment can increase nitrogen compound content, there are differences between the sink capacities of cyanogenic compounds in the vacuole and photosynthetic pigments in the chloroplast. As CNglcs are synthesised and could be stored in the leaf tissues in limited amounts, an excess may be translocated and stored in the root tissues. We found that in our trial, the cyanide-equivalent content in the leaves (L-HCN) had a lower variation than in the roots (R-HCN), even though the amount of CNglcs accumulated in the leaves was much higher than that in the roots. On the contrary, the chlorophyll and carotenoid contents in the leaves (CHLO and L-CA) seemed to increase under certain circumstances such as with high nitrogen fertilisation. As a result, the cyanide-equivalent content in the roots (R-HCN) could be altered with various nitrogen use efficiency of cassava breeding lines, and the chlorophyll and carotenoid contents in the leaves. However, there is a non-significant positive correlation between the carotenoid content in the root (R-CA) and leave (L-CA) in this trial. This result could be due to different expression levels of phytoene synthase isozymes, the key enzyme in carotenoid synthesis in the leaves and roots (Arango et al., 2010). It was also found in this trial that the chlorophyll content in leaf (CHLO) at 9.37±1.93mg cm^{-2}, ranging

from 6.19-13.54mg cm^{-2} and carotenoid content in leaf (L-CA) at 2.15±0.33mg cm^{-2}, ranging from 1.54-2.97mg cm^{-2}, had lower coefficients of variation compared to the carotenoid content in root at 3.94±1.76 µg g^{-1}, ranging from 2.00 to 7.77 µg g^{-1}, which showed a noticeably higher variation of flesh colour, varying from white to cream and to yellow, which is similar to the report from the previous studies with the root carotenoid variation from 1.02 to 10.40 µg g^{-1} (Chavez et al., 2005). In this study, however, the chlorophyll and carotenoid measurements were carried out only in selected breeding lines.

The multiple regression or the path analysis showed the contribution of cyanide-equivalent content in leaf (L-HCN), chlorophyll content in leaf (CHLO), the tuberous root-knot symptom scoring (TRK) to the cyanide-equivalent content in the root (R-HCN) with R^2 = 52.20 (Table 3). Byju and Anand (2009) reported that under high nitrogen condition, the leaf tissue and chlorophyll content were abundant because nitrogen is an important macronutrient for plant growth that contributes to chlorophyll and CNglcs molecules. In our trial, however, the cyanide-equivalent content in root (R-HCN) showed a high positive correlation with the chlorophyll content in leaf (CHLO) and carotenoid content in leaf (L-CA) and was higher than the correlation with the cyanide-equivalent content in leaf (L-HCN).

The path analysis showed that both the cyanide-equivalent content in leaf (L-HCN) and chlorophyll content in leaf (CHLO) had a direct effect on the cyanide-equivalent content in root (R-HCN) with a low indirect effect through other traits, while the tuberous root-knot symptom scoring (TRK) had an indirect effect on the cyanide-equivalent content in leaf (L-HCN), which was mainly through the chlorophyll content in leaf (CHLO) (Figure 3). This implies that the effects of the root-knot disease scoring on cassava growth by reducing the nitrogen uptake capacity caused lower chlorophyll content in leaf (CHLO) and carotenoid content in leaf (L-CA), and consequently a lower cyanide-equivalent content in root (R-HCN).

The symptom scoring of cassava bacterial blight (CBB) and disease caused by the root-knot nematodes with fibrous root-knot (FRK) and tuberous root-knot (TRK) symptom scorings in this trial also showed higher coefficients of variation, which could be of a benefit in studying the correlations between the agronomic and disease-resistant traits. For disease symptom scoring analysis, cassava bacterial blight (CBB) showed no significant correlation with any agronomic traits in our findings.

Gleadow and Møller (2014) stated that cyanogenesis or the release of toxic HCN from endogenous CNglcs is an effective defense against generalist herbivores but less effective against fungal pathogens. Efforts are underway to genetically engineer CNglcs into some crops as a pest control measure, whereas in other crops, efforts are directed toward their removal to improve food safety. However, Lertsuchatavanich et al. (2014) reported a high positive correlation between the root CNglcs content

(R-HCN) and cassava bacterial blight (CBB) resistance. For the root-knot disease, the root-knot symptom scorings had no significant correlation with any agronomic traits but the score of inhibited growth of tuberous root-knot showed a negative correlation with the root starch content (SC) and cyanide-equivalent content in root (R-HCN) and the cyanide-equivalent in leaf (L-HCN) and carotenoid content in leaf (L-CA) (Table 2). Furthermore, in the locations where there is no incidence of root-knot disease, the tuberous root-knot (TRK) symptom scoring would not be included in the stepwise path analysis. The standardised regression analysis carried out also showed that the cyanide-equivalent content in leaf (L-HCN) and carotenoid content in leaf (L-CA) contributed significantly to the cyanide-equivalent content in root (R-HCN) with R^2 = 55.06 (Table 3).

As shown in the path analysis with the tuberous root-knot (TRK) symptom scoring (Figure 3), the path analysis without the tuberous root-knot (TRK) symptom scoring (Figure 4) also showed that both the cyanide-equivalent content in leaf (L-HCN) and carotenoid content in leaf (L-CA) had a direct effect on the cyanide-equivalent content in root (R-HCN), with a low indirect effect through one another agronomic trait.

CONCLUSION

Eighty-three cassava breeding lines were used for the clonal selection trial carried out from May 2012 to May 2013. As a consequence of the variation evaluation of the 17 agronomic traits chosen for further determination, the multiple regression analysis employing the path analysis to select appropriate agronomic traits of Thai cassava lines yielded an evidence that the cassava lines, with high root yield and high CNglcs content in the roots (Kasetsart 50, Huai Bong 60, Huai Bong 80, and Rayong 5) and the cultivars with low CNglcs content in the roots but low root yield (Kolok, Rayong 2, and Ha Na Thi) used in this trial, had a significant correlation between the root yield and low CNglcs content in the roots.

This finding provided a future promising achievement in cassava breeding programme for high root yield but low CNglcs content in the roots. It could be speculated that some cassava lines with high root yield and low CNglcs in the roots would be eventually achieved. The CNglcs and carotenoid contents in the leaves may be indicative traits for screening for low CNglcs content in the roots, as evident from the correlations obtained in the trial. Moreover, it is also evident from the data analysed that the cassava plants, with high leaf weight (LW), high stem weight (SW), high starch content (SC), high first branch height (FBH) and high CNglcs content in leaf (L-HCN), contributed significantly to the high root weight (RW). However, the CNglcs content in root (R-HCN) and in leaf (L-HCN) did not show a correlation with cassava bacterial blight (CBB) and fibrous root-knot (FRK) symptom scorings. It was indicated that an artificial inoculation might be required for the disease symptom scoring in the future experiments. The relatively high scoring of the tuberous root-knot

(TRK) symptom might contribute to the low CNglcs content in leaf (L-HCN) as a consequence of some factors that decreased the plant growth by limiting the nutrient transport. Nevertheless, there is a need for additional data to be acquired and generated from further trials and evaluations in other locations with various environmental regimes for the reliable verification of the multiple regression models obtained from the path analysis carried out in our present investigation.

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