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Early Nitrogen Applications Promote the Initiation of Sweetpotato Storage Roots

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Abstract

A number of published studies have focused on the effects of N application timing on sweetpotato yield. However, none of them examined the influence of the timing of N fertilisation on the initiation of storage roots (SRs), which largely determines the number of tubers per plant and substantially affect the yield. A pot experiment was conducted in sand culture to evaluate the influence of N application timing (N applied on the planting day, 3, 7 and 14 days after transplanting (DAT), denoted T1, T3, T7 and T14, respectively, and a no N applied control T0) on the plant acquisition of N and the initiation of SRs of 'Orleans' cultivar. A previously-determined optimal N rate for the SR formation (Hoagland solution with 100mg/L N) in the experimental system was used. Rapid SR formation was found to require N during the first week after transplanting. No or late N application delayed the initiation of SRs and reduced the number of SRs. No or delayed N application inhibited the formation of regular vascular cambium (RVC) and anomalous cambium (AC) and led to an increase in lignification of stele cells leading to significantly higher proportion of lignified roots. By contrast, plants supplied with N within 7 DAT had a higher rate of SRs and lower rate of lignified roots. Also, earlier N application promoted total plant and root growth measured as higher biomass and storage root weight, as well as higher N accumulation in plants and higher nitrogen recovery efficiency (NRE). Results suggested that the SR formation required N during the first week after transplanting. Therefore, moderate N should be available in soil before or on planting day.

Keywords: sweetpotato, nitrogen application timing, storage root initiation

1. Introduction

Nitrogen (N) fertilisation has been demonstrated to improve production and quality of sweetpotato (Duan et al., 2019; Frahm et al., 2002; Hammett & Miller, 1982). An adequate supply of N is necessary for the growth of above-ground parts and for photosynthetic activity required for storage root (SR) development (Kays, 1985). Nitrogen availability also affects the formation of SRs and finally contributes to SR accumulation of this crop (Kim et al., 2002). Deficient or excessive N application inhibits the formation of SR, resulting in reduced yield potential of sweetpotato (Dong, 2021; Villordon & Franklin, 2007). Obviously, large amount of N application in the early stage of plant development results in poor synchronisation between the supply of N and plant demand. Nitrogen application timing is a critical part of the management plant. Previous studies mainly focus on its effects on yield of crops and nitrogen use efficiency of crop at harvest rather than certain stages of plant development. Proper N management can maximise the use of the nutrients and minimise the contamination of excessive N fertiliser (*e.g.*, to ground water). This can be achieved by the examination of crop nutrient needs and the assessment of nutrient supply to match the needs. Appropriate N management especially N application timing is beneficial for improving plant growth and contributing to crop yield.

Nitrogen application timing has been demonstrated to affect the development and yield of many crops, as N requirements are different in various stages of plant growth. Some vegetative crops such as carrots, cabbage and onion require a small amount of N for plant growth during early stage development as indicated by slow accumulation of N in plants (Salo, 1999). After that, they require higher N supply until harvest (Salo, 1999; Westerveld et al., 2006). In potato, N application before or on planting day promoted early tuber development and nitrogen use efficiency (Alva, 2004). Early N application also improved the grain yield of wheat (Fowler & Brydon, 1989). Therefore, adding N at the wrong time may reduce N fertiliser use efficacy as it may not use by crops and could be leached.

Nitrogen application timing has been studied in sweetpotato with the focus on the yield of this crop. Some previous studies suggested that a single N application resulted in significantly higher yield than split applications (Ankumah et al., 2003; Schultheis et al., 1995). However, another study in Louisiana indicated that applications of N split between pre-transplantation and 28 days after transplanting (DAT) increased the SR marketable yield when compared to single applications before transplantation or at 21, 28, 35 and 45 DAT (Villordon & Franklin, 2007), and the late application of N 28 DAT or later reduced the yield of the crop. Philip and Warren (2005) suggested that N application within week 2 or week 3 after planting provided a higher marketable SR yield of 'Beauregard" in Virginia compared to pre-planting or at 4-5 weeks after planting.

Although some studies reported the influence of N application timing on the SR yield of sweetpotato (Phillips et al., 2005; Villordon et al., 2009a), none of them focused on its effect on the formation of SRs as a key yield determinant. In a related experiment (Dong, 2021) insufficient or excessive N supply were both shown to inhibit or delay the formation of anomalous cambium (AC), whose appearance constitutes the formation of SRs. In a sand culture, the application of N at rate of 100 mg/L increased the percentage of SRs and reduced lignified roots. However, that experiment did not determine when plants started to require N for their SR formation. Therefore, the main objectives of this study were (1) to evaluate the effect of N application timing on anatomical features of sweetpotato roots during the early stage of SR initiation, which may significantly affect SR number, thereby the final yield, but was largely absent in literature, (2) to identify the best N application timing for 'Orleans' to promote the formation of SRs, and (3) to assess the effects of N application timings on the allocation of N in different parts of the plant during the initiation of SRs.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

From 20 October to 8 December 2018, the study was conducted in a glasshouse at the Queensland Department of Agriculture and Fisheries Bundaberg Research Facility (24°50′54″S; 152°24′14″E, Queensland, Australia). The shade cloth roof (approximately 30% shade) was closed to reduce the inside temperature during the middle of the day. The average daily maximum and minimum temperatures were 32.9 °C and 20.5 °C, respectively and the average daily maximum and minimum relative humidity were 86.9% and 43.1%, respectively. Plastic pots of 20 cm in diameter and 27 cm in height were used in the present experiment. Each pot was filled with 4 L of washed river sand. Tap water was added to the pots to field capacity three days before transplanting in order to achieve the same moisture in the sand in all pots. 'Orleans' transplants were cut from a local commercial nursery farm (McCrystal Agricultural Service Pty, Ltd.). All cuttings used for the experiment were healthy and uniform, were at least 20 cm long, and had five fully opened leaves. One cutting was planted horizontally in each pot with three nodes below the sand surface and two fully opened leaves above-ground.

2.2 Experiment Design

The study consisted of five treatments with different dates of N application, including none, on planting day, and on 3, 7 and 14 DAT (hereafter T0, T1, T3, T7 and T14, respectively). The experimental design was a complete randomized design (CRD) (Appendix A). There were multiple harvests over the growing period on 10, 21, 35 and 49 DAT. Six plants from each treatment were sampled at each harvest. Roots were washed in tap water to remove sand with minimum damages (Appendix B). Three plants were used for observation of root anatomy and morphology. The other three plants were used to analyse N acquisition. Biomass and yield for the last harvest were assessed based on these three plants. In total, four harvests were conducted over the experimental period totalling 120 plants (5 N application timing treatments × 4 harvests × 6 plant per harvest). In addition, eight plants per treatment were grown for back up purposes in case of death or abnormal growth, so in total 160 pots were prepared. Hoagland's lacking N solution (Hoagland & Arnon, 1950) was utilised for T0 treatment. The other treatments were supplied with the modified Hoagland's solution with 100 mg/L N added in the form of ammonium and nitrate (50% each). The nutrient solution with N was applied to plants regularly from treated dates. Plants were watered with the same amount of nutrient to field capacity every other day with the amount

varying from 120 to 180 mL pot^{-1} depending on plant growth and weather conditions. For the N application timing treatments, plants were watered with N lacking nutrient solution every two days from planting to dates treatment started; after that they were watered with Hoagland's solution with 100 mg/L N every other day till the end of the experiment. The total N applied for treatments T0, T1, T3, T7 and T14 over 49 days were 0, 485, 470, 420 and 400 mg $plant^{-1}$, respectively. A dripping irrigation system was utilised to water plants with tap water (with undetectable N level) on the days without nutrient solution supply if needed.

2.3 Measurements and Data Collections

2.3.1 Anatomical Observations of AR Development

Three plants from each treatment were used for anatomical observations on 10, 21, 35 and 49 DAT. The adventitious root (AR) count for individual plants was recorded on each sampling date. Transverse sections for a single root at around 3-4 cm from the proximal end of the root were prepared using free-hand sectioning using sharp razor blades (Villordon et al., 2009c). Sections were stained with Toluidine Blue O 0.05% to observe the structure under the microscope (Eguchi & Yoshinaga, 2008). Images of sections were taken using a Nikon DS-L2 camera (Nikon corporation, Tokyo, Japan) under an Olympus CX31 microscope (Olympus corporation, Tokyo, Japan) to classify the development stages of ARs.

The number of protoxylem elements (Appendix C) was observed in the first observation at 10 DAT. The other features such as initial regular vascular cambium (IRVC), completed regular vascular cambium (RVC), appearance of anomalous cambium (AC) and lignification of more than 50% of lignified cells (LC) were observed at four sampling times. The development of these anatomical features was described by Wilson and Lowe (1973).

Based on root anatomical characteristics (Appendix D) described by Wilson and Lowe (1973), ARs are classified into initiated SRs, pencil roots (PRs) and lignified roots. The approach to distinguish these three types of ARs based on anatomical characters was suggest as:

- Those roots with limited cambial activity around the central metaxylem and one or more protoxylem connected to the metaxylem cells by a strand of lignified tissues are classified as PRs (Villordon et al., 2012; Wilson & Lowe, 1973) (Appendix E).
- Roots with the continued activity of vascular cambium and AC develop into SRs. In such roots, circular AC is observed around the central metaxylem cells and protoxylem elements. In some roots without the central metaxylem, the initiation of primary cambium is associated with meristematic activity in the pith cells and the AC formed around the protoxylem elements (Villordon et al., 2012) (Appendix E).
- Adventitious roots that developed heavily lignified steles (more than 50% lignified stele), xylem rays, a broad secondary cortex and limited secondary phloem are classified as lignified roots (Appendix E).

2.3.2 Morphological Characteristics of Roots

Roots from three plants for anatomical observations were collected and used to investigate the morphological characteristics on 10, 21, 35 and 49 DAT. Epson Perfection V700 Photo Scanner (Seiko Epson, Nagano, Japan) was used to achieve root images. Acquisition images were analysed using the WinRHIZO Pro software (version 2012a; Regent Instruments Inc., Quebec, Canada). Data for the total root length, average root diameter and total root volume were extracted from the analysis.

2.3.2 Nitrogen Acquisition in Sweetpotato

Three plants for each treatment were harvested at 10, 21, 35 and 49 DAT to determine N acquisition. Fresh vine and root samples for each plant were collected after harvesting and kept separately in paper bags. The fresh and dried weights of samples were recorded. Samples were dried in a preheated oven at 90 °C for 90 minutes and then converted to 70 °C for an additional 48-72 hours to a constant weight (Maness, 2010). Samples were stored in a -80 °C freezer after drying until extraction. The concentration of C and N in different parts of plants was analysed using TruMac® Carbon/Nitrogen Instrument (LECO Corporation, Michigan, USA).

Nitrogen recovery efficiency (NRE) was calculated based on the average N accumulated in untreated plants (N_O) and fertilised plants (N_{FP}) and the amount of N fertiliser applied (N_F) (Zvomuya et al., 2003):

$$NRE\ (\%) = [(N_{FP} - N_O)/N_F] \times 100 \quad (1)$$

2.4 Statistical Data Analysis

The experimental data recorded from each harvest were subjected to standard analyses of variance using the one-way ANOVA procedure of the IBM® SPSS® software statistical package (version 25; IBM, New York,

USA). As different plants were sampled in each harvest, a two-way ANOVA was used to analyse the effect of the main factors and the interaction between treatments and harvesting times. This analysis allowed for testing the global effects of N application timing, time and the interactive effect of N application timing by time over the study period. All data in percentage were arcsine-transformed to analyse data in SPSS. The AR count and data for CN analysis were transformed using log 10 and square root transformation, respectively. For significant values, means were separated by Turkey HSD. Differences at the P value ≤ 0.05 was regarded as a test of statistical significance. Graphs were produced using SigmaPlot® software package (version 14; SYSTAT Software, Inc, California, USA).

3. Results

3.1 Effects of N Application Timings on Sweetpotato Anatomical Root Features

There was no significant difference in the number of ARs among N application timing treatments at various harvesting times (Table 1). The total AR number from three subterranean nodes varied from 10 to 13. In general, the number of ARs in all treatments peaked at 21 DAT and remained stable until the last harvest on 49 DAT. The main effect of N application timing and interactive effect of N application timings by harvesting time were not significantly different on the AR count ($P > 0.05$). However, the main effect of harvested time was statistical significance ($P < 0.001$).

Table 1. Effects of N application timing on adventitious root number at different sampling times

Treatment	10DAT	21DAT	35DAT	49DAT	*P* value
T0	10.7±0.7	11.7±0.7	11.7±0.3	11.7±0.3	NT: $P = 0.85$
T1	11.0±0.6	12.7±0.9	11.3±0.3	11.7±0.3	T: $P < 0.001$
T3	10.3±0.3	12.3±0.7	11.7±0.3	11.0±0.6	NT × T: $P = 0.97$
T7	10.3±0.7	12.3±0.3	11.7±0.3	11.3±0.3	
T14	10.3±0.7	12.0±0.6	11.0±0.6	11.7±0.3	
P value	0.923	0.85	0.674	0.674	

Note. The values are indicated as mean±standard error (SE) (n = 3). ANOVA results are based on log-transformed data. Two-way ANOVA results, including the effect of N application timing, sampling time and N application timing by time are shown. *P* values from one-way ANOVA analysis for each sampling date are presented within columns.

Abbreviations: NT = Nitrogen application timing; T = Time.

In this experiment, the number of protoxylem elements in roots on 10 DAT varied from four to ten. The arrangement of them was classified into three groups (Figure 1). Results showed that there was no statistically significant effect of N application timing on the distribution of protoxylem numbers among treatments ($P > 0.1$). A combination of pentarch and hexarch comprised around 70% to 85% of the total root number. A small percentage of roots with tetrarch stele were found in the T0 and T7 treatments. Protoxylem element number for the rest of the roots were higher polyarch steles (more than six steles).

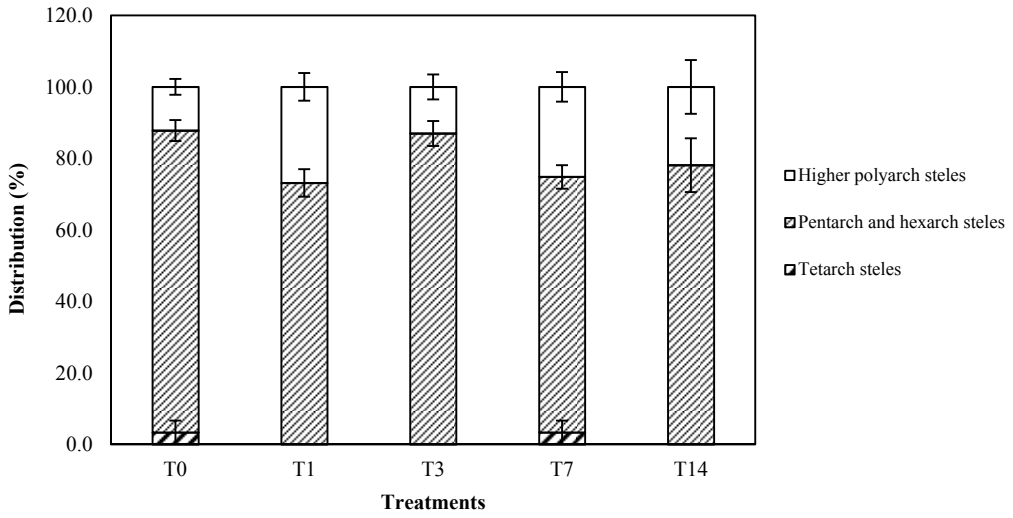

Figure 1. Distribution of AR protoxylem element number in 'Orleans' as affected by N application timings sampled at 10 DAT; the error bars indicate the SE of the mean value (n = 3)

The no cambium (NC) development in ARs was only observed at 10 DAT (Table 2). Nitrogen application timing had a significant effect on the percentage of root without cambium. The highest rates of roots with NC development were recorded in the T0 and T14 treatments, suggesting delayed development of cambium. The development of IRVC was found in roots during the first three weeks after transplanting. The percentage of roots with IRVC decreased over time. There was no significant effect of N application timing on the formation of IRVC on both 10 and 21 DAT.

In this experiment, RVC was observed throughout the study period (Table 2). When the effects of N application timing on the formation of RVC at different times were separately analysed, there were significant differences among treatments at all sampling dates except at 49 DAT, when RVC in all treatments declined to below 20%. The T0 and T14 treatments had the lowest rate of roots with RVC development on 10 DAT and the highest rate in the next two observation at 21 and 35 DAT. No difference was found on the effect of N application timing on RVC formation between T1, T3 and T7 treatments over the period.

Under our experimental conditions, the appearance of AC was found in roots at 21 DAT in all treatments (Table 2). Both T0 and T14 treatments showed the lowest percentage of roots initiating AC during this time. The rate of roots with AC increased over time in all treatments and reached the highest point at 49 DAT. During this time, AC was observed in around 65% of roots in the T1, T3 and T7 treatment. The percentage of roots with AC development in the T1, T3 and T7 treatments were always significantly higher than those for T1 and T14 treatments in all three harvesting times between 21 and 49 DAT ($P < 0.001$). Therefore, the application of N during the first week after transplantation promoted the formation of SR while no N supply or late application had the opposite effect.

Adventitious roots with more than 50% of LC were characterised between 21 and 49 DAT (Table 2). The effect of N application timing on the percentage of roots that developed more than 50% stellar lignification showed no significant difference between treatment at 21 and 35 DAT. However, T0 treatment had the highest rate at 49 DAT followed by T14 treatment, respectively, at 54.3% and 39.9%. A significantly lower rate of roots with more than 50% LC was observed in the T1, T3 and T7 treatments in the same period.

Table 2. Effects of N timing application on the development of anatomical features of root in different sampling times

Cambia	Treatment	10 DAT	21 DAT	35 DAT	49 DAT	ANOVA
IRVC (%)	T0	46.7^{b}±3.3	11.2^{b}±2.1			NT: $P = 0.891$
	T1	54.0^{a}±4.5	11.0^{b}±0.5			T: $P < 0.001$
	T3	51.2^{a}±6.9	10.7^{b}±3.3			NT × T: $P = 0.571$
	T7	45.4^{b}±7.2	16.0^{a}±3.6			
	T14	51.9^{a}±6.1	13.5^{ab}±3.4			
	P value	0.702	0.803			
RVC (%)	T0	22.2^{b}±4.0	48.7^{a}±2.9	28.5^{a}±2.5	5.8±2.9	NT: $P = 0.16$
	T1	39.9^{a}±5.1	26.3^{b}±1.7	14.6^{b}±2.8	3.0±3.0	T: $P < 0.001$
	T3	42.1^{a}±4.1	29.1^{b}±5.9	14.4^{b}±3.2	3.0±3.0	NT × T: $P < 0.001$
	T7	42.0^{a}±1.3	27.1^{b}±3.1	19.9^{b}±2.6	6.1±3.0	
	T14	22.6^{b} ±2.6	39.1^{ab}±3.5	30.2^{a}±1.8	8.6±0.3	
	P value	0.004	0.006	0.003	0.542	
AC (%)	T0		19.8^{b}±1.6	37.1^{c}±2.4	39.9^{c}±1.8	NT: $P < 0.001$
	T1		47.4^{a}±3.3	61.6^{ab}±3.6	68.4^{a}±3.4	T: $P < 0.001$
	T3		46.9^{a}±8.4	65.7^{a}±1.0	66.8^{a}±1.8	NT × T: $P = 0.851$
	T7		48.7^{a}±1.3	59.8^{bc}±3.6	64.6^{a}±1.0	
	T14		25.1^{b}±1.2	48.5^{c}±1.5	51.5^{b}±1.5	
	P value		0.002	< 0.001	< 0.001	
LC (%)	T0		20.3±3.6	34.3 ±4.9	54.3^{a}±2.4	NT: $P < 0.001$
	T1		15.3±3.6	23.7 ±3.5	28.5^{c}±2.5	T: $P < 0.001$
	T3		13.3±2.1	19.9±2.6	30.2^{bc}±1.8	NT × T: $P = 0.072$
	T7		8.1±4.8	20.2±6.0	29.3^{c}±2.0	
	T14		22.3±5.6	21.3±3.1	39.9^{b}±1.8	
	P value		0.190	0.156	< 0.001	

Note. The table presents the mean values followed by standard errors (SE) (n = 3). Means followed by different letters are significantly different ($P < 0.05$) within columns. ANOVA results are based on arcsine-transformed data. Original data is given in the table.

Abbreviations: IRVC = Initial regular vascular cambium; RVC = Complete regular vascular cambium; AC = Anomalous cambium; LC = lignified cells.

The effect of N application timing on the percentage of SRs was significantly different among treatments at different sampling times (Figure 2). Both insufficient N supply and late N application at 14 DAT reduced the rate of SRs. On the other hand, application of N within the first week after transplantation increased the formation of SRs in comparison to other treatments. At 49 DAT, the highest rate of SRs was recorded in T1 and T3 treatments followed by the T7 treatment. During this time, 59.8% and 64% of roots were SRs in the T1 and T3 treatments, timing treatments respectively. As demonstrated by the two-way ANOVA, the main effect of N and sampling time was significant on the percentage of SRs while the interactive effect of treatment by time was not statistically different.

Around 3%-9% of the ARs developed into PRs across the treatments (data not shown). Nitrogen application timings had no effect on the formation of PRs in all three observations and there was no significant interaction of treatment by time.

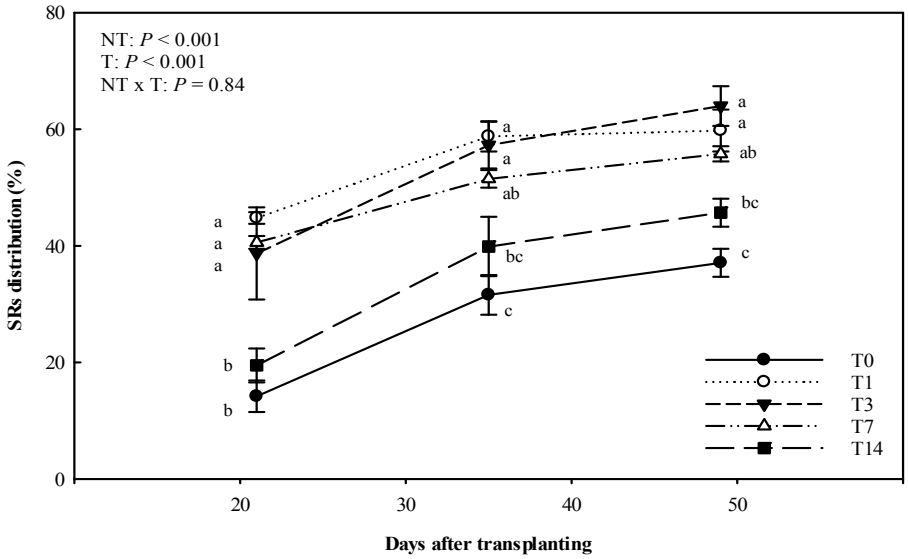

Figure 2. Effects of N application timing application on the initiation of SRs of 'Orleans' on different sampling dates

Note. Values are indicated as mean±SE (n = 3). ANOVA results are based on arcsine-transformed data. Two-way ANOVA results, including effect of N level, sampling time, and N level by time on anatomical development are shown. Different letters are significant different among treatments on single sampling dates using one-way ANOVA (Tukey's HSD, $P < 0.05$).

Abbreviations: SRs = storage roots; NT = nitrogen application timing; T = time.

3.2 Effects of N Application Timings on Root Morphology, Plant Performances and Yield of Orleans

In this study, Orleans roots were examined for the morphological root structures four times at 10, 21, 35 and 49 DAT (Table 3). The total root length increased over time in most treatments except T0. As there was no N supply in the T0 treatment, the total root length increased by 21 DAT and then decreased. Although there was no difference found on the effect of N application timing on this root parameter at 10 DAT, significant differences among treatments were recorded in the other three observations. No difference was found between T1 and T3 treatments during the study time and those treatments had a significantly higher total root length in all sampling times in comparison to other treatments. Delayed N application tended to reduce total root length, and the more delayed, the stronger the effect. The root volume followed a similar pattern to the total root length. By contrast, there was no difference on the effect of N application timing on root diameter of Orleans at different sampling times (Table 3).

Table 3. Effect of N application timings on the total root length, root diameter and total root volume of 'Orleans' on different sampling dates

	Treatment	10 DAT	21 DAT	35 DAT	49 DAT	ANOVA
TRL (cm)	T0	507±41	822$^{c±}$61	694$^{c±}$55	441$^{c±}$35	NT: $P < 0.001$
	T1	452±15	1479$^{a±}$93	1690$^{a±}$54	1497$^{a±}$68	T: $P < 0.001$
	T3	431±14	1377$^{a±}$63	1771$^{a±}$103	1633$^{a±}$86	NT × T: $P < 0.001$
	T7	525±18	1226$^{ab±}$28	1478$^{a±}$84	1359$^{ab±}$74	
	T14	473±40	916$^{bc±}$90	1042$^{b±}$108	1122$^{b±}$64	
	P value	0.19	< 0.001	< 0.001	< 0.001	
RD (mm)	T0	0.77±0.02	0.72±0.02	0.76±0,02	0.86±0.01	NT: $P = 0.09$
	T1	0.82±0.01	0.75±0.02	0.75±0.01	0.92±0.04	T: $P < 0.001$
	T3	0.81±0.03	0.72±0.01	0.75±0.01	0.87±0.01	NT × T: $P = 0.55$
	T7	0.78±0.01	0.76±0.02	0.76±0.01	0.86±0.03	
	T14	0.83±0.01	0.75±0.03	0.79±0.01	0.87±0.04	
	P value	0.12	0.62	0.12	0.49	
RV (cm^3)	T0	5.0±0.9	8.7$^{b±}$0.5	9.6$^{c±}$0.8	7.3$^{c±}$0.8	NT: $P < 0.001$
	T1	5.1±0.5	21.4$^{a±}$1.8	35.3$^{a±}$1.4	36.2$^{a±}$3.2	T: $P < 0.001$
	T3	5.0±0.5	17.6$^{a±}$1.7	32.3$^{a±}$1.2	34.4$^{a±}$1.8	NT × T: $P < 0.001$
	T7	5.6±0.3	17.0$^{a±}$1.6	27.9$^{a±}$1.4	30.8$^{abs±}$1.4	
	T14	4.6±0.5	10.3$^{b±}$0.5	20.0$^{b±}$2.3	23.6$^{b±}$1.2	
	P value	0.81	< 0.001	< 0.001	< 0.001	

Note. The table presents the mean values followed by standard errors (SE). ANOVA results are based on square root transformed data. Two-way ANOVA results, including the effect of N application timing, time and interactive effect of N application timing by time are shown.

Means followed by different letters are significantly different ($P < 0.05$) within columns using one-way ANOVA (Turkey's HSD, $P < 0.05$).

Abbreviations: TRL = Total root length; RD = Root diameter; RV = Root volume; NT = Nitrogen application timing; T = Time.

Results from this experiment showed that all treatments with N applications significantly increased the dry above-ground and root weight compared to the T0 treatment at 49 DAT (Table 4). The highest and lowest SR weights were recorded in the T1 and T0 treatments, respectively. The earlier N was applied, the larger and heavier storage roots yielded.

Table 4. Effect of N application timings on biomass weight, SR length, SR diameter and SR yield at 49 DAT

Treatment	ADW (g/plant)	RDW (g/plant)	SRL (mm)	SRD (mm)	FSRW (g/plant)
T0	1.6$^{c±}$0.09	4.8$^{d±}$0.4	42.2$^{c±}$6.4	9.4$^{b±}$0.7	16.0$^{ds±}$2.0
T1	13.4$^{a±}$0.60	23.1$^{a±}$1.3	105.9$^{a±}$4.3	17.7$^{a±}$0.7	125.3$^{a±}$6.9
T3	9.9$^{b±}$0.67	18.4$^{b±}$0.9	102.8$^{a±}$4.6	14.5$^{a±}$1.0	94.7$^{ab±}$8.3
T7	10.1$^{b±}$0.26	17.7$^{b±}$0.6	91.7$^{ab±}$3.4	15.7$^{a±}$0.6	83.2$^{b±}$5.7
T14	9.7$^{b±}$0.32	12.9$^{c±}$1.0	78.0$^{b±}$2.3	14.8$^{a±}$2.0	57.0$^{c±}$4.8
P value	< 0.001	< 0.001	< 0.001	< 0.001	0.003

Note. The table presents the mean values followed by standard errors (SE) (n = 3). ANOVA results are based on square root transformed data and original data is presented in the table.

Means followed by different letters are significantly different ($P < 0.05$) within columns using one way ANOVA (Turkey's HSD).

Abbreviations: ADW = Above-ground dry weight; RDW = Root dry weight; SRL = Storage root length, SRD = Storage root diameter and FSRW = Fresh storage root weight.

3.3 Effects of N Application Timing on N Acquisition in Sweetpotato During the SR Formation

Results from the present experiment showed that the N concentration in vines and roots were affected by N application timing applications and significant differences were found among treatments (Figure 3A; Figure 3B). The concentration of N in no N application (T0) had the lowest value in both vines and roots. In vines, four treatments with N supply on various dates had no statistical difference in all observations except at 21 DAT. During this time, T1 and T3 treatments had the highest N concentrations, followed by T7 and then T14. No significant effect was found on the N concentration in roots among four treatments including T1, T3, T7 and T14 at 21 and 49 DAT. In general, the earlier N was added, the more N was acquired by the plant by 49 DAT (Figure 4). Delayed N application initially led to low N in vine but vine N content became normal after N was applied. The T1 treatments had the highest value of both vine and root N acquisition in all sampling times. Based on three harvesting times at 21, 35 and 49 DAT, the two-way ANOVA results showed that the main effects of N application timing and sampling time were significant on the total N in vine, root and the whole plant ($P < 0.001$). Also, the interactive effect of N application timings and time was significant ($P < 0.001$).

The highest C:N ratio in both vines and roots was found in the T0 treatment, which was significantly higher than those of other treatments in all observations (Figures 3C and 3D). The C:N ratio in vines of the T0 treatment increased clearly over the time from around 20 at 10 DAT to approximately 50 in the final samplings (Figure 3C). However, that of other treatments remained stable during the study period. In roots, four treatments treated with N had similar N content in all harvesting times, which was significant lower compared to T0 treatment (Figure 3D).

Results from the present experiment showed that the effect of N application timings was significantly different on the NRE in all sampling times (Figure 5). The highest and lowest NRE were observed in the T1 and T14 treatments, respectively.

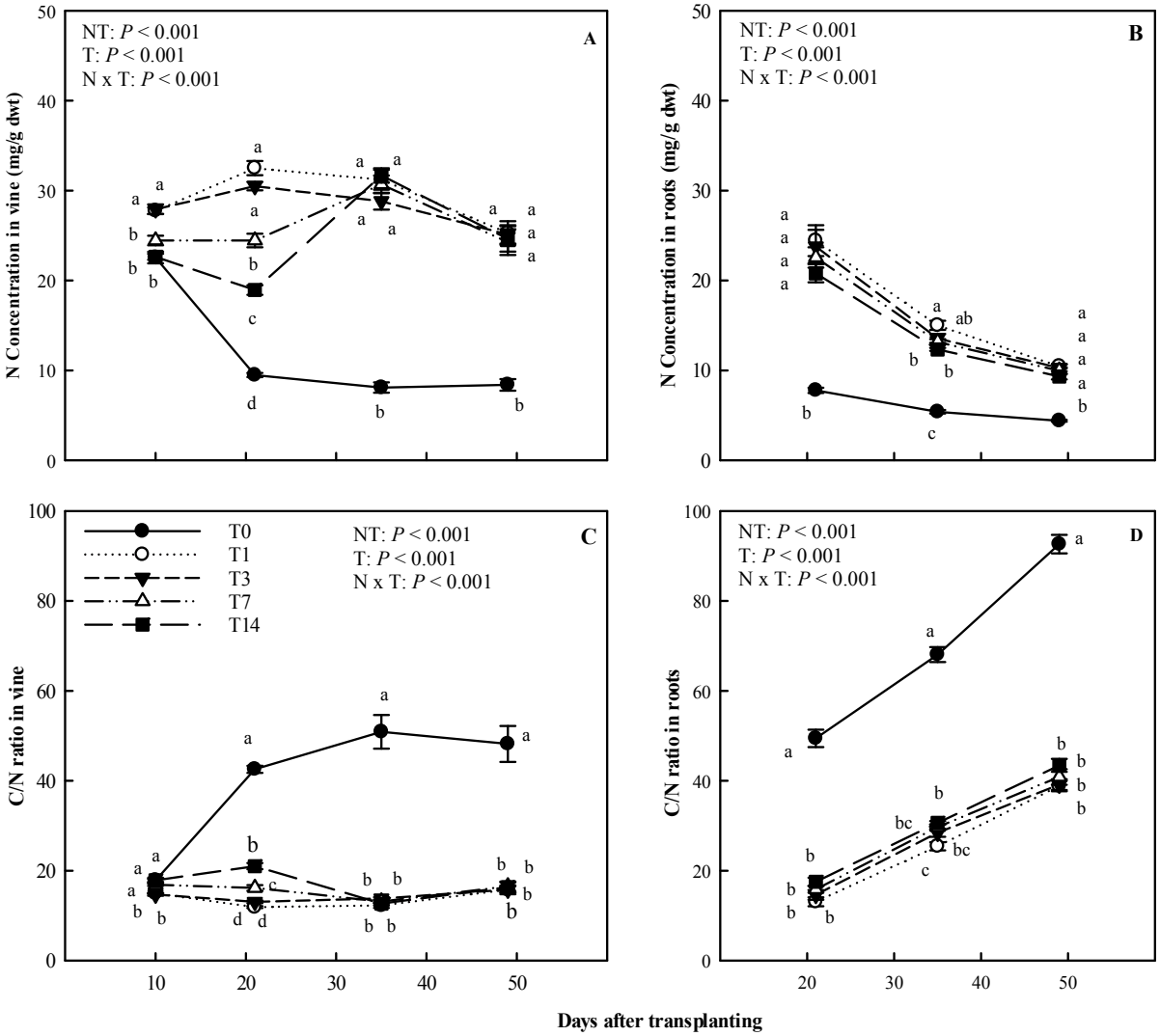

Figure 3. The effect of N application timings on the N concentration in (A) vines; in (B) roots; and on the C:N ratio in (C) vines in (D) roots

Note. ANOVA results, including effects of N application timing, harvesting date, and N application timing by time are shown. Different letters indicate significant different among treatments on the same harvesting date (Turkey's HSD).

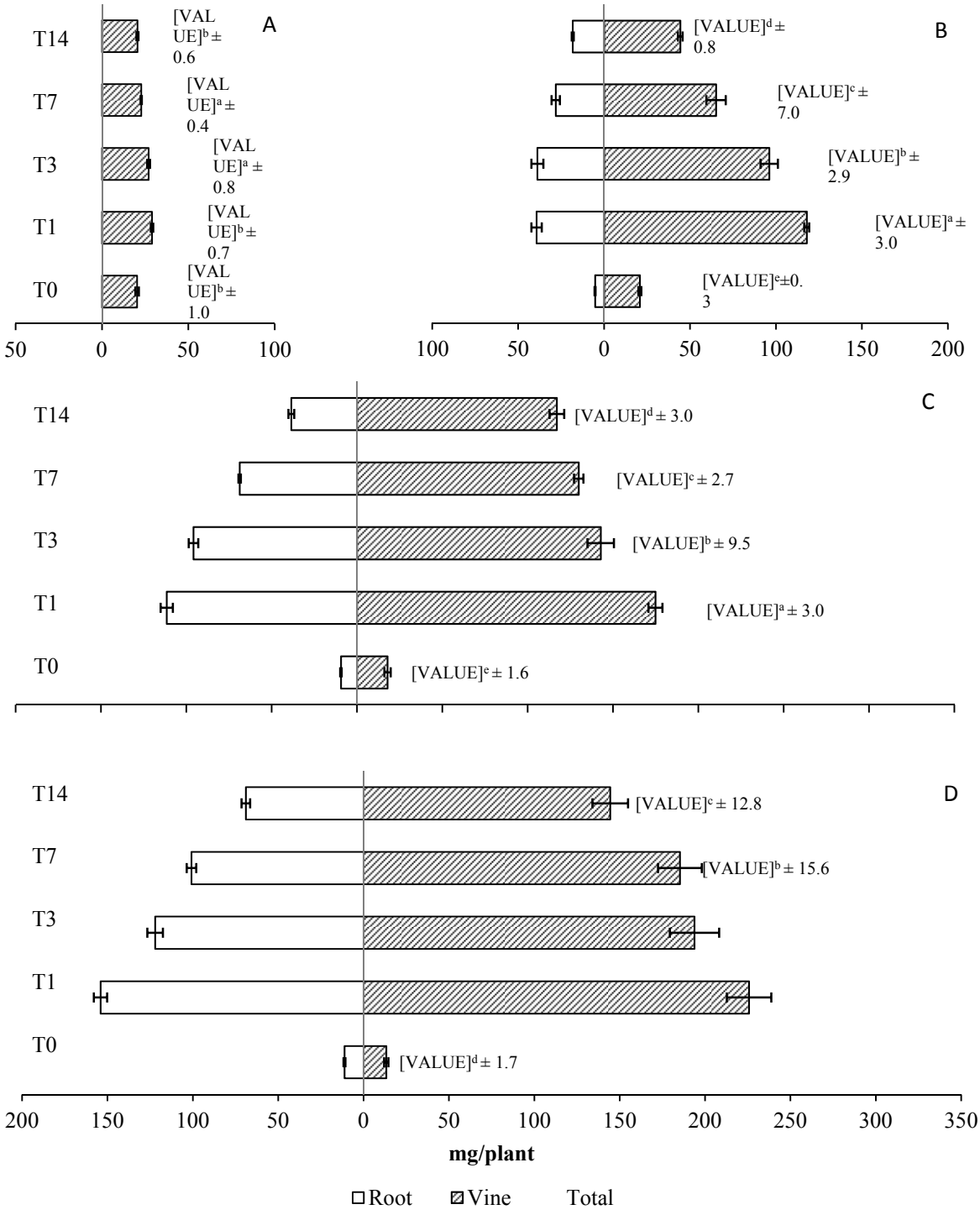

Figure 4. Effect of N application timings on the acquisition of N (mg) at various sampling times: (A) 10 DAT; (B) 21 DAT; (C) 35 DAT and (D) 49 DAT

Note. Numbers are mean value of total N accumulated in the whole plant (including vine and root) followed by SE. Means followed by a different letter are significantly different among N application timings (Turkey's HSD, $P < 0.05$).

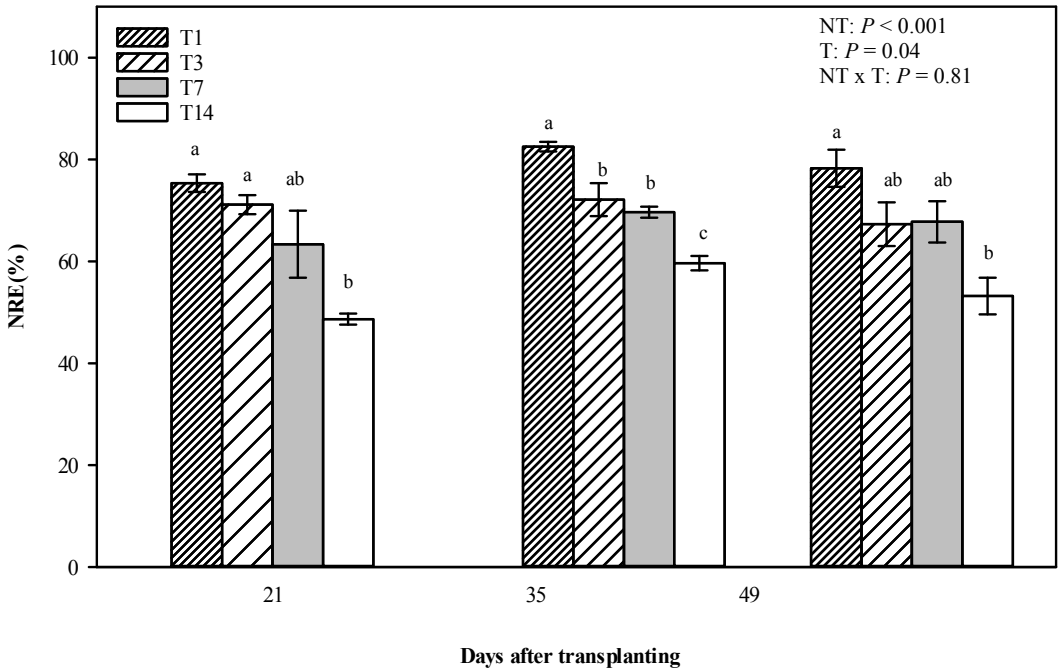

Figure 5. The efficiency of N use in sweetpotato under different N application timings

Note. Values are indicated as mean±SE (n = 3). ANOVA results including the effect N application timing, sampling time, and N application timing by time interaction are shown. Means followed by different letter are significant different among N application timing treatments (Turkey's HSD, $P < 0.05$).

Abbreviation: NT = N application timing; T = Time.

4. Discussion

4.1 Effect of N Application Timing on Anatomical Features of 'Orleans'

To the best of our knowledge, the effect of N application timing on anatomical structures of sweetpotato roots is examined for the first time in this study. In this experiment, N application timing had no effect on the number of ARs per plant. The AR number increased between 10 and 21 DAT in all treatments. This finding is similar to results in previous studies for some American and Indian cultivars (Villordon et al., 2009c; Wilson & Lowe, 1973). The arrangement of vascular cylinders in ARs was stated to relate to SR formation (Belehu et al., 2004; Togari, 1950; Villordon et al., 2009c; Wilson & Lowe, 1973). Most of the roots were pentarch and hexarch, which accounted for a combination of 70-85% of total roots. This result was similar to findings for Beauregard (Villordon et al., 2009c), Atacama (Belehu et al., 2004) and some Indian cultivars (Wilson & Lowe, 1973). Overall, N application timing had no effect on the AR number and the arrangement of vascular cambium in roots, so the effect on SR formation rate generally could be interpreted as the impact on SR number.

Orleans roots sampled at four sampling times showed varying anatomical features (Appendix B) related to root thickening. In sweetpotato, the thickening of roots started as the initiation of the IRVC from undifferentiated primary cambium cells between the protoxylem and phloem (Villordon et al., 2009c). Then, the meristematic activity of vascular cambium produces secondary xylem and phloem and gradual completion of RVC. N application during the first week after transplanting (T1, T3 and T7 treatments) had no effect on IRVC, the percentage of roots with RVC in early stage (10 DAT) was significantly increased (Fig. 2B). Therefore, sweetpotato cultivar Orleans appeared to need N supply to promote the formation of cambium in roots from planting.

The appearance of AC around protoxylem elements and central metaxylem cells was the first clear sign of SR formation (Villordon et al., 2009a; Wilson & Lowe, 1973). The time frame for this event varied widely among cultivars and took place from 7 to 91 DAT (Ravi & Indira, 1999). Under our experimental conditions, the SR initiation was first observed in roots at 21 DAT in all treatments. This is in line with results of Villordon et al., (2009b) for Beauregard variety under glasshouse conditions. Nearly 50% of roots exhibited AC appearance in

the T1, T3 and T7 treatments at 21 DAT, which was significantly higher than that of T0 and T14 treatments. In the next two observations, those three treatments also had the higher rate of roots with this anatomical feature than T0 and T14. In addition, treatments T1, T3 and T7 had significantly lower rates of lignified roots than others. These results suggested that application of N during the first week after transplantation promoted the initiation of SRs and inhibited lignification. No N supply reduced the formation of AC while late N application delayed AC initiation, resulting in lower SR initiation and increased rate of absorbing roots, which might enhance N absorption as an acclimation to low N availability. Nutrient deficiency has been reported to increase root/shoot ratio (Chapin, 1980) or favour root growth (Ericsson, 1995).

Treatments T1, T3 and T7 promoted the formation of cambial cells during the very early stage of root development. Then, they continued to stimulate the formation of AC and supressed root lignification. As a result, more SRs were initiated in those treatments compared to T0 and T14. Possible mechanisms related to the development of cambium in roots would be that the application of N during the first week supplied the optimum N level for cambium formation and development. Plants in T0 and T14 treatments experienced insufficient N supply, which has been demonstrated to reduce the formation and activity of cambium (Dong, 2021). Previous studies suggested that cambial activity or cell division was regulated by cytokinin level in plants (Kakimoto, 2003; Matsumoto-Kitano et al., 2008). Cytokinin level generally increase in response to N and P (Matsumoto-Kitano et al., 2008; Samuelson et al., 1992) and reduce in insufficient nutrient conditions (Yang et al., 2001). In such adverse conditions, the cambium activity is inhibited (Matsumoto-Kitano et al., 2008). This suggests that cytokinin may act as regulator of cambium development. In our study, delayed N application retards the synthesis of cytokinin, so that AC formation is inhibited. In addition, auxin is produced in young shoots and is transported to roots depending on source-sink relation (Aloni et al., 2006), regulate cambium activity (Wang, 2020). Cambial activity and secondary growth are halted when shoot tips are removes (Wang, 2020). Moreover, high levels of auxin are found in cambium region of plants (Uggla et al., 1996). This phytohormone is also closely related to N concentration (Kiba et al., 2011).

The effect of N application timing on the distribution of SRs was significant in all observations during the study time. The higher rates of SRs were observed in T1, T3 and T7 treatments. This could be associated with the formation and activity of the cambium, as application of N during the first week after transplanting of Orleans promoted the formation of RVC and AC as well as inhibited lignification of roots. None or delayed N application at 14 DAT reduced the percentage of SR initiation and increased the number of lignified roots. It was demonstrated in previous studies that insufficient N supply reduced the number of sweetpotato SRs (Njoku et al., 2001; Okpara et al., 2009; Taranet et al., 2017). It is notable that by 49 DAT, root differentiation was largely completed, so the reduction of SR number due to the absence or delayed application of N after planting could be permanent and potentially impact yield.

The distribution of PRs was not affected by N application timings in our experiment. This type of root develops from thick roots under inconducive conditions for SR formation or from thin roots (Kays, 1985). In general, sweetpotato has a minority rate of PRs, so it does not attract much focus from researchers. Moisture and soil properties were documented as driving factors associate to the formation of PRs (Kays, 1985). For example, dry and compacted soil are favour conditions for PR initiation.

4.2 Effects of N Application Timing on Root Morphology, Plant Performances and Yield of Orleans

At the beginning, available N in cuttings may be sufficient for root growth, so no significant effect of N application timings was observed on root morphology at 10 DAT. In the next three samplings, three treatments (T1, T3 and T7) had higher total root length and root volume than other treatments. This indicates that early N application is essential to stimulate root growth. In another study, the optimum level of N supply at the rate of 50 kg ha^{-1} increased the total root length of lateral roots (Villordon et al., 2013). They also suggested that further increasing N applications up to 200 kg ha^{-1} did not result in greater lateral root length. Results in maize indicated that the total root length and root volume increased with increasing nitrate supply ranging from 0.04 to 4 mM (Wang et al., 2005). In addition, plants in no or delayed N treatments were grown under insufficient N condition in the first 14 days. They both had lower above- and below-ground biomass, as well as smaller SR size at 49 DAT. This result confirmed that insufficient N supply inhibited the growth of sweetpotato (Okpara et al., 2009; Osaki et al., 1995).

In contrast to our results, previous studies observed that delaying N application 2 to 5 weeks after transplanting increased SR production of sweetpotato (Mulkey et al., 1994; Phillips et al., 2005; Villordon et al., 2009b). A possible reason for the difference could be the difference of available N in growing substrates. Our experiment was conducted in river sand with very poor nutrients whereas other studies were carried out in soil under field

conditions. Sweetpotato required moderate levels during SR initiation and maximum N uptake was during 23 and 40 DAT (Villordon et al., 2009b), so N available in soil might be adequate for SR formation in some literature.

4.3 Effects of N Application Timing on N Accumulation During the SR Formation of 'Orleans'

As we used the same rate of N at 100 mg/L in a nutrient solution to apply to sweetpotato, treatment T1 generally received higher N supply over the whole experiment period, resulting in higher N accumulation than others. The response of N concentration in plants was positively related to N supply rates in numerous previous studies (Dordas & Sioulas, 2009; Osaki et al., 1995; Villagarcia et al., 1998; Zotarelli et al., 2009). The C:N ratio was significantly lower in the treatments with N at either application date because of high N acquisition. A similar effect was found in other plant species such as potato and wheat (Mittelstrass et al., 2006; Rahimizadeh et al., 2010). N acquisition in vines and roots in this study was similar to observation in another study where the amount of N accumulation increased from planting until 100 DAT (Osaki et al., 1995).

Earlier N application timing treatment had a higher NRE than the delayed N supply timings. This finding is in line with previous results in another crop such as corn or potato. Those studies found that delayed N application resulted in lower nitrogen recovery efficiency of crops (Jung et al., 1972; Millard & Robinson, 1990; Walsh et al., 2012). One possible reason for this could be that plants need N for their growth, and so plants that were supplied with N earlier could start their development earlier than those in the delayed treatments, which promoted N acquisition over the course of the experiment. In our study, the significantly higher dried above-ground biomass and root biomass was recorded in the earlier N fertilisation treatments as compared to the delayed N application treatments. This suggested that when N application was delayed, the growth of the N-starved crop was not able to catch up even after N was added. Another possible reason leading to lower NRE in delayed N treatment would be that delayed N application plants formed fewer SRs and more lignified roots while the growth of lignified roots required fewer N, which may inhibit the need and acquisition of N. It was reported that in the early stage of plant development, N was taken up into the canopy and then relocated to the growing of tubers (Millard & Robinson, 1990). The majority of N in plants was stored in harvested parts rather than other part of plants (Olson & Kurtz, 1982). Therefore, N application timing affected NRE in sweetpotato, with higher NRE in the earlier N application.

5. Conclusion

Results from the current experiment found that both deficient and delayed N application would inhibit SR formation in sweetpotato by reducing the formation of RVC and AC and promoting the lignification in ARs. Nitrogen application within the first week after transplantation regardless of whether it is on planting day or 7 DAT (T1, T3 and T7 treatments), increased the distribution of SRs and reduced the number of lignified roots. Those three treatments had higher N accumulation in vines and roots and higher NRE. In addition, those treatments had the highest root length and volume over the study time and greatest SR yield at 49 DAT. Our results suggest a moderate level of N is required for sweetpotato SR initiation from transplantation to promote the formation of the maximum SR number per plant. Early application of an appropriate level of N also stimulates vines and root growth of sweetpotato. Ideally, in agronomic practice, adequate (but not excessive, Togari, 1950; Wilson, 1973) level of available N should be established in soil before or when cuttings are planted.

Acknowledgements

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Appendix A

Planting sweetpotato in pots

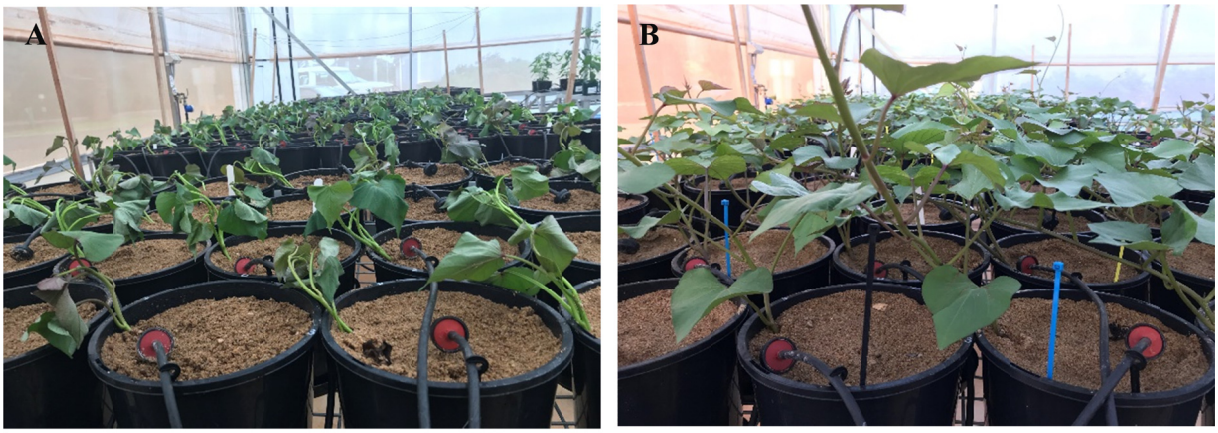

Figure A. Experimental set up with dripping irrigation system. (A) after planting and (B) three weeks after planting

Appendix B

Sweetpotato plant with visual storage roots

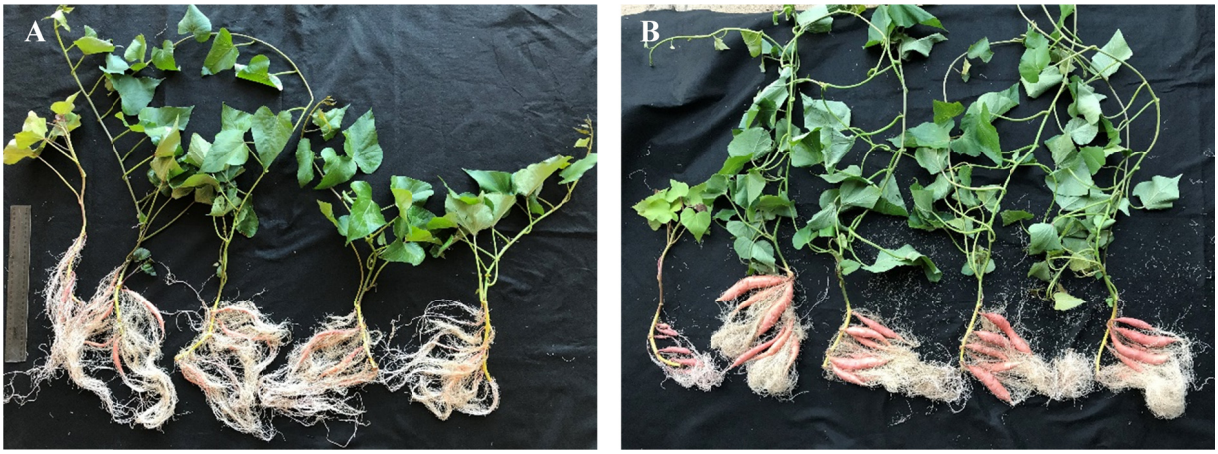

Figure B. Sweetpotato plant with visual storage root. (A) three weeks after planting and (B) seven weeks after planting. From left to right T0, T1, T3, T7 and T14

Appendix C

Transverse sections of roots

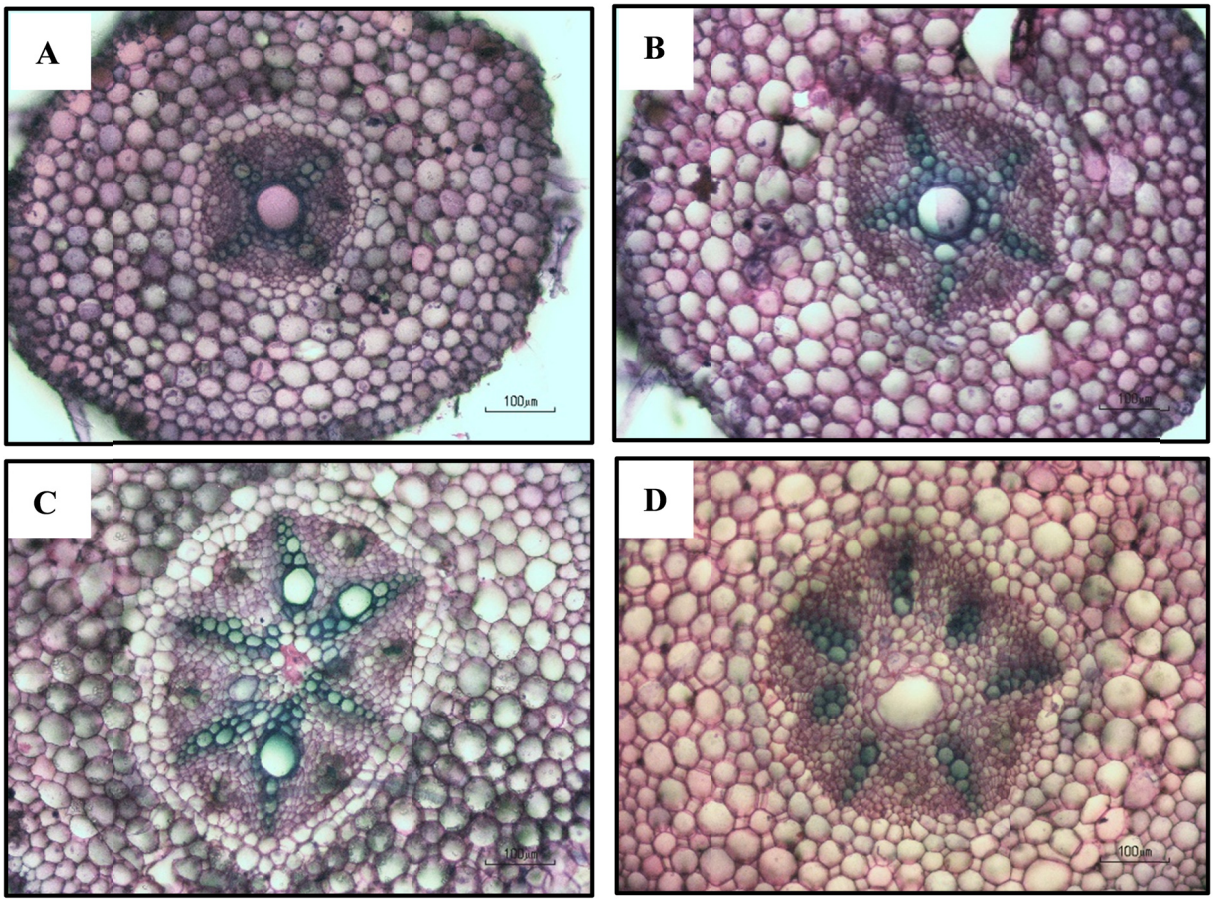

Figure C. Transverse section of sweetpotato root (A) section with tetrach steles; (B) Section with pentarch steles; (C) Section with hexarch steles and (D) Section with higher polyarch steles

Appendix D

Development of cambium in roots

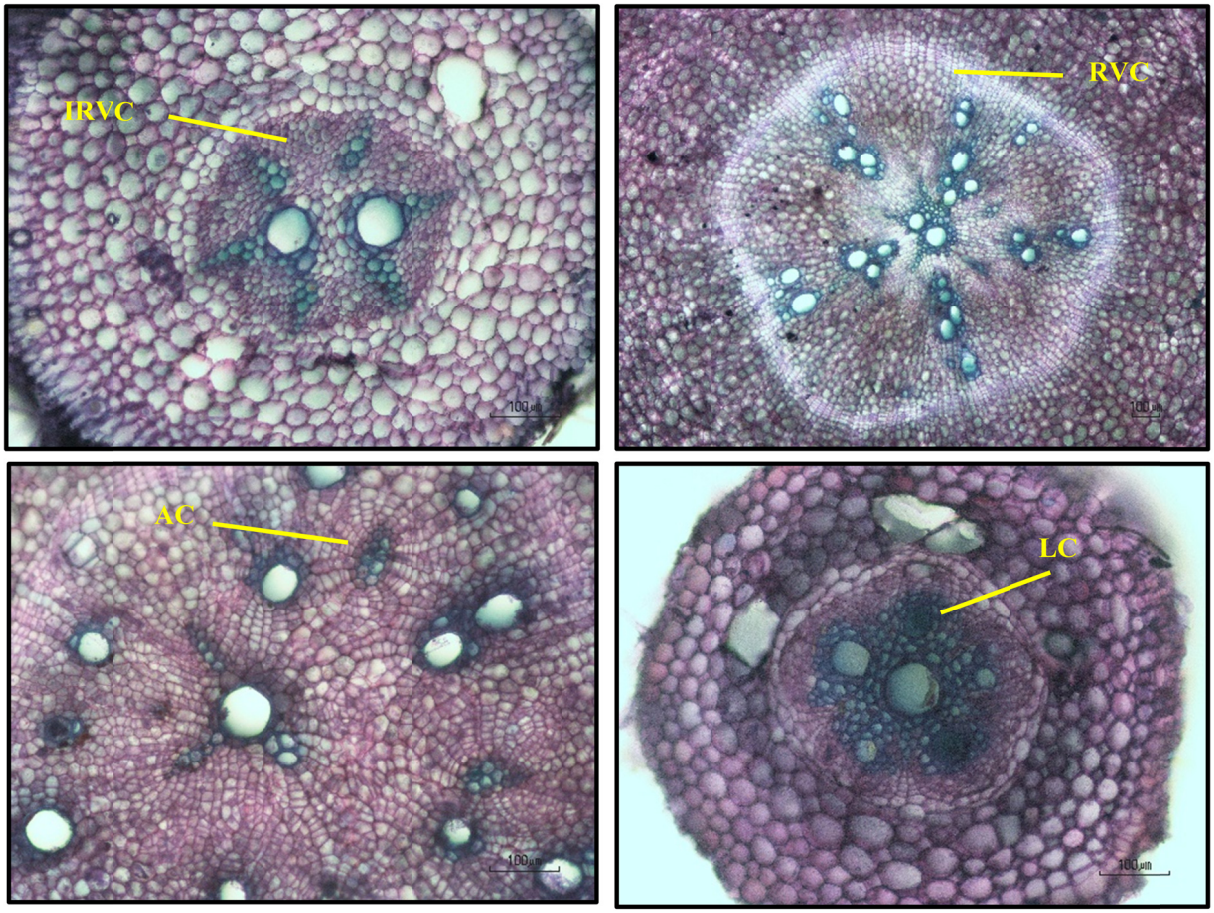

Figure D. Cambium development stages in adventitious roots

Note. Abbreviation: IRVC = Initial Regular Vascular Cambium; RVC = Complete Regular Vascular Cambium; AC = Anomalous Cambium; LC = Lignified Cells.

Appendix E

Transverse section of roots at 21 DAT

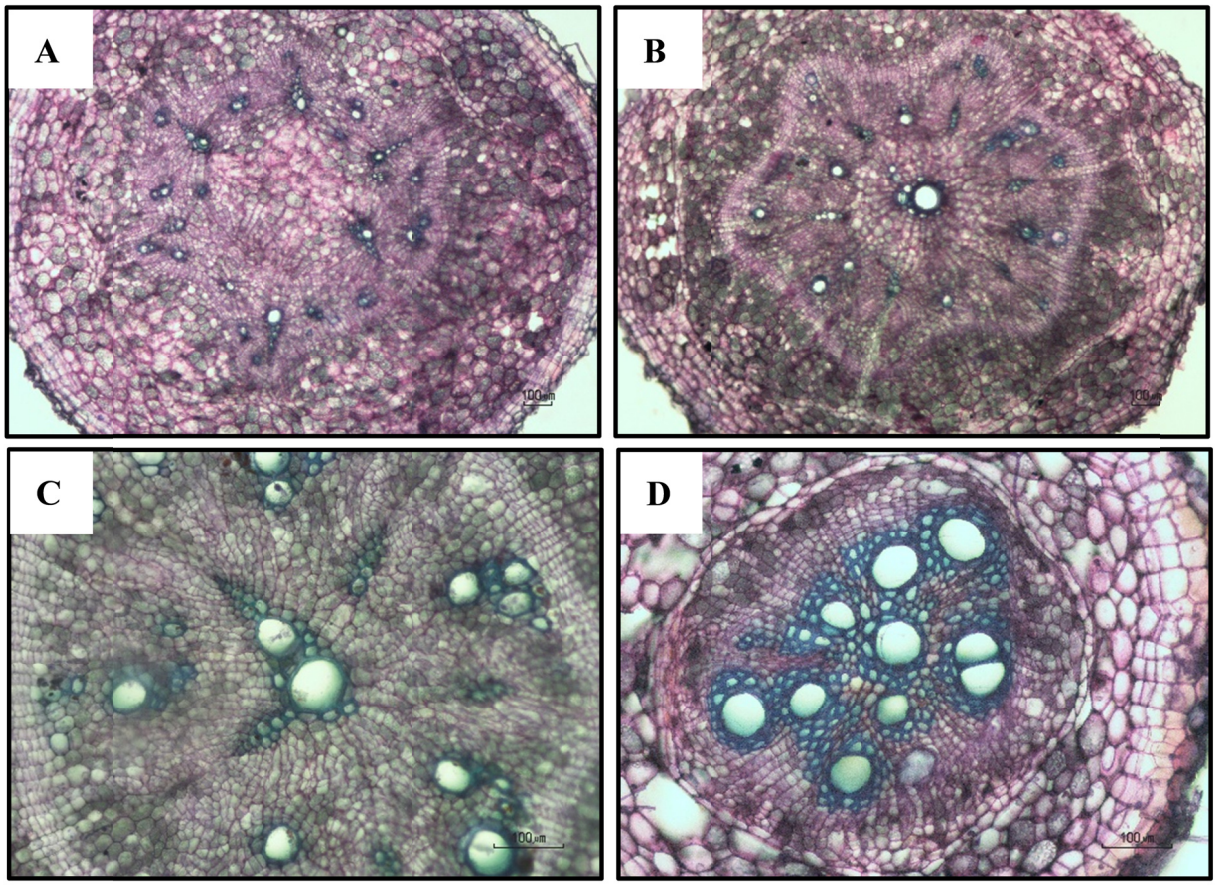

Figure E. Micrograph transverse sections of adventitious roots at 21 DAT. (A) initiated SR without central metaxylem, AC surrounds xylem elements; (B) initiated SR with AC surrounding central metaxylem and xylem elements; (C) PR with two protoxylem elements connected to the central metaxylem and some AC developed around protoxylem elements and the central cell. (D) lignified root with more than 50% of lignified steles as indicated by the green colour