Understanding agronomic factors that affect the initiation and development of sweetpotato (*Ipomoea batatas* (L.) Lam.) storage roots—the role of nitrogen fertilisation and organic soil amendments

by

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Thesis abstract

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a major root crop that is widely grown throughout the world. In Australia, sweetpotato is one of the few very profitable and rapidly expanding vegetable crops. The sweetpotato industry is experiencing remarkable growth, with rapid growth in recent decades. However, storage root (SR) size and shape are important for consumer acceptance in developed countries, including Australia, where SRs with an excessively large or small size are rated as low quality with low commercial value. This determines the marketable yield and profit of growers. Agronomical factors have been reported to influence initiation and development of SRs, which affects the size, number and evenness of SRs over time. Environmental factors cannot be manipulated on a large scale. Therefore, this project focused on the influence of nitrogen (N) fertilisation and some local organic amendments to these processes. Also, the chemical changes inside the plants, including soluble sugar and starch, as well as the accumulation of N, were examined during SR initiation.

Preliminary experiments were conducted using different soilless cultures to investigate the development of SRs. Three growing methods, nutrient film technique, fine sand and washed (coarse) river sand, were utilised. Results demonstrated that sweetpotato could be grown in the nutrient film technique systems as some SRs formed in the system. However, the observations of SR initiation and development were obstructed as SRs developed under the nutrient film and formed odd shapes. Similarly, fine sand culture was not suitable as it resulted in delayed SR formation and promotion of lignified roots. Washed river sand culture was suitable for SR initiation and development, and was utilised in further studies to examine the initiation of SRs.

Results suggested that both deficient (N0) and high (N200) rates of N inhibited the formation of primary cambium at 10 days after transplanting (DAT) and then anomalous cambium (AC) at 21 DAT. Therefore, the initiation of SRs was delayed in those N conditions. Both N0 and N200 treatments had a significantly lower SR rate at 21 DAT whereas both 50 and 100 mg/L N treatments promoted the formation of SRs during this period. Application of N at 100 mg/L was optimal for SR formation as suggested by the highest percentage of SRs during 21 and 56 DAT. In this experiment, treatment N200 had the highest percentage of roots with cambium development in the earliest observation and the highest percentage of initiated SRs between 21 to 56 DAT. This treatment also

had the highest starch accumulation in roots during the first 35 DAT. However, sweetpotato required more N after SR formation as indicated by faster growth, higher N acquisition, highest efficiency of N use after 35 DAT and higher carbohydrate accumulation in roots in the N200 treatment. This study indicates that moderate N fertilisation level should be maintained for a few weeks to promote SR formation, and then further N fertiliser should be applied to improve SR development.

A pot trial was conducted for Orleans variety to evaluate the effects of different N fertilisation timings on SR initiation, the accumulation of non-structural carbohydrates in plants and the acquisition of N in sweetpotato during the formation of SRs. Both no and delayed N application till 14 DAT inhibited the formation of regular vascular cambium (RVC) and AC during the early stage of adventitious root development. Then, those treatments promoted the lignification of stele cells, resulting in a significantly higher rate of lignified roots. However, plants supplied with N within the first week after transplanting demonstrated significantly higher rates SRs and lower rates of lignified roots. Furthermore, earlier N application promoted plant and root growth as indicated by higher biomass and SR weight, more non-structural carbohydrate and N accumulation in plants, and higher N recovery efficiency. The study indicated that moderate N should be available in soil before or on planting day to promote SR initiation.

A pot experiment was set up to investigate the impacts of poultry manure (PM) and sugarcane trash (SCT) on available soil N and total N in dermosol soil and then examined the effects of these products on the initiation of Orleans sweetpotato SRs. The PM treatments increased soil available N and total N whereas SCT application had significantly lower available N in soil. The SCT application at both rates promoted SR initiation and reduced lignification compared to PM and chemical fertilisers. By contrast, both PM applications inhibited the initiation of SRs and promoted lignified roots. Application of PM at 66 tons ha^{-1} enhanced vine growth and reduced root growth. Both SCT applications maintained shoot growth and promoted root growth. This indicates that SCT should be used for sweetpotato to promote SR initiation and high PM rates should not be applied to sweetpotato as they inhibit SR initiation. This result is in line with our first pot experiment and demonstrate soil organic amendment affects sweetpotaot SR initiaion by changing soil available N.

Our study provides agronomic indication that moderate N supply level should be maintained from planting for a few weeks to promote SR initiation, and then another

application of N supplied to improve SR development. Soil organic amendments can affect SR initiation due to their impacts on N availability in soil. Therefore, the type and rate should be carefully considered before application.

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This paper has not been submitted for an award by another research degree candidate (Co-Author), either at CQUniversity or elsewhere.

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Thesis Declaration

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Abbreviations

A	Amendment treatment
AC	Anomalous cambium
ADW	Above-ground dried weight
AR	Adventitious root
ARs	Adventitious roots
ANOVA	Analysis of variance
C	Carbon
DAT	Days after transplanting
FSRW	Fresh storage root weight
IRVC	Initial regular vascular cambium
LC	Lignified cells
MCW	Methanol : Chloroform : Water
N	Nitrogen
NC	No cambium
NFT	Nutrient film technique
NL	Nitrogen level
NRE	Nitrogen recovery efficiency
NSC	Non-structural carbohydrates
NSCR	Non-structural carbohydrates in roots
NSCV	Non-structural carbohydrates in vine
NT	Nitrogen application timing

PM	Poultry manure
PRs	Pencil roots
RD	Root diameter
RDW	Root dried weight
RS	Total root surface
RT	Root tips
RV	Total root volume
RVC	Regular vascular cambium
SCT	Sugarcane trash
SE	Standard error
SR	Storage root
SRt	Starch in roots
SRs	Storage roots
SRD	Storage root diameter
SRL	Storage root length
SS	Soluble sugar
SSR	Soluble sugar in roots
SSV	Soluble sugar in vine
SV	Starch in vine
T	Time
TRL	Total root length

Publications during candidature

‘No publications’

Chapter 1. Introduction and rationale

1.1. General introduction

Root and tuber crops such as cassava, sweetpotato, yam, potato and taro are widely grown throughout the world, including in most developing countries, and are important to the agricultural industries and food security of more than 100 countries (GCP21, 2015). They are a major component in the diet of more than two billion people and contribute to animal feed as well as to processing industries (IITA, 2016). The annual world production of root and tuber crops in 2017 was estimated at 887 million tons (MT) consisting of 388 MT of potatoes, 292 MT of cassava, 113 MT of sweetpotato, 73 MT of yams and 21 MT of taro and other aroids (FAOSTAT, 2019). Root and tubers crops are the third most important group of food crops to humankind, after cereals and grain legumes, and are used as a staple or subsidy food for around a fifth of the world population (Chandra, 2006). For example, the consumption of root and tuber crops is over 200 kg capita^{-1} year^{-1} in ten sub-Sahara African countries including Angola, Central African Republic, Congo, Ghana, Mozambique, Nigeria, Rwanda, Benin, Côte d'Ivoire and Togo (FAOSTAT, 2019), with a total population of approximately 400 million (World Bank, 2019). Therefore, improving the production of root and tuber crops may contribute to a substantial reduction in calorie deficiencies for 70% of populations in developing countries (Westby, 2002).

In Australia, root and tuber crops such as potatoes, sweetpotato, taro, beetroot and parsnips have been cultivated for many years. Potato and sweetpotato are the two major crops that are presented in FAO's statistics. In 2018, the total potato growth area was 29,740 hectares and production was approximately 1.2 million tons, and the total sweetpotato growth area and production was 1,928 hectares and 70,204 tons, respectively (FAOSTAT, 2019). The consumption of potato was 18.0 kg capita^{-1} year^{-1}, whereas that of sweetpotato was only 3.7 kg (Horticulture Innovation Australia, 2019). However, both the consumption and price of sweetpotato have been increasing in the past decade (Gething et al., 2012). This has stimulated the sweetpotato industry to increase production to meet the demand of consumers.

As an increasingly important vegetable crop in Australia, sweetpotato commodities have an estimated gross value of approximately $80 million AUD (ASPG, 2020). It is one of the few very profitable and rapidly expanding vegetable crops in Australia (Gething et al., 2012). Queensland is the biggest producer of sweetpotato and contributes to more than 70% of the total production, the majority of which is grown in Bundaberg (ASPG, 2020), and supplies all of the major Australian markets, as well as the processing industry (DAF, 2014).

The sweetpotato industry is a significant regional employer, providing work for 4000–5000 people annually (ASPG, 2020). The industry is experiencing remarkable growth, with sales growing by approximately 20% per year since 1998 (Wolfenden et al., 2014). Hence, the crop has state significance and increasing national importance.

Sweetpotato are vegetatively propagated via vine cuttings which are from mature healthy plants (Truong et al., 2011). Australian sweetpotato growers use an annualise storage root (SR)/plant bed system to produce planting materials (Horticulture Innovation Australia, 2015). Cuttings are produced in a seedbed from SRs (Loader et al., 1999) and can be harvested at least four cuts from a bed until sprout appearance and vigour decline (Horticulture Innovation Australia, 2015). Generally, cuttings from 20-50 cm long are used for planting (Horticulture Innovation Australia, 2015). Tip cuttings or back cuttings can be used for planting (Traynor, 2006). Half of cuttings are buried under soil (Traynor, 2006). According to Loader et al. (1999), mineral fertilisers in the mixture of NPK are used to apply for sweetpotato at rate of 100 kg N, 40 kg P and 120kg K ha^{-1} (Loader et al., 1999). Establishment fertilisers including 40% of N, 100% P and 50-60% K of the total fertilisers over the whole cropping cycle can be applied before or after planting up to 10 DAT. Then, supplementary fertilisers are supplied at week 7 or week 8. In addition, organic soil amendments such as poultry manure and mill mud are suggested to apply for sweetpotato (Loader et al., 1999).

In Australia, the introduction of virus-free planting material and associated research on seed bed technology and agronomy has promoted rapid industry expansion during the last decade, with the improvement of yield being 17% per year (Wolfenden et al., 2018). The use of virus-free planting vines or sprouts of optimal length, in combination with best practice planting techniques and agronomy, have improved yields by up to 80% (Coleman et al., 2006). However, the size and shape of the SRs are important for consumer acceptance, and many crop management practices are aimed at optimising the yield of commercially acceptable roots.

1.1.1. Root systems of crops

In most plants, the formation of the primary root is the first sign of seed germination. This type of root starts as the radicle. The first root axis, which develops from cell laid down in the seed, is called the tap root in dicotyledonous plants. If the tap root is damaged, replacement roots that take over the functions of this root are called by the same name. Basal

roots are subsequent root axes that arise from mesocotyl or hypocotyl and shoot-borne roots are roots developing from shoot tissues above-ground (Gregory, 2007) (see Figure 1.1a). In the monocotyledonous plants, the primary root grows for a limited period of time and then growth ceases. This is often associated with the formation of lateral and adventitious roots (ARs) that form a fibrous root system (Aloni et al., 2006). The first root and other roots arising from the scutellar node are called the seminal axes. They are called crown, basal or ARs (Gregory, 2007) (Figure 1.1b).

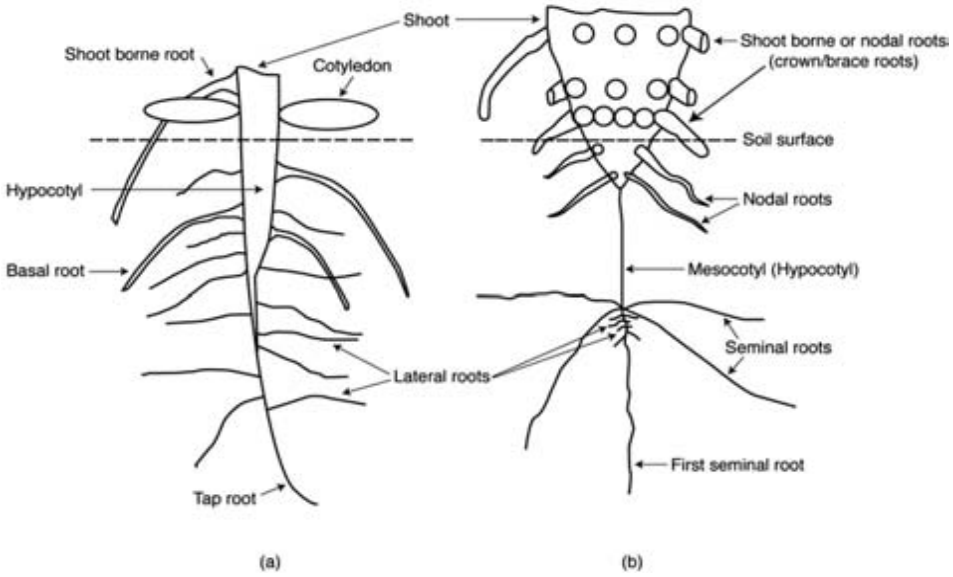

Figure 1.1. Diagrammatic representation of generic (a) dicotyledonous, and (b) monocotyledonous plants. From Gregory (2007).

The root system is a critical part of the crop in terms of function and biomass proportion. The two main functions of root systems of plants are the acquisition of soil-based resources (mainly water and nutrients) and for anchoring the plants (Fitter, 2002). Other functions such as storage, synthesis of growth regulators, reproductions and dispersal could be seen as secondary, although they may also be important. There are two types of roots: (1) roots formed from the embryo and (2) roots formed from consecutive nodes on shoots. The former is referred to the primary and seminal root in maize (Hochholdinger, 2009; Lynch & Brown, 2012) (Figure 1.2), or tap and primary roots in common bean (Lynch & Brown, 2012). The latter usually referred to ARs, such as basal roots in beans, nodal roots in cereals (Lynch & Brown, 2012) and ARs in sweetpotato, potato, cassava and yam (Villordon et al., 2014).

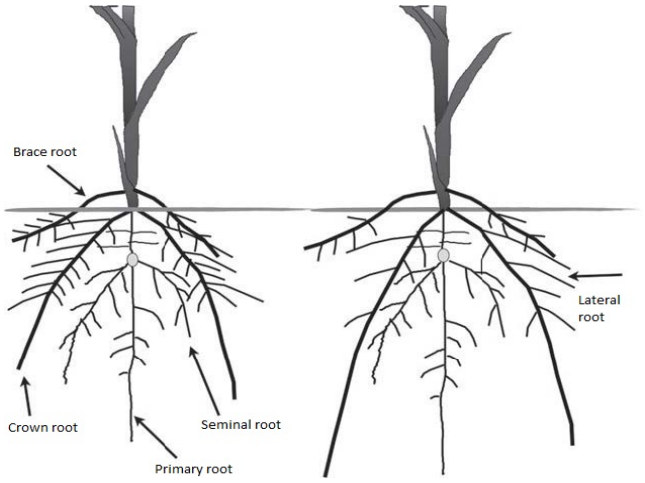

Figure 1.2. Maize root system showing lateral root development. From Lynch and Brown (2012).

Genetic, physiological and environmental factors determine the elongation, distribution, lateral branching and longevity of root systems (Gregory, 2011; Lynch & Brown, 2012). The depth of rooting is different among species even under the same growth conditions (Canadell et al., 1996; Hamblin & Hamblin, 1985). The root systems and their architecture can be affected by a number of intrinsic and extrinsic factors that influence the formation and development of lateral roots (Nibau et al., 2008). Root system architecture is also affected by external environmental conditions such as temperature, soil moisture, nutrition and microbial activities (Liu et al., 2014).

Endorgenous hormones were reported to affect the growth of the root systems by regulating cell division, cell growth and cell differentiation. Auxin controls cell division and cell elongation in roots (Ding & Friml, 2010; Perrot-Rechenmann, 2002). The linkage between lateral root development and auxin is recorded (Casimiro et al., 2003; Reed et al., 1989). Indole-3-acetic acid (IAA) is necessary for lateral root initiation and development (Casimiro et al., 2003) and it also promotes the formation of adventitious roots (Sorin et al., 2005). However, auxin application inhibits cell elongation of root cell layers resulting in root growth inhibition (Perrot-Rechenmann, 2002). Similarly, gibberellin work as regulator in root growth and cell division. In roots, cytokinin determines root meristem size and root growth by controlling meristematic cell differentiation (Perilli et al., 2002).

Root growth and architecture are strongly affected by the plant's nutrition and external available nutrients including N, phosphate and sulfate. In the model root systems of Arabidopsis, N in the form of ammonium (NH_4^+) increased lateral root initiation and branching while N as nitrate (NO_3^-) stimulated the elongation of lateral roots (Lima et al., 2010). The application of N had been reported to increase root length, root surface area, root volume and root fresh weight in rice (Nada et al., 2019). In potato, root architectures such as surface area and root length were reported to associate with the acquisition of N in the form of NO_3^- (Sattelmacher et al., 1990). High level of NO_3^- reduced lateral root number and length in potato (Joshi et al., 2016). In a recent study, the total root length of potato in field conditions was decreased when plants did not receive any N application or excessive N supply (Milagres et al., 2019). Increasing N as NO_3^- application from 0.5 to 12 mM for cassava in pot conditions increased dry mass of absorbing roots and SRs weight (Cruz et al., 2003). Nitrogen application at rate of 50 or 100 kg ha^{-1} increased lateral root length and lateral root number in sweetpotato compared to without N supply (Villordon et al., 2013). A recent study showed that during the formation of SRs, medium N application at rate of 75 mg kg^{-1} dried soil increased root dried weight and root volume (Li et al., 2021).

The relationship of root architecture and yield in vegetatively root and tuber crops was reported rarely in literature. Tuber yield of potato is positively correlated with total root weigh but negatively correlated with basal root length (Wishart et al., 2013). A study on sweetpotato of Villordon et al. (2012) determined the important of lateral root architecture during the SR initiation as it is one of factors that affect the initiation of SRs. In this study, SRs had greater lateral root count, lateral root surface and lateral root length than PRs and lignified roots.

1.1.2. Root structure

1.1.2.1. Primary structure

The anatomy of roots is structurally complex and differs between and within plant species. There are three types of tissue systems in the primary stage of root growth: (1) the epidermis (a specific absorbing tissue containing root hairs) (Bibikova & Gilroy, 2002); (2) the cortex that usually occupies the largest volume of most roots and consists of vacuolated parenchyma cells (Gregory, 2007); and (3) the vascular tissue systems (forms a central cylinder or hollow cylinder around a central pith) (Enyi, 1977). These three tissue systems

form numerous cell types such as meristem, xylem, phloem and many others that are visible in transverse and longitudinal sections (see Figure 1.3).

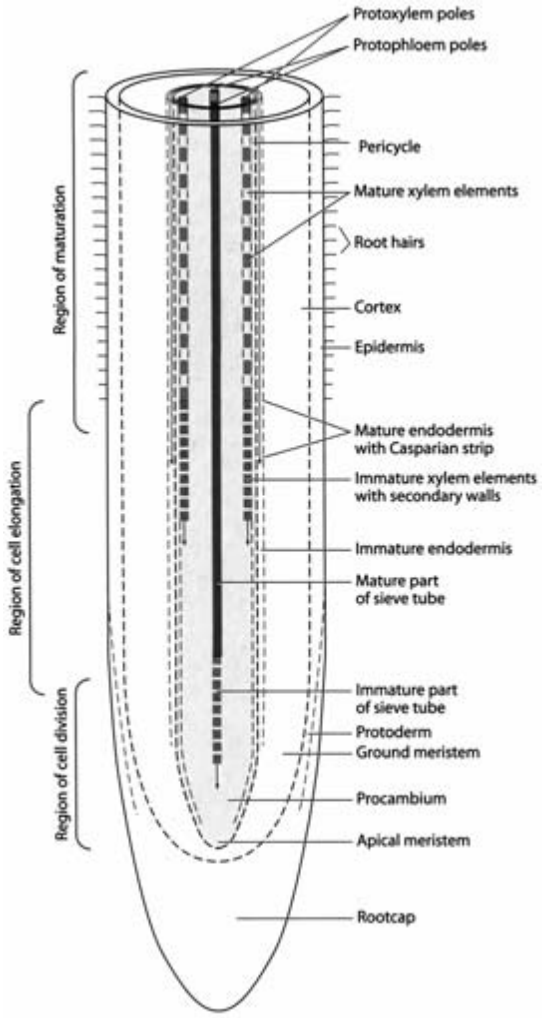

Figure 1.3. Diagrammatic representation of the early stages of primary development of a root. From Gregory, 2007.

The epidermis is the outer layer of roots and is usually composed of thin-walled, elongated cells (Wieckowski & Schiefelbein, 2013). It is also where the water and minerals enter through mass flow, root interception and diffusion. The root hair cells and non-hair cells compose the root epidermis in most vascular plants (Dolan, 1996). The epidermal cells that produce root hairs are called trichoblasts, whereas the epidermal cells without capacity to generate root hairs are called atrichoblasts. There are three types of epidermal patterns in plants (Dolan, 1996): (1) all epidermal cells can produce root hairs (found in some monocotyledons, nearly all dicotyledons and most ferns) (Cormack, 1935; Dolan & Costa, 2001); (2) two types of cells at different lengths compose the epidermis and only the shorter

cells can produce root hairs (common in the oldest land plants such as Lycopsida, Equisetum or Selaginella, in some monocotyledonous species and in individual families of dicotyledonous plants) (Bibikova & Gilroy, 2002; Dolan, 1996); and (3) a striped pattern where files of root hair and non-hair cells are present (found in (Dolan & Costa, 2001; Pemberton et al., 2001).

The cortex, which lies between the epidermis and vascular tissues, is the predominant part of the primary root and comprises primarily parenchyma cells (Gregory, 2007). In monocotyledonous roots, the cortex may exist during the whole life of the roots. However, in dicotyledonous roots with significant secondary thickening it is gradually replaced by the periderm. In some secondary thickening roots, the cortex may persist for a long time and undergo secondary development (Lux et al., 2004). The cortical cells have a large number of interconnections through the cell walls and plasmodesmata that connect the protoplasm of each cell (Roberts et al., 2003). The cortex is composed of three layers that are the hypodermis (exodermis), the endodermis and the storage parenchyma (mesodermis) in between (Lux et al., 2004). The hypodermis and endodermis form the outermost and innermost layers of the cortex, respectively (Peterson, 1989). They are both derived from the ground meristem and characterised by the formation of Casparian bands in anticlinal walls of their cells (Ma & Peterson, 2003). The mid-cortex layers consist of thin-walled, living parenchyma cells, which store starch and other substances (Lux et al., 2004).

The vascular tissue and one or more layers of non-vascular tissue, the pericycle, which surrounds the vascular tissue, comprise the vascular cylinder (stele) (Gregory, 2007). In most dicotyledonous roots, the central vascular cylinder forms a core of primary xylem (Esau, 1977). There are strands of primary phloem between ridges. In many monocotyledons, when the xylem does not show differences in the centre of roots, a pith of parenchyma or sclerenchyma that is parenchyma with secondary walls is present. The number of xylem ridges differs between species and among species (McCully, 1999). The first mature xylem elements are those next to the pericycle, then those closer to the centre with the typically wider metaxylem elements. Phloem presents a centripetal order of protoxylem nearest the pericycle and metaphloem nearer the centre (Gregory, 2007).

1.1.2.2. Secondary structure

Secondary growth of roots is usually absent from most monocots. However, this system is characteristic of gymnosperms and of most dicots. It consists of the formation of

secondary vascular tissues and periderm that derives from cork tissue. This results in the thickening of roots by adding vascular tissues. The secondary vascular tissues divide and expand in the radical direction (Esau, 1977). The secondary growth of roots is driven by the cambium, which consists of two morphologically distinct cell types: fusiform initials and ray initials. The fusiform initials produce all longitudinally oriented cells, including tracheids, vessel elements, fibres, axial parenchyma cells, sieve elements and companion cells, and the ray initials give rise to all radially oriented cells. They are both thin-walled, highly vacuolated cells (Chaffey, 2002).

The secondary growth of roots begins when procambial cells that remain undifferentiated between primary xylem and primary phloem, divide parallelly into the vascular cambium, including secondary xylem and secondary phloem (Chaffey, 2002). Two or more regions of cambial activity will be initiated depending on the number of xylem and phloem groups presenting in roots. Cambium quickly surrounds the core of xylem due to the dividing of pericycle cells opposite the protoxylem elements. The vascular cambium opposite the phloem strands produces secondary xylem inwards, so the strands of primary phloem are moved outwards. Simultaneously, the cambium opposite the protoxylem is active and dividing, resulting in circular cambium and separating primary phloem and xylem (Gregory, 2007) (Figure 1.4).

Periderm is a protective layer derived from cork cambium after the formation of secondary xylem and phloem. Pericycle cell division increases the number of pericycle cell layers (Esau, 1977; Gregory, 2007). There are three types of tissues constituting the periderm of roots: cork cambium, cork and phelloderm. Cork cambium is formed to produce a layer of cork on the outer surface and phelloderm is formed towards its inner surface (Gregory, 2007).

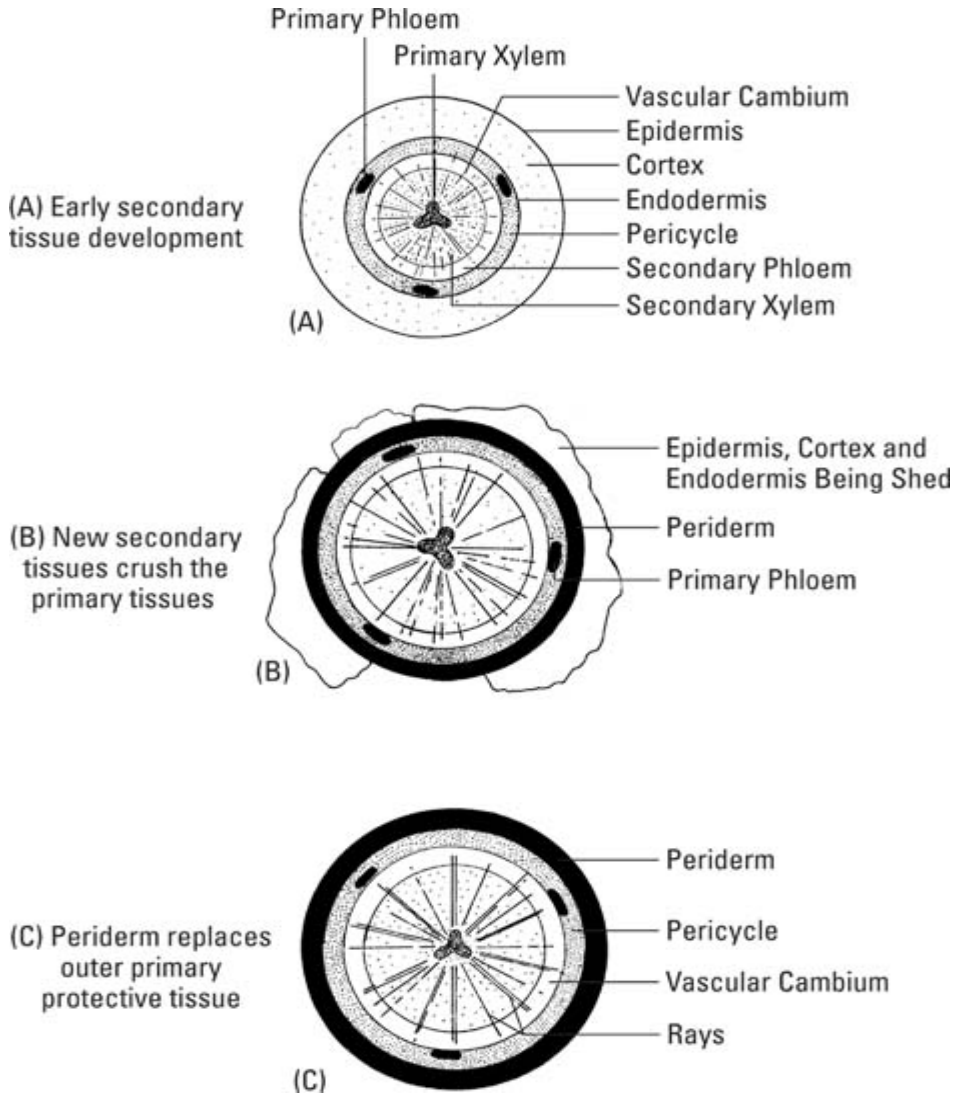

Figure 1.4. Diagram of stages in secondary growth of dicotyledonous roots. From Cliffsnotes (n.d.).

1.1.3. Root systems of sweetpotato

Based on the origin, the root system of sweetpotato is divided into: (1) ARs developed from the underground nodes of a vine cutting after transplanting, and (2) lateral roots arising from existing roots. The ARs are generated from undamaged preformed root primordia located on the nodes and the cut ends (callus or wound roots) of the stem; they then grow rapidly and develop into fibrous roots and some further develop into storage roots (SRs) (Belehu et al., 2004). Hence, the number of preformed root primordia per node determines the number of ARs. The ARs of sweetpotato are subdivided into three different types of roots, being primary fibrous roots, pencil roots (PRs) and SRs (Figure 1.5), whereas the

lateral roots are subdivided into primary, secondary and tertiary roots (Kays, 1985). The fibrous roots absorb nutrients, water and anchor plants, whereas the SRs store photosynthetic products (Huaman, 1999).

Based on the external morphology, the sweetpotato ARs may be separated into 'thick' and 'thin' roots. While the thick roots arise from the nodal region, the others develop from the internodal area of the underground stems (Kays, 1985). Togari (1950) indicated that young thick roots would develop into SRs in a conducive environment or become primary fibrous roots in unfavourable conditions, such as with high nitrogen (N) or low soil oxygen. Young thin ARs would develop into either primary fibrous roots or PRs (Togari, 1950). Adventitious roots can be subdivided into thick pigmented SRs which would develop to SRs, thick pigmented PRs that do not form SR and thin white fibrous roots (Lowe & Wilson, 1974b). All three types of roots give rise to lateral fibrous roots.

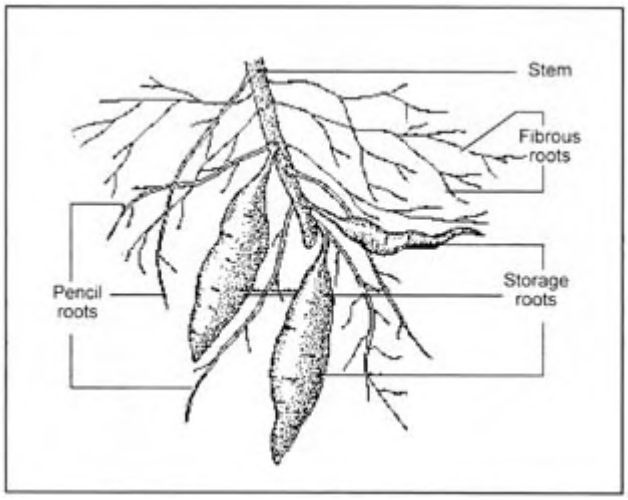

Figure 1.5. Types of roots in sweetpotato plant. From Huaman, 1999.

1.1.3.1. Fibrous roots

Fibrous roots are interpreted as those roots that are generally less than 5 mm in diameter and that originated from thin ARs (Chua & Kays, 1981; Togari, 1950). Roots that have a tetrarch arrangement of primary vascular tissues or those from the internodal region of a subterranean vine stem cutting are likely to be fibrous roots (Truong et al., 2011). They may arise from thick roots, which are pentarch, hexarch or septarch in the arrangement of their

primary vascular tissue under adverse conditions. The fibrous roots and lateral roots are branched to form a dense network to take up water or nutrients (Belehu et al., 2004).

1.1.3.2. Pencil roots

Pencil roots are thicker than fibrous roots but thinner than SRs. These roots show heavy lignification with xylem rays, a broad secondary cortex and limited secondary phloem. They are normally between 5 and 15 mm in diameter (Kays, 1985; Togari, 1950; Wilson & Lowe, 1973) and develop mainly from young thick ARs under conditions that are not conducive for the development of SRs (Belehu et al., 2004). According to Wilson and Lowe (1973), during the early-stage development of PRs, there are no external differences between fibrous roots and PRs. The restricted activities of the vascular cambium in thick ARs produce a heavily lignified stele, resulting in the failure of tuberous development (Wilson & Lowe, 1973). This leads to uncompleted lignification and the roots remain unthickened. As these roots are thin and elongated, they do not meet market needs (Wolfenden et al., 2014). Hence, the high number of PRs reduces marketable yield of crop and are not favoured by growers.

1.1.3.3. Storage roots

The sweetpotato SRs develop from the underground vine (stem) cuttings that are used as the crop planting materials. Nearly 90% of sweetpotato SRs are from the ARs that are initiated from the root primordia in the stem within the first week after transplanting (Villordon et al., 2009c). Adventitious roots originating from the damaged preformed root primordia cannot develop into SRs, so the number of undamaged, preformed root primordia determines the number of SRs per node (Belehu et al., 2004). Thus, the number of ARs that will be induced to form SRs largely determines the yield of sweetpotato.

The SRs are generally more than 15 mm in diameter and develop from thick ARs (Kays, 1985; Togari, 1950). In these roots, there is no lignification of the cells between the protoxylem poles and the central metaxylem (Togari, 1950) or limited lignified cells (LC) (Artschwager, 1924; Wilson & Lowe, 1973). The increase in the number and size of cells contributes to the growth of SRs, whilst the accumulation of photosynthates contributes to SR weight (Wilson, 1977).

Agronomically, the most important organ of sweetpotato is its tuberous SR, which is used as a staple food source or as animal feed (Posas, 1989). The yield of sweetpotato is determined by the number of plants per hectare, the number of SRs per plant, and the shape

and size of each SR at harvest. The density of plant in the field is well manipulated by commercial practices (Meyers et al., 2014). Hence, the formation and growth of SRs are key factors that affect the yield. The number of SRs produced per plant determines both the yield and quality of this crop (Makunde et al., 2017). The wide variability of SR yield between individual plants of the same variety or among cultivars has been documented (Lowe & Wilson, 1974b; Togari, 1950). The shape and size of each SR at harvest determine the marketable yield of the crop. Therefore, increasing the number of SRs of a certain shape and size is a critical method to maximise marketable yield of the crop (Meyers et al., 2014).

1.1.3.4. Lateral roots

The lateral roots of sweetpotato develop on all ARs (Kays, 1985). Some of them emerge from the base of damaged preformed root primordia (Belehu et al., 2004). The primary lateral roots arise from the ARs while the secondary laterals develop from the primary, and the tertiary laterals are from the secondary laterals. They will develop to form the fibrous root system to absorb water and nutrients.

1.1.4. The anatomical structure of young adventitious sweetpotato roots

In transverse sections of young roots, there are several groups of vascular tissues, separated from the cortex by an endodermis. The vascular tissues are arranged radially with the xylem and phloem strands intervening (Artschwager, 1924). The number of protoxylem points varies between five and ten (Villordon et al., 2009c; Wilson & Lowe, 1973). The arrangement of the central cylinder in some roots is tetrarch or higher polyarchy, while a minority of roots are fasciated roots that have double steles or a sinuous band of xylem (Wilson & Lowe, 1973) (Figure 1.6A, 1.6B and 1.6C). Around 80–90% of the total numbers of roots were pentarch or hexarch (Villordon et al., 2009c). The development of protoxylem elements is completely centripetal, resulting in connection to central metaxylem cells (Figure 1.6D). Partial centripetal development of primary xylem elements is often observed, with one protoxylem element connected to central cells and the others separated by parenchymatous cells (Wilson & Lowe, 1973).

Figure 1.6. Transverse sections of sweetpotato roots at 10 DAT. (A) Section with tetrarch steles; (B) Section with septarch steles and central metaxylem; (C) Section with hexarch stele without central metaxylem; and (D) Section with hexarch steles and some protoxylem elements connected to the central metaxylem.

The development of protoxylem in some roots is incomplete. This results in a central pith of thin-walled parenchymatous cells containing a central metaxylem (Wilson & Lowe, 1973). Whereas the outer tracheary elements at each pole show the representation of protoxylem, the inner metaxylem shows the representation of two or three large metaxylem elements in the central part of the vascular cylinder (Villordon et al., 2009c). The primary xylem elements are sometimes connected together to be a continuous cylinder surrounding the parenchymatous pith (Wilson & Lowe, 1973). The protophloem lies between the protoxylem strands and within the youngest circle of protoxylem element. A parenchymatous sheath intervenes between phloem and xylem. A single layer of pericycle separates xylem and phloem from the endodermis (Artschwager, 1924).

The outer layer of roots, which consists of thin-walled elongated cells, is the epidermis. In sweetpotato, the root epidermis that is later replaced by a periderm covers the peripheral cells. A broad band of tissues composed of large cells with conspicuous intercellular spaces is formed from root cortex (Artschwager, 1924).

1.1.5. The initiation and development of sweetpotato storage roots—a review

Storage root initiation is the process by which ARs differentiate into SRs under proper conditions. This can be observed by root anatomical investigation as early as 13 days after planting (DAT) (Arnold et al., 2013). The formation and development of SRs is a complex process relating to various steps, including the cessation of root elongation, increase of radial growth, formation of primary and secondary vascular cambia, initiation and development of anomalous and interstitial cambia, and accumulation of starch as well as protein (Desai, 2008; Ravi & Indira, 1999). Adventitious roots occur during the first week after transplanting, playing an important role for the initiation and development of SRs. A study in American varieties showed that nearly 90% of SRs at 60–65 DAT were traced from ARs developed during 5–7 DAT (Villordon et al., 2009c).

1.1.5.1. Anatomical development of adventitious roots into storage roots

1.1.5.1.1. The development of primary cambia in adventitious roots

Anatomically, the ARs first initiate the primary cambia within the parenchymatous region, separating the protoxylem from the protophloem (Wilson & Lowe, 1973). These cambium cells are connected to form a complete cylinder through the division of the single layered pericycle. At first, the outline of this cylinder is irregular due to the radial arrangement of the vascular tissues and the increase of cells in the pericycle opposite the phloem points. After that, the cambia produce more cells rapidly in the inner region that contributes to the development of the regular cylinder (Artschwager, 1924). Villordon et al. (2009c) stated that the meristematic activity of the vascular cambium led to the formation of the secondary xylem and phloem and gradually a regular vascular cambium (RVC) (cambial ring).

1.1.5.1.2. The development of secondary cambia in adventitious roots

The secondary cambia are formed around the protoxylem elements and central cells (Artschwager, 1924; Wilson & Lowe, 1973). In roots with the central metaxylem cell, the

differentiation of vascular cambia is accompanied by the formation of primary anomalous cambium (AC) around the central metaxylem and protoxylem elements. In roots without central metaxylem cells, the formation of primary cambia is related to meristematic activity in the pith cells and the initiation of primary AC around the protoxylem elements (Wilson & Lowe, 1973). The AC developing around the central cell or primary xylem elements is called interstitial or lateral cambia, respectively (Togari, 1950).

The appearance of secondary AC is the first signal of the formation of SRs (Villordon et al., 2009c; Wilson & Lowe, 1973). There is no external morphological feature to indicate that SR formation has taken place (Wilson & Lowe, 1973). The formation of AC is in various tissue types. They develop around the central cell and primary xylem elements, within xylem derived from vascular cambium or primary AC. Sometimes, they even form independent vascular groups (Artschwager, 1924; Wilson & Lowe, 1973). Activities of all cambia result in the formation of thin-walled, starch-storing parenchyma cells (Wilson & Lowe, 1973). Physiologically, the sign of SR initiation is the accumulation of photosynthates (predominantly starch) (Chua & Kays, 1982).

1.1.5.1.3. Anatomical development of storage roots and pencil roots

Anatomical structures of roots associated to the formation and development of SRs are described in some studies. The SRs usually develop from the young, thick, primary roots that have pentarch or hexarch steles and enlarged apical meristem (Togari, 1950) or from ARs with a pith and centrally located metaxylem element (Artschwager, 1924). The presence or absence of the central metaxylem element of sweetpotato roots is due to the variation in the differentiation of primary cells (Wilson & Lowe, 1973). The thick ARs with no or slight lignified development of cells between the protoxylem points and the central metaxylem can form SRs (Kays, 1985). Young, thin ARs with a tetrarch stele and a central core of metaxylem elements without central pith (Artschwager, 1924) or a small apical meristem (Togari, 1950) cannot develop into SRs. Uniform, thickened roots with completed lignification of the stele were unable to form SRs (Togari, 1950). An AR, with a typical septarch stele without the central metaxylem elements, had the potential to develop into SRs (Belehu et al., 2004). Togari (1950) documented that the lignification prevents the development of the vascular cambium, so the balance between LC and cambial activity determined the formation of SRs. This can be used to explain why roots cannot become thickened if the cells between the protoxylem elements and the central cell become lignified in the early differentiation processes (Artschwager, 1924).

In some thickened roots (up to 5 mm in diameter), the activity of cambia leads to the lignification of steles and abnormal activity of anomalous cambium (AC) leads to the production of storage parenchyma (Wilson & Lowe, 1973). Such roots have no potential to be SRs and will develop into fibrous roots with completed centripetal development of protoxylem elements without central pith (Togari, 1950).

The growth of PRs was described by Wilson and Lowe (1973). Early meristematic activity associated with SR initiation in some roots resulted in uniform thickening of the entire root. Meristematic activity and expansion of cells in the stellar parenchyma contributed to the activity of the vascular cambium in these roots. Sometimes, cambia formed around primary xylem points and meristematic activity took place in cells around the central metaxylem. This completely separated protoxylem elements from the central cell. In some roots, one or more protoxylem elements often connected to the metaxylem cell by a strand of LC. The activity of the vascular cambia in such roots was limited, leading to production of a heavily lignified pencil root.

1.1.5.2. The origin and initiation of storage roots

Most sweetpotato SRs are normally initiated from the ARs that undergo sudden changes in their growth patterns (Firon et al., 2009). Only a small proportion of SRs develop from lateral roots, which arise from existing roots (Kays, 1985). The initial sign in the growth of the SRs is the continued activity of the vascular cambium and anomalous primary and secondary cambia to form thin-walled, starch storing parenchymatous cells (Villordon et al., 2009c; Wilson & Lowe, 1973). The activity of those cambia results in localised swelling of the root, which is the first externally visible sign of SR development. The contribution of vascular cambium and AC in producing storage parenchyma varies among cultivars (Wilson & Lowe, 1973). During the SR growth, none, or only a small proportion, of the cells between the protoxylem points and the central metaxylem become lignified (Belehu et al., 2004). The important feature of SR development is differentiation of storage parenchyma from the vascular cambium (Wilson & Lowe, 1973).

The reports about the time when SR initiation starts are inconsistent. Ravi and Indira (1999) reviewed and stated that the formation of sweetpotato SRs varies in cultivars between 7 and 91 DAT. Some research reported that SRs formed from 35 to 60 DAT (Agata, 1982; Enyi, 1977; Wilson, 1982), whilst another stated that SR formation occurred within 7–21

DAT (Du Plooy, 1990). Recently, a study indicated that the formation of SRs started from 19–21 DAT (Villordon et al., 2009c).

1.1.5.3. The effects of agronomical and environmental factors on storage root initiation and development

Agronomical factors were demonstrated to influence the initiation and development of SRs. The effect of different soil moisture levels on the initiation and development of SRs were evaluated in the literature. The response of sweetpotato to soil moisture was different dependent on the variety (Gajanayake et al., 2013). They suggested that the optimal soil moistures for the formation of SRs were 63% and 75% field capacity for cultivars Beauregard and Evangeline, respectively. Other studies showed that the optimal moisture for root development of Atacama and Mafutha 80% field capacity (Belehu & Hammes, 2004; Bok, 1998). Soil moisture below 40% field capacity suppressed root development (Belehu & Hammes, 2004). Gajanayake and Reddy (2013) suggested that the number of SRs, time to total 50% SR formation (using cross section to determine) and maximum SR number were affected by moisture regimes. They also revealed that the formation of SRs was delayed under drought conditions.

In addition, the relationships of temperature to root formation and growth were also evaluated. Both air and soil temperature control the development of shoots and SRs (Ravi et al., 2009). A study recorded that the optimum root growth for Atacama cultivar was achieved when plants were exposed to air temperature at 24°C (Belehu & Hammes, 2004). The maximum rate of SR formation (the rate of SRs and ARs) for Beauregard was reached at 29.5°C, whereas the optimum temperature for maximum number of SRs was 25.3°C (Gajanayake et al., 2014b). They also indicated that growth temperature above 26.5°C had a detrimental effect on the growth of SRs. By investigating the effect of temperature during mid and late-season, Gajanayake et al. (2015) revealed that the optimal temperature for SR development, which finally resulted in highest yield, was 25.6°C. In contrast, higher temperatures promoted the growth of above-ground parts, leading to a reduction in yield of SRs (Gajanayake et al., 2015b). The optimal night temperature for sweetpotato SR initiation and development is 15–25°C (Truong et al., 2011). Night air temperature above 25°C (Chatterjee & Mandal, 1976; Nakatani, 1988) and below 15°C (Ngeve et al., 1992) suppresses the formation of SRs.

The effect of light on sweetpotato storage roots was investigated. A study for three cultivars, including TI-155, GA120 and Georgia Jet, showed that plants under continuous light produced more SRs than those with less than 12 hours of light (Bonsi et al., 1992). Long light periods (14 hours) increased SR yield of sweetpotato (Porter, 1979). Plants under natural days (11.5–12.5 hours) produced greater SR yield than those given short days (8 hours) or long days (18 hours) (McDavid & Alamu, 1980). By contrast, shorter light periods (9 hours a day) increased the number of SRs compared to longer periods (16 hours) (Biswas et al., 1989).

Fertilisers were determined to influence sweetpotato SRs. Potassium (K) had a positive impact on SR quality and yield of sweetpotato (El-Baky et al., 2010; Fujise & Tsuno, 1967: Liu et al., 2013). El-Baky et al. (2010) found that the increasing of K application levels significantly increased the SR dried biomass. Many studied showed that the optimum rate of K for sweetpotato was around 150 kg ha^{-1} (Njoku et al., 2001; Uwah et al., 2013; Wang et al., 2020). However, sweetpotato required up to 300 kg K ha^{-1} in poor soil (Jian-wei et al., 2001). The application of K increased the SR yield by increasing the number of SRs and the average SR weight, so K application played somewhat in the formation of SRs (Wang et al., 2020).

Sweetpotato has been reported to require low level of phosphorus (P) (Djazli & Tadano, 1990). However, the response of sweetpotato cultivars to phosphorus (P) was variable. Rashid and Waithaka (1985) reported that all P levels from 0 to 350 kg ha^{-1} did not have any significant effects on the growth of vine or SR yield of both sweetpotato cultivars, Musinya and Gikanda. High level of P in soil was reported to supress SR development (FAO, 1994). In a recent study, P application in the form of super phosphate or crystallizer phosphate at the rate of 500 kg ha^{-1} reduced the yield of sweetpotato (Kareem et al., 2020). However, most of the research revealed that P significantly increased the total and marketable yield of the crop (Abd-El-Fattah & Abd-El-Hamed, 1997; El-Sayed et al., 2011; Montanez et al., 1996). Other fertilisers such as N were also investigated in this crop (Arnold et al., 2013; Fichtner & Schulze, 1992; Schultheis et al., 1995; Villagarcia et al., 1998).

1.1.6. Storage root yields as affected by nitrogen fertilisation

Nitrogen is an essential element in structural proteins, enzymes, chlorophyll, nucleic acids and other organic compounds, all of which are necessary for structural and metabolic functions of plants, and for photosynthesis in particular (Deckard et al., 1984; Morot-Gaudry, 2001). The importance of N for plant growth and productivity of crops has

been recorded (Atkinson et al., 2010, Hosseini-Bai et al., 2012). Nitrogen influences critical physiological processes such as photosynthesis and the activity of cambium that are related to the yield and quality of SRs (Hall & Rao, 1999). In photosynthesis, N has a crucial role in the structure of photosynthetic pigments and enzymes (Hall & Rao, 1999). Nitrogen deficiency leads to adverse effects on plant photosynthesis, resulting in reduction of yield (Reich et al., 1998). Nitrogen affects the formation of SRs of sweetpotato. Excessive inorganic N supply (NO_3^-) for the crop was likely to decrease the activity of cambium and increase lignification of root tissues (Togari, 1950). This could induce ARs developing into non-storage roots.

There are some studies addressing the effects of N rates on yield of sweetpotato. Research on this topic addressed the effect of N on yield of sweetpotato and created a wide range of inconsistent and contradictory results (Phillips et al., 2005). Some studies stated that N application did not affect the yield of crops (Guertal & Kemble, 1997; Schultheis et al., 1995). However, others indicated that the application of N resulted in the increase of SR yield of sweetpotato, with the magnitude of the yield rise varying in different research. A study in Cuba showed that the application of 70 kg N ha^{-1} significantly raised the SR yield of 90-510 cultivar by nearly 20% (Frahm et al.). Another study in America revealed that the yield could increase by more than 70%, with the application of N at 120 kg ha^{-1} or 240 kg ha^{-1} for MD810 and Jemel cultivars, respectively (Villagarcia, 1996). One study showed that the optimum N rate for Beauregard variety in Virginia, USA, was 28 kg ha^{-1} under normal precipitation and 56 kg ha^{-1} under wet conditions (Phillips et al., 2005). Further research in Louisiana on the same cultivar revealed that the optimal N rate under normal conditions was 50 kg ha^{-1} (Mulkey et al., 1993). This indicates that the optimal N rate for this crop depends on multiple factors such as cultivar, soil quality and climate conditions.

A number of fertiliser experiments have been conducted to examine the influence of N application timing on the yield components, including total yield and marketable yield of sweetpotato. Nitrogen timing affected root size distribution of the crop (Phillips et al., 2005). The application of N at 2–3 weeks after transplanting increased the marketable yields; N application at 4–5 weeks after transplanting reduced the percentage of US #1 grade roots (premium grade) and increased that of canner roots (SRs with diameter from 1-1.75 inches). Using the same rate of N application, a single N application yielded higher marketable roots than split applications (Ankumah et al., 2003; Schultheis et al., 1995), although split N application might increase the use efficiency of N (Guertal & Kemble, 1997). In a study by

Villordon and Franklin (2007), the split of N applications (before planting and 28 DAT) resulted in the increase of yield as well as marketable yield of Beauregard cultivar. By contrast, the application of N for sweetpotato after 28 DAT reduced the yield of the crop.

The effects of different N sources on sweetpotato were variable. A trial in a sandy loam soil indicated that N in the forms of urea ($CO(NH_2)_2$ and ammonium nitrate (NH_4NO_3) did not affect the yield of sweetpotato (Ankumah et al., 2003). However, three sources of N, ammonium nitrate ($NH_4NO_{3)}$ calcium nitrate ($Ca(NO_3)_2$) and sodium nitrate ($NaNO_3$), showed different results when used on Jewel cultivars. Sweetpotato supplied with sodium nitrate produced a lower yield than that of NH_4NO_3 and $Ca(NO_3)_2$ (Hammett & Miller, 1982). A recent study indicated that application of NH_4^+ significantly increased the number of ARs and SRs, and produced the highest SR yield (Makunde et al., 2017).

Several studies have found that N supply affected the development of vegetative and storage organs in plants (Hammett et al., 1984; Kays, 1985; Rufty et al., 1988). They showed that the response of root growth to N supply depended on genotype and developmental stages. Knavel (1971) concluded that there was a positive correlation between N fertilisation and growth of vine. He showed that the components of SR yield such as root length and size during the first two months of growth were affected by N application. Moderate N deficiency reduced shoot growth and enhanced root development (Fichtner & Schulze, 1992). However, a high level of N stimulated top growth and inhibited root initiation of sweetpotato (Acock & Garner, 1984; Hartemink et al., 2000; Villagarcia et al., 1998). Hence, sweetpotato requires a certain amount of N (the concentration of N in leaves is no less than 2.2%) to promote the growth of shoots and photosynthesis, which provides photo-assimilates for SR development (Kays, 1985).

There was little information about the influence of N fertiliser on root initiation and development of sweetpotato. Results from these studies were inconsistent. Field research of Beauregard cultivar showed that the increase of N fertiliser in the form of granular urea from 50 to 100 kg ha^{-1} increased the number of ARs by 65% (Villordon et al., 2013). Villagarcia et al. (1998) concluded that the high level of nitrate (NO_3^-) at 30 DAT reduced root initiation and diverted sugars to top growth. Hence, the application of N at a high rate may maintain top growth during SR formation and this may lead to irreversible lignification. The switching from a high rate of N to a low rate promoted SR development (Villagarcia et al., 1998).

1.1.7. Applications of soil amendments in agriculture – an overview

Soil amendments are materials that can be added to the soil to improve physical and chemical properties of soil (Davis & Wilson, 2000). This aims to provide a better environment for the growth of roots and supply nutrients for plants. Soil amendments are classified into two categories: organic and inorganic amendments. Organic amendments originate from biomass whereas inorganic amendments are mined or synthetic.

There are several types of organic soil amendments that are often used to apply to crops, including green manure, animal manure, compost and, more recently, biochar. The properties of these amendments are influenced by feedstock sources and other factors (Singh et al., 2010). For example, pH values can range from nearly neutral to highly alkaline. Organic amendments may contain plant nutrients and act as organic fertilisers (Davis & Wilson, 2000). Organic residues are derived from animal or plants and are considered as slow-release fertiliser (Avnimelech, 1986). Nutrients from these substances are released from the decomposition process by microbial activities. This process depends on organic materials and the soil environment (Avnimelech, 1986). They are also a crucial source of organic matter in soil (Davis & Wilson, 2000), and this amends soil structure to create places accommodating water, air and soil organisms.

Application of soil amendments to soil has several advantages. For plants, organic soil amendments are organic sources of N, P and K (Hue & Silva, 2000). They can release mineral nutrients immediately after application (chicken manure) or several months to years after application (wood products). For soil, the use of organic amendments has been shown to improve the physical and chemical properties of soil (Bulluck Lii et al., 2002; Tejada et al., 2006), or soil biological activities and crop viability (Hulugalle et al., 1986; Nakamura et al., 2007). The changes in soil properties were consistent with the amendments used in the research, which were usually alkaline and had high carbon (C) content. Soil amendments have various ways to improve soil properties. They can increase soil organic matter content, which improves soil compaction and water infiltration, as well as increases nutrient holding capacity (Bulluck Lii et al., 2002; Davis & Wilson, 2000).

More importantly, organic matter provides critical food and energy sources for fungi, bacteria and soil fauna (Davis & Wilson, 2000). Compost application can benefit soil by decreasing the rate of infiltration and buffering soil pH changes to prevent acidification (Stamatiadis et al., 1999). In other research, repeated compost applications

increased water content, reduced bulk density and changed pH at greater depths (Tester, 1990). Other forms of organic amendments have also proved to improve physical and chemical soil properties (Bulluck Iii et al., 2002; Chan et al, 2008; Van Zwieten et al., 2010). Charcoal had positive effects on soil properties by increasing pH values, total N and available phosphorus pentoxide contents, and cation exchangeable capacity, and reducing the contents of exchangeable aluminium (Yamato et al., 2006). In addition, organic waste improved properties of saline soil by increasing soil structural stability and reducing the percentage of exchangeable sodium (Tejada et al., 2006).

The application of soil amendments has been demonstrated to improve crop productivity. Soil amendments increased yield of many crops such as radish, tomato, peanut, sweetpotato and corn (Agyarko et al., 2013; Bulluck Lii et al., 2002; Chan et al., 2008; Yamato et al., 2006). The application of organic soil amendments could improve crop productivity by its influence on photosynthesis (Wang et al., 2014) or by enhancing soil water storage (Streubel et al., 2011). The dry matter yield of radish increased 42–96% dependent on the type of amendments (Chan et al., 2007). Biochar application doubled or tripled the pod yield of peanut in ferrosol and redoxi-hydrosol soil (Xu et al., 2015). By contrast, soil amendment may not affect the yield of crops in when the level of nutrients in amendments is low, or the amendment is carbon-based in N deficiency condition (e.g. sugarcane residual, wood-made biochar). Although wood vinegar showed a trend to increase the yield component of soybean, the difference of crop yield from the control was insignificant (Pangnakorn et al., 2009). Without N application, green waste biochar did not improve the yield of radish (Nakamura et al., 2007).

In addition, the influences of soil amendments on microbial community and plant health have been widely observed. This led to increased recycling of nutrients trapped in biomass residue (Steinbeiss et al., 2009). The number of beneficial micro-organisms in soil was affected positively by applying compost waste, cattle manure and yeast-derived biochar (Bulluck Lii et al., 2002; Steinbeiss et al., 2009). Organic amendments such as manure and compost that are rich in N may reduce soil-borne diseases (Bailey & Lazarovits, 2003). This is due to subsequent microbial decomposition or the release of allelochemicals generated during the amendment product storage (Bailey & Lazarovits, 2003).

Different forms of organic soil amendments have been applied for many crops such as soybean, peanut, melon, tomato, radish, corn, and cowpea (Bulluck Lii et al., 2002; Chan et al., 2007; Yamato et al., 2006). The effect of organic soil amendments on root and tuber

crops has not received much research attention. There are few studies that examine the influence of these products on such crops. Poultry manure at the rate of 10 tons ha^{-1} was shown to improve the yields of cassava by almost 30% (Ojeniyi et al., 2012). Farmyard manure, biochar from cassava stem and biochar from farmyard manure increased cassava yield by 15% in a cassava-peanut intercropping system (Islami et al., 2011). The combination of poultry manure with NPK fertiliser increased the yield of cassava by 66–133% (Ojeniyi et al., 2012). A long term study on potato revealed that the average total yield of potato tuber over seven growing seasons increased by 27% in treatments either with compost at 16 tons ha^{-1} or manure at 12 tons ha^{-1} (Kimpinski et al., 2003). Biochar application had no or negative impacts on the growth and yield of potato (Koga et al., 2017; Liu et al., 2016).

1.1.8. The potential influences of soil amendment on storage root initiation and development of sweetpotato

To date there have been limited studies on the influence of organic soil amendments on sweetpotato in general. Most of them examined the effects of these amendments on the yield of this crop. The application of biochar, manure, green leaf manure and vermicompost had a positive effect on sweetpotato yield (Agyarko et al., 2013; Nedunchezhiyan et al., 2010; Walter & Rao, 2015; Yeng et al., 2012). There were only a few studies that evaluated the impacts of these substances on root development, SR components and other aspects of the crop. Therefore, more work is needed to explain mechanisms that related to SR initiation and yield of sweetpotato.

The application of organic amendments for sweetpotato in sandy soil had a significant improvement on the total SR and total marketable SR yield (Siose et al., 2018). Their results showed that the marketable yield increased by around 120% to nearly 300% over the control. Organic amendments also increased the marketable SR number in this experiment. Higher sweetpotato SR number is indicative of a favourable response to the application of organic amendments. In earlier research work, the SR number and yield of sweetpotato was also found to be influenced positively by poultry manure (Sowley et al., 2015).

By contrast, some research indicated that organic soil amendments had no or negative effects on sweetpotato. Poultry manure, cow manure and farmyard manure had no significant effect on SR number and SR yield of sweetpotato compared to inorganic fertiliser (Adeyeye

et al., 2016). In another study, high rates of chicken manure at 40 to 60 tons ha^{-1} reduced the yield of sweetpotato (Yeng et al., 2012).

Organic amendments improved sugar content and the appearance quality of sweetpotato (Dou et al., 2012). The combination of organic amendments and NPK fertilisers produced higher tuber length, diameter and marketable fresh SR yield compared to the sole application of these substances (Agyarko et al., 2013; Walter & Rao, 2015; Yeng et al., 2012). The application of organic amendments enhanced the nutrient uptake in sweetpotato (Siose et al., 2018; Walter & Rao, 2015). A long-term study of four years in the sweetpotato field showed that amended soil with some types of organic manure promoted physiological N use efficiency and increased the uptake of N, P, K, calcium (Ca) and magnesium (Mg), resulting in an increase in the SR yield (Pan et al., 2019).

The application of organic soil amendments, as mentioned above, could improve the yield of many crops including sweetpotato. This can be explained by the influences of its fertilisaton effect on photosynthesis (Wang et al., 2014). In addition, the application of organic amendments could enhance the properties of soil such as soil moisture or bulk density, which might have a positive impact on the initiation and development of sweetpotato SRs. The high bulk density of soil reduced the formation of SRs (Chua & Kays, 1982; Togari, 1950). The moisture of soil affected the number and length of the root system of sweetpotato (Pardales et al., 2000). Moisture deficit before and during the formation of SRs had detrimental impacts on the final number of SRs and yield (Gajanayake et al., 2013). Commercial organic manure was reported to increase pH in acid soil (Pan et al., 2019). Furthermore, organic amendment applications had positive influences on soil nutrients. For example, poultry manure significantly increased total N, organic matter and exchangeable cations in low nutrient Ghanaian soils (Agyarko et al. (2013). Similarly, organic matter has been shown to enhance N and Mg levels in soil (Agbede et al., 2010).

Organic manure is a natural product that can be used as a sole source of nutrients or integrated with inorganic fertilisers. Nutrient properties vary in such amendments and are mainly determined by the source of products. For example, poultry manure is rich in N, whereas plant manure is rich in organic matters. They release and provide nutrients for plants in the form of organic fertilisers (Davis & Wilson, 2000) at different levels. Therefore, they will affect the initiation and development of sweetpotato SRs differently compared to inorganic amendments.

1.1.9. Limitations and research gaps

The initiation and development of sweetpotato SRs are critical processes that directly influence the yield and marketability of the crop. These processes are affected by a number of factors including genetic, environmental, and agronomical factors. Few studies have been conducted to examine the influence of these factors on SR formation and development, although anatomical changes during formation and development have been detailed (Artschwarger, 1923; Togari, 1950; Wilson & Lowe, 1973). However, there are not many studies that focused on SR initiation and development as affected by nutrient supply. In addition, none of them studied on Orleans cultivar, which is one of the most popular sweetpotato varieties in Australia.

Both N and organic soil amendments have been demonstrated to affect the yield components of sweetpotato, resulting in the influence of the total yield as well as marketable yield. Little information has been found on the influence of N fertiliser on root initiation and development of sweetpotato as discussed above. Similarly, no information is available about the influences of organic matter on those processes in sweetpotato. Knowledge to explain whether these amendments promote or inhibit SR formation and subsequent development is limited. Therefore, this study aims to achieve better understanding of the impact of N and a number of organic soil amendments on the initiation and development of sweetpotato SRs. This knowledge can then be applied in management to manipulate the number of SRs to achieve optimal outcomes for crop production.

1.2. Aims, objectives and research questions

To overcome the above-mentioned limitations, there are some main agronomical practices that can be easily translated into management protocols to optimise the production of sweetpotato. Nitrogen rates, N application timings and a number of organic amendments that affect soil available N were used to investigate the SR formation of Orleans variety.

1.2.1. Aims of the project

This project aims to:

- Suggest the optimum rates and N application timing to promote the initiation of sweetpotato SRs.

- Suggest the types and rates of organic soil amendments on the initiation of sweetpotato SRs.
- Understand the mechanisms by which N factors and organic soil amendments influence the formation of SRs.

1.2.2. Research objectives

1) To explore the growth of sweetpotato in soilless culture to validate a growing method for subsequent trials in the present study.

2) To identify and evaluate in which N rates that the formation of potential SRs would be promoted/inhibited.

3) To identify and evaluate the application timings of N that promote/inhibit the formation of potential SRs.

4) To assess the effects of organic soil amendments in releasing available N into soil in order to identify and evaluate the influence of organic soil amendments on the formation of SRs.

1.2.3. Research questions

The research aims to address these questions as below:

Questions under objective 1:

What is the most suitable growth culture for initiation and development of Orleans root systems for research purposes?

a. How do ARs develop under different growth cultures?
b. How do plants grow in such growth cultures?

Questions under objectives 2 and 3

1) How does plant response vary between different rates of N and application timing?
   a. How do N rates and timings affect the formation of SRs?
   b. What rates of N and timings promote the formation of AC, a sign critical for SR initiation?
   c. What rates of N and timings will result in the highest weight of SRs during SR formation?
2) How do N rates and timings influence the accumulation of starch and soluble sugar in plants?

3) How are total N acquisitions in plants affected by N rates and timings?

Questions under objective 4

1) How does plant performance vary between different organic amendment applications?
a. How do organic amendments affect the formation of SRs?
b. What types and rates of amendments promote the formation of AC, which is a critical sign of SR initiation?
2) How do organic amendments influence the accumulation of starch and soluble sugar in SR plants?
3) How are total C and N in plants affected by organic amendments?
4) How do organic amendments affect soil properties, especially soil available NH_4^+ and NO_3^-?

1.3. The model crop for the experiments

The majority of sweetpotato varieties grown in Australia are the gold-fleshed cultivars with a rose/gold smooth skin, making up to 90% of production (ASPG, 2020). There are some cultivars including Beauregard, Orleans and Bellevue belong to the gold category in Australia (ASPG, 2021). Orleans is similar to Beauregard, which is the most popular cultivar, in terms of flesh flavour, production, disease resistance and plant canopy appearances (LaBonte et al., 2012). However, their SR shapes are more consistent than that of Beauregard, so it is gradually replacing the other and will soon to be the most popular variety to meet market demand. Because of that Orleans is chosen in the present study to assess the influence of N factors and organic amendments.

Chapter 2. Evaluation of sweetpotato root initiation and development under different growth cultures—a preliminary study

2.1. Introduction

Root study has been limited due to the difficulty in root observations and root samplings from the systems of roots in situ (O'Toole & Wolfe III, 1990). There are numerous methods for root studies such as excavation, monolith, auger, profile wall, glass wall, and container methods (Bohm, 2012). The excavation method requires to remove all growing substrates to expose the entire root systems of plants. Chosing suitable growing cultures that minimise damages of root system during the excavation would bring acurate results for researchers (Hoagland & Arnon, 1950). Water culture allows a direct access to the root systems for observations, measurements and periodic sampling (O'Toole & Wolfe III). Sand culture (McCall & Nakagawa, 1970) is also convenient for studying root systems as root systems would be less damaged while they were taken out of sand rather than soil.

The chosen method depends on the aims of the investigator and the crop. For example, solution culture has been used for growing plants to study nutritional disorders or the root morphology of many plants, including lettuce, tomatoes, herbs and potatoes (Adams, 1981; Al-Maskri et al., 2010; Hayden, 2006; Zhao et al., 2012). Sand culture also has been widely used to investigate nutrient requirement or root studies for many crops such as vegetables, beans, cereals, and root and tuber crops (Gajanayake et al., 2013; Robbins, 1946; Villordon et al., 2009c; Yamaguchi, 1935).

2.1.1. Nutrient Film Technique system

Nutrient Film Technique (NFT) is considered a specific form of hydroponic culture in which plants are grown on nutrient solutions. A shallow stream of water flows through the bare roots of the plant in growing channels. In this system, the nutrient solution is continuously recirculated and supplies all of the dissolved nutrients required for plants (Adams, 1981). Plants are provided with the exact nutrients that they need with precise ratios for particular crops (Sheikh, 2006). The root systems are developed in dark conditions and absorbed necessary inorganic ions from the dilute nutrient medium provided (Zobel et al., 1976). They are kept moist by a thin film of nutrient solution with the bottom of the roots exposed to the nutrient solution (Sheikh, 2006). Thus, there is sufficient water and oxygen, which are often limited in conventional soil and water media systems (Nir, 1981).

The system has been tested in many crops such as lettuce, leafy crops, herbs, cucumbers and tomatoes (Sheikh, 2006). The system has been used to grow lettuce and celery

for commercial production (Mohammed & Sookoo, 2016). Plants grown in such systems provide better yield, better fruit quality, better crop handing and better control over the environmental conditions and nutrition requirements (Martinez, 1999, as cited in Gualberto et al., 2002). Tomatoes produced more fruit with better quality in terms of minerals, soluble solids, acidity contents and firmness values (Gormley et al., 1983). The NFT system has been used for root and tuber crops. The effects of nutrient solutions on yield and leaf elemental concentrations of beetroot were examined by using NFT (Egilla, 2012). In addition, NFT systems have been used to produce potato mini-tubers for seed production in Belgium (Rolot & Seutin, 1999). They claimed that tubers from such systems were healthy without any infections.

The systems were used as a method to study root systems of crops. In this case, researchers do not need to excavate root systems of crops but they can directly access the root system for observation (O'Toole & Wolfe III, 1990; Peterson & Krueger, 1988). The NFT systems have been utilised to study root systems for some crops such as tomato, cucumber (Gislerød & Kempton, 1983), lettuce (Al-Maskri et al., 2010), potato (Wan et al., 1994) and sweetpotato (Sherif et al., 1994).

The use of NFT systems is beneficial in a number of ways. Water is used efficiently as it is free from leakages and is only utilised by plants. The nutrition for crops is precisely controlled. Plant roots can be observed without excavation. However, the system requires a high quality pump to avoid disruption of nutrient solution during the growing period. Suitable materials for growing channels are necessary to eliminate the release of phytotoxic compounds into the recirculating solution. Therefore, the cost of installing and maintaining the NFT system is high (Adams, 1981). There are some limitations that need to be considered when using the system, including power dependence, the need to heat the nutrient solution on cold days, the development of diseases and the change of nutrient properties.

2.1.2. Sand culture

Sand culture is a method of growing plants without soil. Physiological studies on the nutrition of plants are often conducted by means of sand culture (Yamaguchi, 1935). Although plant performances in sand are different from those in soil, sand culture has been utilised for different crops, including pastures, legumes, maize, or root and tuber crops (Firon et al., 2009; Othman et al., 1986; Solis et al., 2014). The system has been utilised to investigate the formation of nodules in phasey beans. This substrate has been used for

numerous root studies. There are different types of sands that have been used, including fine sand (Gajanayake et al., 2015a) or coarser river sand (La Bonte et al., 2012; Solis et al., 2014).

In sand culture, nutrients and water are added as needed to supply the plant's growth. They are applied by a dripping irrigation system or over the surface of sand and then allowed to drain off (McCall & Nakagawa, 1970). Nutrients are supplied to plants in the form of solutions (Firon et al., 2009; Gajanayake et al., 2013) or powder/granules (La Bonte et al., 2012; Pardales et al., 2000).

Sand culture is a convenient method for growing plants for root studies. Plant roots are easy to excavate and wash with minimum damage in this growing substrate. It is very inexpensive and is available anywhere to set up research. However, water and nutrients need to be applied frequently to ensure that they are adequate for plant growth as they can easily drain out. Sand is over-heated on hot days, so it will negatively affect plant development, especially the root architecture or root development.

In this study, sweetpotato was tested using the NFT and sand culture. To our knowledge, there are very few studies that using these systems for sweetpotato, and so growing this crop in those cultures to form SRs needed to be investigated. This study aims: (1) to assess the growth of sweetpotato plants and the development of root systems in soilless cultures; and (2) to investigate the formation of sweetpotato storage root in these cultures. We addressed the following questions: (1) How do plan grow in the soilless systems? (2) How do the systems affect the initiation of SRs? And (3) Which system is suitable for SR formation and root observations of sweetpotato?

2.2. Materials and methods

2.2.1. A preliminary experiment in the NFT system

The trial was conducted in a non-temperature-controlled glasshouse at the Bundaberg Research Facility (24°50′54″ S 152°24′14″ E) from 30 October 2017 to 12 February 2018. Mean maximum and minimum air temperatures outside the glasshouse varied between 28–32°C and 18–22°C, respectively. The walls of greenhouse were covered by insect net and well ventilated. The design of the system is shown in Figure 2.1. Clear plastic containers were used to design individual systems with the size of 17 cm (H) x 57 cm (W) x 38 cm (D). They were covered by silver insulation to minimise solution heating in the root zone and

exclude light for root development. A reservoir with the capacity to hold 60 L of the nutrient solution was constructed to feed the system via a PVC pipe. There was a water valve to control the amount of the solution supplied for the system. Sweetpotato cuttings were planted through small holes in the lids of the growing containers (Figure 2.2). Five individual systems were set up with different types of nutrient films, including a weed mat, a capillary watering mat and a hard plastic sheet with small cells on the top to store some solution. The details for each system can be seen in Table 2.1. There was no replication for these testing systems.

The growing channels were placed at an angle on a bench. There was a film of nutrient solution on the bottom of the growing containers. A half strength solution of a commercial hydroponic product, Optimum Grow part A and part B (Growth Technology Ltd, Taunton, Somerset) was utilised to grow the crop. This solution was stored and shaded in a black reservoir and pumped throughout the system by a submarine pump. The solution was supplied continuously to each channel at 120±10 ml min^{-1}, then drained out and returned to the reservoir. The nutrient was renewed every fortnight and the pH value of the solution was adjusted to around 6.0 every 5–7 days. The concentration of nutrients in the Optimum Growth solution is shown in the Appendix 1. One or two cuttings of Orleans (*Ipomoea batatas* (L.) Lam) from a commercial nursery farm (McCrystal Agricultural Service Pty Ltd, Bundaberg) were transplanted into each system with three nodes attached to the nutrient film mat.

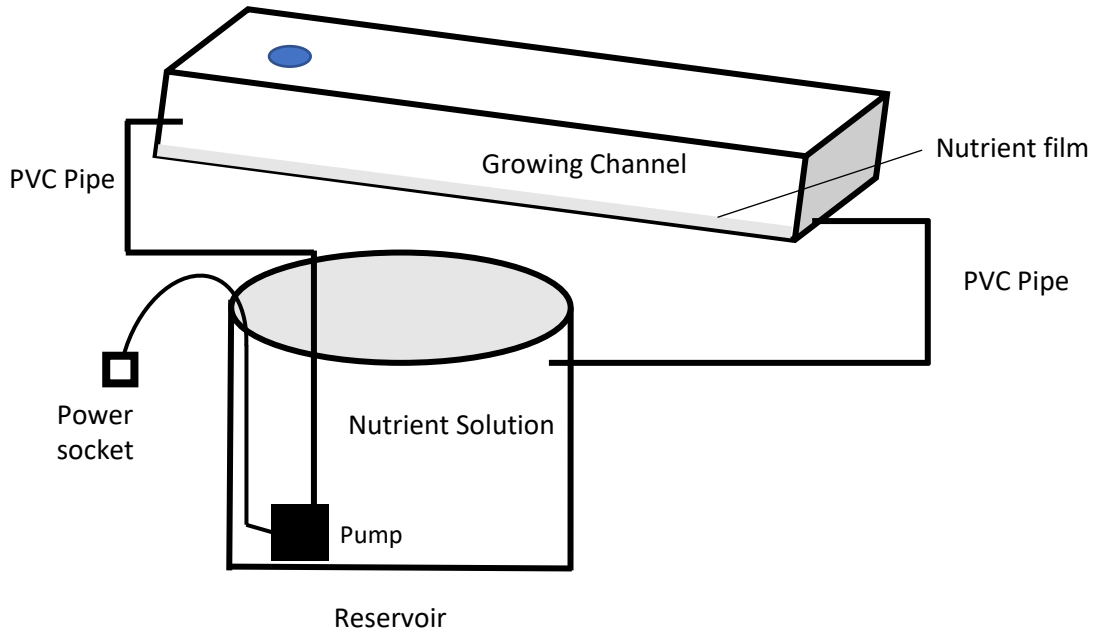

Figure 2.1. Schematic diagram of the NFT system.

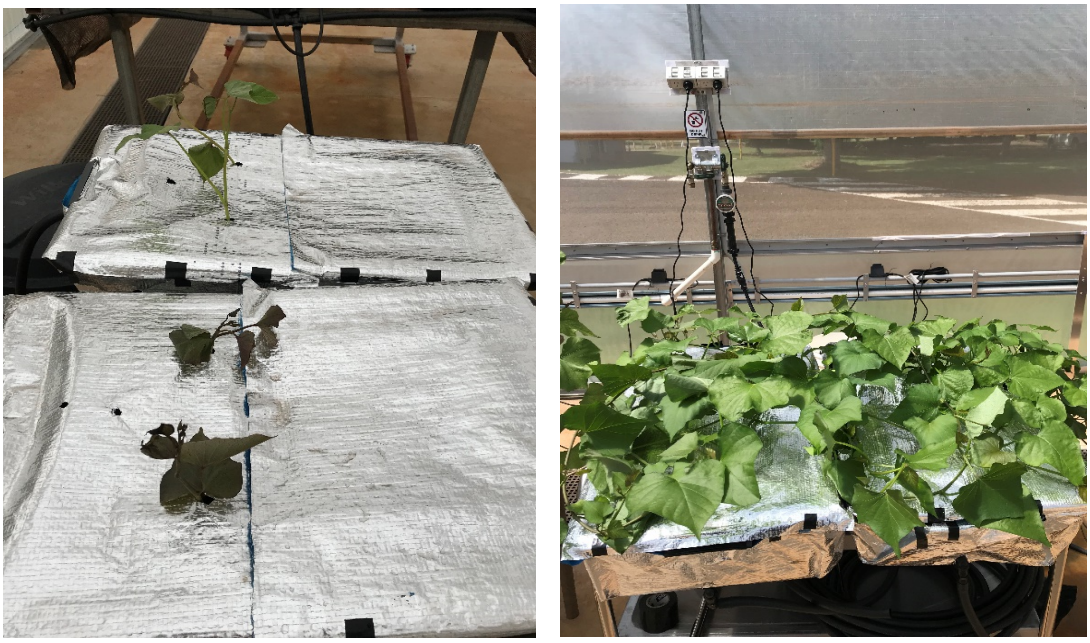

Figure 2.2. The top parts of a NFT system with plants.

Table 2.1. Designed details for NFT systems

Systems	Characteristics of the system
S1	One layer of weed mat on the bottom of the container, one plant
S2	Two layers of weed mat on the bottom of the container, one plant
S3	One layer of capillary mat stayed on top of the styrofoam sheet, one plant
S4	One layer of capillary mat stayed on top of the styrofoam sheet, two plants
S5	Hard plastic sheet with small cells on the top stayed on top of the styrofoam sheet, one plant

2.2.2. Preliminary experiments in sand culture

2.2.1.1. A preliminary experiment in fine sand

This preliminary experiment was carried out in the same glasshouse (Section 2.2.1) from 30 October 2017 to 22 January 2018 (12 weeks). The sand temperature varied from 31°C to 36°C during the hottest times of the days. Mean of the maximum and minimum air temperatures outside the glasshouse were 27–32°C and 18–21°C, respectively. Black polythene pots were filled with 4 L of sterilised fine sand. Around 65% of fine sand particles were less than 0.1 mm and the rest were 0.1–0.2 mm. Maximum water holding capacity in this sand was 18.8%. The slow-release fertiliser Scotts Osmocote® (15.3% N, 1.96% P,12.6%

K) was utilised for this study and applied on top of the sand surface (15 g pot^{-1} based on the recommendation for pots 15–20 cm in diameter). Water was supplied through a drip irrigation system once or twice a day to field capacity dependent on the weather and the growth of plants.

Cuttings of the sweetpotato (*Ipomoea batatas* (L.) Lam) cultivar Orleans from the nursery field were used in this experiment. Cuttings used in the experiment had six fully opened leaves and were at least 20 cm long. All cuttings were tip cut and from the fourth cut from the planting bed. Three nodal cuttings were planted under the sand and three fully opened leaves were above-ground.

2.2.1.2. A preliminary experiment in river sand

The study was conducted in the same glasshouse (Section 2.2.1) from 12 February to 26 March 2018 (six weeks). The sand temperature varied from 32°C to 35°C. Mean of the maximum and minimum air temperatures outside the glasshouse were 27–31°C and 19–22°C, respectively. Black plastic pots were filled with 4 L of washed river sand. Particle sizes were in the range 0.1–1 mm (around 10% 0.1–0.25 mm, 70% 0.25–0.5 mm and 20% 0.5–1 mm). The maximum water holding capacity was 12.3%. Half strength Optimum Growth nutrient solution (105 mg N, 18 mg P and 135 mg K) was watered every two days with the amount ranging from 60 ml pot^{-1} to 150 ml pot^{-1} dependent on plant growth. The same irrigation system (Section 2.1.1.1) was used to supply water for plants. One Orleans cutting was planted in each pot with three buried nodes in the sand.

2.2.3. Measurements and data collections

2.2.3.1. The NFT trial

The initiation and development of sweetpotato roots were visually observed each day in the first week and then once since the week after. Days for AR initiation (presence of at least one AR with minimum length = 0.5 cm in each plant) (Villordon et al., 2009a), number of ARs per plant at 7, 21 and 35 DAT, and days for first ARs that went through the mat were recorded. The total numbers of SRs were counted at harvest (105 DAT). Data for each system were collected from two plants for the system S4 and from a single plant for others system. As there was only one or two plants for each system, data was not analysed statistically. In the system S4, means were calculated based on two plants.

2.2.3.2. The experiment in the fine sand

Three random plants were sampled every week for 8 weeks. They were dug out of the sand very carefully to avoid root damage, and then roots were washed in tap water to remove all sand. All ARs were serially cross-sectioned to investigate the anatomical roots using free hand sectioning and stained with Toluidine Blue O (Eguchi & Yoshinaga, 2008). The total ARs for sectioning observations during the 8 weeks was 278. The observation was recorded in sections at around 3–4 cm from the proximal end (Firon et al., 2009). Transverse sections for root anatomy were prepared by hand with the use of sharp razor blades. The thickness of sections was around 8-15 µm and were not oblique. Several sections were cut at the same time and transferred to deionised water in petri dishes. The thinnest sections were selected to stain and then the anatomy was observed under an Olympus CX31 microscope (Olympus Corporation, Tokyo, Japan). Images of sections were taken using a Nikon DS-L2 camera (Nikon Corporation, Tokyo, Japan).

Sections were stained with 0.05% Toluidine Blue O (ProSciTech, Queensland, Australia) for one minute by placing a drop of the staining solution on the section (O'Bien & McCully, 1981). Then the stain was removed using a piece of filter paper. Sections were rinsed in running tap water until there was no excess stain around the sections (O'Brien & McCully, 1981). A drop of water was added over the section and a glass cover was put on top. Meristem elements show as blue or blue-green under this stain. Some main anatomical features, including the number of protoxylem elements, initial development of RVC, completed RVC, appearance of AC and lignification of more than 50% of the stele cells, were observed. The number of ARs was recorded for each plant. Protoxylem pole numbers were classified into tetrarch, pentarch, hexarch, septarch, octarch, ennearch and decarch.

The root architecture of the sweetpotato was examined every week for the first eight weeks after transplanting using an Epson Perfection V700 Photo Scanner (Seiko Epson, Nagano, Japan). The WinRHIZO software (version 2012a; Regent Instruments Inc., Quebec, Canada) was used to analyse the root images. After sectioning, ARs were placed into perspex trays placed onto the scanner bed to acquire images. Roots were divided into smaller sections to make sure they could spread out in the trays.

Data were collected for an individual plant and mean values were calculated based on the three harvested plants. Data for the total root length, total root surface, root volume and total root tips were log-transformed ($\log_{10}(x)$) before analysis. One-way ANOVA using SPSS

statistical package (version 25; IBM, New York, United States) was applied to analyse the data. Each harvest was considered as a treatment. All the post hoc tests were conducted with Turkey HSD. Graphs were produced using SigmaPlot® software (version 14; SYSTAT Software, Inc., California, United States).

2.2.3.3. The experiment in the washed river sand

Five random plants were sampled every week for the first seven weeks after transplanting. They were dug out of the sand very carefully to avoid root damage, and then roots were washed in tap water to remove all sand. All ARs from the sampled plants were used for root anatomy and architecture as described in Section 2.2.3.2. Data collections and calculations were also done as stated in Section 2.2.3.2. Data was analysed by one-way ANOVA using the IBM® SPSS® software statistical package (version 25; IBM, New York, USA) and each plant was one replication. Means for each harvest were compared using Turkey HSD. SigmaPlot®software (version 14; SYSTAT software, Inc., California, United States) was used to produce graphs.

2.4. Results and discussions

2.4.1. The growth of sweetpotato in the NFT system

The NFT system with the thick capillary mat (S3 and S4) could hold more water than others (S1, S2 and S5). Therefore, plants in the S3 and S4 systems generated ARs earlier. Under experimental conditions, plants in the NFT were observed to generate a minimum of one AR at 3 DAT for the systems with the capillary mat and 4 and 5 DAT for the systems with the weed mat and plastic sheet, respectively (Table 2.2). This was similar to observations by Villordon et al. (2009a) for the Beauregard sweetpotato cultivar. The ARs generated within 24 hours in a previous study when root primordia of vines were exposed to a humid atmosphere in polyethylene bags (Belehu et al., 2004).

After a few days, more ARs formed and some of them went through the mats except for the plant in the system with the specific hard plastic mat. The ARs in the S4 system started to go through the mat at 6 DAT, while those in the S3 were later at 7 DAT. It was observed that ARs in the system with the weed mat stayed on top of the mat longer than others. The ARs in S1 and S2 started to go through the mat at 14 DAT (Table 2.2). Unexpectedly, some ARs in the S1 and S2 systems and most of those in S3 and S4 went

through and developed under the mat (Table 2.2), resulting in SRs forming under the nutrient film and these could not be observed from the top. All ARs in S5 stayed and developed on top of the mat due to the hard-plastic sheet. It was estimated that around 90% of the sweetpotato root systems stayed under the mat in the S3 and S4 systems, compared to 70% and 65% of roots in the S1 and S2 systems, respectively (Table 2.2).

Table 2.2. Sweetpotato root initiations and development in the NFT system

Systems	*ARs initiated (DAT)*	*ARs went through nutrient mat*	
		DAT	% of the root system at harvesting
S1	4	14	70
S2	4	14	65
S3	3	7	90
S4	3	6	90
S5	5	0	0

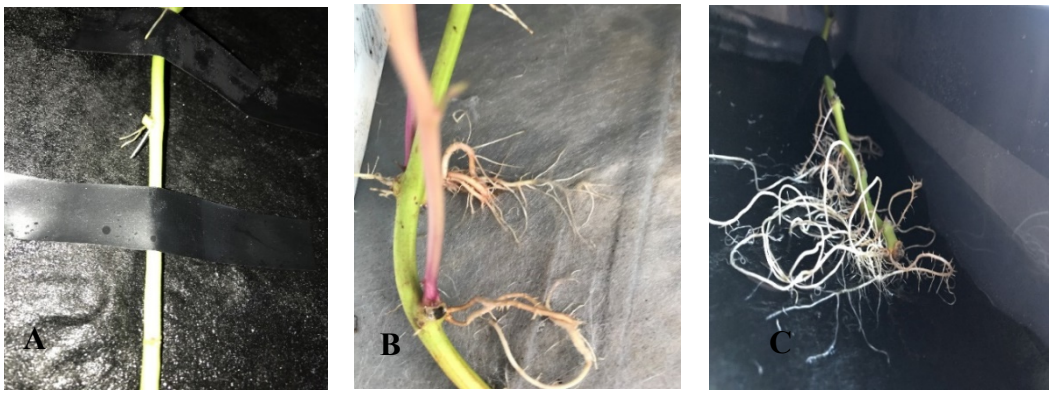

Figure 2.3. Initiation and development of the sweetpotato root system in NFT: (A) AR initiation; (B) the development of ARs in the system with capillary mat; and (C) the development of ARS in the system with weed mat.

The AR counts varied between 7 and 13 at 7 DAT depending on the system. The number of ARs increased from day 7 after transplanting to 21 DAT and were stable after that (Table 2.3). This trend differed from the findings of Villordon et al. (2009c) for the Georgia variety where the AR number dropped significantly from 7 DAT. However, the trend was similar to the results of the Beauregard cultivar in the same study (Villordon et al., 2009c).

The number of SRs per plant at harvesting varied from 1 to 6 (Table 2.3). The formation of SRs is affected by many factors, including moisture (Gajanayake et al., 2014b).

The water holding capacity of different types of nutrient films were not the same (data not shown). This resulted in different moisture levels for the root zone and likely affected the differentiation of ARs. Only one SR was formed in the S5 system and the maximum number of SRs was recorded in S3, with 6 SRs forming. The number of SRs initiating in the system with the capillary mat tended to be higher than those with the weed mat.

Table 2.3. The number of ARs and SRs in the NFT system

Systems	Number of Ars			SRs	
	7 DAT	21 DAT	35 DAT	Number	Fresh weight (g plant^{-1})
S1	7	9	8	2	174
S2	9	11	11	3	280
S3	12	14	14	6	475
S4	11	14	14	4	256
S5	7	10	9	1	38

The maximum fresh weight of SRs per plant was recorded in the S3 system and the minimum was exhibited in S5 where the plastic sheet was utilised as a nutrient film for the system. There were two plants in the S3 and the average of SRs was 256 g plant^{-1}. Total SR weight for this system was highest at 512 g. This could be due to the water holding capacity of the mat. Observation indicated that the capillary mat was thicker and absorbed the nutrient solution better than the weed mat. However, there was some water under the capillary mat and all SRs developed under the mat (Figure 2.4). In the system with the weed mat, some SRs went through the mat while the others stayed on top.

Figure 2.4. Sweetpotato roots in the NFT system: (A) Above the nutrient film; and (B) below the nutrient film.

Results from the present experiment indicate that sweetpotato could develop in the NFT system. However, the root system was not studied from the top as they formed and stayed beneath the nutrient film. Although the growing channel size was enough for SR development, the growth of SRs was abnormal as shown by their odd shapes (Figure 2.4B). Further study is needed to find out the causes for this problem.

2.4.2. The growth of sweetpotato in the fine sand culture

The AR counts increased from 9.3 roots per plant at 7 DAT to around 12 at 28 DAT (Figure 2.5). Then, they remained stable by 56 DAT. These results were similar to the findings of Villordon et al. (2009b) with the Beauregard cultivar. The trend was opposite in another variety, Georgia Jet, with AR counts decreasing by 28 DAT. However, they both had similar numbers of ARs at 26 to 35 DAT (Firon et al., 2009).

The number of primary xylem elements ranged between 4 and 8 (Figure 2.6). The arrangement of the central cylinder in roots was mainly pentarch or hexarch, which accounted for 75% of the total number of Orleans roots. Root central cylinders with a vascular system consisting of tetrarch and septarch arrangement were 14.3% and 7.1%, respectively. There was a minority of roots (3.6%) with octarch arrangement of the central cylinder. It has been well documented that sweetpotato roots were normally pentarch or hexarch (Artschwager, 1924; Firon et al., 2009; Wilson & Lowe, 1973).

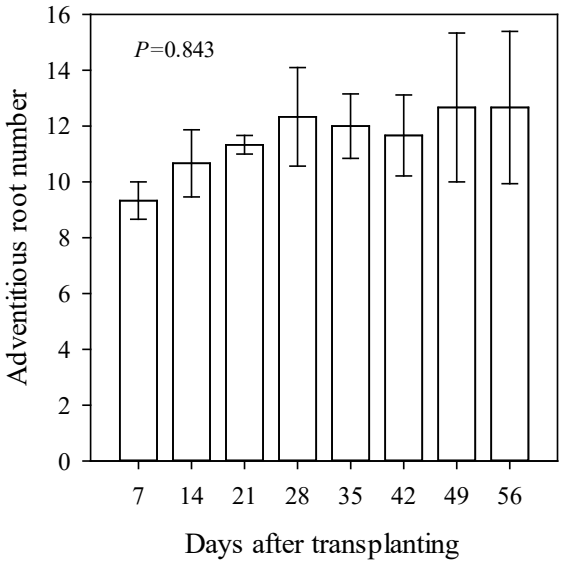

Figure 2.5. AR number of Orleans at various sampling dates. ANOVA results are based on log-transform data.

Values are indicated as mean ± SE (n=3)

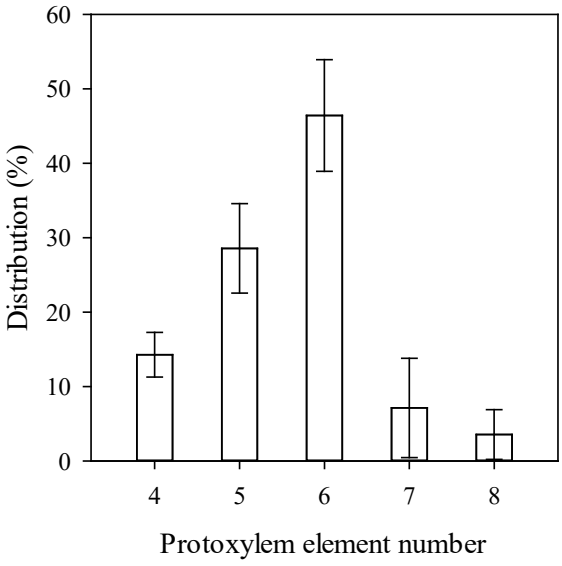

Figure 2.6. Distribution of AR protoxylem element number in Orleans at 7 DAT.

Values are indicated as mean ± SE (n=3)

Root sections showed various anatomical features of root development (Figure 2.7). In this experiment, initial RVC (IRVC) was found as early as 7 DAT, while RVC was observed at 14 DAT. The earliest lignified roots were detected at 14 DAT. The percentage of lignified roots increased sharply from 12.2% at 14 DAT to 93.3% by week 8. The percentage of LC at 35 DAT was about 64%, which doubled that of Beauregard for the same time in a study in the USA (Firon et al., 2009). There was a minority of roots developing AC that started to be observed at 35 DAT in 2.8% of the total root counts (Table 2.4). The one-way ANOVA showed that the distribution of no cambium (NC), IRVC and LC were significantly different over the sampling dates ($P<0.05$). By contrast, there were no significant differences found on the distributions of RVC and AC over the time ($P>0.05$).

Gajanayake et al. (2014) found that SRs of Beauregard were visible at about 20 DAT. This is similar to another study by Villordon et al. (2009b) with the same cultivar. The SRs were formed at 19 DAT in greenhouse conditions. The possible reason for late forming SRs and a low rate of this type of roots could be a lack of oxygen in the root region. Water is drained too slowly and proper aeration is difficult to obtain in this growing media (McCall & Nakagawa, 1970). It has been reported that the deficiency of oxygen in the rooting zone depresses growth and yield of dryland species (Drew, 1997). Another study demonstrated that low oxygen concentration reduces the number of SRs and the yield of sweetpotato (Chua &

Kays, 1981). They found that only 0.8 SR was produced in treatment with 2.5% oxygen while that of 21% oxygen was 6.2 per plant.

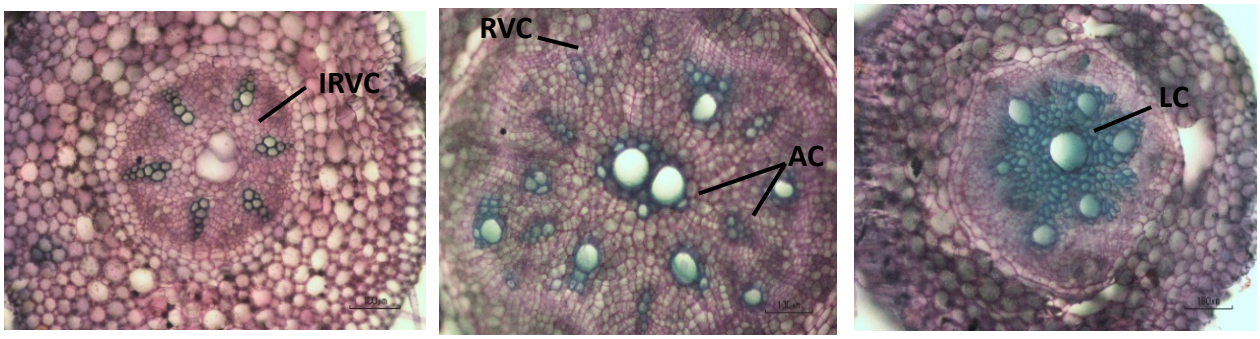

Figure 2.7. Micrograph of transverse sections of roots with different anatomical structures.

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

Table 2.4. Anatomical features of Orleans roots in fine sand culture

Sampling dates (DAT)	Distribution of anatomical features (%)				
	NC	IRVC	RVC	AC	LC
7	42.5^{a}±3.8	57.5^{a}±3.8	0	0	0
14	28.8^{b}±3.0	43.5^{a}±1.8	15.5±2.6	0	12.2^{c}±1.6
21	20.7^{bc}±3.3	26.3^{b}±4.4	17.4±4.6	0	35.6^{c}±5.9
28	12.9^{cd}±1.6	13.7^{bc}±3.6	10.3±1.6	0	63.1^{b}±3.1
35	10.9^{cd}±1.8	8.1^{c}±4.2	14.2±3.4	2.8±2.8	64.0^{b}±5.5
42	5.1^{d}±2.6	2.4^{c}±2.4	22.5±3.4	2.4±2.4	67.6^{b}±8.8
49	0	5.2^{c}±2.9	15.6±2.9	3.3±3.3	75.9^{ab}±3.0
56	0	0.0	1.9±1.9	4.8±2.6	93.3^{a}±1.2
P value	0.008	0.003	0.113	0.368	0.003

Table presents the mean values followed by standard errors (SE). Data were log-transformed ($\log_{10}(x)$) to analyse in SPSS and original data is presented in the table.

Abbreviation: NC = No cambium; IRVC = Initial regular vascular cambium; RVC = Complete regular vascular cambium; AC = Anomalous cambium; LC = Lignified cells

Sweetpotato root morphology characteristics in fine sand culture were recorded at a 7-day interval over a period of 56 days from transplantation (Table 2.5). In our study, the

diameter of the root slightly decreased but there was no significant difference over time (P>0.05). However, the total root length, total root surface area, total number of tips and total root volume increased over the given period. The ANOVA results showed significant effects of time on these features (P<0.01). The values for these parameters were higher than those in a recent study (Chen et al., 2017). The experimental conditions might explain this difference as root morphological characteristics were affected by many factors such as root zone temperature (Banoc et al., 1999), fertiliser applications (Chen et al., 2017) or soil moisture (Pardales et al., 2000).

The last harvesting was conducted at 84 DAT. There were no visible SRs found in all three plants harvested at 84 DAT, although the initial formation of AC was recorded at 35 DAT. Some PRs of less than 5 mm in diameter were collected (data not shown) as results of root thickening. This means that the activity of that cambium was limited, leading to heavy lignification to form PRs (Wilson & Lowe, 1973).

Our experiment in fine sand culture showed that the formation of SRs was delayed until 35 DAT. Intensive lignification was observed in almost all roots (93%) at 56 DAT. Fine sand was an unfavourable medium for SR development, resulting in no SR observed at 84 DAT. Our observations indicated that fine sand in pots was impacted after watering a few times and some water from the underneath part could not drain out.

Table 2.5. Root morphology and physiological characteristics of sweetpotato roots in fine sand culture

Sampling date (DAT)	TRL (cm)	RS (cm^2)	RD (mm)	RV (cm^3)	RT (number)
7	146^{c}±7	61^{d}±9	0.97^{a}±0.03	1.4^{c}±0.2	170^{d}±31
14	474^{c}±68	382^{cd}±69	0.82^{b}±0.02	7.9^{bc}±1.2	861^{cd}±165
21	1276^{b}±152	782^{bc}±65	0.82^{b}±0.06	15.8^{b}±1.3	1848^{cd}±326
28	1614^{b}±114	1008^{b}±49	0.77^{b}±0.01	19.3^{a}±2.9	2602^{bc}±192
35	2481^{a}±197	1802^{a}±121	0.75^{b}±0.02	33.9^{a}±2.9	4900^{b}±417
42	2628^{a}±89	1847^{a}±109	0.76^{b}±0.01	35.0^{a}±1.9	5184^{ab}±410
49	2647^{a}±190	1708^{a}±132	0.76^{b}±0.01	36.2^{a}±3.0	5938^{ab}±446
56	3010^{a}±77	1954^{a}±273	0.77^{b}±0.01	40.5^{a}±4.4	6907^{a}±751
P value	0.000	0.001	0.000	0.001	0.002

Table presents the mean values for each sampling date followed by standard error (SE) (n=3). Data excluding RD were log-transformed ($\log_{10}(x)$) to analyse in SPSS and original data is presented in the table.

Abbreviations: TRL = Total root length; RS = Total root surface; RD = Root diameter; RV = Total root volume; RT = Total number of root tips.

2.4.3. The growth of sweetpotato in the river sand culture

The AR number of Orleans grown in sand culture was highest at 21 DAT, then it stabilised at around 13 roots by 35 DAT (Figure 2.8). The data for this study was higher than that in other studies. Villordon et al. (2009b) recorded that the maximal AR counts of Beauregard across two nodes were approximately 6.5 at 26 DAT. In the same experiment for another cultivar, the figure for Georgia Jet was about 12 at 7 DAT. The number of ARs in sweetpotato depends on the number of preformed root primordia which are presented in sets of 4–10 close to the leaf bases on vine cuttings (Belehu et al., 2004).

The number of primary xylem poles varied between 4 and 10. Around 85% of Orleans ARs were pentarch or hexarch while the rest of the roots were tetrarch, septarch, octarch or decarch (Figure 2.9). These results are in line with findings for Beauregard (Firon et al., 2009) and some West Indian cultivars (Wilson & Lowe, 1973).

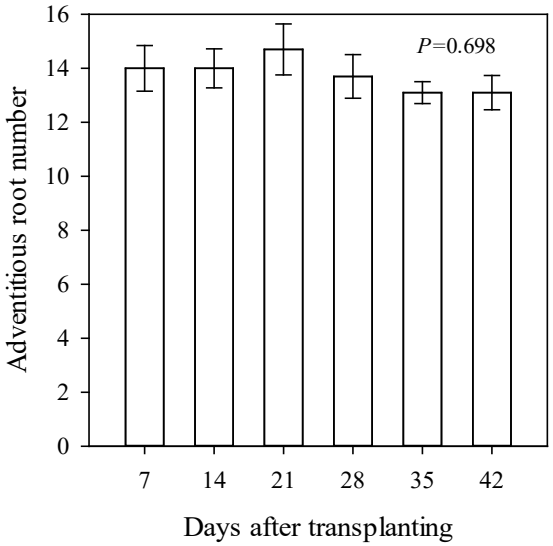

Figure 2.8. AR numbers of Orleans in river sand culture at various sampling dates. ANOVA results are based on Log-transform data.

Values are indicated as mean ± SE (n=5)

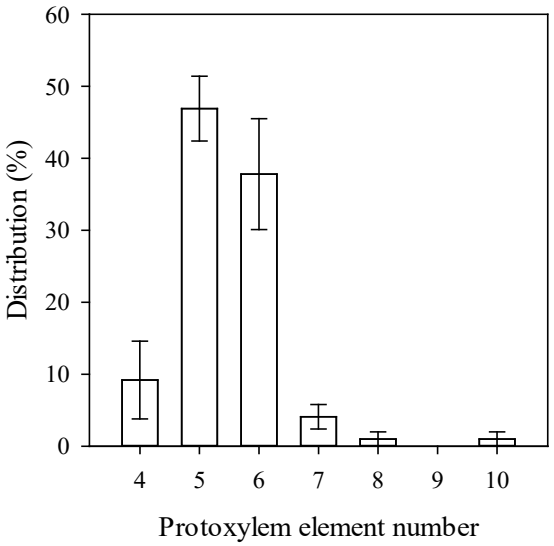

Figure 2.9. Distribution of protoxylem element numbers of Orleans in river sand culture at 7 DAT.

Values are indicated as mean ± SE (n=5)

The anatomical features of ARs were investigated every 7 days for 6 weeks after planting (Figure 2.10). Our observations indicated that the percentage of roots with no cambium, IRVC and RVC development decreased significantly over the time (P<0.01). There was no root without cambia or IRVC development from 42 DAT. The lignification of more than 50% stele cells started at 14 DAT, then it rose significantly in the next few weeks (P<0.01) and reached 44.6% of the roots by 42 DAT. Those roots did not develop into SRs. The AC was initially observed at 21 DAT with 13.6% total roots initiating these cambia. A significant increase in the percentage of roots with AC development was observed, rising to nearly 50% of the roots at 42 DAT. The activity of those cambia contributes to the root thickening (Artschwager, 1924; Wilson & Lowe, 1973). The first clear sign of the SR formation is the appearance of AC surrounding the primary and secondary xylem (Togari, 1950; Wilson & Lowe, 1973). The time frame for the appearance of AC in this study was similar to the findings of Villordon et al. (2009b) and Gajanayake et al. (2014) for the Beauregard cultivar in glasshouse conditions.

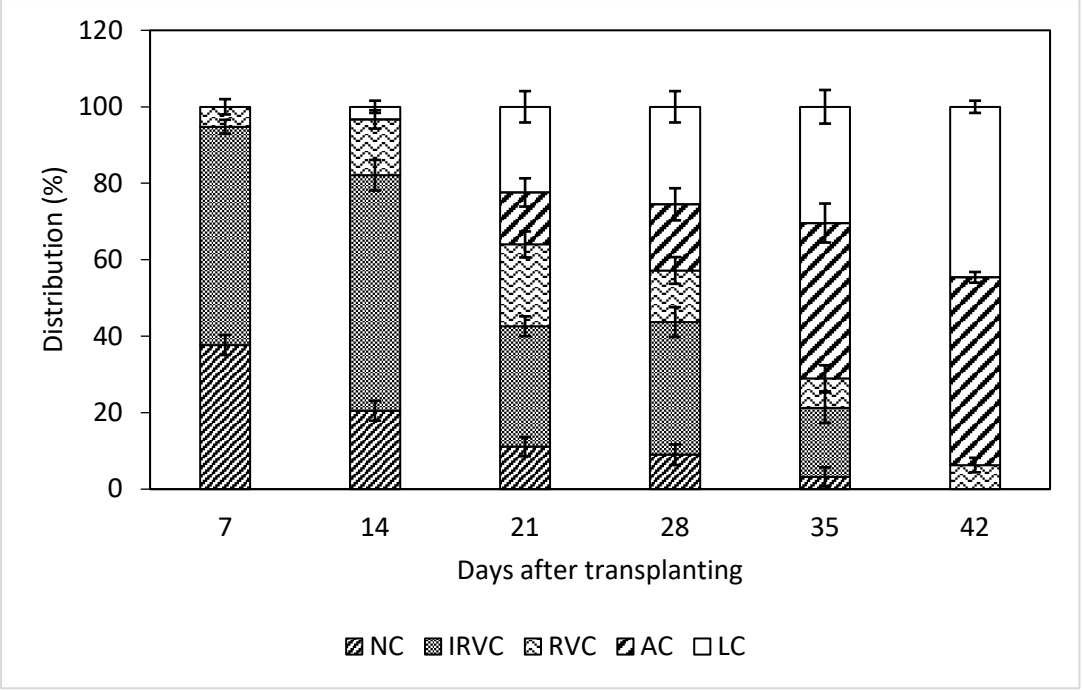

Figure 2.10. Distribution of anatomical features of sweetpotato roots developing in river sand culture at different sampling times. Values are indicated as mean ± SE (n=5).

Abbreviations: NC = No cambium; IRVC = Initial regular vascular cambium; RVC = Complete regular vascular cambium; AC = Anomalous cambium; LC = Lignified cells.

Table 2.6. Root morphological characteristics of sweetpotato roots in river sand culture

Sampling date (DAT)	TRL (cm)	RS (cm^2)	RD (mm)	RV (cm^3)	RT (number)
7	184^{d}±20	82^{d}±6	0.67^{ab}±0.005	1.3^{d}±0.1	241^{d}±14
14	715^{c}±68	448^{c}±42	0.66^{b}±0.005	7.4^{c}±0.7	1142^{c}±89
21	964^{bc}±102	681^{bc}±106	0.68^{ab}±0.013	11.6^{bc}±2.0	1537^{bc}±180
28	1165^{b}±85	768^{bc}±73	0.64^{b}±0.007	12.2^{bc}±1.1	1848^{bc}±163
35	1296^{b}±63	1001^{b}±58	0.64^{b}±0.008	16.0^{b}±0.8	2212^{b}±127
42	1868^{a}±131	1562^{a}±110	0.72^{a}±0.018	27.9^{a}±2.5	3201^{a}±270
P value	0.000	0.000	0.027	0.000	0.000

Table presents the mean values from four different plants for each sampling dates followed by standard error (SE) (n=4).

Abbreviations: TRL = Total root length; RS = Total root surface; RD = Root diameter; RV = Total root volume; RT = Total number of root tips.

The characteristics of Orleans roots in the river sand culture are recorded in Table 2.6. In this experiment, values for all root morphology increased over the observational time,

excluding root diameter (RD), which fluctuated around 6.5 mm. However, RD increased in the last week to 0.72 mm by 42 DAT. This might be due to the formation and development of SRs. The one-way ANOVA results found that there were significant changes in all morphological characteristics over the time (P<0.05) due to the growth and development of root systems over the time.

Our observations revealed that the development of plants in river sand is normal (Figure 2.11). At the end of the experimental period, 7 plants were harvested at 42 DAT. Potential SRs (including PRs and SRs) with a diameter of more than 5 mm were collected. Harvesting data showed that the number of SRs was 5.1 per plant. The average length and diameter of SRs was 96.8 mm and 11.4 mm, respectively.

Figure 2.11. Development of Orleans in river sand culture: (A) Plants at 21 DAT; (B) Plant root system at 21 DAT (scale bar represents 30 cm); and (C) Orleans root system at 42 DAT.

2.5. Conclusions

Our preliminary experiment indicated that a small number of SRs was produced in NFT systems. However, most of the root systems, including SRs that developed under the nutrient film, formed odd shapes. Therefore, observations of SR initiation and development were obstructed. More work is required to develop and scale up the system to maximise the number of SRs and control the root development. Time was limited for this project, so we decided to use another soilless method for our study.

In the fine sand culture, SR initiation time was delayed until 35 DAT. Then, those roots with initiated SR signs developed into PRs and no SRs were observed at 84 DAT. In

previous studies, SRs were produced when sweetpotato were planted in pots filled with find sane (Gajanayake et al., 2015a; Gajanayake et al., 2014b). The suppression of SR formation and development in the current experiment may be due to the lack of oxygen in root zones and unfavourable moisture levels for growth under ourexperimental conditions. Both previous experiments using fine sand were conducted in SPAR chambers, so all growing conditions were controlled. By contrast, our experiment was carried out in an uncontrolled greenhouse with temperature, humidity and light depending on weather conditions, under which more irrigation was required to maintain the media temperature not overheated.

The river sand culture was suitable for our aims of SR initiation and development. In this growing method, SR initiation was observed at 21 DAT, similar to previous studies. Nearly 50% of root developed AC at 42 DAT. The total number of visual SRs from three nodes was 5.1 per plant. This growing culture could be used for growing Orleans cultivar for root studies.

Chapter 3. The effects of nitrogen rates on sweetpotato storage root initiation and development

Abstract

Nitrogen is an essential element for plant growth and therefore an adequate supply must be available for optimal crop production. Both insufficient and excessive use of N have been demonstrated to have detrimental agronomic effects. In sweetpotato, some studies have demonstrated the strong influence of N on the SR yield, but little information is available on the relationship between N application levels and the initiation and development of sweetpotato SRs. There has not been a comprehensive study that investigated root anatomical changes, the accumulation of non-structural carbohydrates (soluble sugar and starch), and N acquisition during SR formation. A pot experiment was conducted in Central Queensland, Australia, in 2018 to examine how N promoted or inhibited the formation and development of SRs. Cuttings of Orleans were grown in river sand culture supplied with modified Hoagland nutrient solution at four different rates of N, 0 (N0), 50 (N50), 100 (N100) or 200 (N200) mg/L of N. In the early stage of AR development, both deficient (N0) and high (N200) rates of N inhibited the formation of primary cambium at 10 DAT and then AC at 21 DAT. As a result, the formation of SRs was delayed in those conditions. Significantly lower SR formation rates in N0 and N200 treatments than that of N50 and N100 were recorded at 21 DAT, as was lower N recovery efficiency in treatment N200. By contrast, the 50 and 100 mg/L N treatment promoted the formation of SRs. However, plants required more N after SRs were formed, as suggested by faster growth, higher N acquisition, highest efficiency of N use after 35 DAT and higher carbohydrate accumulation in roots in the N200 treatment. Our study provides agronomic indication that moderate N supply level should be maintained for a few weeks to promote SR initiation until most SRs are formed (35 to 49 DAT in our experimental conditions), and then further N fertiliser should be supplied to improve SR development.

3.1. Introduction

Nitrogen is one of the most critical elements for plant growth and optimum yield of crops (Atkinson et al., 2010; Bai et al., 2012; Taranet et al., 2017). Nitrogen promotes growth and development of leaves, stem and other vegetative plant parts (Leghari et al., 2016). Nitrogen is an essential constituent of protein which is associated to all vital processes in plants. It is also an essential constituent of chlorophyll, the pigment present in many major parts of the plant body to enable the process of photosynthesis (Leghari et al., 2016). Therefore, N plays an important role in agriculture through its impact on the yield and quality of crops (Massignam et al., 2009).

Suitable N supply is critical for crops to achieve optimum production. The effects of N rates have been studied in various crops and show a fundamental role in increasing productivity of many crops including wheat, rice and cotton (Ali et al., 2000; Malik et al., 2014; Nadeem et al., 2010). However, both deficient and excessive use of N can result in adverse agronomic effects. Deficiency of N often causes typical symptoms of chlorosis and red/purple spots on leaves, and reduces growth and yield of plants (Leghari et al., 2016). For example, in root crops, it was found that sweetpotato yield reduced under N deficiency (Osaki et al., 1995; Phillips et al., 2005). In comparison, excessively high N supply levels also have negative effects on tuberisation and yield of root and tuber crops (Hammes & Beyers, 1973; Hill, 1984). A recent study concluded that the overuse of N reduced the total SR yield of sweetpotato (Taranet et al., 2017). High rates of N application may promote the growth of vines rather than SR development (Nedunchezhiyan et al., 2012), leading to low fertiliser efficiency when N is overused. Hence, the use of appropriate N rates is an effective measure to increase the SR yield.

Root crops such as sweetpotato, cassava and yam are widely grown around the world and are the third most important group of food crops in global food and energy security (Chandra, 2006). Sweetpotato can be grown all year round and are an important vegetable crop in Australia (ASPG, 2020). The utilisation of N fertilisation is one of the main factors that contributes to increasing the production of sweetpotato (Taranet et al., 2017). Thus, numerous research studies in this crop showed that the application of N increases the root yield compared to without N application. Previous studies on the N requirement of sweetpotato mainly focus on the influence of this element on the final yield of this crop (Hammett et al., 1984; Kelm et al., 2001; Phillips et al., 2005). It has been demonstrated that

the response of sweetpotato to N application varies from location to location and from year to year (Taranet et al., 2017; Villordon et al., 2009b). The rates of N to produce maximum yield of sweetpotato varied from none to 240 kg N ha^{-1} (Guertal & Kemble, 1997; Hartemink et al., 2000; Phillips et al., 2005; Villagarcia, 1996), depending on growth conditions. In a 3-year study using Beauregard cultivar in Virginia, USA, the requirement of N to produce the maximum marketable yield varied from 28 to 56 kg ha^{-1} depending on rainfall (Phillips et al., 2005). Hartermink et al. (2000) found that the highest marketable yield was obtained with the application of 100 kg N ha^{-1} in a study using Markham variety in Papua New Guinea. However, Guertal and Kemble (1997) reported that there was no response of sweetpotato to N application up to 108 kg ha^{-1}.

A few previous studies investigated the influence of N on the formation and development of SRs. The SR initiation determines the number of SRs per plant, which contributes to the yield of sweetpotato. High levels of N supply supressed the initiation of SRs as it affected the balance between cambial development and lignification in roots, which determines the differentiation of ARs into SRs (Gifford et al., 2008; Togari, 1950). Wilson (1973) detected SR formation in sand culture of two sweetpotato cultivars. The results showed that NO_3^- - N levels from 21 to 105 mg/L in sand culture produced tubers and uniformly thickened roots, whereas 210 mg/L N treatment produced very few tubers. In another study, high rates of fertiliser reduced the number of SRs grown in greenhouse pots (Acock & Garner, 1984). Nitrogen deficiency also inhibited the formation of SRs (Si et al., 2018). Their results showed that the number of SRs was significantly lower under deficient N conditions. The formation of SRs is one of the main factors contributing to the final yield of the crop as it determines the number of SRs forming per plant (Ma et al., 2015).

There have been limited studies focusing on the effects of N fertiliser rates on the initiation and development of sweetpotato SRs. None of them examined the anatomical development in combination with non-structural carbohydrate (soluble sugar and starch as storage) change and the acquisition of N during SR initiation. Therefore, the aims of this study were: (1) to investigate the formation of sweetpotato SRs with different N supply levels, and (2) to assess the effects of these N levels on the accumulation of soluble sugar and starch as well as the acquisition of N in plants during the formation of SRs. We specifically addressed the following questions: (1) How does N application level affect SR initiation? (2) Does N supply level affect the accumulation of soluble sugar and starch related to the formation of SRs? and (3) How is N acquisition in plants affected by N supply level? The

Orleans cultivar was used in this study as it is one of the most popular varieties in Australia and has consistent yields for early, middle, or late-season plantings (La Bonte et al., 2012). In the research reported here, the influence of N rates on the initiation of SRs was evaluated in a glasshouse trial.

3.2. Materials and methods

3.2.1. Plant materials and growth conditions

The experiment was conducted in a glasshouse at Bundaberg Research Facility (24°50′54″ S 152°24′14″ E) from 23 April 2018 to 11 June 2018. The average daily maximum and minimum temperatures during this period inside the glasshouse were 27.8°C and 15.7°C, respectively, and the average daily maximum and minimum relative humidity was 79.4% and 49.3%, respectively. Washed river sand was used as a growth substrate for the experiment. Healthy uniform cuttings of Orleans of at least 20 cm length with five fully opened leaves were utilised to examine SR initiation. Two leaves from the cut end of the cuttings were removed before planting. One cutting was planted horizontally in each pot with two nodes buried under the sand and three fully opened leaves above-ground.

3.2.2. Growth medium preparation

Black polythene pots of 15 cm in diameter and 30 cm in height with small holes on the bottom to drain water were used in this experiment. Each pot was filled with 4 L of washed river sand. Tap water was added to the pots to achieve field capacity three days before transplanting.

3.2.3. Experiment design

The experiment consisted of four treatments with different rates of N supply, being 0, 50, 100 and 200 mg/L in nutrient solution (hereafter N0, N50, N100 and N200, respectively), with multiple harvests during the entire experimental period. The pots were arranged in a completely randomised design (CRD). Each time, six plants were harvested for measurements. Three replicate plants were used for anatomical observations and morphological analysis of the roots, and the other three were used for carbohydrate and CN analysis. In total, five harvests were conducted over the experimental period and included 120 plants (4 treatments x 5 harvests x 6 plants per harvest). Eight additional plants per

treatment (32 plants in total) were grown for backup purposes in case death or abnormal growth occurred.

3.2.4. Sampling dates

The interval between each harvest was between 7 and 14 days. The first harvesting was 10 DAT, and then plants were sampled at 21, 35 and 49 DAT. Plants were dug up carefully to minimise root damage and washed in tap water to remove all sand. Final harvesting was conducted at 56 DAT for root anatomical study and yield purposes.

3.2.5. Nutrient solution preparation and application

Hoagland's modified solution was utilised in this experiment as the only source of nutrients for the plants (Hoagland & Arnon, 1950) as the amount of nutrient in washed river sand is negligible. Molar stock solutions were made separately and stored in different bottles. Distilled water was used to prepare stock solutions. These stock solutions were diluted in tap water immediately before application to plants. A supplementary solution was used to supply micronutrients. The concentration of nutrients in the N free solution after dilution was as follows: 195 mg/L K, 31 mg/L P, 100 mg/L Ca, 208 mg/L sulfur (S), 49 mg/L Mg, 0.5 mg/L boron (B), 0.5 mg/L manganese (Mn), 0.05 mg/L zinc (Zn), 0.02 mg/L copper (Cu), 0.01 mg/L molybdenum (Mo), and 2 mg/L iron (Fe). Nitrogen in the form of NH_4NO_3 was then added to the N lacking nutrient solution to make up the 50, 100 and 200 mg/L of N in each solution. The protocol for Hoagland's solution preparation is shown in the Appendix 2.

Plants were watered with these solutions to field capacity every two days. The amount of nutrient solution for each pot varied from 80 to 150 ml over time dependent on plant age and weather conditions. The total amount of N applied for N0, N50, N100 and N200 treatments over the study period were 0, 193, 386 and 772 mg $plant^{-1}$, respectively. During the entire experimental period, the sand moisture was kept at field capacity level by adding tap water into the pots. As pots were put into saucers, the amount of water application was sufficient to soak all sands in pots without excessive water in the saucers.

3.2.6. Measurements and data collections

Anatomical observations

Three plants from each treatment were sampled at 10, 21, 35, 49 and 56 DAT. The number of ARs was recorded for each plant. For each sampling time, all ARs from individual plants were serially cross sectioned using sharp razor blades (Villordon et al., 2009c). Sections from each root were prepared and transferred to deionised water in a petri dish. Samples used for microscopic observation were cut very thinly and were not cut obliquely. The thinnest cross sessions were chosen for staining with Toluidine Blue O 0.05% to observe the anatomical features (Eguchi & Yoshinaga, 2008). The observations were recorded from sections at around 3–4 cm from the proximal end of roots (Villordon et al., 2009c). Sections for all ARs were observed for each plant.

Sections were stained for one minute and rinsed in running water until there was no excess stain around the sections (O'Brien & McCully, 1981). 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 determine the AR developmental stages. Some main anatomical features of roots were examined from those photos. The number of protoxylem elements was observed at 10 DAT. Protoxylem element numbers at this stage were classified into tetrarch, pentarch, hexarch, septarch, octarch, ennearch and decarch. The other features such as initial development of IRVC, RVC, AC and LC (lignification of more than 50% of the steles) were observed throughout the sampling times.

Classifications of AR development stage

Adventitious roots initiate primary cambium, which connects to form IRVC and gradually, RVC (Villordon et al., 2009b; Wilson & Lowe, 1973; see Figure 3.1 for illustration). In some ARs, the differentiation of the vascular cambium was accompanied by the appearance of AC around the central metaxylem and protoxylem elements and this constitutes the formation of SRs (Belehu et al., 2004; Villordon et al., 2009c; Wilson & Lowe, 1973). In some other roots, the activity of vascular cambium results in heavy lignification of the stele cells (Togari, 1950; Villordon et al., 2009c; Wilson & Lowe, 1973). Those roots develop into fibrous roots. Therefore, the activity of cambium determines the development of ARs to be SRs, PRs or lignified roots. The development of cambia in ARs is described briefly below (See Figure 3.1).

In this experiment, classification of AR development was based on criteria described by Wilson and Lowe (1973). They classified ARs into three stages: initiated SRs, PRs and lignified roots. Initiated SRs were ARs in which circular AC was observed around the central

metaxylem and around the primary xylem poles (Figure 3.2A). For the initiated SRs without the central metaxylem cell, primary cambia were associated with meristematic activity in the pith cells and AC was formed around primary xylem elements (Figure 3.2.B). Adventitious roots were classified as PRs when at least one of the protoxylem points connected to central metaxylem by a strand of LC and some meristematic activity was around the central metaxylem (Figure 3.2C). Lignified roots were ARs that developed heavily lignified steles, xylem rays, a broad secondary cortex and limited secondary phloem (Figure 3.2D). In this study, those roots with more than 50% lignified stele were considered lignified roots. Therefore, the classification of sweetpotato roots in the early stage was mainly based on the anatomical changes, especially on the cambial development (Figure 3.2).

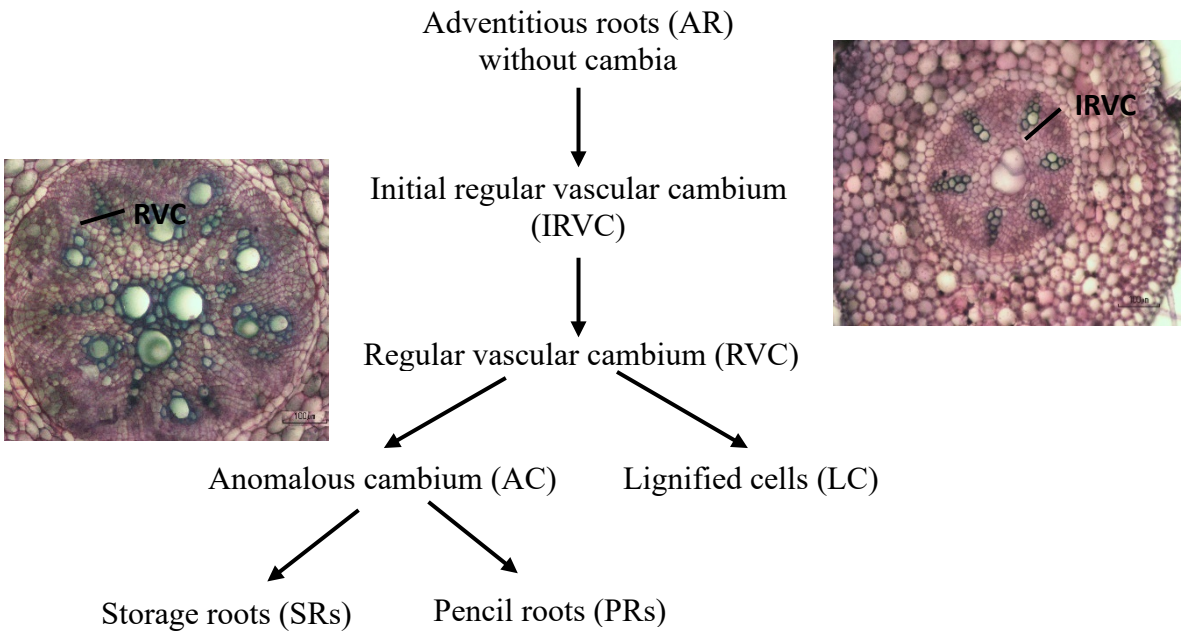

Figure 3.1. Cambial development stages to form different types of roots based on descriptions of Wilson and Lowe (1973).

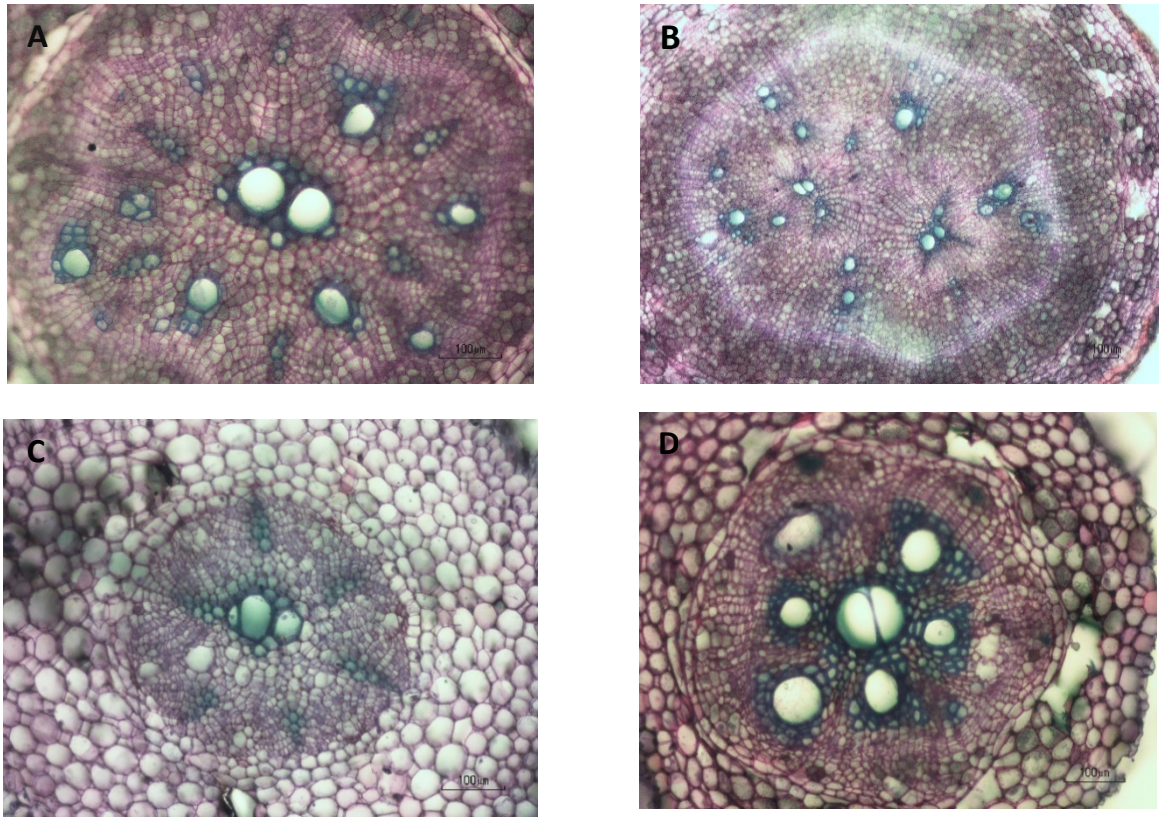

Figure 3.2. Micrograph transverse sections of AR development stages at 21 DAT: (A) initiated SR with AC surrounding the central metaxylem and xylem elements; (B) initiated SR without central metaxylem, AC surrounds xylem elements; (C) Pencil root with one of protoxylem elements connected to the central metaxylem and some AC developed around protoxylem elements and the central cell; (D) lignified root with more than 50% of stele cell lignification as indicated by the green colour.

Morphological characteristics of roots

Roots from plants for anatomical observations were collected individually. The morphological characteristics of sweetpotato roots were detected at 10, 21, 35 and 49 DAT for the entire system of roots using an Epson Perfection V700 Photo Scanner (Seiko Epson, Nagano, Japan). The WinRHIZO Pro software (version 2012a; Regent Instruments Inc., Quebec, Canada) was utilised to analyse root images. Adventitious roots were floated in a transparent tray and scanned to acquire images. Where necessary, roots were divided into smaller sections to make sure they could all spread out in the tray. The following characteristics were recorded: total root length, average RD, and total root volume.

Soluble sugar and starch contents in vines and roots of sweetpotato

Three plants from each treatment were harvested at 10, 21, 35 and 49 DAT for soluble sugar and starch analysis. 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 to stop enzymatic sugar conversion in tissues, 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.

Soluble sugar and starch contents in both vines and roots were extracted by methanol:chloroform:water (MCW) solution (Dickson, 1979; Rose et al., 1991). Overall, samples were extracted three times by MCW solution. They were centrifuged for 10 minutes at 3000 x *g* to separate the supernatant (soluble sugar) from the pellet (starch). Then, the supernatant was placed in a refrigerator while the pellet was placed in a fume hood overnight to dry. The next day, perchloric acid (35%) was added to the tubes containing the dried pellet to extract starch.

The measurements of soluble sugar and starch were conducted the day following extraction. The colorimetric phenol-sulfuric acid assay was used to determine the concentrations of sugar and starch (Dubois et al., 1956). The soluble sugar supernatant was taken from the fridge, and measured for the volume of the water:methanol fraction (soluble sugars) using the graduation on the centrifuge tube (generally 6.5 mL). A 6-point standard curve for glucose for the range 0–100 µg mL^{-1} was prepared. The determination of glucose content was done with three repetitions. The absorbance of samples was recorded at a wave length of 490 nm using a spectrophotometer. The amount of sugar and starch in each sample was determined using the standard curve. The concentrations of sugar or starch in samples were calculated accordingly.

Nitrogen acquisition in sweetpotato

Dried samples from three plants for carbohydrate analysis were also used to determine N acquisition. The concentration of C and N in different parts of the plants was analysed using TruMac® Carbon/Nitrogen Analyser (LECO Corporation, Michigan, USA).

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

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

3.2.7. Statistical data analysis

All data recorded from each sampling event were analysed using one-way ANOVA using the IBM® SPSS® software statistical package (version 25; IBM, New York, USA). Because different plants were sampled in each harvest, two-way ANOVA (rather than repeated measure ANOVA) was used to analyse the interactive effects of N treatments and sampling times on anatomical root features, morphological characteristics of roots, soluble sugar and starch accumulation, N acquisition, and the number of initiated SRs. This analysis allowed for testing the global effects of N levels, time and the interactive effect of N levels by time over the study period. To test the specific effects of N treatments at specific times, one-way ANOVA was also used to assess the effect of N treatments for each harvest. All data in percentages were arcsine-transformed for analysis in SPSS. The numbers of ARs were transformed using log 10 while other data were square root transformed in SPSS. All of the posthoc tests were conducted with Tukey HSD. A *P*-value of less than 0.05 was regarded as statistically significant. Graphs were produced using SigmaPlot® software package (version 14; SYSTAT Software, Inc., California, USA).

3.3. Results

3.3.1. Effects of N rates on sweetpotato anatomical root features

There was no significant effect of N treatments on the AR count summed from two subterranean nodes of transplants. Those root from the cut end and upper nodes were excluded from our study. The total AR number of Orleans ranged roughly between 7 and 10 (Table 3.1). Most AR counts increased in the first three weeks, and then decreased before remaining stable from 35 DAT. The two-way ANOVA showed that both the main effect of N levels and interactive effect of N treatment x time were not significant ($P>0.05$). However, the main effect of harvesting time was significant differences ($P<0.001$).

Table 3.1. The effects of N supply levels on the number of ARs per plant in different sampling times

Treatment	10 DAT	21 DAT	35 DAT	49 DAT	56 DAT	ANOVA
N0	7.3±0.3	9.0±0.6	8.7±0.3	8.3±0.3	8.7±0.3	N: $P = 0.71$
N50	7.3±0.3	9.3±0.7	8.7±0.3	8.7±0.3	8.7±0.3	T: $P < 0.001$
N100	7.7±0.3	9.7±0.3	8.7±0.7	9.0±0.6	8.3±0.3	N x T: $P = 0.88$
N200	7.3±0.7	8.7±0.3	9.3±0.3	8.7±0.3	9.0±0.6	
P value	0.802	0.574	0.642	0.743	0.743	

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 level, sampling time and N level by time are shown. *P* values from one-way ANOVA analysis for each sampling date are presented within columns.Abbreviations: N = N level; T= Time.

The number of protoxylem elements in Orleans roots at 10 DAT ranged between 4 and 9. There was no significant effect of N rates on the arrangement of the vascular cylinder. The arrangement of the central cylinder was mainly pentarch and hexarch, which accounted for a combined 68–82% of the total ARs at this stage (Figure 3.3).

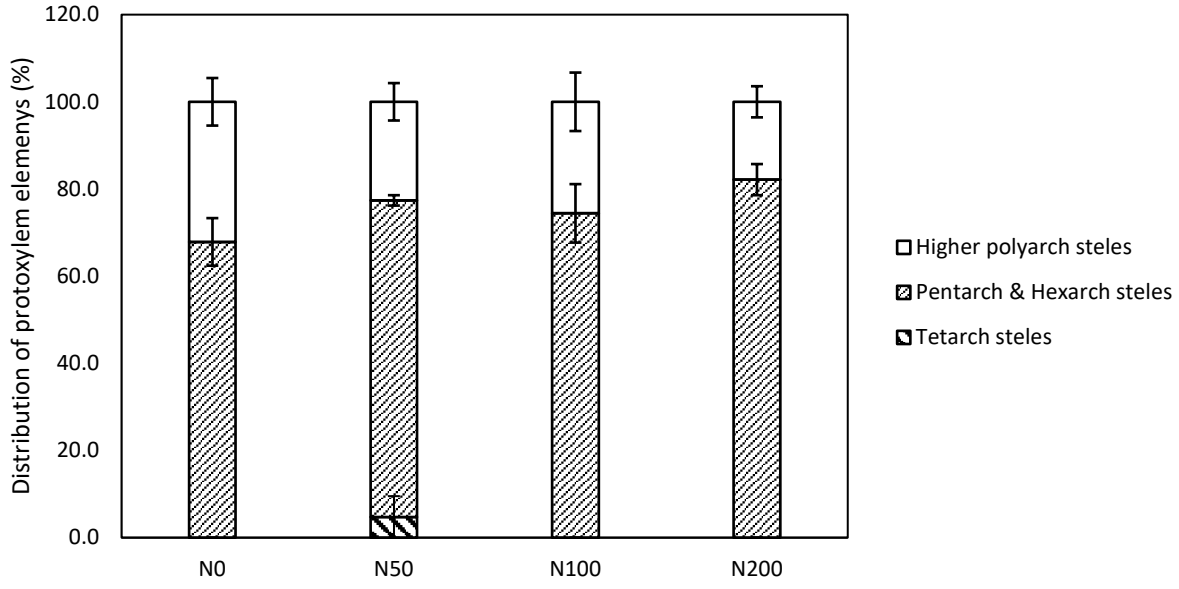

Figure 3.3. Distribution of AR protoxylem elements in Orleans sampled at 7 DAT; the error bars indicate the SE of the mean value. The error bars indicate the SE of the mean value (n=3)

Different anatomical structures were observed at certain harvesting times (Figure 3.4). Adventitious roots without cambium were only observed at 10 and 21 DAT and IRVC were

found in roots at 10, 21 and 35 DAT (Figure 3.5A; Figure 3.5B). The activity of vascular cambium led to the formation of RVC from 21 to 49 DAT (Figure 3.5C). The other structures, such as AC and LC, were detected from 21 DAT until the last harvest (Figure 3.5D; Figure 3.5E). These structures appeared sequentially as a natural process of root development.

The nitrogen application levels showed a significant impact on the development of vascular cambium. In the early stage of root growth, N50 and N100 promoted cambium formation by increasing the percentage of roots with IRVC development at 10 DAT (Figure 3.5B). In contrast, N0 and N200 treatments inhibited the initiation of vascular cambium as indicated by a significantly higher rate of roots forming IRVC than other treatments. As a result, a higher rate of roots without cambium was observed in those treatments.

The distribution of roots with IRVC and RVC decreased over the time (Figure 3.5B; Figure 3.5C). At 10 DAT, a significantly higher rate of roots developing IRVC and RVC was observed in the N50 and N100 treatments. In the next sampling time, N50 and N100 continued to promote cambial activity as a higher rate of roots with RVC and AC at 21 DAT (Figure 3.5C; Figure 3.5D).

The distribution of roots with AC formation in all treatments increased until 49 DAT, and then remained stable (Figure 3.5D). The N100 treatment showed the highest percentage of roots with AC development on all sampling dates while the control showed the lowest rate. At 21 DAT, the percentage of roots observed for AC initiation in the N200 treatment was similar to that of the control treatment. However, anomalous cambium development\increased sharply and showed no significant differences compared to other N treatments at 49 and 56 DAT. Therefore, the N200 treatment delayed the formation of AC at 21 DAT, but after that it promoted AC development. In general, the N application increased the rate of roots with AC formation between 21 and 56 DAT.

There were no significant differences among treatments for the percentage of roots with more than 50% of LC at 21 and 35 DAT (Figure 3.5E). However, a significantly higher rate of lignified roots in N0 was observed at 49 and 56 DAT.

Basically, in N0 treatment cambium formation and development were all inhibited, and lignification was promoted from day 49. However, N50 and N100 treatments enhanced the initiation of vascular cambium and AC throughout SR formation. The N200 treatment

reduced the formation of procambium during the early stages, but it started to promote the AC from day 35 to a similar extent than N50 and N100 treatments.

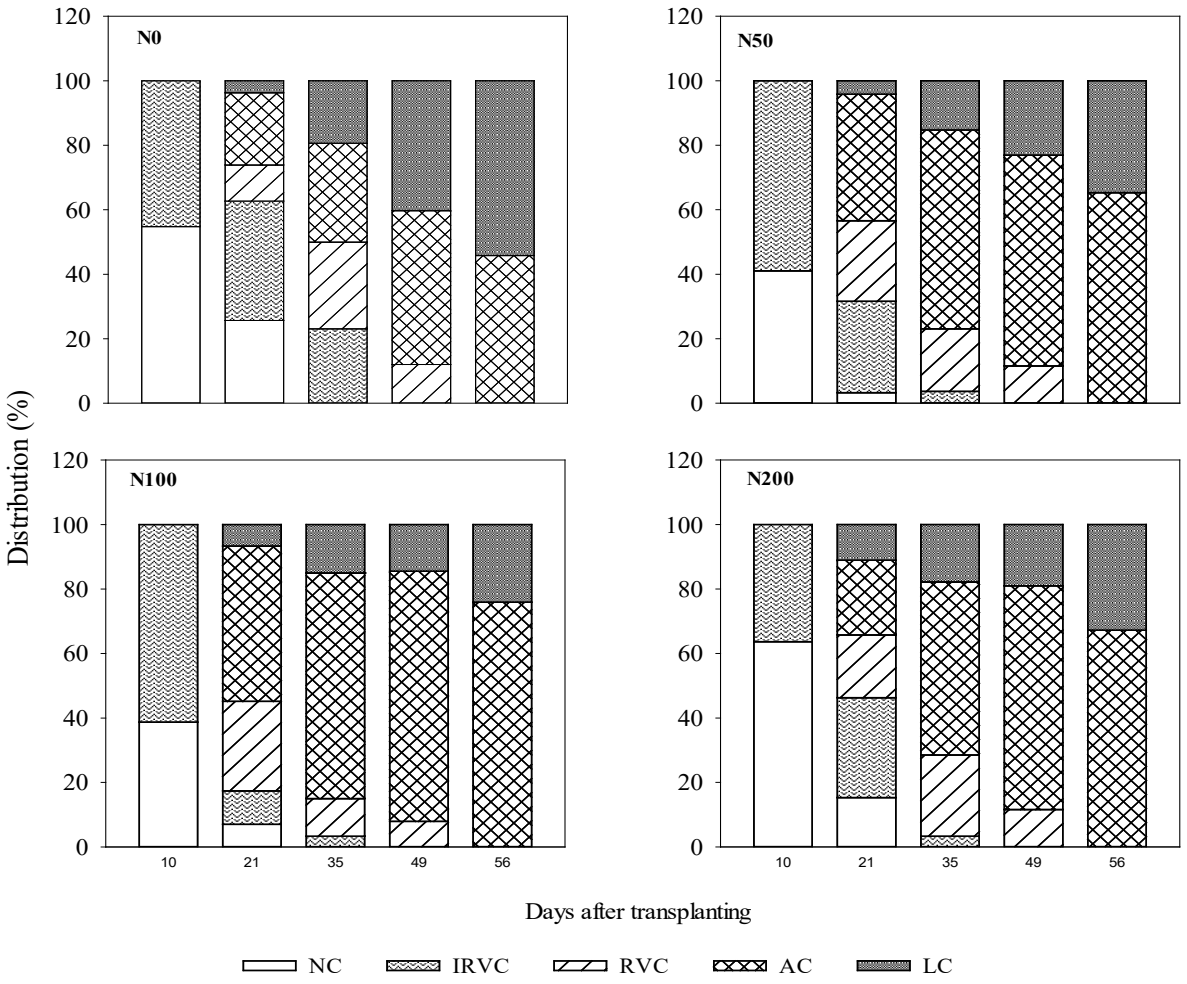

Figure 3.4. Effects of N supply levels on the development of anatomical features of roots on different sampling dates.Values are indicated as mean (n=3).

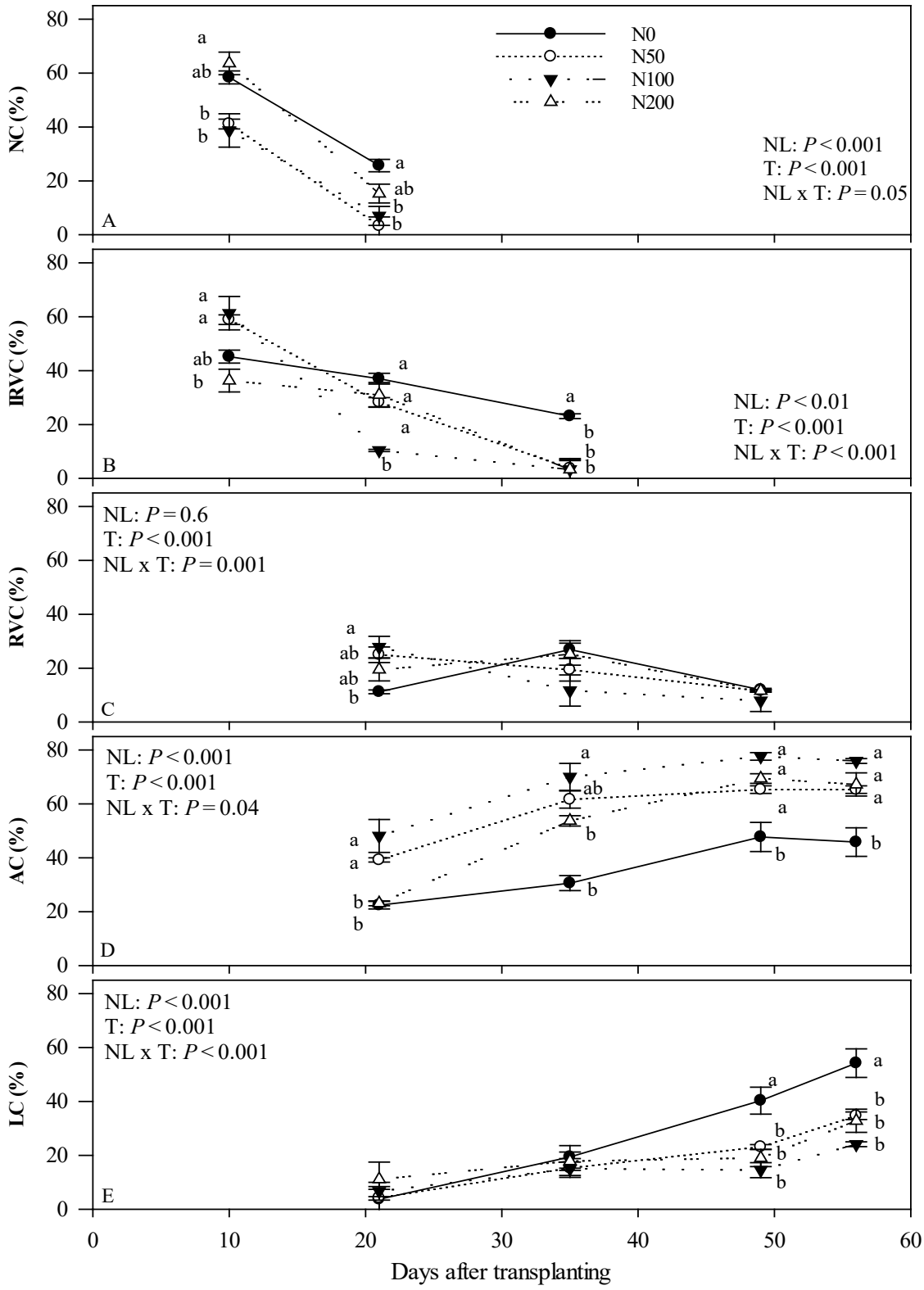

Figure 3.5. Effects of N supply levels on the development of anatomical features of roots at different sampling times.

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 significantly different among treatments on single sampling dates using one-way ANOVA (Tukey's HSD).

Abbreviations: IRVC = Initial regular vascular cambium; RVC = Complete regular vascular cambium; AC = Anomalous cambium; LC = Lignified cells; N = N level; T = Time.

In the present experiment, effects of N application levels on the formation of SRs were statistically different among treatments, with the SR rate increasing over the time (Figure 3.6A). The N100 treatment showed the highest rate of SRs compared to other treatments and the lowest was observed in the treatment N0. The treatment N200 had a similar rate of SR to the N0 treatment at 21 DAT, but it increased noticeably and was significantly higher than N0 from day 35. After that, it passed above that of N50. However, the percentage of SRs in N200 treatment was still lower than for the N100 treatment.

The results showed that the difference on the percentage of PRs among N treatments was not statistically significant (Figure 3.6B). The rate of PRs increased over the time and reached the highest rate at the final sampling. The distribution of PRs varied from 4 to 7% at 21 DAT and then increased to approximately 10-15% at 56 DAT. The main effect of time on PR was significant (P=0.003).

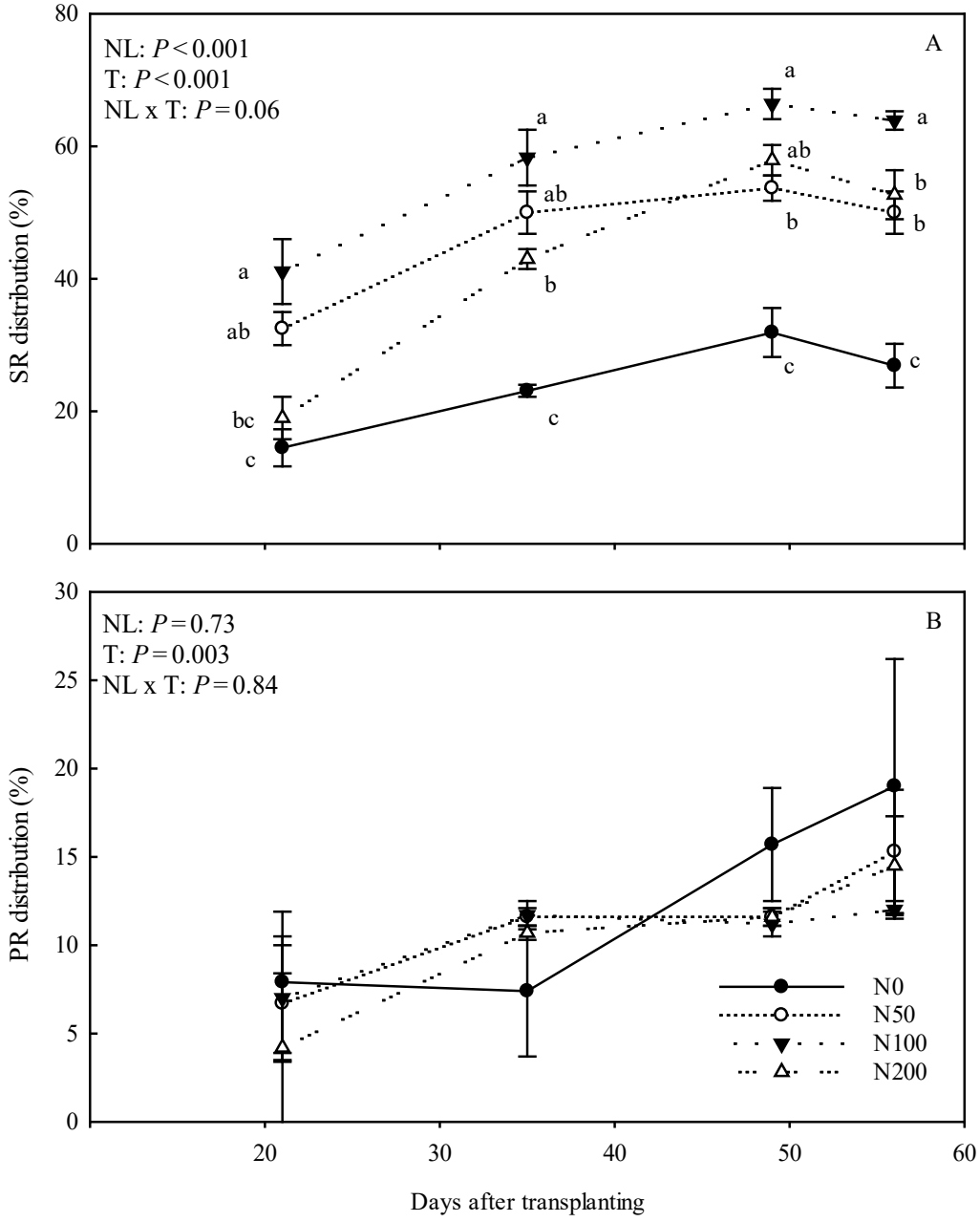

Figure 3.6. Effects of N supply levels on the formation of SRs and PRs over time.

(A) Distribution of initiated SRs; (B) Distribution of PRs. 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 SR and PR are shown. Different letters are significantly different among treatments on each sampling dates using one-way ANOVA (Tukey's HSD, $P<0.05$). The values are indicated as mean ± SE (n=3).

Abbreviations: SR = Storage root; PR = Pencil root; N = N level; T = Time.

3.3.2. Effects of N rates on root morphology, plant performances and yield of sweetpotato

The total root length increased over time for N treatments whereas N0 showed a similar trend by 35 DAT and then reduced slightly in the next two observations (Table 3.2). Significant differences among treatments were observed on all sampling dates except 10 DAT. The N0 treatment showed the lowest average root length while the N100 and N200 treatments showed the highest average root length. Two-way ANOVA results showed that the interactive effect of N and time was significant for total root length (Table 3.2). Similarly, the total root volume followed a similar trend to the total root length with the figures increasing over time, and higher rates of N treatments had higher values.

The diameter of Orleans roots was affected by N treatments after 35 DAT (Table 3.2). The effects of N rates on RD for each harvest were statically different at 49 and 56 DAT. At the final sampling time, the N200 had the highest RD (largely due to the faster growth of SRs) while the N0 had the lowest. The average diameter of roots for each N treatment on different harvesting dates was significantly different. Two-way ANOVA also showed the main effect of N levels and time were significant different on the RD (P<0.001). However, the interactive effect of N level and time was no significant (P=0.37).

All N addition treatments showed significantly higher growth parameters (above-ground dry weight and root dry weight) compared to N0 (Table 3.3). Higher levels of N had the higher weight of above-ground and root biomass, as well as larger SRs. The N200 treatment had the highest SR weight at 34.8 g plant^{-1}; although it was not statistically higher than that of the N100 treatment.

Table 3.2. Effect of N supply levels on the total root length of Orleans on different sampling dates

	Treatment	10 DAT	21 DAT	35 DAT	49 DAT	56 DAT	ANOVA
TRL (cm)	N0	257±16	363^{b}±26	424^{b}±26	352^{c}±23	272^{c}±20	NL: P<0.001
	N50	257±22	602^{a}±44	846^{a}±117	774^{b}±39	755^{b}±31	T: P<0.001
	N100	233±12	524^{a}±16	1014^{a}±15	1020^{a}±56	1034^{a}±44	NL x T: P<0.001
	N200	260±20	504^{a}±42	861^{a}±46	1025^{a}±45	1189^{a}±31	
	P value	0.69	0.01	<0.001	<0.001	<0.001	
RD (mm)	N0	0.99±0.02	0.73±0.02	0.69±0.02	0.69^{b}±0.01	0.70^{c}±0.01	NL: P<0.001
	N50	1.02±0.03	0.71±0.01	0.70±0.01	0.72^{b}±0.01	0.73^{bc}±0.01	T: P<0.001
	N100	1.02±0.06	0.74±0.01	0.74±0.01	0.77^{ab}±0.03	0.76^{b}±0.02	NL x T: P=0.37
	N200	1.05±0.07	0.76±0.03	0.74±0.01	0.83^{a}±0.01	0.85^{a}±0.01	
	P value	0.83	0.23	0.09	<0.001	<0.001	
RV (cm^3)	N0	1.7±0.1	3.2^{b}±0.1	4.5^{b}±0.2	3.4^{c}±0.2	3.1^{d}±0.2	NL: P<0.001
	N50	1.8±0.1	5.5^{a}±0.2	9.7^{a}±0.5	11.3^{b}±0.5	11.7^{c}±0.5	T: P<0.001
	N100	2.1±0.2	6.1^{a}±0.3	14.1^{a}±0.3	17.4^{a}±0.4	18.2^{b}±0.5	NL x T: P<0.001
	N200	2.1±0.2	6.0^{a}±0.2	13.1^{a}±0.3	20.9^{a}±0.6	23.2^{a}±0.5	
	P value	0.12	<0.001	<0.001	<0.001	<0.001	

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

Means followed by different letters are significantly different (P<0.05) within columns (Tukey's HSD, one-way ANOVA). Two-way ANOVA results, including effect of N level, sampling time and N level by time are shown in the last column.

Abbreviations: TRL = Total root length, RD = Root diameter, RV = Root volume; NL = N level; T= Time.

Table 3.3. Effect of N supply levels on above-ground dry weight, root dry weight, SR length, SR diameter and SR weight at 56 DAT

Treatment	ADW (g plant^{-1})	RDW (g plant^{-1})	SRL (mm)	SRD (mm)	FSRW (g plant^{-1})
N0	1.1^{d}±0.1	0.5^{d}±0.1	51.2^{b}±3.1	4.2^{c}±0.4	4.3^{c}±0.8
N50	3.3^{c}±0.2	2.0^{c}±0.1	92.0^{a}±5.0	5.7^{b}±0.1	17.9^{b}±0.9
N100	5.9^{b}±0.3	3.6^{b}±0.2	108.5^{a}±12.2	7.8^{ab}±0.7	27.5^{ab}±2.1
N200	9.3^{a}±0.2	6.0^{a}±0.3	120.8^{a}±7.4	8.2^{a}±0.4	34.8^{a}±2.2
P value	<0.001	<0.001	0.001	0.001	<0.001

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 (Turkey's HSD, one-way ANOVA).

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

3.3.3. Effects of N rates on soluble sugar and starch accumulation in Orleans plants

3.3.3.1. Effect of N rates on the concentration of soluble sugar and starch

The concentration of soluble sugar and starch in both vines and roots were the highest in the N0 treatment (Figure 3.7). While vine soluble sugar concentration of N0 increased, that of other treatments decreased during SR initiation (Figure 3.7A). However, soluble sugar concentration in roots followed a different pattern, with an increasing trend over the study time in all treatments (Figure 3.7B). At 49 DAT, the highest concentration of soluble sugar in roots was observed in N0 at around 100 mg g^{-1}, followed by the N200 treatment, while N50 and N100 treatments had similar concentrations. Root starch concentration grew over the time in all treatments (Figure 3.7D). Vine starch concentration increased clearly over time in treatment N0 while it remained stable in the N100 and N200 treatments (Figure 3.7C).

Non-structural carbohydrates (NSC) in plants are mainly sugar and starch, so it has been calculated by summing soluble sugar and starch. The NSC concentration in vines and roots followed similar patterns to starch, which accounts for about 80% of NSC. The N0 treatment showed the highest concentration of NSC in both vines and roots, compared to other treatments (Figure 3.7E; Figure 3.7F), suggesting the suppression of photosynthate formation in SRs due to N deficiency. There was no statically significant difference found in the NSC concentration between the N100 and N200 treatments.

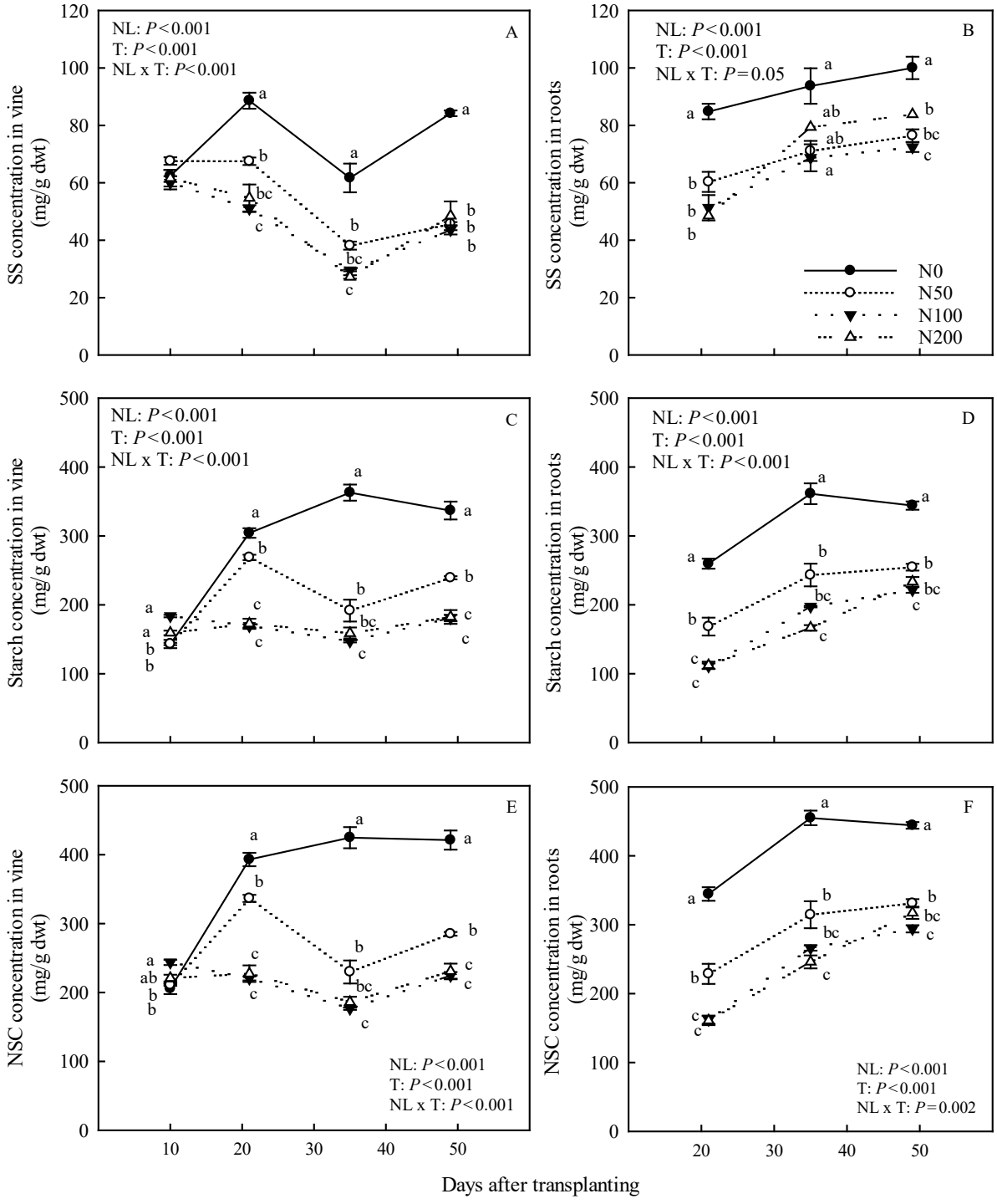

Figure 3.7. Concentration of carbohydrate as affected by N rates.

(A) Soluble sugar concentration in vines; (B) Soluble sugar concentration in roots; (C) Starch concentration in vines; (D) Starch concentration in roots; (E) Carbohydrate concentration in vines; and (F) Carbohydrate concentration in roots.

Values are indicated as mean ± SE (n=3). ANOVA results are based on square root transformed data. Two-way ANOVA results, including effects of N level, harvesting date, and N level by time interaction on SS, starch and NSC concentrations are shown. Different letters indicate significant

difference among treatments on the same harvesting dates using one-way ANOVA (Tukey's HSD, $P<0.05$).

Abbreviations: SS = Soluble sugar; NSC = Non-structural carbohydrates; N = N level; T = Time.

3.3.3.2. Effect of N rates on the soluble sugar and starch accumulation in plants

As the root biomass at 10 DAT was insufficient for analysis, these carbohydrates were analysed at three sampling dates starting from 21 DAT. In general, the total soluble sugar and starch, as well as NSC accumulation, increased over the time in all treatments except the N0 treatment (Figure 3.8). The accumulation of these carbohydrates was positively related to N application levels in most observations, with the lowest and highest amount in N0 and N200, respectively.

In the first three weeks after transplanting, the total soluble sugar content in vines was similar in all treatments (Day 10: $P = 0.51$; Day 21: $P = 0.15$) (Figure 3.8A; Figure 3.8B). After that, the effects of N application levels on vine soluble sugar were significant (Figure 3.8C; Figure 3.8D). There was also a significant interactive effect of N supply levels and harvesting time on soluble sugar in vines (Table 3.4). In contrast, vine starch accumulation was significantly different among treatments over this study period. In the final sampling, the N200 treatment had significantly higher NSC in vines and roots than those in other treatments and the untreated N treatment had the lowest NSC with only 71 mg plant^{-1} (Figure 3.8D). In roots, the soluble sugar and starch accumulation followed a similar trend of N supply treatment to vines. The N200 treatment had the greatest amount of soluble sugar, starch and NSC on all sample dates, except 21 DAT when N0 had higher NSC accumulation than other N treatments.

Despite the total NSC accumulation in vines being generally higher than that in roots, the photosynthate storage shifted from vines to roots over the time, except for N0, as indicated by the decreasing vine to root NSC ratio (Table 3.5). There was no distinct change in this ratio between vines and roots for the N0 treatment. However, this ratio was reduced noticeably in other treatments from 5–7 at 21 DAT to around 1.5 at 49 DAT in all N added treatments. No significant differences were found on the carbohydrate vine/roots ratio at 35 DAT (P=0.13). The two-way ANOVA results showed that the effects of N levels, harvesting times and the interactive effect of N and time on the total NSC in vines, roots and ratio of vine and roots were statistically significant.

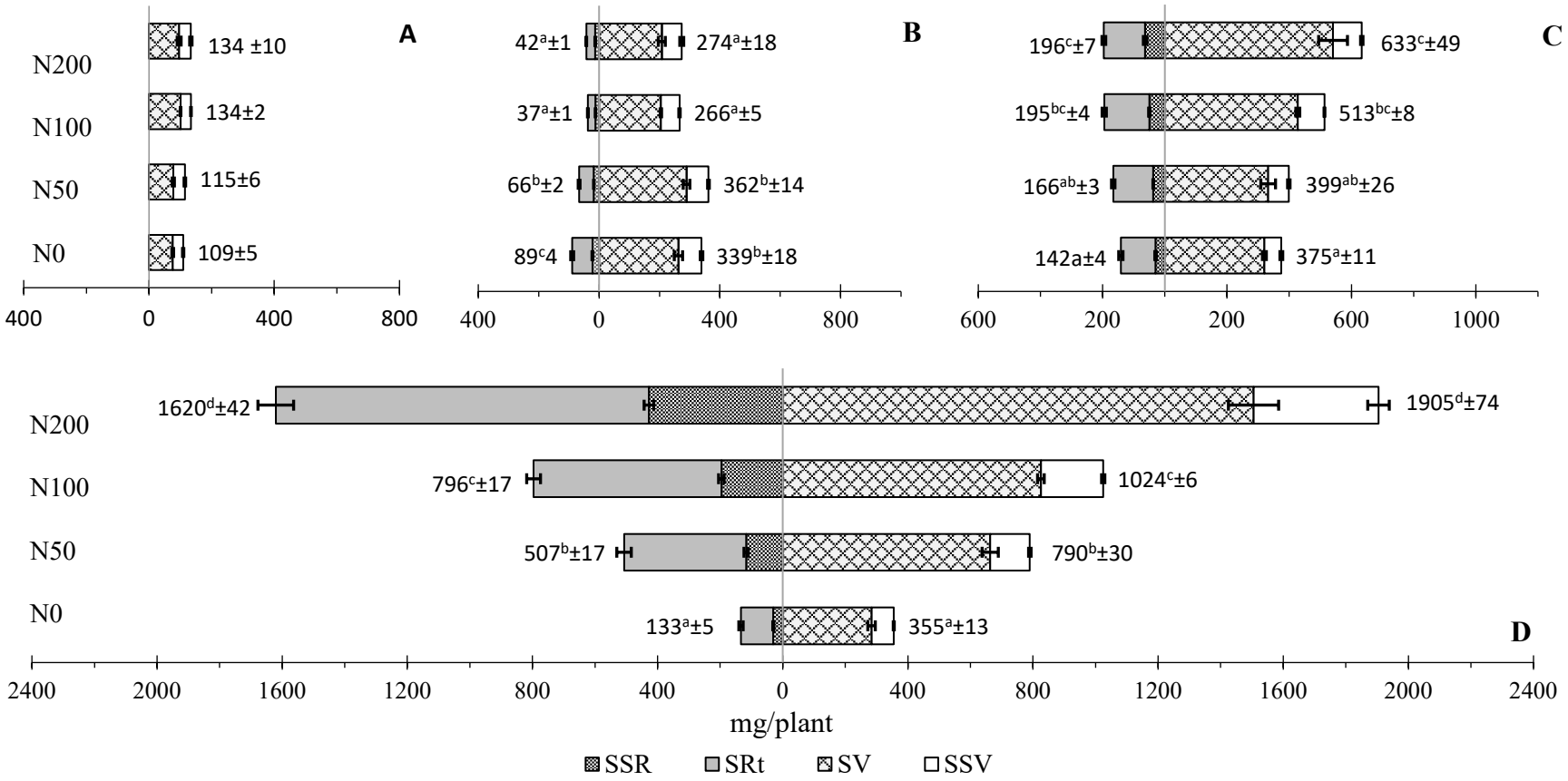

Figure 3.8. Effect of N treatments on the accumulation of soluble sugar and starch in plants at various sampling times. (A) 10 DAT; (B) 21 DAT; (C) 35 DAT; and (D) 49 DAT.

The x-axis represents the soluble sugar (SS)/starch and the y-axis represents the treatments. Numbers in the left and right are mean values of total NSC (SS + Starch) in roots and vines, respectively, followed by standard error (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letters are significantly different among treatments (Tukey's HSD, $P<0.05$).
Abbreviations: SRt = Starch in roots; SSR = Soluble sugar in roots; SSV = Soluble sugar in vine; SV = Starch in vine.

Table 3.4. The main and interactive effects of N treatments and time on the accumulations of soluble sugar, starch and NSC in sweetpotato plants

Factor	SSV	Starch in vine	NSC	SSR	Starch in roots	NSCR
N level	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N level x time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Two-way ANOVA results are based on square root transformed data.

Abbreviations: SSV = Soluble sugar in vines; SSR = Soluble sugar in roots; NSCV = Non-structural carbohydrates in vine; NSCR = Non-structural carbohydrates in roots.

Table 3.5. The effect of N treatments on the vine to root NSC ratio on different sampling dates

Treatments	21 DAT	35 DAT	49 DAT	ANOVA
N0	3.9^{b}±0.5	2.7±0.2	2.7^{a}±0.1	NL: $P = 0.02$
N50	5.5^{ab}±0.3	2.4±0.1	1.6^{b}±0.1	T: $P < 0.001$
N100	7.2^{a}±0.2	2.6±0.1	1.3^{bc}±0.1	NL x T: P <0.001
N200	6.5^{a}±0.6	3.3±0.4	1.2^{c}±0.1	
P value	0.003	0.13	<0.001	

The table presents the mean values followed by standard errors (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letters are significantly different (Tukey's HSD, P<0.05) within columns (one-way ANOVA). The two-way ANOVA results, including N level, time and interactive effect of N level by time are shown in the last column.

Abbreviations: NL = N level; T= Time.

3.3.4. Effect of N supply levels on Nitrogen acquisition N in plants

The N concentration in vines and roots was significantly different among treatments at P<0.001. Overall, the concentration of N in all of the N-added treatments increased over the study period while that in the N0 treatment followed an opposite trend (Figure 3.9A; Figure 3.9B). A significantly higher N acquisition in both vines and roots was observed in all added N treatments compared to N0 on each sampling date (Figure 3.10).

Nitrogen supply treatments have negative or no effect on C:N ratio in the present experiment. While the C:N ratios in vines and roots of the N0 treatment increased noticeably during the growth period, other treatments decreased slightly or remained stable. The highest C:N ratio in both vines and roots was found in the N0 treatment (Figure 3.8C; Figure 3.8D). At 49 DAT, the N200 treatment had the lowest C:N ratios in both vine and roots.

Significantly a higher ratio was observed in the treatment N100 and then N50 compared to other treatments.

Nitrogen recovery efficiency (NRE) increased over the study time in all treatments with N (Figure 3.11). The N200 treatment had the lowest NRE at 21 and 35 DAT, while the N100 treatment had the highest rate on all sampling dates. At 49 DAT, NRE in the N100 and N200 treatments were the same and significantly higher than N50.

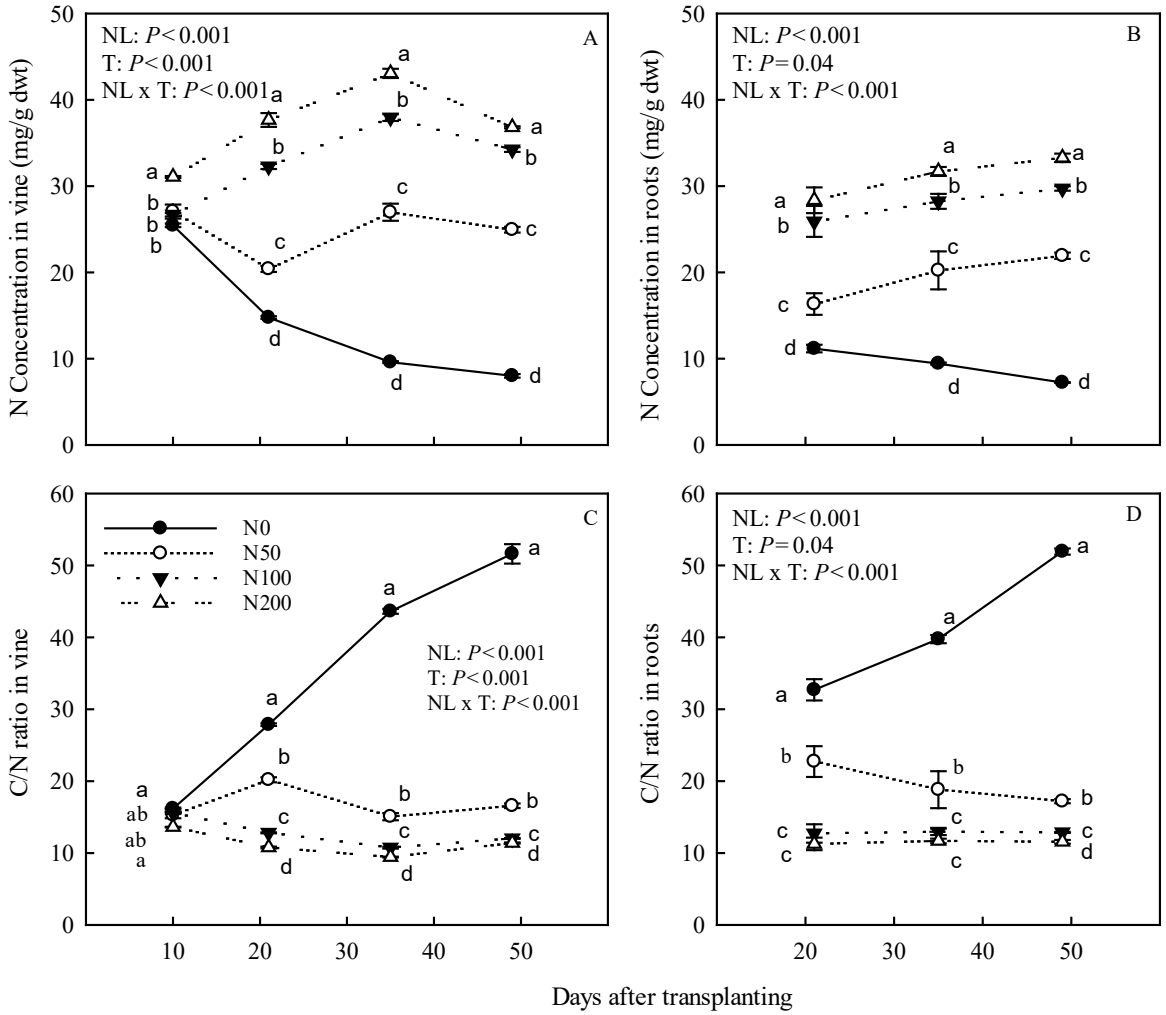

Figure 3.9. N concentration in sweetpotato and C/N ratio as affected by N supply levels. (A) Values of N concentration in vines; (B) N concentration in roots; (C) C:N ratio in vine; and (D) C:N ratio in roots are mean ±SE (n=3).

ANOVA results for N concentration in both vines and roots are based on square root transformed data. Two-way ANOVA results, including effects of N level, harvesting date, and N level by time are shown. Different letters indicate significant difference among treatments on the same harvesting date using one way ANOVA (Tukey's HSD, $P<0.05$).

Abbreviations: NL = N level; T = Time.

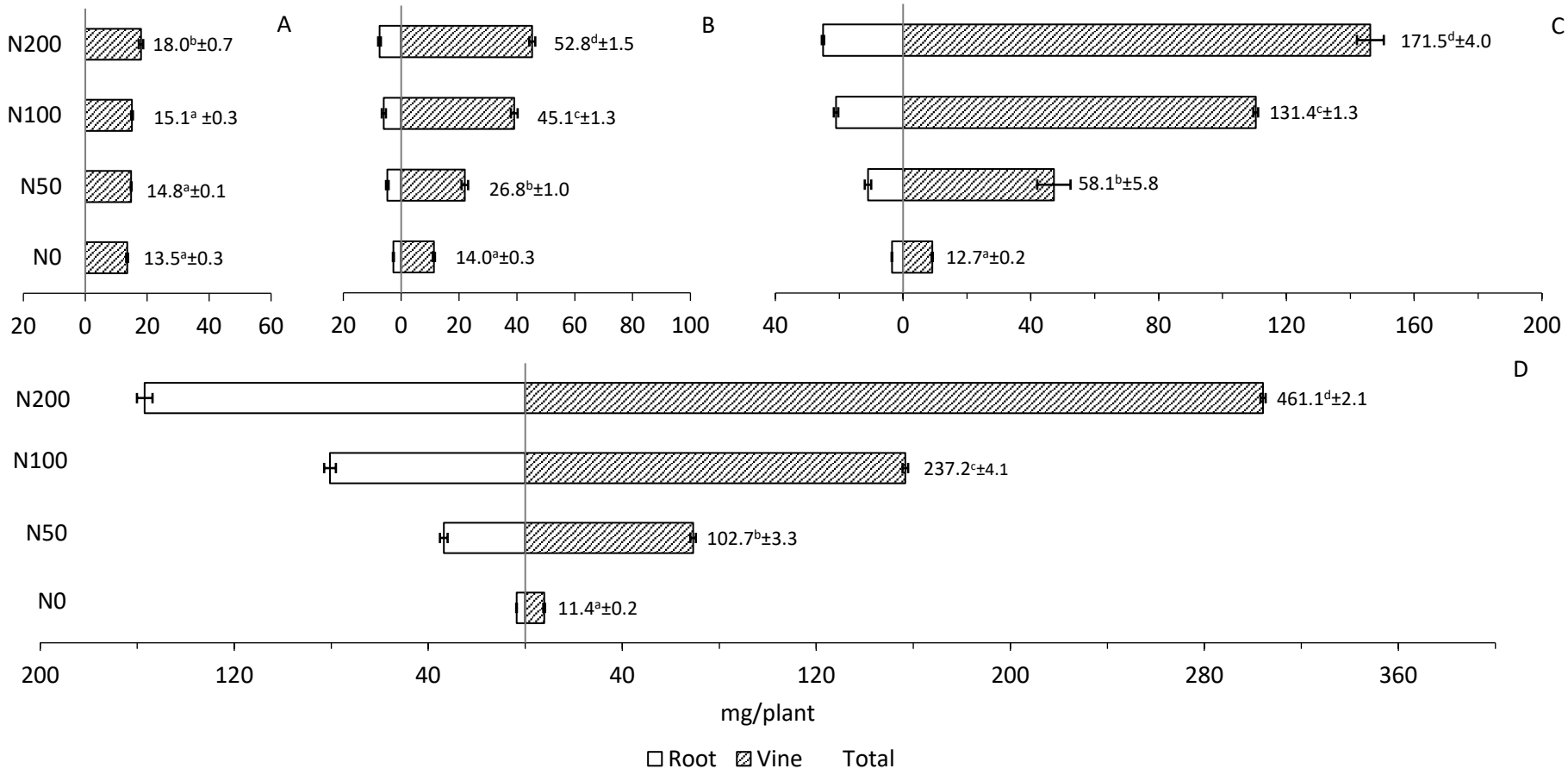

Figure 3.10. Effects of N treatments on the acquisition of N (mg plant^{-1}) in vines and roots on (A) 10; (B) 21; (C) 35; and (D) 49 DAT.

The x-axis represents the N acquisition and the y-axis represents the treatments. Numbers are mean values of total N acquisition of the whole plant (vine + root) followed by standard error (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letter are significantly different among treatments (Tukey's HSD, $P<0.05$) using one-way ANOVA.

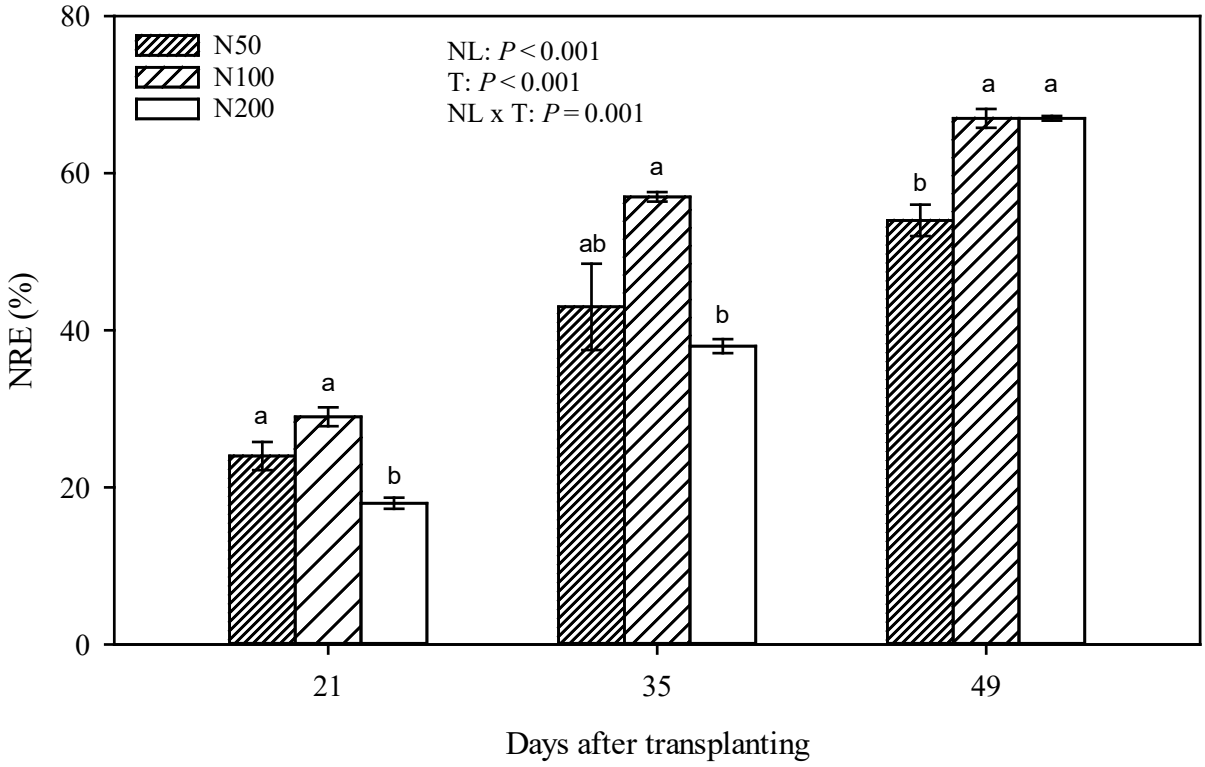

Figure 3.11. The efficiency of N use in sweetpotato under different N supply levels.

Values are indicated as mean ± SE (n=3). ANOVA results are based on arcsine-transformed data. Two-way ANOVA results including the effect N level, time, and N level by time interaction are shown. Means followed by different letters are significantly different among treatments using one-way ANOVA (Tukey's HSD, $P<0.05$).

Abbreviations: NL = N level; T = Time.

3.4. Discussion

3.4.1. Effects of N rates on sweetpotato anatomical root development

In our study, N application at different levels did not affect the AR count. Similarly, in a previous study no difference in the number of ARs was found between untreated N and application of 50 kg N ha^{-1} (Villordon et al., 2013a). Also, in another experiment, four NO_3^- levels of 1–50 mM did not affect the number of ARs (Kim et al., 2002). This may be due to ARs originating from preformed root primordia or wound calluses on the stem (Belehu et al., 2004; Isbell, 1931). Our research focused on SR initiation, and so only ARs developed from nodal regions were examined. It was recorded that the preformed root primordia were presented in sets of 4–10 (Belehu et al., 2004) and the number differed between nodes (Ma et al., 2015). For this reason, the most uniform

cuttings based on the length, diameter and number of fully opened leaves were carefully selected for planting. This helped to reduce the variation of AR numbers between cuttings. The number of ARs increased in the first three weeks and then decreased. A similar trend was reported in previous studies across different cultivars. The AR numbers in Beauregard reached a peak at 26 DAT (Villordon et al., 2009c). In another study, Wilson and Lowe (1973) documented that the root count of some West Indian sweetpotato varieties peaked at 14 to 28 DAT and subsequently decreased. The result on the number of ARs in this experiment indicated that there was no effect of N levels on this parameter under our experimental conditions.

In this experiment, the arrangement of protoxylem elements in Orleans ARs were mainly pentarch and hexarch, which comprised 72–86% of the total ARs at 10 DAT among different treatments. This finding was in line with previous experimental results in Beauregard cultivar (Villordon et al., 2009c) and in some West Indian sweetpotato cultivars (Wilson & Lowe, 1973). This was slightly different from Georgia Jet cultivar where around 90% of ARs were hexarch and septarch steles (Villordon et al., 2009c). According to Artschwager (1924) and Togari (1950), those roots with hexarch and septarch steles will develop into thick roots and some of them would further develop into SRs. In contrast, ARs with tetrarch steles would develop into thin roots being either fibrous roots or PRs (Belehu et al., 2004; Togari, 1950). Therefore, the majority of Orleans ARs were pentarch and hexarch and they were not affected by N supply levels.

Our results suggest that N200 treatments inhibited cambial formation in the early stage of root initiation, with 63.7% of total roots being without cambial development at 10 DAT. The lower levels of N treatments (N50 and N100) had the higher percentage of roots with cambial development than the others at this stage. Therefore, a low rate of N was required to promote the formation of cambium after transplanting. Furthermore, all N treatments had lower rates of the lignified roots compared to the control at 49 and 56 DAT. After that, a higher N application level was needed as plants grew. It has been documented that excessive N application in combination with reduced aeration decreased the activity of cambium and promoted LC (Togari, 1950), as cambial activity is controlled by cytokinin (Matsumoto-Kitano et al., 2008; Wang, 2020), which was demonstrated to be associated with N (Samuelson et al., 1992; Sattelmacher & Marschner, 1978). Cytokinin levels generally increase in response to N and P (Matsumoto-Kitano et al., 2008; Samuelson et al, 1992) and reduce in insufficient

nutrient conditions or water stress (Yang et al., 2001). In such adverse conditions, the cambial activity is inhibited (Matsumoto-Kitano et al., 2008). This suggests that cytokinin may act as regulator of cambium development. 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 removed (Wang, 2020). This phytohormone is also closely related to N concentrations (Kiba et al., 2011). Present experimental findings indicated that the moderate N supply levels promoted the initiation and development of vascular cambium and inhibited the lignification of roots.

Under our experimental conditions, the SR initiation of Orleans was observed at 21 DAT, with the appearance of AC in some roots. This is similar to the findings of Villordon et al. (2009c), who stated that the SR initiation in pots was 19 and 21 DAT for Beauregard and Georgia Jet, respectively. However, a previous report suggested that SR formation started at 42 DAT (Onwueme, 1978). A recent study on Beauregard stated that SR formation in greenhouse conditions was observed at 28 DAT (Taranet et al., 2017). Ravi and India (1999) reviewed the time frame for sweetpotato SR initiation and found that it varied widely from 1 to 13 weeks. These variations of SR initiation dates may be caused by different experimental conditions and varieties in these studies. For example, Villordon et al. (2009c) concluded that the formation of AC in Beauregard was earlier than for Georgia Jet. At 21 DAT, the percentage of roots with AC in N50 and N100 treatments were significantly higher than that of the control and N200 treatment. Then, the rate of roots with AC developments increased in the next 14 days and there were no significant differences among N treated levels, but they were higher than that of the untreated control at 49 DAT. A moderate N application is required for ARs to develop properly into SRs (Meyers et al., 2014). The maximum rate of initiated SR of Orleans in all treatments peaked at 49 DAT. In line with the present finding, Lowe and Wilson (1974) concluded that the maximum SR number was achieved during weeks 7 and 8 after transplanting for all study cultivars except I62. All three treated N levels had a higher percentage of initiated SRs than the control. Similarly, application of N in the form of NH_4^+ or amid at a rate of 60 kg ha^{-1} significantly increased the number of SRs (Si et al., 2018). Our study indicated that SR initiation date for Orleans would be about 21 DAT, which was not affected by N supply levels, but it may change under different growing conditions.

According to our results, possible mechanisms related to initiation of SRs would involve an optimal rate of N that promotes the formation of cambium, resulting in the thickening of ARs. Nitrogen contributed continuously to the activity of these cambial cells in repeated divisions to form secondary cambium and AC in SRs. Low N supply may have reduced cambial activities, and then produced a limited number of cambium but more lignified stele cells.

3.4.2. Effects of N rates on sweetpotato root morphology, plant performances and yield

Results showed that N application positively affected all root parameters of Orleans, including total root length, RD and total root volume after 10 DAT. In the first 10 DAT, available N in plants may be adequate for them to grow, so there were no significant effects of N on root characteristics among treatments. The N0 treatment had the lowest values of these root parameters over four observations at 21, 35, 49 and 56 DAT. In a previous study on Beauregard cultivar, the first-order lateral root and the second-order lateral root length increased by 78% and 2878%, respectively, in a treatment with 50 kg N ha^{-1} (Villordon et al., 2013a). Nitrogen application also increased the total root length of rice, corn and potato (Jian-Bo et al., 2010; Sattelmacher et al., 1990; Wang et al., 2005). The root volume also grew with the N application in potato (Sattelmacher et al., 1990). In a split-root experiment, N fertilised treatment increased the lateral root volume by 161% (Villordon et al., 2013b). Our results suggest that N supplemental treatments started to affect root diameter at 49 DAT, after most SRs formed. This may be due to the development of SRs, leading to higher N demand for growth. Knavel (1971) stated that N influenced the sweetpotato root size. Low amounts of N supply were demonstrated to inhibit root growth (Kim et al., 2002) and insufficient N application for sweetpotato resulted in the formation of small and fine roots (Gifford et al., 2008; Martí & Mills, 2002).

There was little information about the role of root development in SR formation of sweetpotato. The association between lateral root development, which is a major determinant of root architecture, with SR formation in Beauregard sweetpotato has been studied. Villordon et at., (2012) stated that SRs at 20 DAT had greatest lateral root count, lateral root length and lateral root surface area compared to PRs and LC. They also indicated that those lateral root traits for PRs were much higher than that of LC. Villordon

et al., (2014) indicated that successful lateral root development is associated with the reduction of lignification in the adjacent tissues of the ARs and promotes the formation of sweetpotato SRs from ARs. Our study confirmed that N supply could improve root growth and this improvement could be found after day 10 from planting. Nitrogen application from 100 to 200 mg/L in solution would provide greater improvement on all root parameters.

In this experiment, we confirmed that the growth of sweetpotato was positively regulated by N supply. Both above- and below-ground dry matter of plants in the N0 treatment were much lower than other N treatments. Similar results were found in a previous study, which showed the deficiency of N supply inhibited the dried weight accumulation of shoots and SRs and subsequently reduced the total biomass of plants (Osaki et al., 1995). According to Okpara et al. (2009), N applied at rates from 40 to 120 kg ha^{-1} significantly increased the dry shoot biomass weight of sweetpotato compared to untreated N.

There was a significant increase in SR yield at 56 DAT as affected by N supply. The application of N from 50 to 200 mg/L increased the SR weight. Similarly, Kelm et al. (2001) indicated that the application rates of N from 0.4 to 2 g pot^{-1} increased SR yield of both studied cultivars. Also, in another experiment, dry-biomass of SRs in glasshouse increased with increasing N supply from 30 to 230 kg ha^{-1} (Taranet et al., 2017). In field conditions, N application at 75 kg ha^{-1} significantly increased the SR yield while a higher level of 150 kg N ha^{-1} had no effect (Duan et al., 2019). Hence, the present experiment confirmed the roles of N in producing sweetpotato productivity.

Although high levels of N inhibited the formation of SRs in the early stages as discussed in Section 3.4.1, it is necessary for the growth of SRs after they are formed. Treatment N200 delayed cambial formation and development during 10 and 35 DAT, resulting lower rate of SRs during this period. However, it promoted the formation of SR from 49 DAT and then increased the SR weight. This can be explained by the requirement of N at different developmental stages of the sweetpotato. A recent study by Taranet et al., (2017) reported that the application of N at 0–230 kg ha^{-1} had no effect on SR biomass during the first six weeks after transplanting. After that, the treatments with high N application rates (130–260 kg ha^{-1}) showed a rapid increase in the dry SR yield during week 6 and week 12 after transplanting. Another study in sandy loam soil reported that application of N at either 75 or 150 kg ha^{-1} reduced the number of SRs compared to

no N application, but it significantly improved the SR yield of sweetpotato cultivar Jishu25 (Duan et al., 2019). Therefore, a moderate level of soil available N promoted the initiation of SRs. However, the growth of SRs required more N as the higher rate applications improved the SR yield.

3.4.3. Effects of N rates on soluble sugar and starch accumulation in plants

In this experiment, N application affected the soluble sugar concentration in vines and roots of Orleans. Soluble sugar concentration in shoots and roots without N supplement were generally higher than those that were N treated. This finding is in line with the results of Villagarcia and Collins (1988) on MD810 and Jewel cultivars. They found that treatment with 2 mM N gave a significantly higher concentration of soluble sugar in vines than those with 8 mM or 14 mM during 30–72 DAT. Si et al. (2018) found that application of N had lower sucrose content during the early growth of the cultivars Shangshu 19 and Juxu 23. However, we did not find any published work that explained the effects of N on the total soluble sugar accumulation in sweetpotato. Results in this experiment showed that the accumulation of soluble sugar in vines and roots increased with N availability, suggesting a general promotive effect of N on sweetpotato growth and photosynthate accumulation.

The starch accumulation in vines and roots responded differently to N supplemental levels at various sampling dates. Root starch concentration kept increasing whereas vine starch concentration did not increase for all N treatments. Obviously, when N is available, roots grow over the time and become a sink that accumulates starch for storage. Low N availability inhibited this process. The concentration of starch in vines in the N0 treatment increased over the time and was higher than those of any N supply levels. The values for N treated plants fluctuated during the study period. However, the total starch accumulations in both above- and below-ground parts for treatment N0 were much lower than for other treatments. Another experiment found that the lowest N level (1 mM) had the highest concentration of starch in vines and roots (Kim et al., 2002). Villagarcia and Collins (1988) found that sweetpotato responded differently to N during the growing period among cultivars. Application of N at rates of either 75 or 150 kg ha^{-1} for Jishu 25 significantly increased the total starch content in mature SRs by around 10% compared to the untreated control (Duan et al., 2019). Therefore, N application of 50 to

200 mg/L in solution promoted starch accumulation in plants and the starch content in both vine and roots were positively related to N supply rates.

In this experiment, the concentration of both soluble sugar and starch increased with time in roots but were largely stable in vines in all N treatments. In contrast, NSC concentrations increased in both roots and vines for N0. This result suggests that when N is available, SR development gradually creates a sink of carbohydrates for storage proposes. The total NSC accumulated in plants was also positively related to N supply rates. This is in line with results in other crops such as corn and sweet sorghum. High rates of N application had resulted in the highest plant biomass, but lowest carbohydrate concentration in tissues (Almodares et al., 2009). Normally, plants temporarily store carbohydrate in leaves before retranslocation to other parts (Paul & Foyer, 2001). Low N availability inhibited plant growth and thereby translocation of photosynthates, leading to the accumulation of carbohydrates in vines. The inhibition of photosynthate transport was indicated by the consistent ratio of vine/root NSC in the N0 treatment over the whole experiment, whereas this ratio reduced rapidly when N was available, indicating active translocation of photosynthates to SR.

3.4.4. Effects of N on N acquisition on sweetpotato

The results from the present experiment showed that the total amount of N in vine or the whole plant in N0 decreased over the study period. For the treated N levels from 50 to 200 mg/L (N50, N100 and N200), N acquisition in both vines and roots kept increasing and the higher level of N supply had the higher value. In a previous study, application of N at 2, 8 and 14 mM of NO_3^- increased positively with the acquisition of N in the plant (Villagarcia et al., 1998). In that study, the N content in the plant treated with 2 mM was 48 mmol $plant^{-1}$, while that of the treatment with 14 mM was 142 mmol $plant^{-1}$, which was almost three times as much. Furthermore, in the same experimental conditions, there were differences in the N acquisition among cultivars. Villargarcia et al. (1998) observed that with the same supplemental level of N the Jewel variety had a lower N content in plants than MD810. In another study, deficient N conditions reduced the amount of N accumulated in shoots, roots and SRs of sweetpotato (Osaki et al., 1995).

There was an increase in the NRE of Orleans over time and the N200 treatment had lower values of NRE at 21 and 35 DAT. This confirms that plants did not require much N during the early stages. In a previous study on two sweetpotato cultivars,

Villargarcia et al. (1998) suggested that low rates of N application resulted in higher NRE and that the utilisation of N increased over the study period. In a different study, N application levels were negatively related to NRE of wheat (Haile et al., 2012). However, NRE was highest at 49 DAT, and the N100 and N200 treatments had a similar rate of NRE and this was higher than the N50 treatment. A possible reason for this would be that plants accumulated more N for SR development, as suggested by more root N accumulation.

3.4.5. Agronomic indication of N application for sweetpotato

Results from our experiment suggested that sweetpotato required adequate N levels to form SRs. The formation and division of cambial cells in ARs were strongly affected by N supply. Deficient N or a high rate of N inhibited the formation of cambium during SR formation. After that, a high rate of N was likely to improve the SR development as suggested by high carbohydrate accumulation in SRs. Therefore, moderate N supply levels should be maintained in the soil for a few weeks (5–7 weeks for Orleans cultivar under our experimental conditions) to promote the formation of the most SRs before further application of N fertiliser to boost SR growth. Appling high rates of N fertiliser before or immediately after planting should be avoided because the initiation of SR could be suppressed and the fertiliser use efficiency will remain low.

Previous studies suggested that the number of SRs would not increase much after initiation in the early stage. Nearly 90% of SRs for Beauregard and Georgia Jet developed from ARs that were formed within the first week after transplanting (Villordon et al., 2009c). The maximum SR number of six West Indian sweetpotato cultivars was established by week 8 (Lowe & Wilson, 1974a), and for Beauregard cultivar by week 4 (Gajanayake et al., 2014a). Furthermore, the SR number per plants is important to determine the yield of the crop (Ma et al., 2015). Thus, maximising the number of SRs during the early stages will potentially increase production of sweetpotato. It also helps to produce more consistent and suitable SR size, which is essential for crop profit margins, at least in developed countries such as Australia, where SRs with excessively large sizes are rated as low quality with low commercial value. Starting with high N could lead to the risk of resulting in fewer, large SRs that may reduce the income of famers. In this case, careful fine-tuning of N fertilisation schemes would help to produce a more profitable sweetpotato crop.

3.5. Conclusions

Our study found that N level treatment did not affect the number of ARs in Orleans sweetpotato cultivar, but both deficient and excessive N supply would inhibit the formation of SRs. Application of N at 100 mg/L significantly promoted the formation of cambium in young sweetpotato ARs and improved the percentage of initiated SRs. The N100 treatment demonstrated the highest percentage of roots with cambium development in the earliest observation and the highest percentage of initiated SRs between 21 to 56 DAT. Further, this treatment had the highest starch accumulation in roots during the first 35 DAT. The N200 treatment inhibited the formation of cambium in the early stage development of ARs and delayed AC development at 21 DAT. Although the rate of ARs with AC formation of N200 caught up to that of the N100 treatment during 35 to 49 DAT, the percentage of SRs in the N200 treatment was still significantly lower than that of N100. However, both the highest storage root weight and root starch accumulation were observed in the N200 treatment from day 49, despite lower SR numbers than in N100. Also, NRE in N200 was lowest at 21 and 35 DAT but became highest at 49 DAT. Insufficient N supply reduced the cambial initiation during the first 10 DAT and suppressed the formation of AC after that, resulting in fewer SR formations. Therefore, sweetpotato variety Orleans required different amounts of N supplement for optimal SR formation during certain growing periods after planting. Our study suggests that moderate soil available N should be maintained for about five weeks to promote the initiation of adequate numbers of storage roots, before further N fertilisation should be applied to boost SR growth.

Chapter 4: Effect of N application timing on the initiation of sweetpotato storage roots

Abstract

Nitrogen has been demonstrated to improve the production of crops. Nitrogen requirements in plants are associated with plant growth and crops may need more N during certain developmental stages. Choosing the right time for N application is critical to improve N use efficiency and to assure healthy crop development. 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 timing of N fertilisation on the initiation of SRs, which determines the number of tubers per plant and substantially affect the yield. A pot experiment was conducted in Bundaberg, Queensland, to evaluate the influence of different N application timings on the initiation of SRs of Orleans cultivar. The accumulation of carbohydrate and the acquisition of N during SR formation were also assessed in this study. Nitrogen fertilisation at the rate of 100 mg/L in the form of a nutrient solution, which was proved to be effective to promote SR initiation, was used to supply plants in a sand culture system. Five different timings of N applications, nil N supply (T0), and with N applied on the planting day, 3, 7 and 14 DAT (accordingly, T1, T3, T7 and T14, respectively), were included in the experiment. Results from the experiment suggested that the SR formation required N during the first week after transplanting. No or late N application delayed the initiation of SRs. During the early stage of AR development, no or delayed N application till 14 DAT inhibited the formation of RVC and AC. Then, the lignification of stele cells increased, leading to a significantly higher proportion of lignified roots. However, plants supplied with N within 7 DAT had a higher rate of SRs and a lower rate of lignified roots. Also, earlier N application promoted plant and root growth as suggested by higher biomass and storage root weight, more NSC (the sum of soluble sugar and starch) and N accumulated in plants, and higher NRE. The study provided agronomic indicators that moderate N should be available in soil before or on planting day.

4.1. Introduction

Nitrogen 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 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 (Villordon & Franklin, 2007; also see Chapter 3). 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 is beneficial for improving plant growth and contributing to crop yield.

Nitrogen fertilisation timing has been demonstrated to affect the development and yield of many crops, as N requirements are different in the 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 harvesting (Salo, 1999; Westerveld et al., 2006). Therefore, adding N at the wrong time may reduce N fertiliser use efficacy as it is washed away or not used by crops.

Nitrogen fertilisation timing has been studied in many crops. In potato, N application before or on planting day promoted early tuber development and nitrogen use efficiency (Alva, 2004). Four sweetpotato cultivars, including Georgia Jet, TU-82-155, TU-1882 and Rojo Blanco, were used to examine the influence of timing of N fertilisation on the yield (Ankumah et al., 2003). The results suggested that a single application of 90 kg N ha^{-1} at 20 DAT resulted in significantly higher yields than four split applications of the same amounts starting from 20 DAT over 20 day intervals. Similarly, another experiment in North Carolina on Beauregard variety showed that the highest marketable yield was achieved with a single application at either 21, 28 or 35 DAT compared to split applications during 10–21 DAT or 21–35 DAT (Schultheis et al., 1995). However, a study in Louisiana for the same cultivar indicated that the split application of N at pre-transplantation and 28 DAT increased the SR marketable yield

when compared to single applications before transplantation or on 21 DAT (Villordon & Franklin, 2007). They also suggested that late application of N after 28 DAT reduced the yield of the crop. Philip and Warren (2005) suggested that N application after 2 or 3 weeks from 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 recorded the influence of N application timing on the SR yield of the crop, none of them focused on its effect on the formation of SRs and how this affected the yield. Furthermore, the initiation and development of SRs was also related to carbohydrate accumulation and N acquisition in plants. In our previous study (see Chapter 3), the results showed that insufficient or excessive N supply inhibited the formation of AC associated with the initiation 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, we did not determine when plants started to require N for their SR formation. Therefore, the main objectives of this experiment were: (1) to evaluate the effect of N fertilisation timing on sweetpotato during the early stage of SR initiation in a glasshouse trial; (2) to identify the best N fertilisation timing for Orleans to promote the formation of SRs; and (3) to assess the effects of N application timing on the accumulation of carbohydrate and acquisition of N in different parts of the plant during the initiation of SRs. In particular, we addressed the following questions: (1) Do N fertilisation timing applications affect the anatomical development in roots that results in SR initiation? (2) How are soluble sugar and starch accumulations in plants affected by N fertilisation timing? (3) Do N fertilisation timings influence the N acquisition in plants?

4.2. Materials and methods

4.2.1. Plant materials and growth conditions

The experimental trial was conducted in a glasshouse at Bundaberg Research Facility (24°50′54″ S 152°24′14″ E) for seven weeks in early summer from 20 October to 8 December 2018. The shade mat roof (around 30% shade) was closed in order to reduce the inside temperatures 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. Washed river sand was used as the growth medium for the experiment. Orleans transplants were

obtained from a commercial nursery farm. 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.

4.2.2. Growth medium preparation

Black 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.

4.2.3. Experiment design

The experiment consisted of five treatments with different started N timing applications including none, on planting day, and on 3, 7 and 14 DAT (hereafter T0, T1, T3, T7 and T14, respectively). There were multiple harvests over the growing period on 10, 21, 35 and 49 DAT. The experimental design was a complete randomised design (CRD). Six plants from each treatment were sampled at each harvest. Three plants were used for observation of root anatomy and morphology. The other three plants were used to determine soluble sugar and starch accumulation, as well as N acquisition. In total, four harvests were conducted over the experimental period totalling 120 plants (5 N fertilisation timing treatments x 4 harvests x 6 plant per harvest). In addition, eight plants per treatment were grown for back up purposes in case of death or abnormal development, so in total 160 pots were prepared. Hoagland's modified solution lacking N (Hoagland & Arnon, 1950) was utilised for the T0 treatment. The protocol for preparation of the solution is explained in Appendix2. The other treatments were supplied with the modified Hoagland's solution with 100 mg/L N added in the form of NH_4^+ and NO_3^- (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} dependent on plant growth and weather conditions. For the N fertilisation timing treatments, plants were watered with N free nutrient solution every two days from planting to treated dates. 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 on the days without nutrient solution supply if needed.

4.2.4. Sampling date

The first sampling date was on 10 DAT, and then plants were sampled on 21, 35 and 49 DAT. Six plants for each treatment were uprooted on a sampling date. They were divided into two sets with three plants each for: (1) root examination; and (2) soluble sugar, starch and CN analysis. Plants were dug up carefully to minimise root damage and washed in tap water to remove all sand.

4.2.5. Measurements and data collections

Anatomical observations

Three plants from one set of the experiment were used for anatomical observations on 10, 21, 35 and 49 DAT. The AR count for individual plants was recorded on each sampling. All roots were collected individually and placed in deionised water and sectioned as soon as possible to ensure reduced damage. 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). The procedure for staining sections was described in Chapter 3.2.6. 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 was observed in the first observation at 10 DAT. The other features such as IRVC, completed RVC, appearance of AC and more than 50% LC were observed at four sampling times.

Classifications of AR development stage

Based on root anatomical characteristics described by Wilson and Lowe (1973), ARs are classified into initiated SRs, PRs and lignified roots. 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). 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).

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. An Epson Perfection V700 Photo Scanner (Seiko Epson, Nagano, Japan) was used to achieve root images as described in Chapter 3.2.6. 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.

Soluble sugar and starch contents in vines and roots of sweetpotato

The second set of experiments including three plants from each treatment were harvested on 10, 21, 35 and 49 DAT. Fresh vine and root samples from each plant were collected separately. They were dried immediately after sampling in a preheated oven at 90°C for 90 minutes to stop enzymatic sugar conversion in tissues, and then converted to 70°C to constant weight (Maness, 2010). Dried samples were stored in a -80°C freezer for chemical analysis. They were ground to a fineness of <0.25 mm and uniformed before analysis. A MCW solution was utilised to extract soluble sugar and starch from plant tissues (Dickson, 1979; Rose et al., 1991). The concentrations of sugar and starch were determined using the colorimetric phenol-sulfuric acid assay (Dubois et al., 1956). The procedures for extraction and analysis were described in Chapter 3.2.6.

Nitrogen acquisition in sweetpotato

Samples from three plants for carbohydrate analysis were also used to determine N acquisition. 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 was calculated based on 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):

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

4.2.6. Statistical data analysis

Data recorded from each harvest were analysed using one-way ANOVA using IBM® SPSS® software statistical package (version 25; IBM, New York, USA) to determine the differences among treatments at particular time. As different plants were sampled in each harvest, two-way ANOVA was used to analyse the global effect of the main factors and the interaction between treatments and harvesting times. All data in percentages were arcsine-transformed to analyse data in SPSS. The AR count and data for carbohydrate and CN analysis were transformed using log 10 and square root transformation, respectively. For significant values, means were separated by Tukey HSD. Difference at the *P* value ≤0.05 was regarded as a test of statistical significance. Regression analysis was conducted in SPSS to detect the relationship between C accumulation and root traits. Graphs were produced using SigmaPlot® software package (version 14; SYSTAT Software, Inc., California, USA).

4.3. Results

4.3.1. Effects of N fertilisation 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 4.1). 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 timings and interactive effect of N application timings by harvesting times 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 4.1. Effects of N fertilisation timing on adventitious root number at different sampling times

Treatment	10 DAT	21 DAT	35 DAT	49 DAT	*P* value
T0	10.7±0.7	11.7±0.7	11.7±0.3	11.7±0.3	NT: 0.85
T1	11.0±0.6	12.7±0.9	11.3±0.3	11.7±0.3	T: <0.001
T3	10.3±0.3	12.3±0.7	11.7±0.3	11.0±0.6	NT x T: 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	

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 4.1). Results showed that there was no statistically significant effect of N fertilisation 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 steles 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).

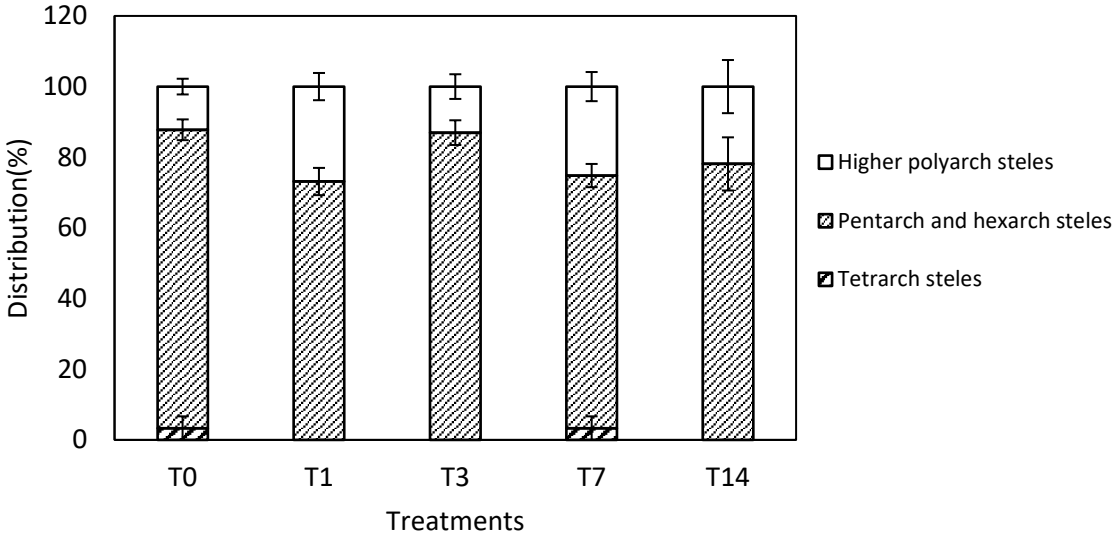

Figure 4.1. Distribution of AR protoxylem element number in Orleans as affected by N application timings sampled at 10 DAT; the error bars indicate the SE of the mean value (n=3).

Results from the present experiment showed that the NC development in ARs was only observed at 10 DAT (Figure 4.2). Nitrogen fertilisation timings 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 (Figure 4.3A). The percentage of roots with IRVC decreased over time. There was no significant effect of N fertilisation timing on the formation of IRVC on both 10 and 21 DAT.

In this experiment, RVC was observed throughout the study period (Figure 4.3B). When the effects of N fertilisation timings 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 observations at 21 and 35 DAT. No difference was found on the effect of N fertilisation 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 (Figure 4.3C). 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% LC were characterised between 21 and 49 DAT (Figure 4.3D). The effect of N application timings 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.

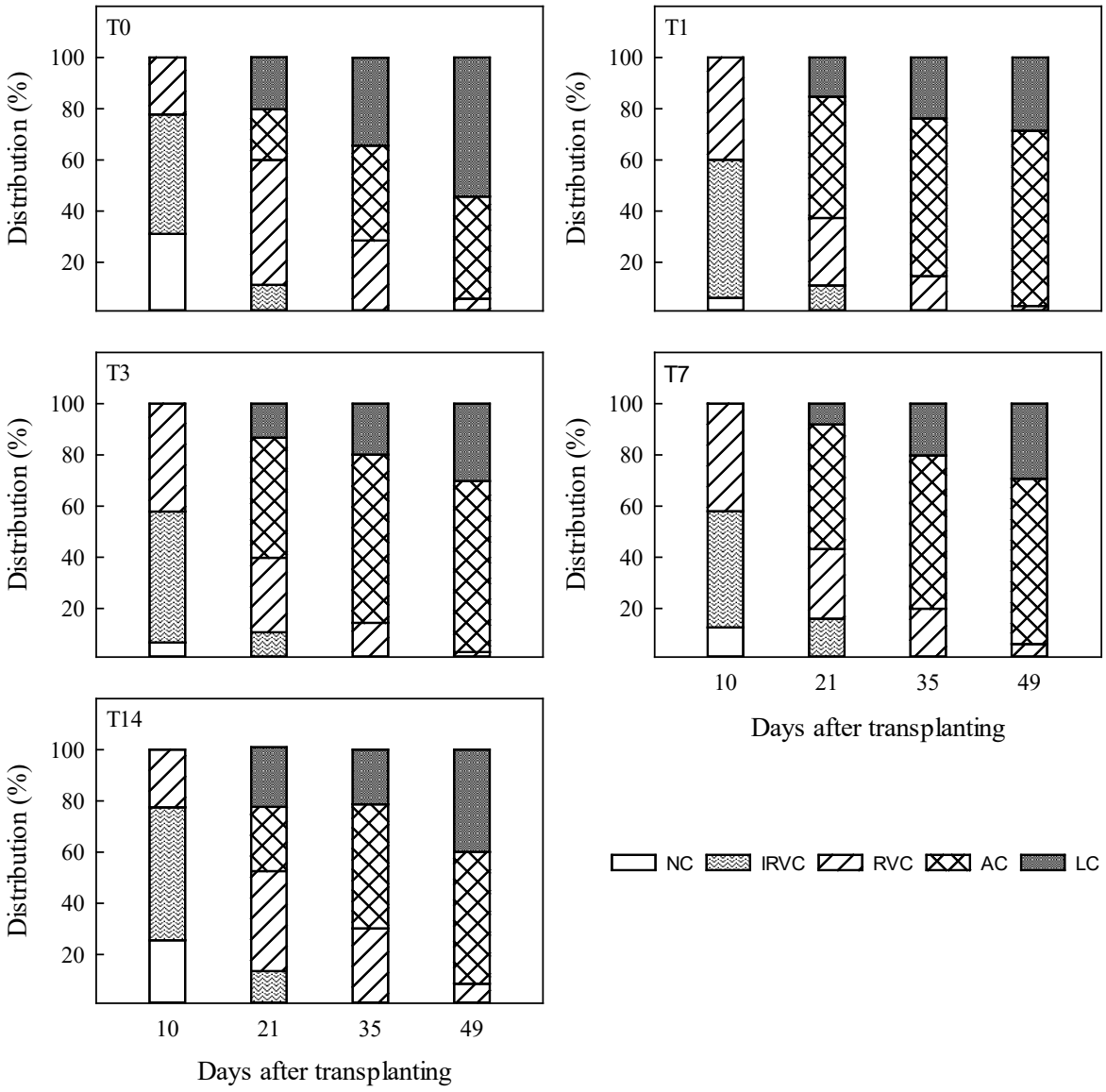

Figure 4.2. Effects of N fertilisation timing on the anatomical distribution in roots over the sampling time. The values are indicated as mean (n=3).

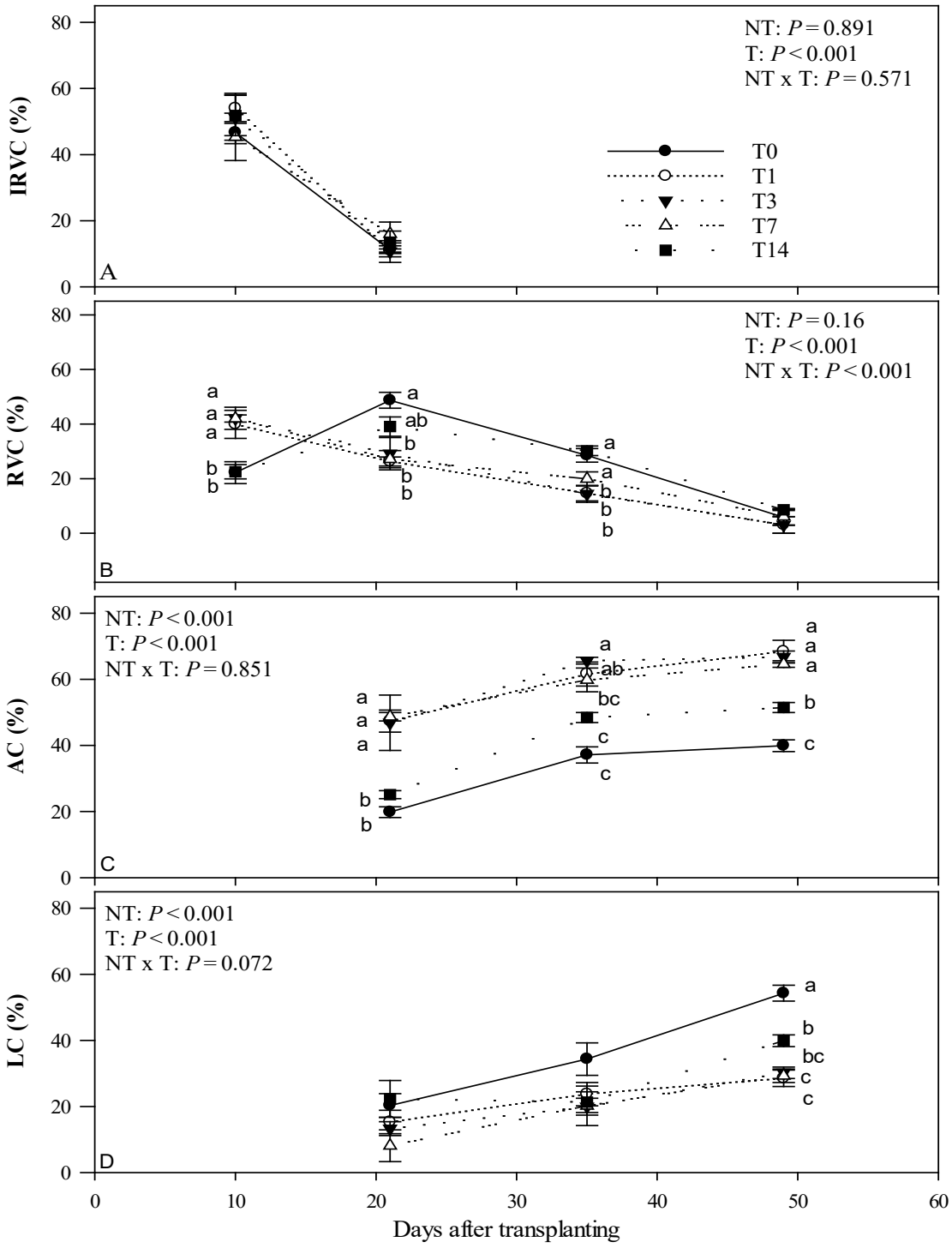

Figure 4.3. Effects of N application timings on the development of anatomical features of roots at different sampling times.

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

Abbreviations: IRVC = Initial regular vascular cambium; RVC = Complete regular vascular cambium; AC = Anomalous cambium; LC = Lignified cells; NT = Nitrogen application timing; T = Time.

In our experiment, the effect of N fertilisation timing on the percentage of SRs was significantly different among treatments at different sampling times (Figure 4.4). 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, respectively. As demonstrated by the two-way ANOVA, the main effect of N application timing treatments and sampling times was significant on the percentage of SRs while the interactive effect of treatments by time was not statistically different.

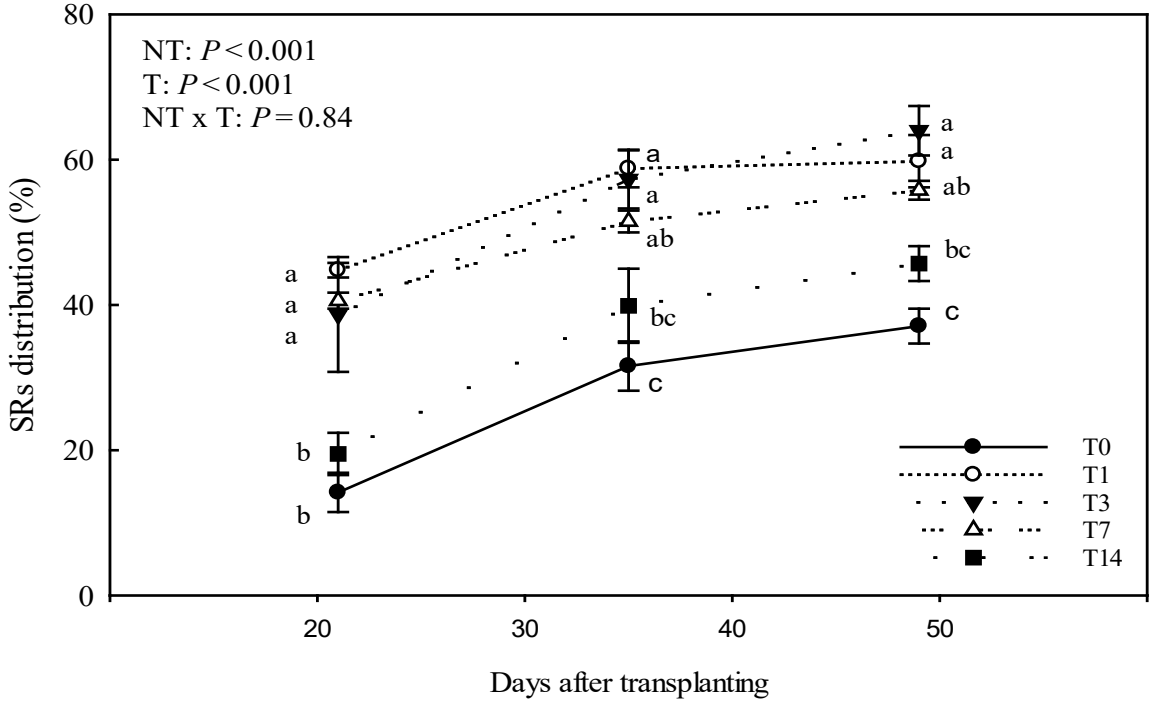

Figure 4.4. Effects of N fertilisation timing application on the initiation of SRs of Orleans on different sampling dates.

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 significantly 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.

Around 3–9% of the ARs developed into PRs across the treatments (data not shown). Nitrogen fertilisation timings had no effect on the formation of PRs in all three

observations. When the data was analysed by two-way ANOVA, results showed that no significant difference was found on the main effect of N application timings, sampling time and interaction of treatment by time.

4.3.2. Effects of N fertilisation 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 4.2). The total root length increased over the 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 fertilisation 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 delay, the stronger the effect. The root volume followed a similar pattern to the total root length.

Regarding the average root diameter, our results indicated that there was no difference on the effect of N fertilisation timing on root diameter of Orleans at different sampling times (Table 4.2) and there was no interactive effect of treatments and time.

Table 4.2. Effect of N fertilisation 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 x 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 x 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 x T: $P < 0.001$
	T7	5.6±0.3	17.0^{a}±1.6	27.9^{a}±1.4	30.8^{ab}±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	

The table presents the mean values followed by standard errors (SE) (n=3). 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(Tukey's HSD).

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 4.3). 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 4.3. Effect of N application timings on biomass weight, SR length, SR diameter and SR yield at 49 DAT

Treatment	ADW (g plant^{-1})	RDW (g plant^{-1})	SRL (mm)	SRD (mm)	FSRW (g plant^{-1})
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

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 (Tukey'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.

The relationship of C accumulation in plants and TRL was highly positively related in all three harvestings, 21 DAT (p<0.001, R^2 = 0.8286), 35 DAT (p<0.001, R^2 = 0.9000), 49 DAT (p<0.001, R^2 = 0.8618). Similarly, there was significant positive relationship between C accumulation and RV over the study period (Figure 4.5). However, no relationship found between the accumulation of C in plants and RD (p>0.01, Figure 4.10).

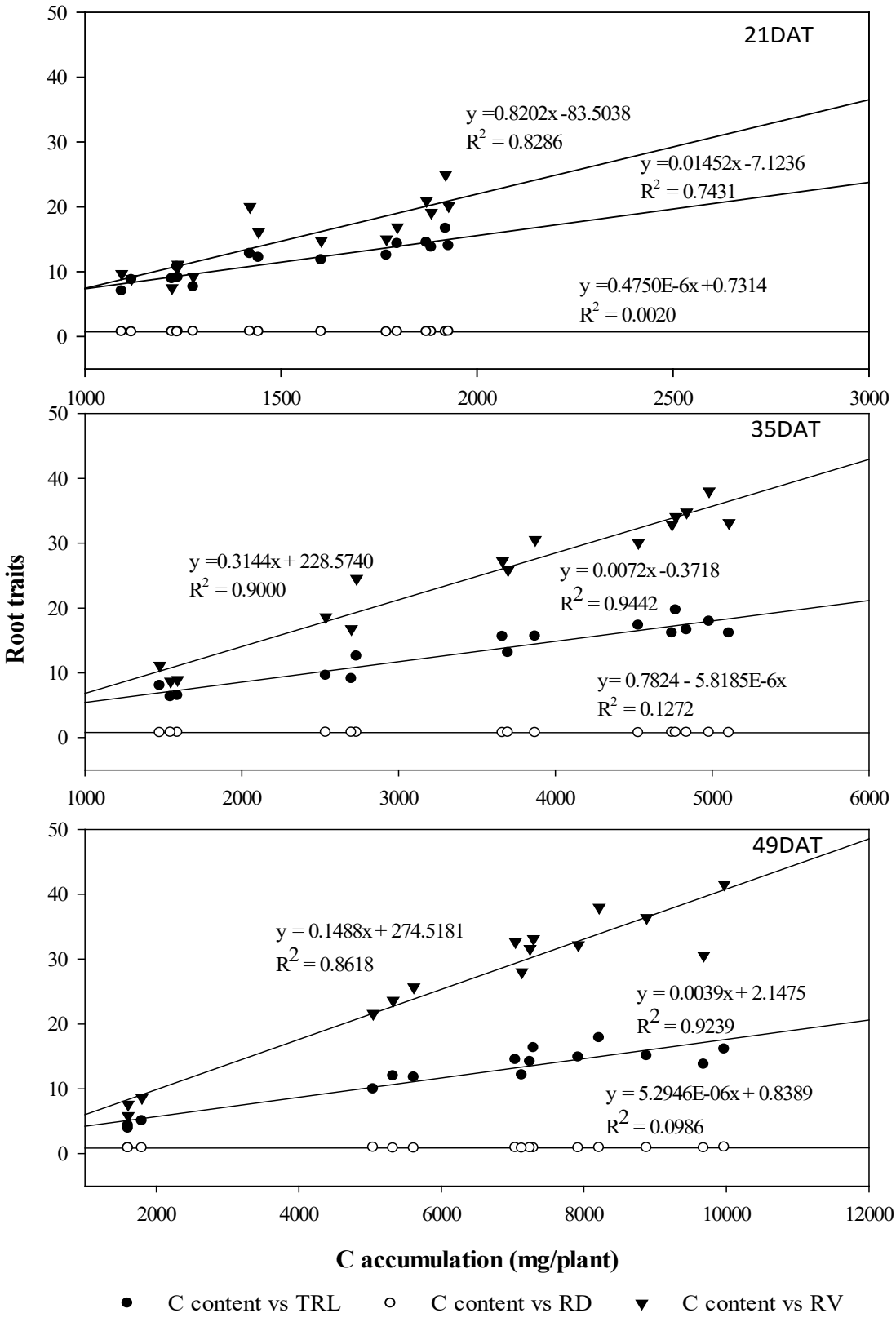

Figure 4.5. Relationship between C accumulation in plant and root traits.

The x-axis represents the total C accumulation in plants and y-axis represents root traits (TRL(x10), RV and RD).

4.3.3. Effects of N fertilisation timing on soluble sugar and starch accumulation in sweetpotato during SR formation

4.3.3.1. Effect of N fertilisation timing on the concentration of soluble sugar and starch during the initiation of SR

During the early period of sweetpotato growth, the four N fertilisation timing treatments reduced the soluble sugar concentration in both vines and roots compared with that in the T0 treatment (Figure 4.5A; Figure 4.5B). The T1 treatment had the lowest concentration of soluble sugar in most samplings. There was not much change in the soluble sugar content in vine (Figure 3.5A). However, the figure for roots increased noticeably in all treatments. All four N fertilisation timing treatments had similar concentrations of soluble sugar in roots during the study period, which was significantly lower than the T0 treatment.

The effects of N application timings on the starch concentration were significant among treatments at all sampling times (Figure 4.5C; Figure 4.5D). In vines, the starch content was highest in the T0 treatment at all sampling dates except 10 DAT. At 10 DAT, T1 and T3 treatments had the highest concentrations of starch at 180 and 191 mg g^{-1} dwt, respectively, which were significantly higher than that of other treatments. At 21 DAT, vine soluble sugar showed a large variation among treatments, and the earlier N was added, the lower the rate of soluble sugar, suggesting availability of N photosynthate use by stimulation of growth. This variation among treatments faded over time. In roots, the highest starch content was observed in the T0 treatment at various harvestings. Other treatments with different N fertilisation timings had lower starch concentration.

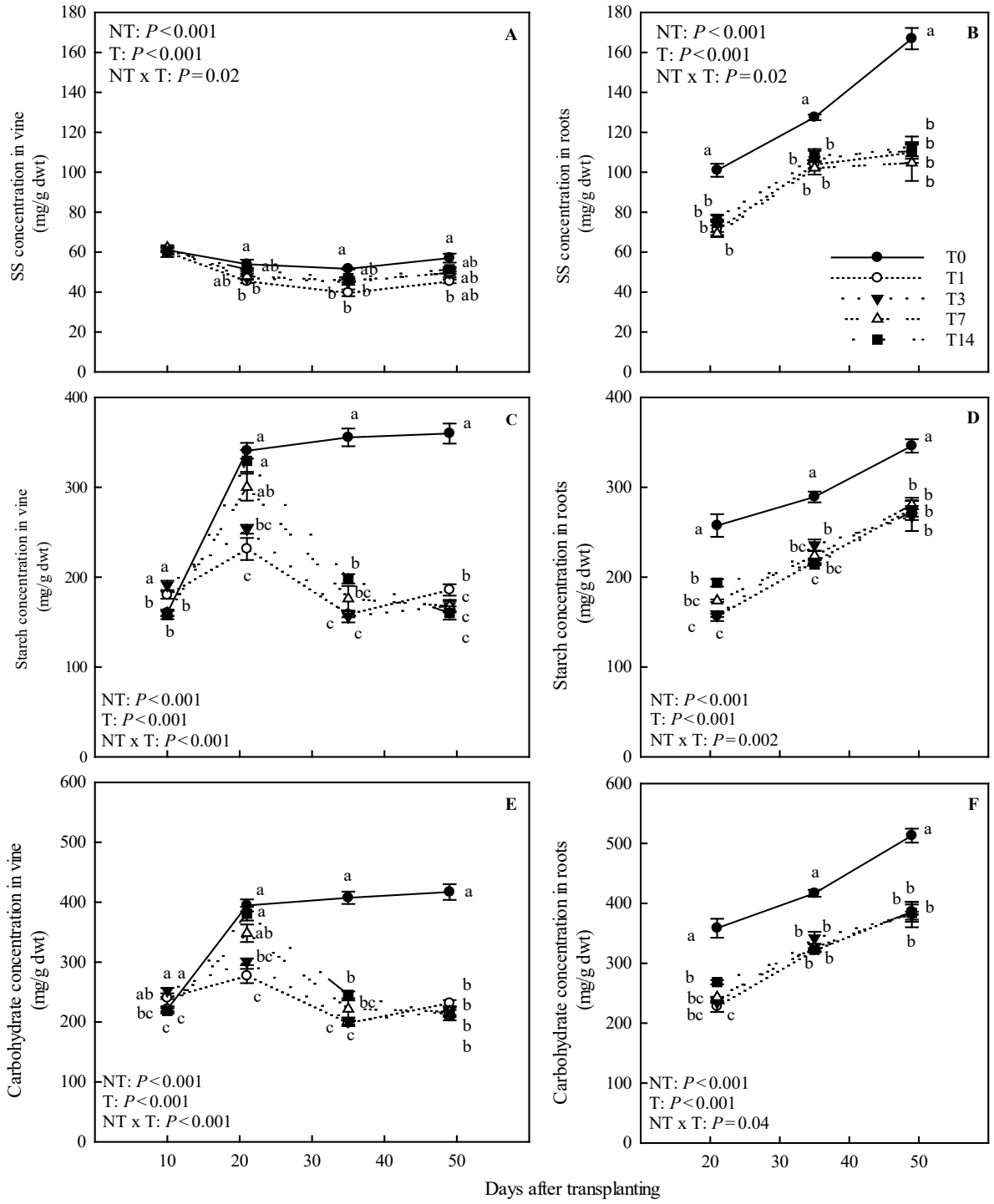

Figure 4.6. Concentration of carbohydrate (mg g^{-1} dwt) as affected by N application timings. (A) Soluble sugar concentration in vines; (B) Soluble sugar concentration in roots; (C) Starch concentration in vines; (D) Starch concentration in roots; (E) Carbohydrate concentration in vines; (F) Carbohydrate concentration in roots.

Values are indicated as mean ± SE (n=3). ANOVA results are based on square root transformed data. Two-way ANOVA results, including effects of N application timing, harvesting date, and N application timing by time interaction on soluble sugar, starch and NSC concentration are shown. Different letters indicate significant differences among treatments on the same harvesting dates using one-way ANOVA (Tukey's HSD).

Abbreviation: SS = Soluble sugar.

In this experiment, total NSC (the sum of soluble sugar and starch) concentration in both vine and roots followed similar patterns to starch content (Figure 4.5E; Figure 4.5F). The total concentration of carbohydrates in vines increased during the first three weeks after transplanting, and then decreased in all treatments except T0 (Figure 4.5E). In roots, increasing trends of the total soluble sugar and starch concentrations were observed in all treatments. Three N fertilisation timings, T1, T3 and T7, had similar carbohydrate concentration in most sampling times during 21 and 49 DAT.

4.3.3.2. Effect of N fertilisation timings on the total soluble sugar and starch accumulation in plants during the initiation of SR

As the amount of dried root at 10 DAT was insufficient for this analysis, soluble sugar and starch accumulated in roots was not examined during this stage. The effect of N fertilisation timing was significant for the total soluble sugar in vines and roots at all sampling times except 10 DAT (Figure 4.6). At 10 DAT, there was no treatment effect on the soluble sugar accumulated in vines (Figure 4.6A). After that the lowest and highest soluble sugar accumulated in vines and roots was recorded in T0 and T1 treatments, respectively (Figure 4.6B; Figure 4.6C; Figure 4.6D). At 49 DAT, there was no statistical difference found on the soluble sugar accumulation among T1, T3 and T7 treatments (Figure 4.6D).

The starch accumulation in the vines and roots increased over the study period. Applications of N from planting to 14 DAT increased the total starch accumulated in plants compared to no N application (T0). In general, the earlier N was applied, the more starch accumulated in the plant by 49 DAT.

The total NSC in both vines and roots also increased over the time in all treatments (Figure 4.6A; Figure 4.6B; Figure 4.6C; Figure 4.6D). The effect of N fertilisation timing on the total NSC accumulation in both vines and roots was statistically significant among treatments in all samplings. During the first three weeks after transplanting, the accumulation of NSC was mainly in vines. More carbohydrate was accumulated in roots compared to that in vine from 35 DAT (Figure 4.6C; Figure 4.6D). Two-way ANOVA results showed that all effects of treatments, sampling times and interaction between them had significant differences on soluble sugar, starch and total carbohydrate accumulation in vines and roots (Table 4.4). The earlier N was added, the more NSC accumulated in the plant.

Table 4. 4. The main and interaction effects of N treatments and time on the accumulation of soluble sugar and starch in vines and roots of sweetpotato

Factor	Vines			Roots		
	SS	Starch	Total	SS	Starch	Total
NT	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NT*T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01

Two way ANOVA results are based on square root transformed data.

Abbreviation: NT= Nitrogen application timing, T=Time, SS= Soluble sugar.

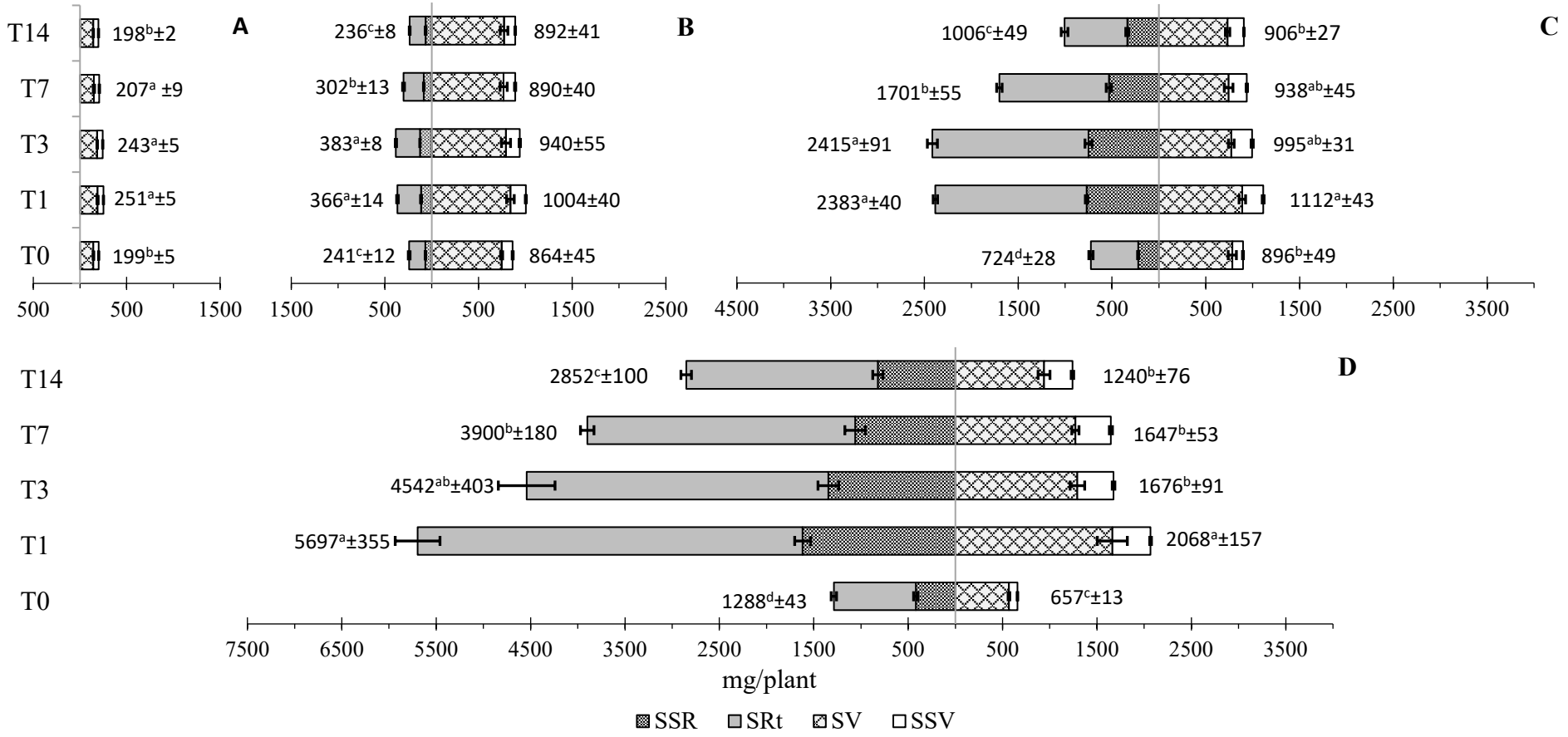

Figure 4.7. Effect of N application timings on the accumulation of soluble sugar and starch in plants at various sampling times. (A) 10 DAT, (B) 21 DAT, (C) 35 DAT and (D) 49 DAT.

The x-axis represents the N acquisition and the y-axis represents the treatments. Numbers in the left and right are mean values of total NSC (SS+Starch) acquisition in roots and vines respectively followed by standard error (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letters are significantly different among treatments using one way ANOVA (Tukey's HSD, $P<0.05$).

Abbreviations: SRt = Starch in roots; SSR = Soluble sugar in roots; SSV = Soluble sugar in vine; SV = Starch in vine.

4.3.4. Effects of N fertilisation 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 4.7A; Figure 4.7B). 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 T3 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 4.8). The T1 treatments had the highest value of both vine and root N acquisition in all sampling times. Based on three harvesting times, the two-way ANOVA results showed that the main effects of N fertilisation timing and sampling time were significant on the total N in vines, roots and plants. 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 (Figure 4.7C; Figure 4.7D). 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 4.7C). 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 4.7D).

Results from the present experiment showed that the effect of N fertilisation timings was significantly different on the NRE in all sampling times (Figure 4.9). The highest and lowest NRE were observed in the T1 and T14 treatments, respectively.

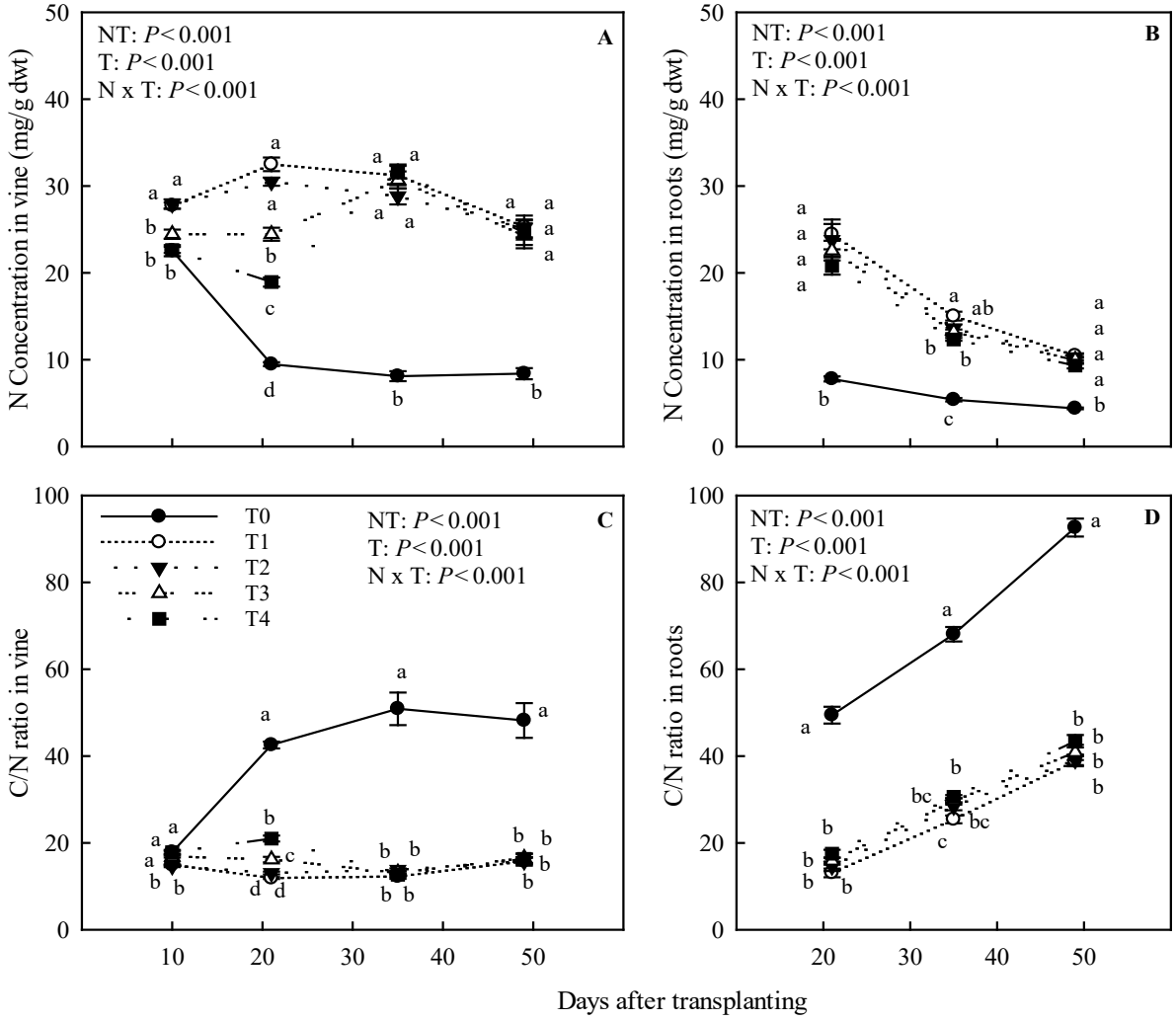

Figure 4.8. The effect of N application timings on the N concentration in (A) vines and in (B) roots; and on the C:N ratio in (C) vines and in (D) roots.

ANOVA results for N concentration for both vines and roots are based on square root transformed data. Two-way ANOVA results, including effects of N application timing, harvesting date, and N application timing by time are shown. Different letters indicate significant differences (P<0.05) among treatments on the same harvesting date using one-way ANOVA (Tukey's HSD).

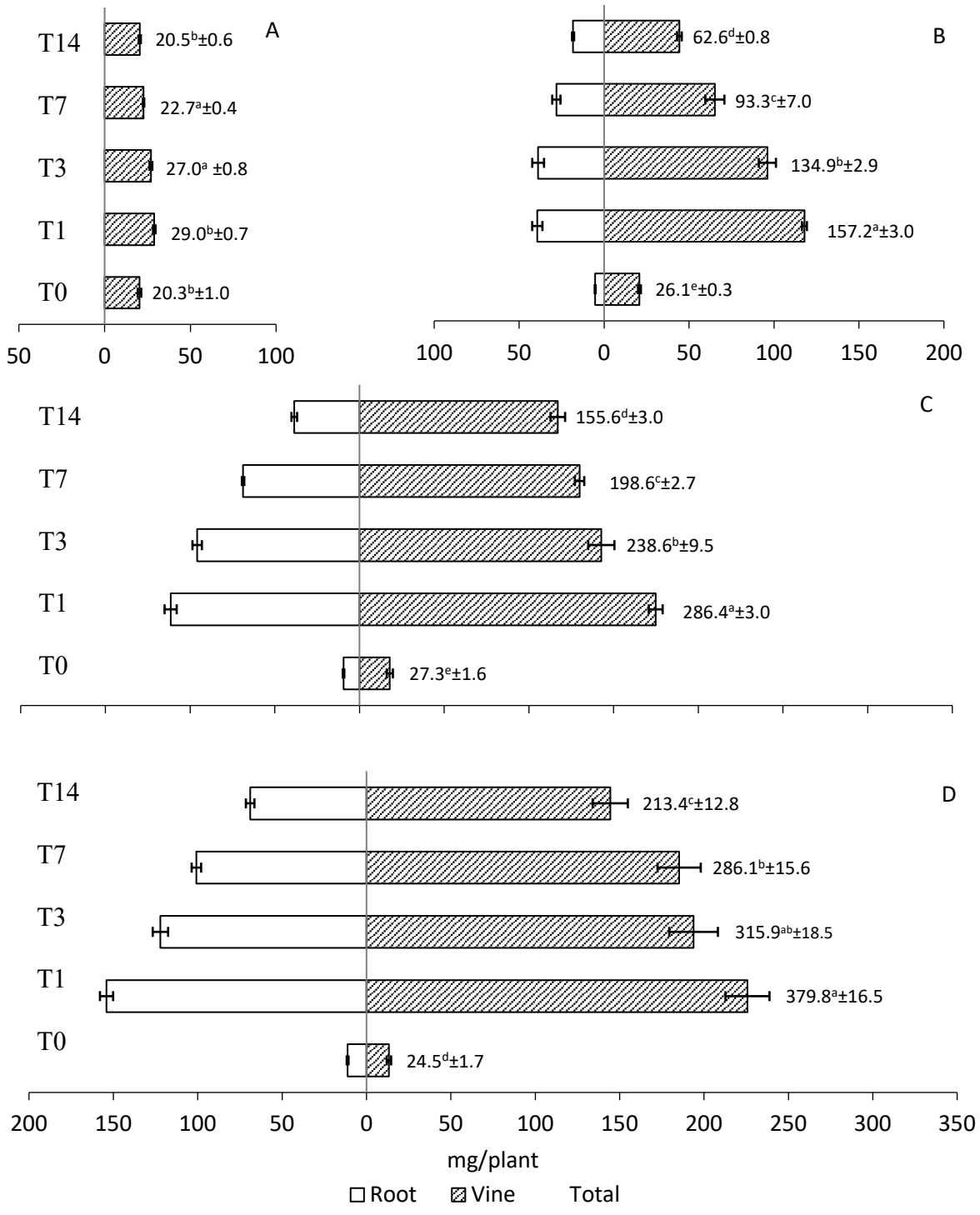

Figure 4.9. Effect of N fertilisation timings on the acquisition of N (mg) at various sampling times: (A) 10 DAT; (B) 21 DAT; (C) 35 DAT; and (D) 49 DAT.

Numbers are mean values of total N accumulated in plants followed by SE (n=3). ANOVA results are based on square root transformed data. Means followed by a different letter are significantly different among N fertilisation timings using one-way ANOVA (Tukey's HSD, $P<0.05$).

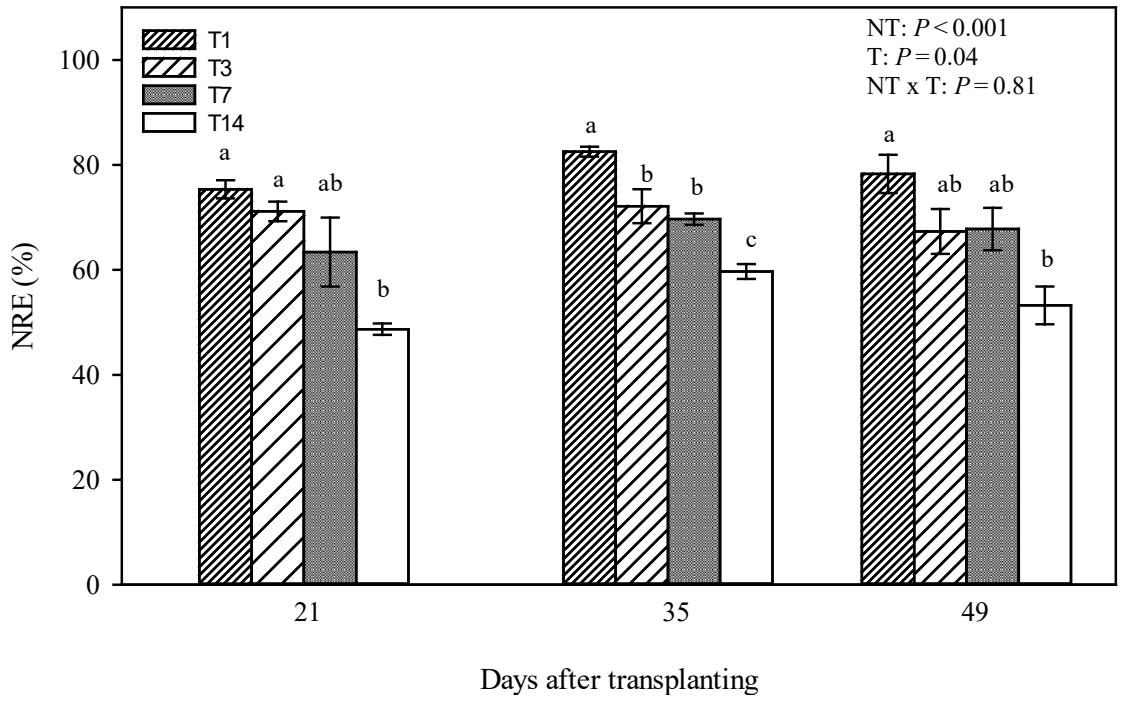

Figure 4.10. The efficiency of N use in sweetpotato under different N fertilisation timings.

Values are indicated as mean ± SE (n=3). ANOVA results are based on arcsine-transformed data. Two-way ANOVA results including the effects of N fertilisation timing, sampling time, and N application timing by time interaction are shown. Means followed by different letter are significantly different among N application timing treatments using one way ANOVA (Tukey's HSD, $P<0.05$)

Abbreviations: NT = N fertilisation timing; T = Time.

4.4. Discussion

4.4.1. Effect of N fertilisation timing on anatomical features of Orleans

In this experiment, N fertilisation timing had no effect on the number of ARs per plant. According to Belehu et al. (2004), the AR number is determined by the number of undamaged and healthy preformed root primordia per node. 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 fertilisation timings had no effect on the AR number and the arrangement of vascular cambium in roots.

Orleans roots sampled at four sampling times showed various anatomical features 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. The IRVC was observed in roots from 10 to 21 DAT and the presence of RVC was recorded between 10 and 49 DAT. In the early stage of root development, no effect of N fertilisation timing was found on IRVC. However, N application during the first week after transplanting (T1, T3 and T7 treatments) significantly reduced the distribution of NC and increased the percentage of roots with RVC. Therefore, sweetpotato cultivar Orleans required N supply to promote the formation of cambium in roots.

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, 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 significant 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 or late N supply reduced the formation of AC, resulting in lower SR initiation. To the best of our knowledge, the effect of N fertilisation timing on anatomical structures of sweetpotato roots is examined for the first time in this study.

In this experiment, treatment 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 (see Chapter 3). Previous studies suggested that cambial activity or cell division was regulated by cytokinin level in plants (Kakimoto, 2003; Matsumoto-Kitano et al., 2008), which could be dramatically stimulated in response to N supply (Kakimoto, 2003; Samuelson et al., 1992) and reduced under nutrient deficiency (Yang et al., 2001).

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 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. No 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 to associate to the formation of PRs (Kays, 1985). For example, dry and compacted soil are favour conditions for PR initiation.

4.4.2. Effects of N fertilisation timing on root morphology, plant performances and yield of Orleans

The timing of N applications had no effect on average root diameter but significant effects on root length and volume between treatments over the study period. This root parameter was calculated based on root diameter of all types of roots in the plant, so the average root diameter would be largely influenced by lateral roots. At the beginning, available N in cuttings is likely to 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 previous 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., 2013a). 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 NO_3^- supply ranging from 0.04–4 mM (Wang et al., 2005).

In the current study, the nutrient solution with the optimum level of N for SR formation at 100 mg/L was applied to plants. Plants in no or delayed N treatments were grown under insufficient N conditions 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). Nitrogen is one of the most important nutrients for plant growth and it affects dry matter accumulation in many species such as potato, carrot and rice (Malik et al., 2014; Mittelstrass et al., 2006; Westerveld et al., 2006).

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 sand with very low 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; see Chapter 3), so N available in soil might be adequate for SR formation in some studies where delayed N application did not affect the initiation of SRs. The relationships of C accumulation in plants and root morphology in this study confirmed that roo t growth is dependent on the accumulation of C. Carbon content was similar between crops and plants (Zhang et al., 2014), so the total content of C in all treatments increased over the study period due to the development of plants which is related to N supplementation. In previous studies, the accumulation of C was correlated with root traits such as root biomass, root diameter and root elongation rates (Rossi et al., 2020).

4.4.3. Effects of N fertilisation timing on NSC and N accumulation during the SR formation of Orleans

Results from this experiment suggested that soluble sugar and starch concentration in both vines and roots were higher in the no N supply treatment (T0 treatment) compared to those of other treatments. Also, delayed N application led to growth limitations and caused

soluble sugar and starch accumulation in vines, as indicated by significantly higher concentrations of soluble sugar and starch in the late N fertilisation timing treatments at 21 and 35 DAT. In this study, plants in the delayed N supply treatment did not receive N before treated days, so the total N added to them was less than plants in the earlier N application treatments. A study of Villargarcia and Collins (1988) on MD810 and Jewel cultivars found that the soluble sugar concentration in vines was negatively associated to N levels and lower rates of N application gave a higher concentration of soluble sugar. In another study, Kim et al. (2002) concluded that higher concentration of starch in vines and roots was found in the lower N treatments, because N limited plant growth and consumption of photosynthates. Similarly, the highest carbohydrate content was obtained under N deficiency in many crops, such as pepper, olive and microalgae (Aloni et al., 1991; Boussadia et al., 2010; Dragone et al., 2011). At 21 DAT, root starch showed similar patterns as that in vine with lower concentration being observed in earlier N application timing treatments. After that, it appears that N application time did not affect NSC concentration. The information here suggests that N is an essential nutrient to carbohydrate accumulation in SR of sweetpotato and is required for plant growth. Numerous studies have found a negative relationship between N and carbohydrate concentration (Araya et al., 2010; Fichtner & Schulze, 1992; Hofstra et al., 1985; Kim et al., 2002).

The earlier N was supplied, the more NSC was accumulated. In this experiment, the no N supply (T0 treatment) had a significantly lower amount of soluble sugar and starch accumulated in vines and roots than in other treatments. This suggested that N promoted sweetpotato plant growth and led to photosynthate accumulation. This finding confirmed the recent results of Duan et al. (2019), who observed that the total carbohydrate accumulated in vines increased significantly after 10 DAT while that of roots grew after 21 DAT. A possible reason for that would be temporary storage of carbohydrate in leaves before relocation to roots after SR formation (Kays, 1985).

Nitrogen fertilisation timing influenced N concentration and total N acquisition in vines and roots, with significantly higher content of N in treatments with applied N. 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. Although total N application of T1 has only about 20% higher than T14, it resulted in almost 100% higher total carbohydrates, 80% higher total N, and 80% higher total biomass. In addition, although N concentration was the same in all treatments, the use efficiency is always lower in T14. All

these suggests delayed N application could cause a damage (e.g. less SRs, fewer lateral roots for nutrient absorption), which could not be compensated even if adequate amount of N is added afterwards. 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). As this highest carbohydrate and lowest N concentration was in the treatment without N supply, the C:N ratio was also highest in this treatment. The C:N ratio was significantly lower in the treatments with N at all application dates 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)

In this study, N acquisition in vines and roots generally increased over time. A similar observation was made in another study where the amount of N accumulation increased from planting until 100 DAT (Osaki et al., 1995). The current study showed that N that accumulated in plants during the study period was mainly allocated to vines. Similarly, N accumulation in carrot SR was negligible until 50–60 days after sowing (Westerveld et al., 2006).

During N limitation, few new leaves are formed due to the reduction of photosynthetic in plants. As the result, the translocation of soluble carbohydrates from vines to roots is reduced significantly (Henry & Raper, 1991). The partitioning of carbon between vines and roots and the utilisation of carbohydrate in sink tissues is altered in N stress conditions (Rufty et al., 1984). Nitrogen uptake by roots depends on the translocation rate of carbohydrates from vines to roots (Raper et al., 1978). The uptake of N is limited during the low translocation rate of carbohydrates from vines to roots (Leslie & Raper, 1985; Raper et al., 1978). Therefore, both NSC accumulation and N acquisition were higher for early N application treatments and were limited in no or delayed N application treatments. In addition, N application level is associated to phytohormone such as cytokinin and auxin (Kiba et al., 2011; Samuelson et al., 1992) that considered to be important regulators of cell division (Matsumoto-Kitano et al., 2008; Wang, 2020). A possible mechanism that influences the formation of SRs would be the combination of phytohormone and the plant physiological process. Proper N application promoted cambial activity and differentiated ARs to become SRs. Simultaneously, appropriate N levels stimulated accumulation of NSC and N in plants and then allocated them from vines to roots.Earlier N fertilisation treatment had a higher NRE than the delayed N supply timings. This finding is in line with previous results in other crops 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. In this experiment, 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 the growth of the crop did not recover quickly after N was added in delayed N application treatments. Another reason would be that delayed N application plants formed fewer SRs and more lignified roots and then the growth of SRs required more N than lignified roots. 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 fertilisation timing affected NRE in sweetpotato, with higher NRE in the earlier N application.

4.4.4. Agronomic indication of N fertilisation timing application for sweetpotato

Results from the current experiment showed that sweetpotato required N from planting to initiate SRs. Delayed N fertilisation application inhibited the formation of vascular cambium in roots and promoted lignified stele cells. This results in a lower rate of SRs and a higher percentage of lignified roots, and may finally affect SR number and yield. Also, early N fertilisation application promoted plant and root growth as indicated by higher biomass, root length and root volume, whereas late application could lead to N limitation. In addition, earlier N fertilisation application enhanced the accumulation of NSC and N in sweetpotato plants while delayed N application appeared to be a limiting factor of carbohydrate relocation to SR.

In combination with results from Chapter 3, it indicated that medium N levels need to be maintained from planting to stimulate SR formation. This experiment was performed in sand culture, which has very poor nutrients. Therefore, in field conditions, soil available N should be tested before planting. If soil has good N availability, probably no N fertiliser is needed when planting, but some fertiliser should be applied if soil is poor in plant available N. Later, a key batch of N fertilisation could be added to stimulate SR growth. It was recorded in a previous field study that a single N application at 21, 28 or 35 DAT resulted in significantly higher marketable yield of Beauregard cultivar compared to split application

made at 10 and 21 DAT (Schultheis et al., 1995). However, field experiments should be conducted in future to test these fertilisation schemes and the final impact on commercial yield. It is also critical to determine the optimal N level to stimulate SR formation in different soil types in future studies.

4.5. 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 total carbohydrate, 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 following 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, adequate levels of available N should be established before or when cuttings are planted in agronomic practice.

Chapter 5: The effects of soil organic amendment applications on sweetpotato storage root formation

Abstract

Organic amendments have been used in agriculture to improve soil properties to provide a better environment for root growth. They have been utilised as a source of nutrients to improve the productivity of many crops, including sweetpotato. Some studies demonstrated the influences of these products on SR development and yield of sweetpotato. However, none of them examined the relationship between organic amendments and the initiation of SRs. A pot experiment was conducted in central Queensland, Australia, in 2019 to investigate anatomical changes in adventitious roots during SR formation. We also examined the effect on NSC, N acquisition in plants, and available and total N in soil. The experiment was designed to elicit if organic amendment affects SR formation by changing soil available N concentration. Cuttings of Orleans were grown in dermosol soil. Available macronutrients in all pots were manipulated to the same level when the experiment was initiated. Two locally available organic amendments including poultry manure (PM) and sugarcane trash (SCT) used in this experiment at different rates. Six treatments were included in the study including unamended soil, unamended soil with chemical fertilisation, PM 22 tons ha^{-1}, PM 66 tons ha^{-1}, SCT 30 tons ha^{-1} and SCT 10 tons ha^{-1}. Results from the study showed that SCT application for sweetpotato at both rates, 10 and 30 tons ha^{-1}, promoted the formation of SRs and reduced lignification compared to other treatments. PM applications from 22 to 66 tons ha^{-1} inhibited SR initiation and enhanced the formation of lignified roots, which appear to be associated with high soil available N caused by PM addition. This is in line with the findings in Chapter 3 and Chapter 4 that there is an optimal soil available N level for SR formation, while both lower or excessive soil available N suppress SR formation. During SR initiation, all amended treatments increased accumulation of NSC and N in plants compared to the control. With PM at 66 tons ha^{-1} promoted vine growth and reduced root growth as indicated by the highest NSC accumulated in vines and the lowest NSC accumulated in roots, as well as the lowest root to shoot ratio compared to other treatments. Both SCT treatments maintained the growth of shoots and promoted root growth as indicated by the highest NSC accumulation in roots and moderate N level in plants. Results from this study provide agronomic indication that the lower rate of PM should be used for amendment of sweetpotato growing soils, or when the higher rate of PM from (~20 tons ha^{-1}) is used, PM

should be amended in soil at least 2–3 weeks before planting. Excessively high rates of PM soil amendment (for example 66 tons ha^{-1}) should be avoided. In contrast, SCT may be applied at a suitable rate (for example 10 tons ha^{-1} in this study) to promote SR formation in sweetpotato, but high rates could lead to immobilisation of N and suppress crop growth.

5.1. Introduction

Organic soil amendments are commonly used in agriculture to improve soil organic matter content and other soil properties, which result in a better environment for root growth (Davis & Wilson, 2000). They are also sources of macronutrients including N, P and K for crops (Hue & Silva, 2000). Some types of organic amendments are commonly used to add in soil such as green manure, crop residue, animal manure, compost and biochar. The application of these amendments has been demonstrated to improve the yield of many crops such as vegetative, grain, root and tuber crops (Agyarko et al., 2013; Bulluck Lii et al., 2002; Chan et al., 2008; Yamato et al., 2006). The effect of organic amendments on root and tuber crops has received less research attention. Some studies examined the influence of these products on such crops. The application of poultry manure (PM) increased cassava yield by around 30% (Ojeniyi et al., 2012). The yield of radish responded differently with types and rates of amendments (Chan et al., 2008). In potato, manure and wood compost were suggested to improve tuber yield (Lynch et al., 2008). Organic amendments have been proven to enhance root growth of crops and result in increased nutrient uptake and yield (Nardi et al., 2002; Opena & Porter, 1999; Yang et al., 2004).

Organic residues such as plant residue and animal manure are widely available in the agriculture industry. Nutrients from these substances are released through the decomposition process by soil microorganisms (Avnimelech, 1986). Some organic amendments such as chicken manure can release nutrients immediately after amending while some others require more time (wood products). The decomposition of organic residues to release nutrients that can be used by plants depends on many factors, such as temperature, moisture, pH, aeration, microbial activities and nutrient levels (Robertson & Paul, 2000). Therefore, decomposition rates of different organic matters varies depending on amendment properties and environmental conditions when it is applied. This leads to variable amounts of nutrients being released into the soil.

The number of storage roots (SRs) varies greatly between individual plants in sweetpotato, leading to variability of yield up to 50% (Villordon et al., 2009c). Obviously, SR yield of the crop is determined by the plant density, SR number and SR size. According to Meyers et al., (2014) the density is adjusted well by commercial practices. As the initiation process of SRs determines the number as well as the size and shape of SRs, it is one of the key factors that affect crop production.

The application of organic amendments was investigated in some previous studies on sweetpotato. Some products such as wheat straw biochar, green leaf manure and PM have been demonstrated to improve SR yield (Agyarko et al., 2013; Liu et al., 2014; Nedunchezhiyan et al., 2010). Applications of organic amendments could stimulate SR development (Isobe et al., 1996) or increase sugar content as well as improve SR appearance quality (Dou et al., 2012). However, none of these studies focused on the influence of these substances on the storage root formation.

In Chapter 3 and Chapter 4, we found that both deficient and excessive N application resulted in inhibition of SR formation. The optimum N for SR formation in Orleans cultivar was 100 mg/L N in solution in sand culture and the crop required N soon after planting to promote the initiation of SR. Organic amendments are a source of N and available N in the soil and may respond differently due to amendment properties and application rates. Therefore, in this study we focused on how N soil availability change caused by soil amendments affects SR formation. Locally available organic amendments were chosen for this study to examine the effects on SR initiation. Queensland is the biggest producer of sugarcane in Australia, accounting for around 95% of Australia's sugarcane (SAR, 2019), and produces a large amount of trash (7–12 tons ha^{-1} $year^{-1}$) (Robertson & Thorburn, 2007). National poultry litter was estimated at around 1.2 million tons per year and expected to increase with industry expansions (McGahan et al., 2013). This study aims: (1) to investigate the influence of organic amendments on sweetpotato SR initiation; and (2) to determine the rate and type of amendments that promote the initiation of SRs. This would help to address the following questions: (1) How do organic amendments affect soil available N and then anatomical changes in roots? (2) Do soluble sugar and starch accumulate in vines and roots related to the initiation of SRs? (3) Do organic amendments affect nitrogen acquisition in sweetpotato?

5.2. Materials and methods

5.2.1. Plant materials and growth conditions

The experiment was conducted in a glasshouse at Bundaberg Research Facility (24°50′54″ S 152°24′14″ E) from 12 May to 1 July 2019. The average daily maximum and minimum temperatures during the study period inside the glasshouse were 26.5°C and 14.3°C, respectively, and the average daily maximum and minimum relative humidity was

87.9% and 48.8%, respectively. A sandy loam soil (Dermosol) (Isbell, 2016) was used for the experiment. Poultry manure blend and SCT were used as soil amendments in this study. Granular fertilisers including urea (46% N as NH_4^+), Nitrocal (15.5% N as NO_3^-), potash (41.5% K) and superphosphate (9.1% P) were added into the soil.

Healthy uniform cuttings of Orleans with six fully opened leaves and at least 20 cm length were utilised to examine SR initiation. Three leaves from the cut end of cuttings were cut off before planting. One cutting was planted horizontally in each pot with three nodes buried under the sand and three fully opened leaves above-ground.

5.2.2. Growth medium preparations

Black plastic pots 20 cm in diameter and 27 cm in height were used in this experiment. Soil (3.5 L) was mixed thoroughly with the respective amount of amendments and fertilisers to fill each pot (Table 5.1). Available nutrients at the beginning of the experiment were adjusted to the same in all pots as N 500 mg, P 1000 mg and K 1800 mg pot^{-1}. The amount of available N coming from soil, organic amendments and mineral fertilisers for treatments are shown in the appendix 3. The amount of P and K supplied for plants in this experiment was close to recommendations for sweetpotato in Australia before or at planting (Loader et al., 1999), and this was similar to the total P and K supplied for sweetpotato when using Hoagland's nutrient solution in the experiments described in Chapter 3 and Chapter 4. Nitrogen available for each plant was around 50% recommendation for basal N fertilisation of sweetpotato (Loader et al., 1999). This was chosen due to the fact that the total N applied for sweetpotato in the seven-week growing period that promoted SR initiation (see Chapter 3) was approximately 340 mg pot^{-1}. Because a key purpose of soil amendment is to increase soil organic C, amendments were applied to achieve two levels of C concentrations in the mixture at 2.16% and 2.55% (from 1.97 of the original soil). Pots were settled in the glasshouse for one week before planting. Tap water was added in the pots to field capacity three days before transplantation to ensure sufficient and similar moisture in all pots.

Characteristics of dermosol soil and organic amendments used for the experiment were analysed at the Environmental Analysis Laboratory, Southern Cross University, Australia (Table 5.2). The soil was acidic (pH = 5.83) with 3.4% organic matter content. Sugarcane trash had the highest organic matter at around 85% and low N content whereas PM had the highest rate of N, which was mainly NH_4^+ . Chemical properties for each

treatment were calculated based on soil, organic amendment properties and synthetic fertiliser addition, except that pH and electrical conductivity were measured.

Table 5.1. Addition of organic soil amendments and fertiliser for each pot (g pot^{-1})

Treatments	*Amendment*	*Nitrogen*	*Phosphorus*	*Potassium*
A0	0	0	0	0
AF	0	0.039	0.096	0.151
PM22	38.5	0.034	0.081	0.106
PM66	115.5	0.024	0.051	0.014
SCT10	17.5	0.038	0.095	0.148
SCT30	52.5	0.038	0.096	0.150

Data for organic amendment presented as the fresh weight of products.

Table 5.2. Chemical characteristics of growth substrate before planting

Parameter	Method reference	Soil	AF	PM*	PM22	PM66	SCT*	SCT10	SCT30
pH	1:5 Water	5.83	5.74	7.71	6.31	6.89	4.51	5.73	5.83
EC (dS m^{-1})	1:5 Water	0.10	0.29	11.61	0.38	0.45	1.66	0.32	0.30
K (mg kg^{-1})	Morgan 1	74	441	19923	441	441	656	441	441
P (mg kg^{-1})	Morgan 1	11	192	6460	192	192	148	192	192
NO_3^- (mg kg^{-1} N)	KCl	9.4	65	200	65	65	2.2	65	65
NH_4^+	KCl	20.4	65	1880	65	65	204	65	65
Sulfur (mg kg^{-1} S)	KCl	11.9	391	3222	313	157	361	391	390
Ca (mg kg^{-1})	Morgan 1	351	690	1170	625	495	547	689	693
Mg (mg kg^{-1})	Morgan 1	229	229	3807	252	297	423	231	234
Organic Matter (% OM)	Total Carbon x 1.75	3.4	3.4	61.2	3.8	4.5	85.3	3.8	4.5
ECEC (cmol+ kg^{-1})	Sum of the exchangeable cation	6.09	6.09	45.1	6.4	6.9	10.8	6.1	6.2
Ca/Mg Ratio	Calcium/Magnesium	1.2	1.2	2.1	1.2	1.2	1.7	1.2	1.2
Zinc (mg kg^{-1})	DTPA	1.9	1.9	353.9	4.0	8.2	13.8	2.0	2.1
Manganese (mg kg^{-1})	DTPA	16	16	203	18	20	50	17	17
Iron (mg kg^{-1})	DTPA	206	206	279	208	211	113	207	208
Copper (mg kg^{-1})	DTPA	0.8	0.8	69.0	1.2	2.1	0.3	0.8	0.8
Boron (mg kg^{-1})	Hot $CaCl_2$	0.36	0.36	19.98	0.48	0.72	4.22	0.38	0.41
Silicon (mg kg^{-1} Si)	Hot $CaCl_2$	35	35	152	36	37	159	35	37
Total Carbon (%)	LECO Trumac Analyser	1.97	1.97	34.95	2.17	2.55	48.72	2.17	2.55
Total Nitrogen (%)	LECO Trumac Analyser	0.09	0.10	3.99	0.12	0.17	0.29	0.10	0.10
C/N Ratio	Total Carbon/Total Nitrogen	23.2	20.7	8.8	18.2	15.3	166.4	22.5	25.9

Soil, PM* and SCT* columns presented measurement values for soil/organic amendments used for this experiment. Other column values are calculated based on original values of soil and amendments.

All results presented as a dried weight. The moisture content of PM was 40.4% and SCT was 6.1%. Soil was sieved and lightly crushed to < 2 mm.

Abbreviations: EC = Electrical conductivity; ECEC = Effective cation exchange capacity.

5.2.3. Experiment design

The experiment consisted of six treatments: unamended, synthetic fertilisers, PM 22 tons ha^{-1}, PM 66 tons ha^{-1}, SCT 30 tons ha^{-1} and SCT 10 tons ha^{-1} (hereafter A0, AF, PM22, PM66, SCT10 and SCT30, respectively) with multiple harvests over the study period. The pots were arranged in a completely randomised design. During each harvest, six plants were harvested for measurements. Three plants were used for anatomical observations and morphological analysis of the roots, and the other three were used for carbohydrate and CN analysis. In total, four harvests were conducted over the experimental period and included 144 plants (6 treatments x 4 harvests x 6 plants per harvest). Eight additional plants per treatment (48 plants in total) were grown for backup purposes in case death or abnormal growth occurred.

5.2.4. Sampling date

The interval time between each harvest was between 10 and 14 days. Plants were sampled at 10, 21, 35 and 49 DAT. Six uniform plants were sampled for each treatment on a sampling date and divided into two sets. Plants were dug up carefully to minimise root damage and washed in tap water to remove all soil.

5.2.5. Measurements and data collections

Available NH_4^+ and NO_3^- in soil

Soil samples were taken from three harvested pots for each treatment at planting, 10, 21, 35 and 49 DAT. They were dried in an oven at 40°C to a constant weight. Then, all samples were stored in a -80°C freezer until analysis. A solution of 0.1 M $MgSO_4$ was used to extract NH_4^+ and NO_3^- in soil samples (Choosang et al., 2018). The concentrations of NH_4^+ and NO_3^- were determined by NH_4^+ and NO_3^- Ion Selective Electrodes (ISEs) (Van London Co., Houston, Texas, USA). Total C and N concentrations in soil samples were analysed using TruMac® Carbon/Nitrogen Analyser (LECO Corporation, Michigan, USA). The C:N ratio was calculated based on their concentration in dried mass basis.

Anatomical changes in ARs

Sampling for root anatomy was performed at 10, 21, 35 and 49 DAT. Three plants from each treatment were dug out carefully and washed in tap water to remove all growing

substrates. The AR count was recorded for each plant. All ARs from an individual plant were harvested and sectioned using sharp razor blades (Villordon et al., 2009c). Sections were cut very thin and were not oblique. Anatomical observations were recorded from sections at around 3–4 cm from the proximal end of roots (Villordon et al., 2009c). Transverse sections of all ARs were stained by Toluidine Blue O 0.05% to observe the anatomy under a microscope (Eguchi & Yoshinaga, 2008). After staining, they were rinsed in water until there was no stain left on the sections (O'Brien & McCully, 1981). Pictures for each section were taken under a microscope to determine anatomical feature development. Some main anatomical characteristics of ARs were investigated using those photos. Protoxylem number was observed from roots at 10 DAT. Other characteristics, including IRVC, completed RVC, appearance of AC and more than 50% of LC were characterised throughout the sampling dates.

Classifying AR development

Adventitious roots were classified into SRs, PRs and lignified roots as described by Wilson and Lowe (1973). Adventitious roots with AC encircling the primary and secondary xylem elements and/or central metaxylems were considered as SRs (Belehu et al., 2004; Villordon et al., 2009b; Wilson & Lowe, 1973). Those roots with at least one of the protoxylem elements connected to the central metaxylems by a strand of LC and with some meristematic activity around the central metaxylem developed into PRs. When more than 50% of stele cells were lignified, these roots would be lignified roots. Details for cambium development in ARs were described in Chapter 3.2.6. Sections were observed under an Olympus CX31 microscope (Olympus corporation, Tokyo, Japan) and images were taken by a Nikon DS-L2 camera (Nikon corporation, Tokyo, Japan).

Root morphology of sweetpotato

The morphological characteristics of roots were detected at 10, 21, 35 and 49 DAT using roots after doing anatomical observations. Roots from each plant were collected individually. An Epson Perfection V700 Photo Scanner (Seiko Epson, Nagano, Japan) was used to scan root images. The WinRHIZO Pro software (version 2012a; Regent Instruments Inc., Quebec, Canada) was utilised to analyse root images. The ARs were floated in a transparent tray and scanned to acquire images. Where necessary, roots were divided into smaller sections to make sure they all could spread out in the tray. The following characteristics were recorded: total root length, average root diameter, and total root volume.

The accumulation of soluble sugar and starch in vines and roots of sweetpotato

Soluble sugar and starch analysis were performed on three plants from each treatment at 10, 21, 35 and 49 DAT. Plants were rooted up carefully to avoid damage and washed in tap water to remove all soil. Shoots and roots for each plant were separated and biomass weight for each sample was recorded. Samples were dried in the preheated oven at 90°C for 90 minutes to stop enzymatic sugar conversion in tissues, and then converted to 70°C to a constant weight (Maness, 2010). After that, samples were stored in a -80$^{\circ C}$ freezer until extraction.

A MCW solution (12:5:3 by volume) was utilised to extract soluble sugar and starch (Dickson, 1979; Rose et al., 1991). The colorimetric phenol-sulfuric acid assay was used to determine the concentrations of sugar and starch (Dubois et al., 1956). The procedure for extraction and analysis was discussed in Chapter 3.2.6.

Nitrogen acquisition in sweetpotato and total N in soil samples

Dried plant samples used for NSC analysis were also used to determine N acquisition. Total N in plant samples was analysed separately using a TruMac® Carbon/Nitrogen Analyser .

5.2.6. Statistical data analysis

All statistical analysis was conducted using the IBM® SPSS® software (version 25; IBM, New York, USA) statistical package. The AR count and percentage data were transformed using $\log_{10}$ and arcsine, respectively, whereas other data were square root transformed in SPSS for analysis. One-way ANOVA was used to test how plants reacted to different organic soil amendments for each harvest. As different plants were sampled in each harvest, two-way ANOVA (rather than repeated measure ANOVA) was used to analyse the global effects of organic amendments, time and interactive effect of amendment and time on anatomical root features, morphological characteristics of roots, soluble sugar and starch accumulation, N acquisition, and the number of initiated SRs. The means were compared using Tukey HSD. A *P*-value of less than 0.05 was regarded as statistically significant. Graphs were produced using SigmaPlot® software package (version 14; SYSTAT Software, Inc., California, USA) and Microsoft® Excel® for Office 365 MSO (Microsoft Corporation, Washington, USA).

5.3. Results

5.3.1. Effect of soil amendment on N concentration in soil samples

Available N and total N in soil were examined over the study period. Results showed that the effects of soil amendments were statistically different in the concentration of soil available N (Figure 5.1). As no N fertilisation was added in the A0 treatment, the lowest N availability was recorded in this treatment. Although initial soil available nutrients were modified to the same level in all amended pots, there were significant differences in the concentration of available NO_3^- and NH_4^+ among amended treatments, especially related to NH_4^+. In the treatments with PM, a higher concentration of NH_4^+ was recorded in those treatments. The highest and lowest mineral N (total NH_4^+ and NO_3^-) was observed in the PM66 and SCT30 treatments, respectively. In all observations, both PM treatments had significantly higher available NO_3^- and NH_4^+ than treatments with SCT. The treatment AF had similar concentration of mineral N to SCT treatments and significantly lower than both PM treatments in the first three weeks. Then, it became higher than that of SCT treatments and lower than that of PM treatments (Figure 5.1).

Regarding the total N in soil, there were significant differences among treatments (Table 5.3). Poultry manure applications increased total N in soil over the study time whereas SCT treatment had a trivial effect on the total N in soil due to the low N concentration. Treatment PM66 had significantly higher total N than other treatments on all sampling dates. By contrast, PM decreased the C:N ratio in soil, whereas SCT increased this ratio (Table 5.4). The two-way ANOVA showed that the main effect of amendment and time was statistically significant on the total N and C:N ratio in soil, while the interactive effect showed no significant difference. Overall, soil total N reduced over time and the C:N ratio increased in all treatments.

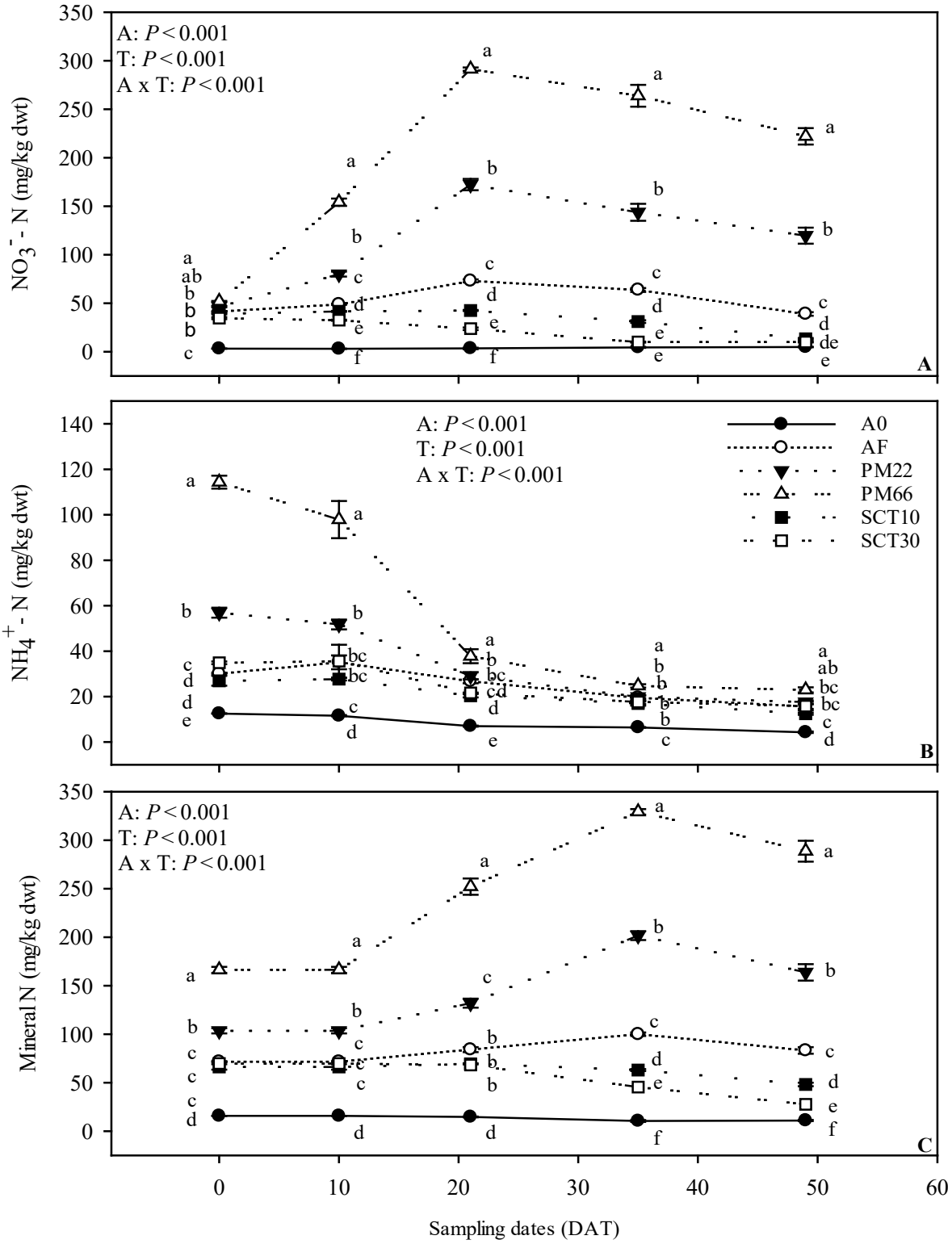

Figure 5.1. Effects of soil amendments on available N in soil (mg g^{-1} dwt) at various sampling times. (A) available NO_3^-; (B) available NH_4^+; (C) mineral N (NO_3^-+ NH_4^+).

Values are indicated as mean ± SE (n=3). ANOVA results are based on square root transformed data. Two-way ANOVA results, including the effect of treatment, time, and treatment by time on SRs and PRs are shown. Different letters are significantly different among treatments on single sampling dates using one-way ANOVA (Tukey's HSD, P<0.05).

Table 5.3. Effects of soil amendments on total N (%) in soil at various sampling times

Treatment	Planting	10 DAT	21 DAT	35 DAT	49 DAT	ANOVA
A0	$0.088^{c}\pm0.004$	$0.087^{c}\pm0.006$	$0.084^{c}\pm0.002$	$0.081^{b}\pm0.005$	$0.079^{b}\pm0.003$	A: $P<0.001$
AF	$0.097^{c}\pm0.004$	$0.094^{c}\pm0.003$	$0.091^{c}\pm0.002$	$0.088^{b}\pm0.009$	$0.082^{b}\pm0.004$	T: $P<0.001$
PM22	$0.118^{b}\pm0.002$	$0.114^{b}\pm0.004$	$0.113^{b}\pm0.007$	$0.105^{b}\pm0.005$	$0.096^{b}\pm0.002$	A x T: $P=0.08$
PM66	$0.173^{a}\pm0.007$	$0.159^{a}\pm0.004$	$0.154^{a}\pm0.005$	$0.135^{a}\pm0.004$	$0.130^{a}\pm0.006$	
SCT10	$0.097^{c}\pm0.004$	$0.097^{bc}\pm0.003$	$0.097^{bc}\pm0.005$	$0.088^{b}\pm0.003$	$0.087^{b}\pm0.002$	
SCT30	$0.099^{bc}\pm0.004$	$0.094^{c}\pm0.001$	$0.096^{bc}\pm0.001$	$0.089^{b}\pm0.005$	$0.088^{b}\pm0.006$	
P value	<0.001	<0.001	<0.001	<0.001	<0.001	

The table presents the mean values followed by standard errors (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letters are significantly different (Tukey's HSD, $P<0.05$) within columns using one-way ANOVA. Two-way ANOVA results, including the effect of treatments, time and treatments by time are shown in the last column.

Abbreviations: A = Amendment treatment; T = Time.

Table 5.4. Effects of soil amendments on C:N ratio in soil at various sampling times

Treatment	Planting	10 DAT	21 DAT	35 DAT	49 DAT	ANOVA
A0	$22.5^{ab}\pm0.6$	$22.6^{ab}\pm1.9$	$22.9^{ab}\pm0.4$	$23.9^{ab}\pm1.3$	$24.2^{abc}\pm0.9$	A: $P<0.001$
AF	$20.5^{bc}\pm0.9$	$21.3^{abc}\pm1.2$	$21.5^{b}\pm0.4$	$22.1^{b}\pm1.4$	$23.6^{bc}\pm1.0$	T: $P<0.001$
PM22	$18.7^{c}\pm1.1$	$18.7^{bc}\pm1.2$	$19.3^{bc}\pm0.9$	$20.0^{b}\pm1.3$	$21.3^{bc}\pm1.1$	A x T: $P=0.99$
PM66	$14.9^{d}\pm0.3$	$16.3^{c}\pm0.9$	$16.7^{c}\pm0.7$	$19.1^{b}\pm2.0$	$19.4^{c}\pm1.3$	
SCT10	$22.5^{ab}\pm0.9$	$22.9^{ab}\pm0.9$	$23.0^{ab}\pm1.0$	$24.4^{ab}\pm1.2$	$26.2^{ab}\pm0.6$	
SCT30	$25.7^{a}\pm0.8$	$27.1^{a}\pm1.1$	$26.8^{a}\pm0.9$	$28.8^{a}\pm0.9$	$28.9^{a}\pm1.3$	
P value	<0.001	0.001	<0.001	0.004	0.001	

The table presents the mean values followed by standard errors (SE) (n=3). ANOVA results are based on square root transformed data. Two-way ANOVA results are shown in the last column. Means followed by different letters are significantly different (Tukey's HSD, $P<0.05$) within columns using one-way ANOVA. Abbreviations: A = Amendment treatment; T = Time.

5.3.2. Effects of soil amendments on root anatomy of sweetpotato

The current experiment showed that applications of mineral fertiliser, PM or SCT had no effect on the number of sweetpotato ARs (Table 5.5). Total AR count from three subterranean nodes varied from around 11 to 15. The AR numbers in all treatments followed a similar pattern, with the highest number at 21 DAT.

Table 5.5. Effect of organic soil amendments on the number of ARs

Treatments	10 DAT	21 DAT	35 DAT	49 DAT	ANOVA
A0	11.0±0.6	13.0±0.6	11.7±0.3	11.3±0.3	A: $P = 0.36$
AF	11.0±0.6	13.3±0.9	11.3±0.3	12.3±0.3	T: $P < 0.001$
PM22	11.7±0.7	14.3±0.7	12.0±0.6	12.0±0.6	A x T: $P = 0.96$
PM66	11.3±0.7	14.0±0.6	11.7±0.3	11.7±±0.3	
SCT10	10.7±0.3	13.7±0.3	11.3±0.3	11.7±0.3	
SCT30	11.0±0.6	14.7±0.7	11.7±0.3	11.7±0.3	
P value	0.88	0.48	0.83	0.58	

The table presents the mean values followed by standard errors (SE) (n=3). ANOVA results are based on $\log_{10}$ transformed data. Two-way ANOVA results, including the effect of soil amendment, sampling time and interactive effect of soil amendment by time are shown. *P* values from one-way ANOVA analysis for each sampling date are presented within columns.

Abbreviations: A = Amendment treatment; T = Time.

The number of protoxylem elements in roots at 10 DAT varied from four to ten. The arrangement of them was classified into three groups (Figure 5.2). There was no statistical significance found on the effect of soil amendments on the distribution of protoxylem number among treatments ($P>0.1$). A large proportion of roots was pentarch and hexarch and accounted for a combination of about 65–85%. A small percentage of roots with tetrarch stele were found in the PM22 and SCT10 treatments.

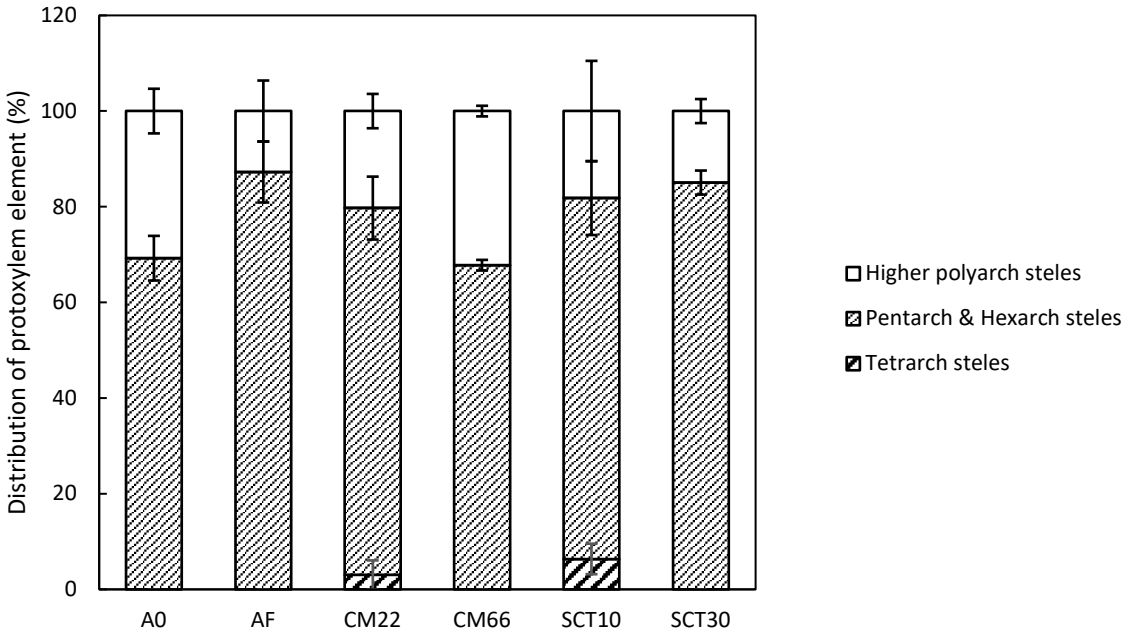

Figure 5.2. Distribution of protoxylem elements as affected by organic soil amendments; the error bars indicate the SE of the mean value (n=3).

Different anatomical features were observed at certain harvesting times (Figure 5.3). For example, NC was observed in roots during 10 and 21 DAT (Figure 5.4A) and IRVC was found in roots at 10, 21 and 35 DAT (Figure 5.4B). The RVC could be seen throughout the study period (Figure 5.4C), while AC and LC were found in roots from 21 DAT (Figure 5.4D; Figure 5.4E).

In the early stage of root development, there was a significant effect of soil amendments on vascular cambium formation. Both treatments with PM reduced the rate of roots with IRVC compared to other treatments (Figure 5.4B). In contrast, a significantly higher percentage of roots with vascular cambium were observed in the SCT10 treatment. As the activity of primary cambium resulted in the development of IRVC and gradually formed RVC, IRVC reduced over time and could not be found in roots at 49 DAT. Meanwhile, RVC increased during 10 and 21 DAT before decreasing until 49 DAT (Figure 5.4C).

The appearance of AC was observed in all treatments from day 21 (Figure 5.3). The formation of this characteristic increased until 49 DAT. The treatment PM66 had a significantly lower rate of roots with this feature compared to the control (A0) and AF,

whereas SCT10 and SCT30 treatments had the highest percentages of root developed AC than other treatments.

The development of LC increased over time in all treatments and there was no significant difference among treatments at 21 DAT. After that, the treatment PM66 promoted LC formation with the highest rate observed at both 35 and 49 DAT. This rate was significantly lower than that of both A0 and AF treatments. However, both SCT10 and SCT30 treatments had the lowest rate of lignified roots varying from around 10% to 28% which was lower than A0 but similar to AF.

Therefore, the formation and development of cambium were all inhibited in both PM22 and PM66 treatments, and lignification was promoted from day 35. By contrast, SCT applications (SCT10 and SCT30 treatments) stimulated the initiation of the vascular cambium and AC throughout the SR formation.

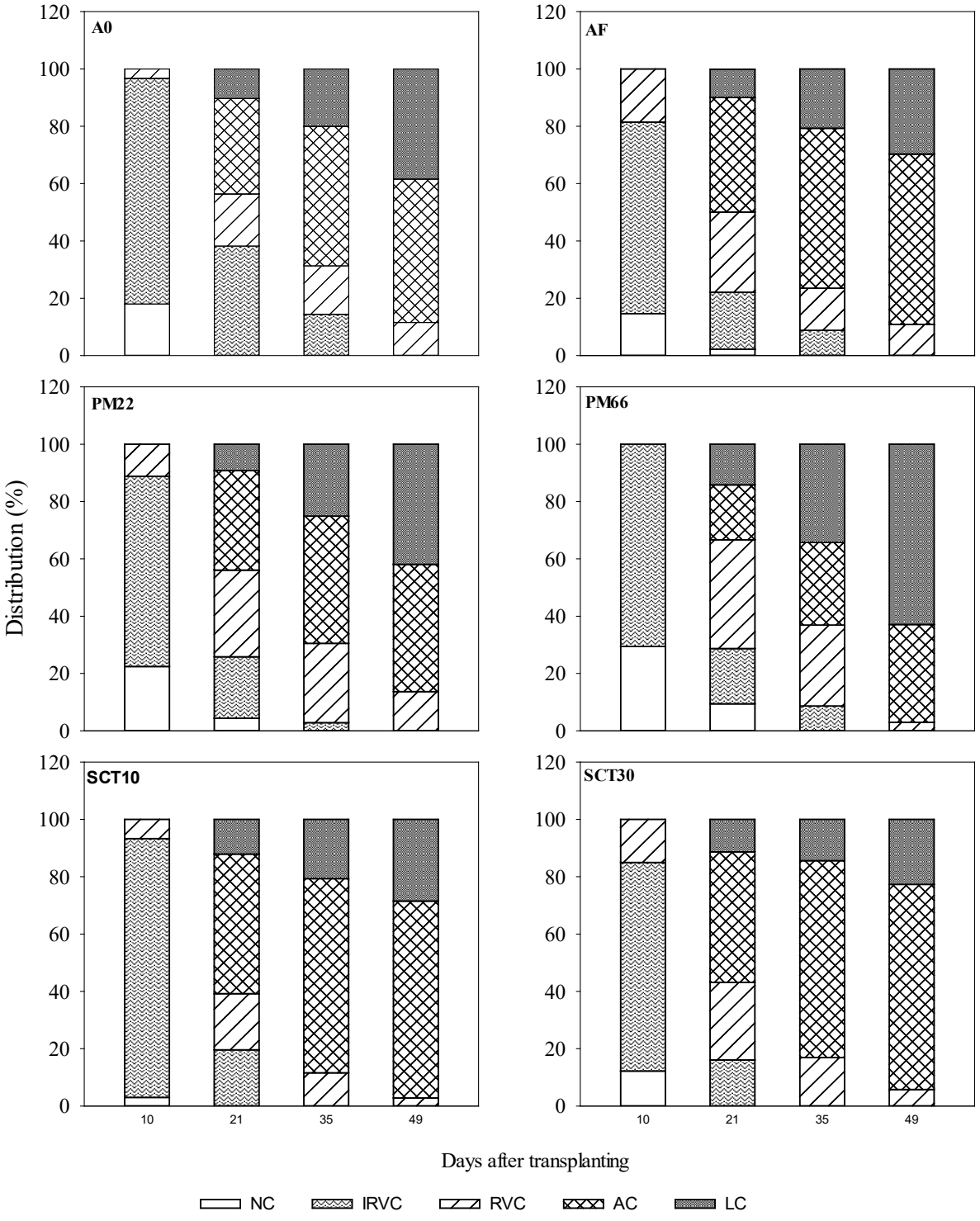

Figure 5.3. Effects of soil amendments on the development of anatomical features in roots. Values are indicated as mean (n=3)

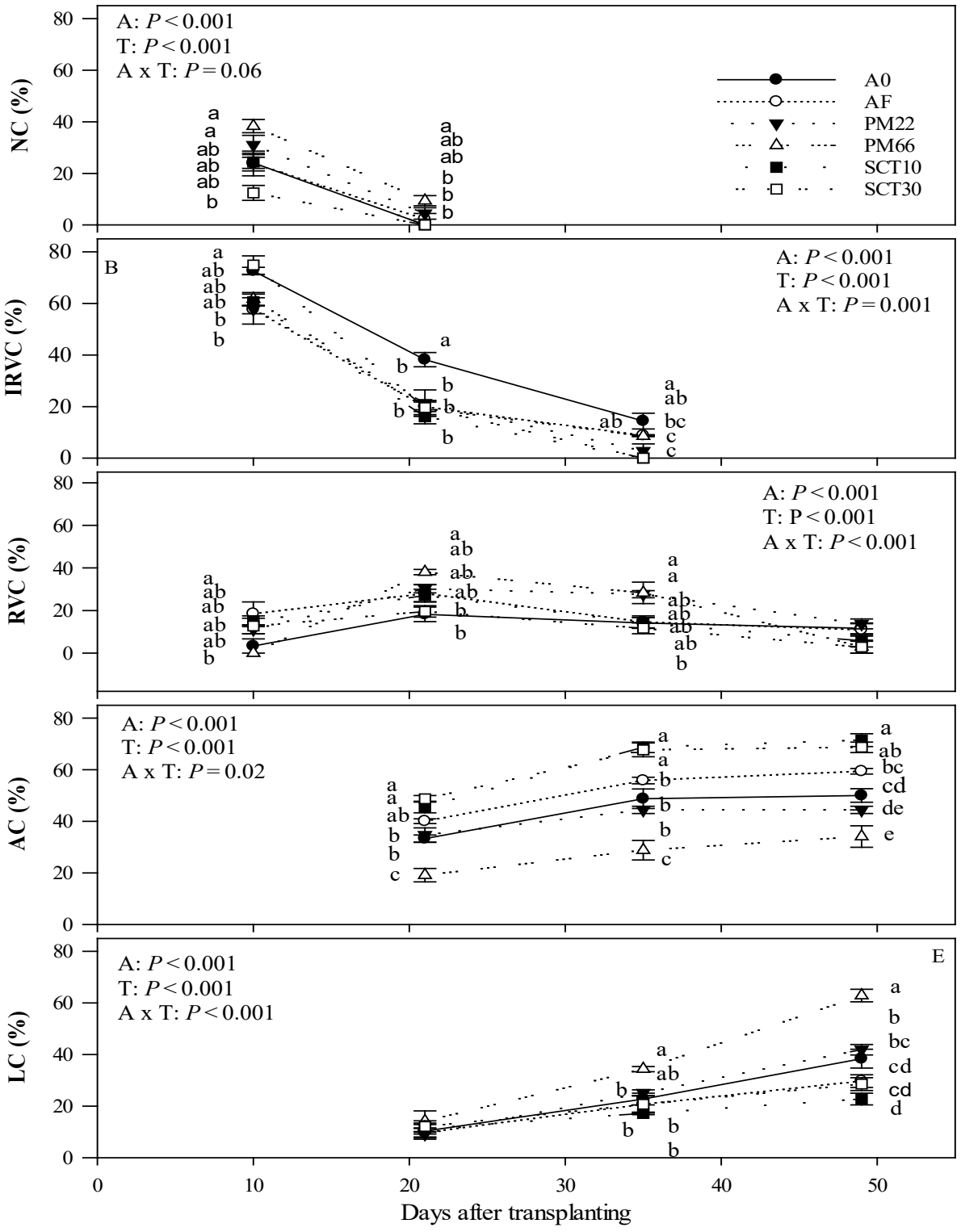

Figure 5.4. Effects of soil amendments on the anatomical feature development at different sampling times.

Values are indicated as mean ± SE (n=3). ANOVA results are based on arcsine-transformed data. Two-way ANOVA results, including effects of treatment, time, and treatment by time on anatomical development are shown. Different letters are significantly different among treatments on single sampling dates using one-way ANOVA (Tukey's HSD).

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

Results from this experiment showed that there were significant effects of soil amendment application on the SR initiation (Figure 5.5A). Both SCT amended treatments had higher rates of SRs compared to A0 and AF. The SCT10 and SCT30 treatments had a similar rate of SRs, which were significantly higher than those of all other treatments. Although AF treatment had a higher distribution of SRs than A0, PM22 and PM66, it was still lower than the SCT10 and SCT30 treatments.

In this experiment, there was no statistical difference in the distribution of PRs (Figure 5.5B). The percentage of PRs increased over time and reached the highest rate at the final sampling. The main effect of time was significant at different sampling times.

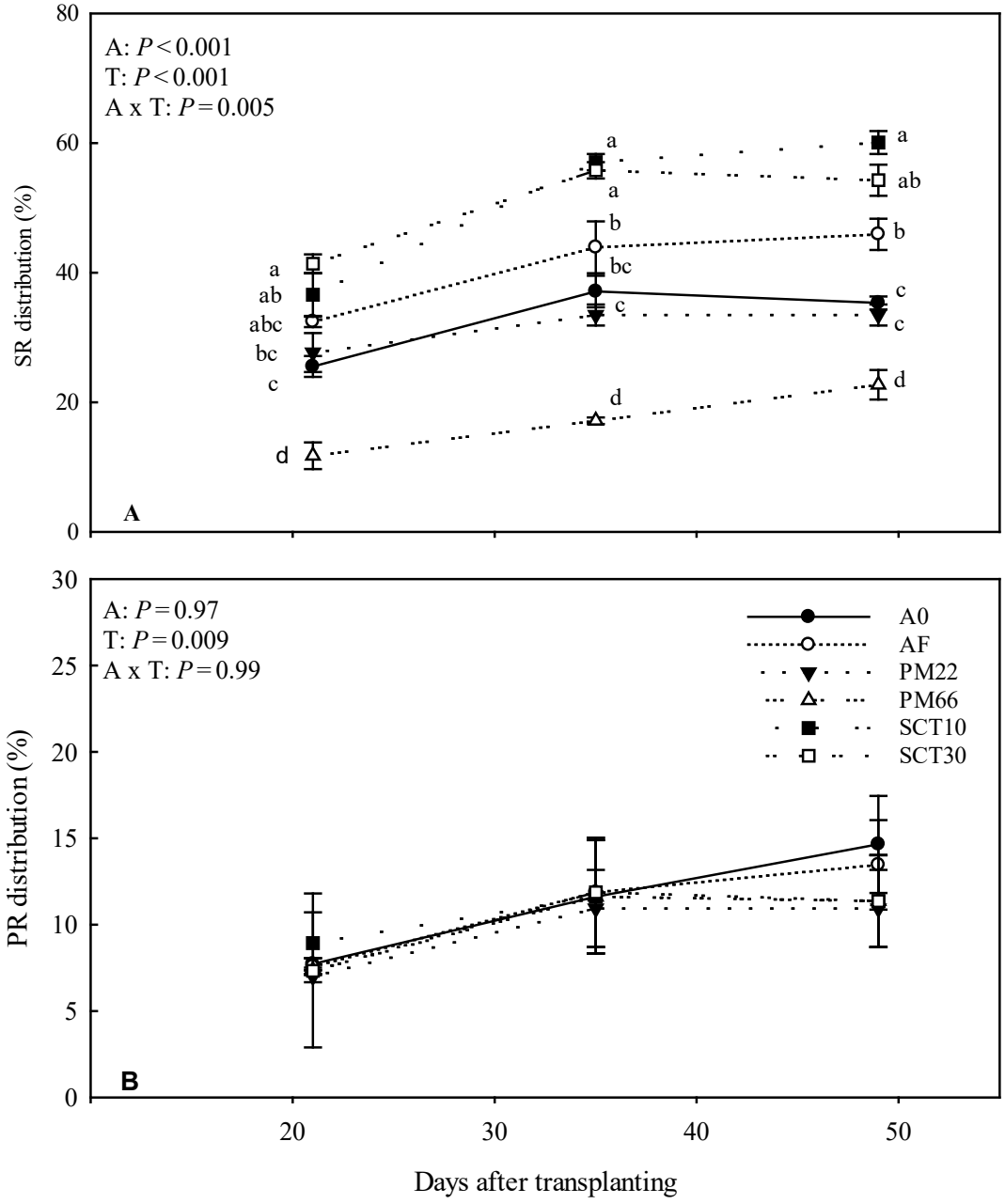

Figure 5.5. Effects of organic soil amendments on the formation of SRs and PRs over time. (A) Distribution of SRs; (B) Distribution of PRs.

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

Abbreviations: SRs = Storage roots; PRs = Pencil roots.

5.3.3. Effects of soil amendments on root morphology, plant performances and storage root weight of sweetpotato

The total root length in all treatments except PM66 increased within the first 35 DAT before slightly decreasing or remaining stable (Figure 5.6A). In the early stages, the lowest total root length was recorded in the PM66 treatment between 10 to 35 DAT. After that, it grew quickly and passed that of A0 treatment to be similar to PM22 and SCT30 treatments. Treatment AF and SCT10 had the highest total root length in most of the sampling times. The total root volume followed a similar pattern to that of total root length, with an increasing trend over time (Figure 5.6C). However, there was no significant difference among the five treatments with soil amendments or fertiliser.

No significant effect was found on the effect of soil amendments on the average root diameter between 10 and 21 DAT (Figure 5.6B). However, amended treatments had greater values at 35 and 49 DAT compared to unamended treatments. The greatest root diameter was observed in SCT10 and SCT30 treatments at 35 and 49 DAT, respectively. Two-way ANOVA results showed that the interactive effect of treatment by time was significant on the total root length, root volume and root diameter.

Results from this experiment showed that all organic amended treatments had significantly higher growth parameters (above-ground dry weight and root dry weight) compared to A0 (Table 5.6). Poultry manure treatments (PM22 and PM66) increased the above-ground weight of plants but reduced the weight of roots. However, SCT application (SCT10 and SCT30 treatments) maintained moderate shoot growth and promoted root weight. Treatments SCT10 and SCT30 had the greatest SR weight compared to other treatments. Although, there was no significant difference among treatments on SR diameter at 49 DAT, a significantly lower value for root length was found in the PM66 treatment. As a result, the lowest SR weight was observed in the PM66 treatment, which was similar to that of A0.

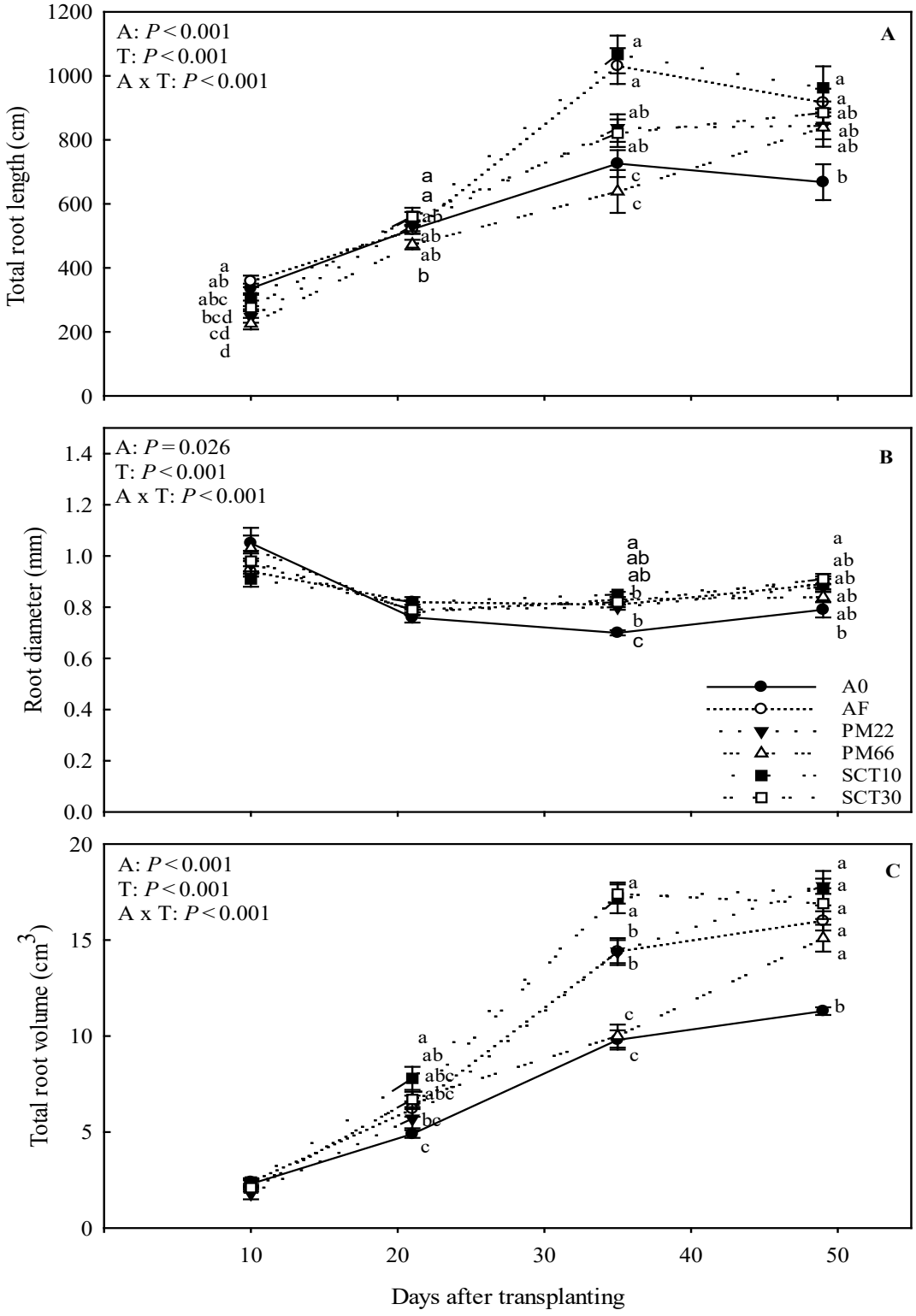

Figure 5.6. Effects of soil amendments on some morphological characteristics of Orleans roots on various sampling dates.

Values are indicated as mean ± SE (n=3). ANOVA results are based on square root transform data. Two-way ANOVA results, including the effect of treatment, time, and treatment by time, on SRs and PRs are shown. Different letters indicate significant differences among treatments on single sampling dates using one-way ANOVA (Tukey's HSD, $P<0.05$).

Table 5.6. Effects of soil amendments on biomass weight, SR length, SR diameter and SR yield at 49 DAT

Treatment	ADW (g)	RDW (G)	SRL (mm)	SRD (mm)	FSRW (g)
A0	1.8^{e}±0.1	1.6^{d}±0.2	81.1^{a}±1.8	7.7±1.0	7.4^{c}±0.3
AF	4.7^{b}±0.1	4.2^{b}±0.2	73.9^{ab}±7.4	7.8±0.6	19.4^{ab}±0.8
PM22	5.0^{bc}±0.2	2.8^{c}±0.2	74.1^{ab}±6.5	9.6±0.8	13.3^{b}±0.8
PM66	6.0^{a}±0.2	1.8^{d}±0.1	50.4^{b}±6.2	8.3±1.2	7.0^{c}±0.5
SCT10	4.2^{c}±0.2	4.6^{a}±0.1	69.5^{ab}±1.7	9.0±0.5	23.6^{a}±1.7
SCT30	3.4^{d}±0.2	4.2^{b}±0.2	74.3^{ab}±5.9	9.3±1.2	22.5^{a}±0.9
P value	<0.001	<0.001	0.02	0.64	<0.001

The table presents the mean values followed by standard errors (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by different letters are significantly different (P<0.05) within columns using one-way ANOVA (Tukey'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.

5.3.4. Effects of soil amendments on soluble sugar and starch accumulation in plants

5.3.4.1. Effect of soil amendment on the concentration of soluble sugar and starch

The highest soluble sugar and starch in vines was observed in A0 treatment in almost all harvestings (Figure 5.7A; Figure 5.7C). The concentration of soluble sugar in vines responded differently, showing a decreasing trend in the A0 treatment and an increasing trend in the SCT10 and SCT30 treatments. In the final sampling, the lowest soluble sugar was recorded in the AF treatment while the lowest starch content was observed in the PM66 treatment. There were increasing trends in root soluble sugar and starch concentration in all treatments except A0 with starch concentration decreasing during 35 and 49 DAT (Figure 5.7B; Figure 5.7D). Treatment PM66 had a significantly lower content of both root soluble sugar and starch in most samplings. In contrast, SCT30 treatment had the highest value in all harvesting except 21 DAT. At 49 DAT, the highest concentration of soluble sugar and starch was observed in SCT30 treatment at around 300 mg g^{-1}.

In this experiment, NSC in plants were calculated by combining values of soluble sugar and starch content (Figure 5.7E; Figure 5.7F). The NSC concentration in vines and roots followed similar patterns to that of starch. The NSC concentration in vines was highest in the A0 treatment over time. At the final harvesting, PM66 treatment had significantly lower vine NSC compared to both A0 and AF. However, the highest concentration of NSC in roots was recorded in the SCT30 treatment at all samplings except 21 DAT. Both SCT treatments had higher root NSC concentration than A0 and AF. The concentration of NSC in

roots for PM22 treatments was higher than A0 but lower than AF while PM66 had lower significant value compared to A0 and AF. The two-way ANOVA showed significant effects of amendments, time and interactive effect between them on NSC concentration in both vines and roots.

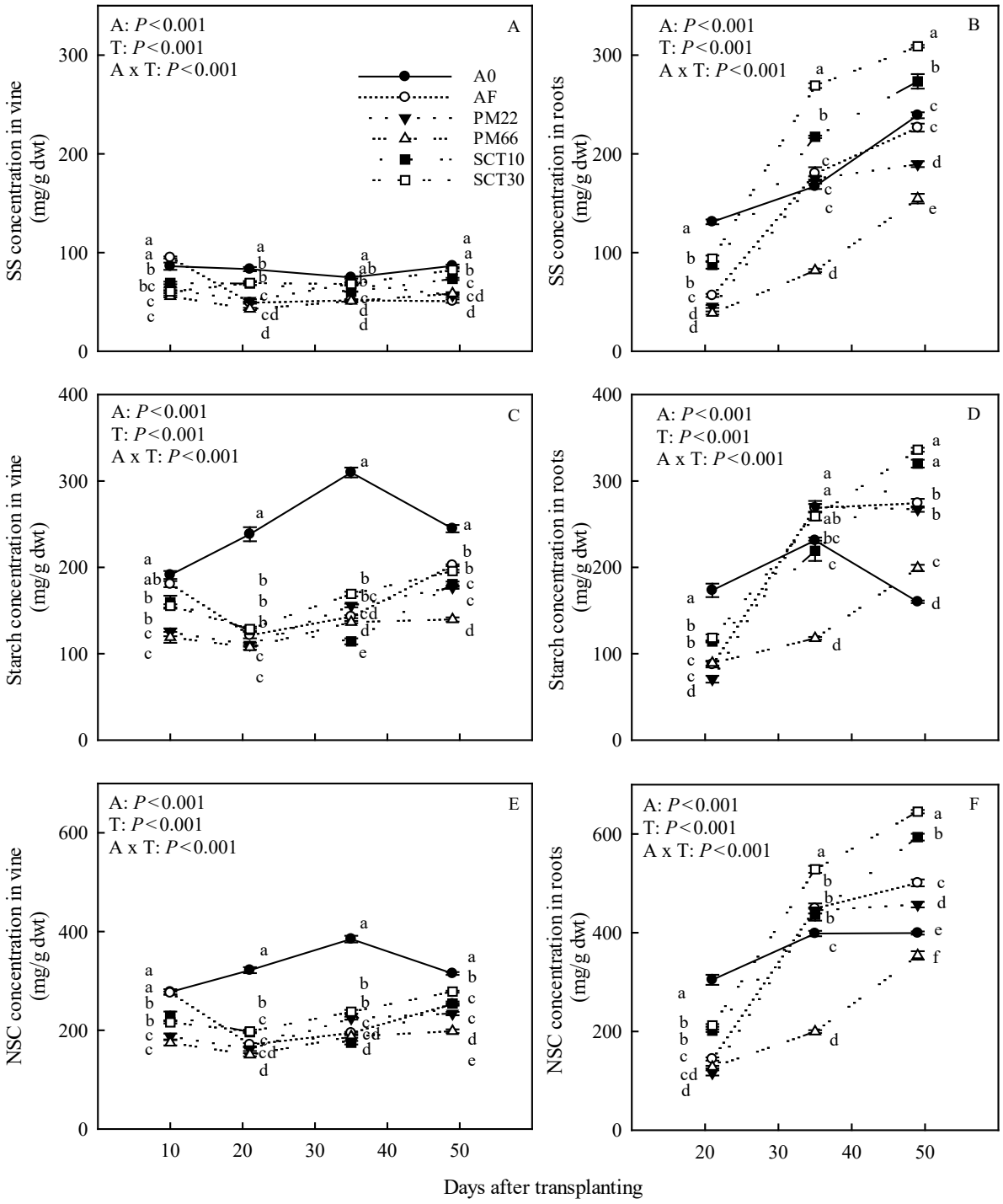

Figure 5.7. The concentration of carbohydrate as affected by organic soil amendment applications. (A) SS concentration in vines; (B) SS concentration in roots; (C) Starch concentration in vines; (D) Starch concentration in roots; (E) Carbohydrate concentration in vines; and (F) Carbohydrate concentration in roots.

Values are indicated as mean ± SE (n=3). ANOVA results are based on square root transformed data. Two-way ANOVA results, including effects of amendments, harvesting date, and treatment by time interaction on SS, starch and NSC concentration are shown. Different letters indicate significant differences among treatments on the same harvesting dates using one-way ANOVA (Tukey's HSD, $P<0.05$)

Abbreviation: SS = Soluble sugar; NSC = Non-structural carbohydrates.

5.3.4.2. Effect of soil amendment on the soluble sugar and starch accumulation in plants

Total soluble sugar and starch in vines were analysed four times at 10, 21, 35 and 49 DAT. As the root biomass at 10 DAT was insufficient for these analyses, they were performed three times starting from day 21. There was a significant difference among treatments on the accumulation of these carbohydrates at all samplings. In general, the total soluble sugar and starch, as well as NSC accumulation, increased over time in all treatments (Figure 5.8).

In the first three weeks after transplanting, the total soluble sugar and starch content in vines and roots were lowest in the PM66 treatment and the highest was in the A0 treatment (Figure 5.8A; Figure 5.8B). At 21 DAT, treatment A0 accumulated 376 mg and 129 mg NSC in vines and roots, respectively. During this stage, the total NSC in vines was higher than that of roots (Figure 5.8B). After that, the A0 treatment had the lowest value whereas SCT10 and SCT30 had the highest values (Figure 5.8C; Figure 5.8D). In the final harvest, the highest vine NSC was observed in the PM66 treatment at 1193 mg $plant^{-1}$ while the highest root NSC was observed in the SCT10 and SCT30 treatments at 2844 mg and 2869 mg, respectively. Both PM22 and PM66 had the significant higher NSC accumulated in roots than A0, but lower than AF. There was also a significant interactive effect of N levels by harvesting time on soluble sugar and starch in both vines and roots (Table 5.7).

Results from the present experiment showed that photosynthate storage reallocated from vines to roots over time in all treatments, as suggested by the decreasing vines to root NSC ratio (Table 5.8). A slight decreasing trend was observed in treatments A0 and PM66, falling from 2.92 and 3.72 at 21 DAT to 1.04 and 1.59 at final sampling. This ratio reduced significantly in other treatments from around 3–5 at 21 DAT to around 0.3–0.6 at 49 DAT.

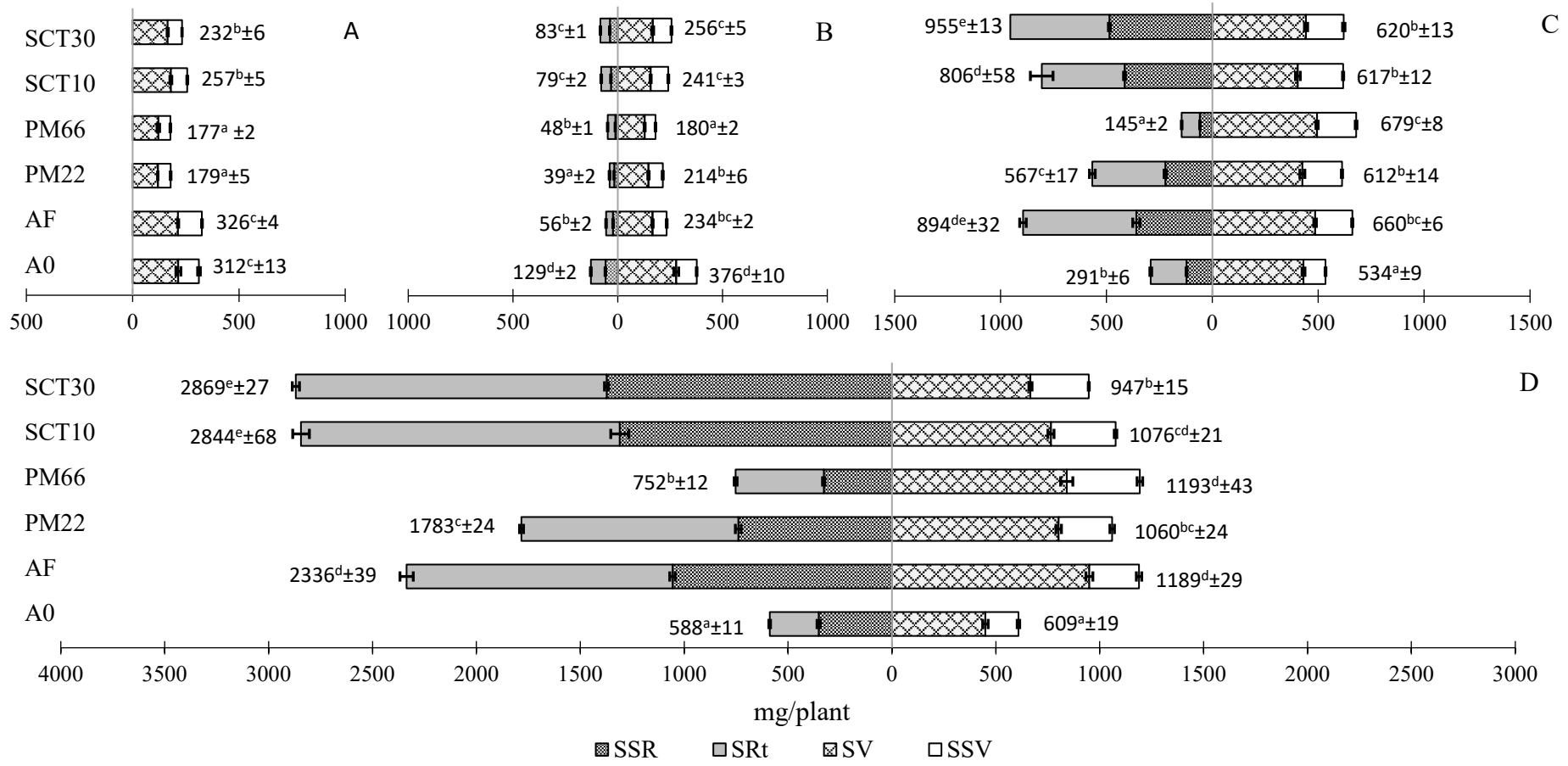

Figure 5.8. Effect of soil amendments on the accumulation of soluble sugar and starch in plants at various sampling times. (A) 10 DAT; (B) 21 DAT; (C) 35 DAT; and (D) 49 DAT.

The x-axis represents the soluble sugar (SS)/starch and the y-axis represents the treatments. Numbers in the left and right are mean values of total NSC (SS + starch) in roots and vines, respectively, followed by standard error (SE) (n=3). ANOVA results are based on square root transformed data. Means followed by a different letter indicate significant differences among treatments using one-way ANOVA(Tukey's HSD, $P<0.05$).

Abbreviations: SR = Starch in roots; SSR = Soluble sugar in roots; SSV = Soluble sugar in vines; SV = Starch in vines.

Table 5.7. The main and interactive effects of amendment application and time on the accumulations of soluble sugar, starch and NSC in sweetpotato plants

Factor	SSV	Starch Vines	NSC	SSR	Starch in roots	NSCR
Amendment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Amendment*time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Two-way ANOVA results are based on square root transformed data.

Abbreviations: SSV = Soluble sugar in vines; SSR = Soluble sugar in roots; NSCV = Non-structural carbohydrates in vines; NSCR = Non-structural carbohydrates in roots.

Table 5.8. The effect of soil amendments on the vine to root NSC ratio on various sampling dates

Treatments	21 DAT	35 DAT	49 DAT	ANOVA
A0	2.92^{c}±0.09	1.84^{b}±0.02	1.04^{b}±0.04	A: P<0.001
AF	4.19^{b}±0.13	0.74^{d}±0.03	0.51^{d}±0.02	T: P<0.001
PM22	5.52^{a}±0.46	1.08^{c}±0.02	0.59^{c}±0.01	A x T: P<0.001
PM66	3.72^{bc}±0.14	4.68^{a}±0.02	1.59^{a}±0.08	
SCT10	3.05^{c}±0.06	0.93^{c}±0.08	0.30^{e}±0.02	
SCT30	3.10^{c}±0.06	0.65^{d}±0.01	0.33^{e}±001	
P value	<0.001	<0.001	<0.001	

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. Two-way ANOVA results, including the effect of soil amendment, sampling time and soil amendment by time are shown. Means followed by different letters are significantly different (Tukey's HSD, P<0.05) within columns using one-way ANOVA.

5.3.5. Effects of soil amendments on N acquisition in plants

Results from this experiment showed that soil amendments had significant effects on N acquisition in plant tissues (Figure 5.9A; Figure 5.9B). The concentration of N in vines and roots decreased over time. Higher N concentrations in both vines and roots were observed in treatments with soil amendments compared to A0. The PM66 treatment had the highest concentration of N in both vines and roots on all sampling dates, which were significantly higher than that of AF treatment. The SCT treatments had lower N concentration in plants than AF, but higher than A0 in most harvests. In general, the higher rate SCT application had a lower N concentration in plants.

There was an increasing trend on the C:N ratio in vines and roots (Figure 5.9C; Figure 5.9D). All treatments with soil amendments had a significantly lower value than the unamended treatment (A0). Among amended treatments, the higher ratio was recorded in treatments with SCT whereas the lower rate was recorded in treatments with PM. At the final harvesting, both PM treatments had significant lower rate of C:N ratio in roots compared to AF treatment.

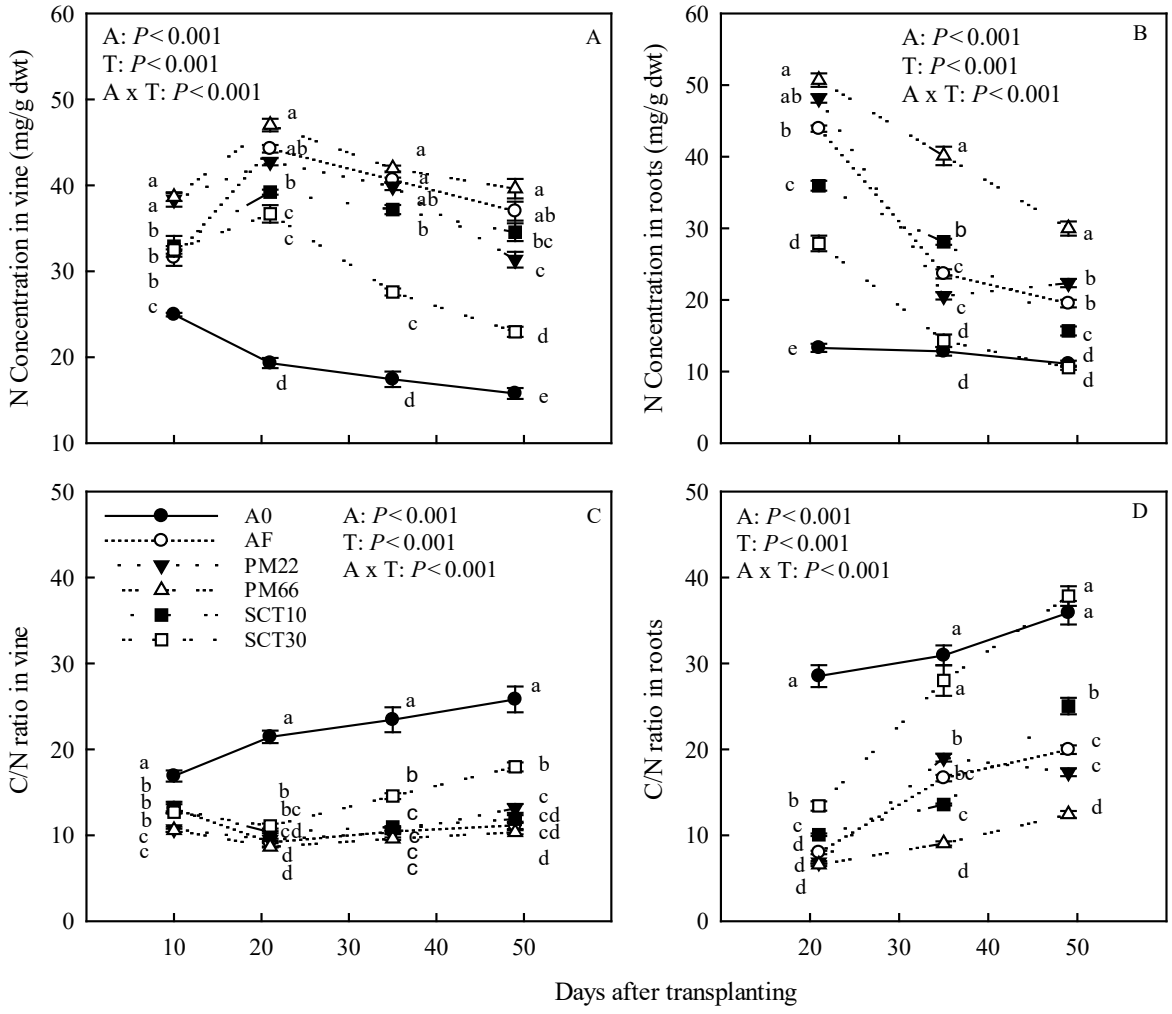

Figure 5.9. N concentration in sweetpotato and C/N ratio as affected by soil amendments. (A) N concentration in vines; (B) N concentration in roots; (C) C:N ratio in vines; and (D) C:N ratio in roots.

Values are indicated as mean ± SE (n=3). ANOVA results for N concentration for both vines and roots are based on square root transformed data. Two-way ANOVA results, including effects of amendments, harvesting date, and amendment by time are shown. Different letters indicate significant differences among treatments on the same harvesting date (Tukey's HSD, P<0.05) using one-way ANOVA.

The total N acquisition in plants increased over time in all treatments (Figure 5.10). The significantly lower N was observed in treatment A0 on all sampling dates and the highest was found in the PM66 treatment in all observations except 21 DAT. There was no significant difference on the effect of PM and SCT application compared to AF at 10 DAT. However, at 49 DAT the accumulation of N in plants of treatment SCT10, SCT30 and PM10 were significantly lower than that of AF treatments. In general, more N was allocated to vines rather than roots, especially for PM66.

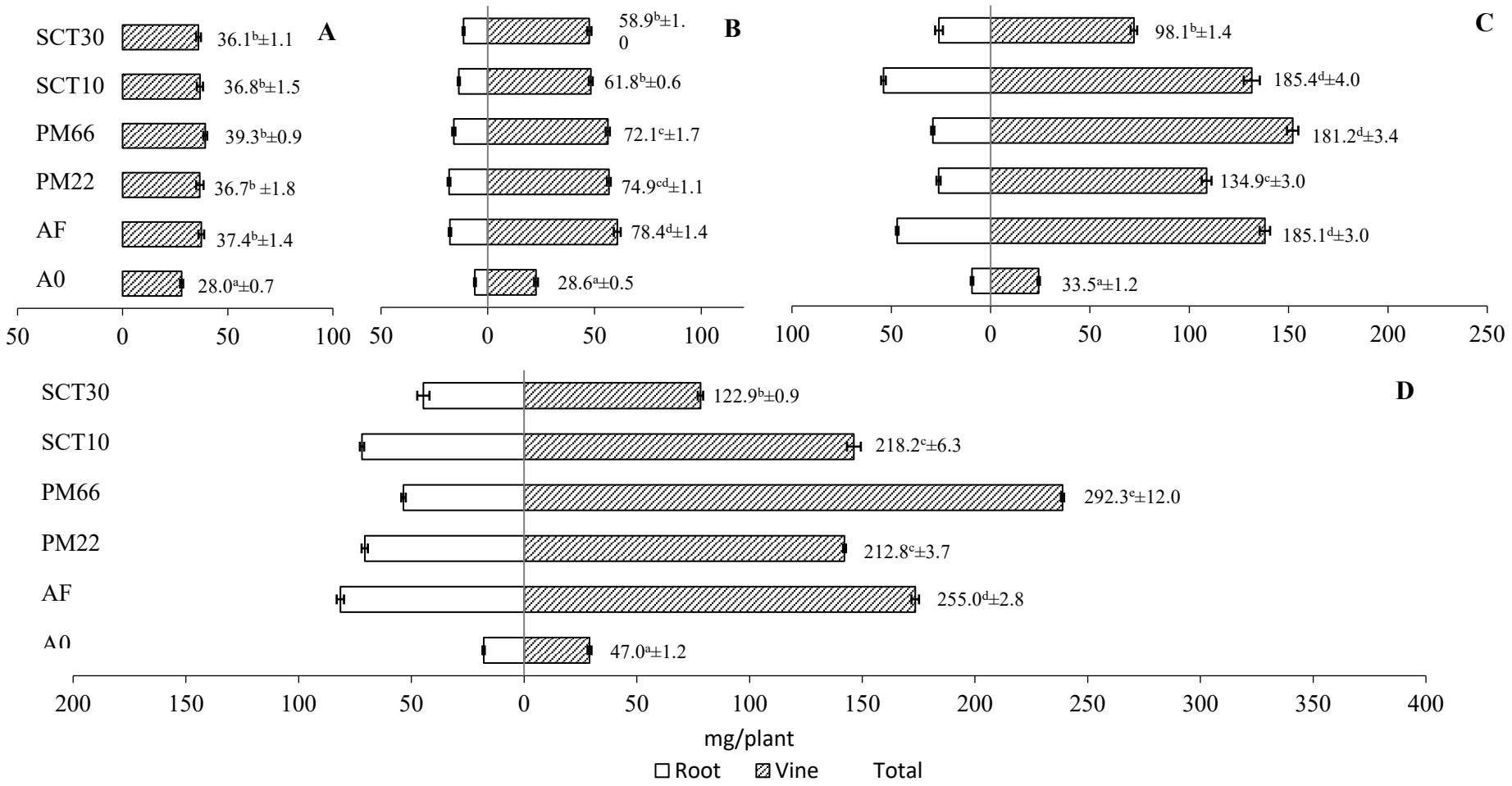

Figure 5.10. Effects of soil amendments on the acquisition of N (mg plant^{-1}) in vines and roots on (A) 10; (B) 21; (C) 35; and (D) 49 DAT.

The x-axis represents the N acquisition and the y-axis represents the treatments. Numbers are mean values of total N acquisition of the whole plant (vines + roots) followed by standard error (SE) (n=3). ANOVA results are based on root transformed data. Means followed by different letters indicate significant differences among treatments using one-way ANOVA (Tukey's HSD, $P<0.05$).

5.4. Discussion

The addition of organic amendments has been used as alternative fertilisers to supply nutrients to plants. They also increase organic matter content which alters soil microorganisms and soil properties as discussed in section 5.1. The experimental trial using organic soil amendments was conducted for only seven weeks as the formation of Orleans sweetpotato SRs peaked at 49 DAT (see Chapter 3 and Chapter 4). In this study, the application of amendments created a gradient of soil-available N. The application of PM released higher levels of available N in soil compared to SCT application. Higher rate of PM application produced higher available N in soil. By contrast, SCT has very high C:N ratio, it when decomposing, it tends to work as a C source. Microbial take C from SCT and N from soil, which reduces soil available N and transfer to microbial biomass. That is why SCT has lower N than AF, although both started with the same level of soil available N. The more SCT added, the stronger this effect. The study focused on how the gradient of soil-available N influenced the SR initiation. The effects of organic amendments on growth and development of roots in a longer-term period should be conducted in the future.

5.4.1. Effect of soil amendment on available N concentration in soil samples

Although all treatments except A0 were adjusted to the same soil available N level before the experiment, the application of PM increased the total content of N and the higher rate of PM application led to the higher available N content in soil due to the rapid decomposition of PM. The N soil availability in the PM treatments was mainly NH_4^+. A high rate of NH_4^+ was observed right after application until six weeks and the availability decreased with time (Hue & Silva, 2000). In line with our results, the use of PM as soil amendments has been reported to improve N available in soil in previous studies (Agyarko et al., 2013; Hirzel et al., 2007; Ojeniyi et al., 2012). The total soil mineral N available in AF treatment was higher than control but lower than PM treatments. This result is in line with previous studies of Agyarko et al. (2013) and Ojeniyi et al., (2012). They reported that the application of PM from 2.5 to 10 ton ha^{-1} in combination with mineral fertilisers increased total N and available soil mineral N compared to sole NPK fertilisers. In contrast, results in this experiment showed that SCT applications lowered these parameters compared to PM treatments and AF treatments (unamended with the

same level of fertilisation addition) due to the immobilisation. Although the SCT amendment slightly increased the total N in soil, it decreased the available N. In line with this result, SCT application at the rate of 3 tons carbon ha^{-1} reduced the available NO_3^- and NH_4^+ in soil compared to the unamended treatment (Muhammad et al., 2011). It has been suggested that plant residues with C:N ratios higher than 30 are expected to result in immobilisation, while those with C:N ratios less than 30 cause N mineralisation (Alexander, 1977). This explains why the concentration of available NO_3^- and NH_4^+ in soil responded differently to different types of organic amendments in our experiment. As the available N in the SCT treatments decreased over the study period and the crop required more N for their development, plants in the SCT30 treatments developed some N deficiency symptoms after 35 days from planting. Therefore, addition of N for sweetpotato grown in amended soil needs to be considered to achieve optimum N concentration for SR initiation.

Compared to the unamended treatment (A0), the addition of PM decreased the soil C:N ratio whereas SCT treatments increased this ratio. In a previous study, cattle manure (C:N ratio 10.2) application also reduced this ratio in soil (Sommerfeldt et al., 1988). However, in a different study, PM (C:N ratio 20) increased soil C:N ratio (Mahmood et al., 2017). This difference is due to the quality of PM especially C:N ratio in the amendment. Organic soil amendment with wood sawdust, waste compost or crop residues generally resulted in a greater C:N ratio in soil compared to unamended treatment (Lozano-García & Parras-Alcántara, 2013; Weber et al., 2007; Wuest & Gollany, 2013).

5.4.2. Effect of organic soil amendment on root anatomy of sweetpotato

Results from this experiment suggest that soil amendments had no effect on the number of ARs over the period of the study. This could be attributed to the uniformity of cuttings used for planting. In a previous experiment, Belehu et al. (2004) indicated that the number of sweetpotato ARs depended on the number of undamaged and healthy preform primordia in the cuttings. The arrangement of the vascular cylinder in roots was mainly pentarch or hexarch in all treatments with a combination of around 65–85% of total roots at 10 DAT. This result is similar to previous studies in some cultivars including West Indian variety, where the majority of roots were pentarch and hexarch (Wilson and Lowe, 1973), Beauregard (Villordon et al., 2009c) and Atacama (Belehu et al., 2004).

In this experiment, soil amendments had a significant effect on the formation of sweetpotato SRs. The SCT10 treatment promoted the initiation of IRVC and RVC in the early stage of root development as indicated by higher rates of roots with these features at 10 DAT and the lowest percentage of roots without cambium development during this period. After that, this treatment continued to promote the formation of AC, and resulted in the highest rate of SRs. The treatment SCT30 showed a similar pattern but a weaker effect than SCT10. This may be due to a stronger N immobilisation effect, which led to some degree of N deficiency. Both SCT10 and SCT 30 increased the SR initiation compared to A0 and AF. Similarly, crop residue additions have been recorded to increase the number of tuber in potato (Verma et al., 2011). Poultry manure application at either rate (PM22 or PM66 treatment) inhibited the formation of cambium as indicated by the highest rate of NC development in roots. In the later stages, those PM treatments promoted lignification in roots and reduced the rate of SR in plants. At the final harvest, the PM66 treatment had a significantly lower rate of SRs in comparision to A0 and AF while PM22 had a similar rate of SRs in AF treatment but lower than that of A0. This might be due to the high concentration of N in soil. Overall, the effect of soil organic amendments on the initiation of SR in this study is consistent with the SR formation pattern in respose to N fertilisation rates detailed in Chapter 1. Therefore, the effect of soil amendments on SR formation is at least partially mediated by the change of soil available N due to soil organic amendments. The results suggested that using organic soil amendments could lead to positive or negative effects on SR initiation of sweetpotato depending on the product's properties.

According to the results from this study, possible mechanisms related to the formation and development of vascular cambium would associate with the nutrients in the organic amendments added into soil. Poultry manure usually contains high amounts of N, especially in the form of uric acid (60–70%) (Hue & Silva, 2000; Nahm, 2003), and the aerobic decomposition of uric acid after amending leads to the high concentration of NH_4^+ in soil (Nahm, 2003; Vogels & Van der Drift, 1976). Therefore, application of PM may result in an increase in the concentration of available N in soil, which was demonstrated to adversely affect the initiation of SRs in previous studies (Gifford et al., 2008; Togari, 1950). Although nutrients in all pots were adjusted to the same level during amending, SCT pots may have lower N concentrations due to the immobilisation of N in conjunction with the decomposition of this amendment. This could result in the optimal

soil available N concentration for SR initiation as indicated by the higher rate of initiated SRs compared to other treatments. Another possible reason for the increase of SR formation in SCT treatments could be due to its effect on the bulk density of the soil and better soil aeration. It has been suggested that high soil bulk density, which is often related to soil compaction, reduced the initiation of SRs (Chua & Kays, 1982).

5.4.3. Effects of organic soil amendments on root morphology, plant performance and yield of Orleans during SR formation stage

Applications of organic amendments had significant effects on all observed root parameters including total root length, average root diameter and total root volume. Generally, the rates of PM amended into soil were negatively associated to these root parameters relative to AF and SCT, which also followed a similar pattern. In the first 35 DAT, the PM66 treatment significantly reduced the total root length and total root volume compared to other treatments. Inhibition of root elongation of crops has been observed under a high ammonium concentration condition (Chen et al., 2013; Li et al., 2010). Adverse effects of animal manure on root development were correlated with the high concentration of in the amendments (Wong et al., 1983). This explains why the lower rate of PM treatment had a higher root length than the higher rate PM application. During the study period, treatment SCT10 promoted root growth, resulting in the highest total root length and total root volume in most observations. This result is also in line with the effect of plant residues on the root growth in different crops (Mandal et al., 2003; Yang et al., 2004). This may be associated with the improvement of microorganism activity in the soil as SCT is rich in organic matter, which is beneficial for soil microbiology. Plants in the treatment SCT30 did not grow as well as SCT10 due to a stronger N immobilisation effect that caused some N deficiency. Our results suggest that all amended treatments started to improve the average root diameter of the crop from 35 DAT. A possible reason for this would be that plants required nutrients for the development of SRs. These nutrients were available in those treatments as they were added into the growth substrate before planting or released from organic amendments.

Both treatments PM22 and PM66 had significantly higher above-ground biomass but lower root biomass at 49 DAT compared to A0 and AF treatments. This means that those treatments promoted shoot growth but inhibited root growth, leading to reduced SR yield. This result is consistent with a previous result on the effect of PM on the biomass

of corn (Hirzel et al., 2007). It could be due to high salt content and N concentration in the amendment, which can damage plant roots when the manure is applied in a large quantity (Hue & Silva, 2000). They also suggested that PM contained N mainly in the form of uric acid, which can be easily converted into ammonia. Ammonia has been known to be toxic for plants as it has detrimental effects on root growth during germination and the early stage of root development (Pan et al., 2016). The growth of vines in sweetpotato is positively related to N fertilisation supply (Knavel, 1971) and higher plant biomass is also due to the high nutrient content in soil (Brouwer, 1962; Grechi et al., 2007). PM used for the current study had ammoniacal N at a rate of 1880 mg kg^{-1} and the total N was 3.99%. Therefore, high N content in PM resulted in the promotion of shoot growth in this experiment.

Treatments SCT10 and SCT30 maintained shoot growth and promoted root growth in this pot experiment and thereby moderate above-ground dried biomass and higher root dried biomass were observed in treatments with SCT. The result could be associated with a very high C:N ratio in the SCT amendment. The activity of soil microorganisms to decompose the material added into soil would slowly release N for plants (Hue & Silva, 2000). Thus, the available N in soil in the SCT treatments was lower in those treatments compared to PM treatments. In addition, SCT10 had higher available N concentration in soil compared to SCT30, which also resulted in greater growth of both roots and vines.

The PM66 treatment changed SR morphology by reducing the SR length at 49 DAT in the present study. This may relate to soil ammonia/ammonium concentration in this treatment as a very high content of ammonia was recorded. Ammonium has been demonstrated to inhibit root elongation in plants (Britto et al., 2001; Chen et al., 2013; Li et al., 2010). The PM66 treatment had an insignificant effect on SR yield during this stage compared to the A0 and AF treartments, and was lower than other treatments. Similarly, PM applications at rates from 20 to 27 tons ha^{-1} in a previous study did not result in a greater yield of cotton compared to the control treatment (Koenning et al., 2003). Similarly, PM application had negative effects on yields of barley, wheat and forage (Lin et al., 2016). However, in an experiment where PM was applied at rate of 10 tons ha^{-1} the yield of cassava increased by around 30% (Ojeniyi et al., 2012). In a different study, PM application at 4.5 tons ha^{-1} improved the SR yield of sweetpotato (Siose et al., 2018).

Therefore, it seems that a very high rate of PM should not be added into the soil as it reduces SR yield of sweetpotato.

The SCT applications in this study increased the SR weight of sweetpotato at 49 DAT. Both SCT treatments, SCT10 and SCT30, had significantly higher SR weight compared to all other amendment treatments including AF treatment. This could be due to the improvement of soil properties. A significantly higher C:N ratio in soil was observed in the treatments with SCT in this pot experiment. Soil C:N ratio is important as it affects soil microbial communities, which are a critical soil functional component. Generally, their alteration could affect the nutrient cycle and soil fertility. Plant residue applications have been reported to reduce the bulk density of soil (Zebarth et al., 1999), which was considered to affect SR initiation and development (Chua & Kays, 1982; Togari, 1950). The application of crop straw at a rate of 7.5 tons ha^{-1} has been reported to improve sweetpotato yield in a previous study (Pan et al., 2019). Hence, our result confirmed the positive effects of plant residues on the yield of sweetpotato.

5.4.4. Effects of organic soil amendments on soluble sugar and starch accumulation of Orleans during SR formation stage

Non-structural carbohydrates, mainly soluble sugar and starch, play an important role in the metabolism process and growth of plants (Wu et al., 2019). The level of NSC in plants reflects their growth status (Housley & Pollock, 1985). The significantly higher concentration of soluble sugar and starch in vines was observed in the A0 treatment. As there was no amendment and fertilisers added into the soil for this treatment, plants suffered nutrient deficiency and their growth was retarded, leading to the accumulation of photosynthates. Both PM treatments had lower soluble sugar and starch content in vines than SCT treatments in almost all samplings. Similarly, the SCT treatments also showed higher NSC than PM treatments. Root NSC in the PM66 treatment was the lowest. These results suggest that a high rate of PM application promoted shoot growth by providing a rich supply of nutrients but poor photosynthate transport to SRs, probably due to the suppression of SR formation. The difference among treatments is possibly due to soil nutrient levels. In this experiment, the higher level of available N in soil was recorded in the treatments with PM compared to SCT. Therefore, PM addition into soil promoted shoot growth but inhibited root growth.

Soil amendment application increased the soluble sugar and starch accumulated in both vines and roots compared to unamended treatment (A0). Similarly, a composted mixture of cotton cake, oil cake and wheat straw applied to tobacco increased the accumulation of soluble sugar and starch in leaves (Song et al., 2016). During the first 21 DAT, the accumulation of NSC in vines was higher than that in roots in all treatments. After that, the content of NSC in vines, however, was lower than that in roots in all treatments except A0 and PM66. This result indicated that the translocation of photosynthates in plants from those two treatments was inhibited, resulting in the accumulation of NSC in vines. Furthermore, the PM66 treatment had the highest NSC accumulation in vines and lowest NSC in roots compared to other treatments. A possible mechanism would be that high rate of PM addition caused high salinity (as indicated by electrical conductivity) and high NH_3^-/NH_4^+ concentration in the soil, which could damage the growth of roots. Then, the decomposition of PM reduced toxicity and plant growth was promoted. This could be the reason why low NSC accumulated in vines during the first 21 DAT and high NSC accumulated in vines after that, as observed in the PM22 and PM66 treatments. Overall, the response of the crop to the application of these amendments was different based on the rates and product's properties.

5.4.5. Effects of organic soil amendments application on N accumulation in plants during SR formation stage

By the end of our experiment, the plant tissue N concentration was the highest in PM66 while lowest in SCT30. A possible reason for this would be the N availability in soil and N uptake. It was concluded that the concentration of N in plants is determined by N uptake and depends on the available N in soil (Pederson et al., 2002). Poultry manure amendment has been recorded to increase N uptake in sweetpotato (Siose et al., 2018), and we see a positive correlation between tissue N concentration and soil available N.

On the final harvesting, the accumulation of N in plant of PM 66 treatment was significantly higher than other treatments and allocated mainly in vines. The SCT30 treatment had lower N accumulated in the whole plant compared to others except for the A0 treatment. Statically significant higher N accumulation in potato was observed in treatment amended with PM at rates of 300–600 kg total N ha^{-1} (Lynch et al., 2008). Similarly, PM application for cassava also resulted in higher N accumulation in plants compared to the control and NPK fertiliser treatments (Ojeniyi et al., 2012). In addition,

organic amendments have been reported to affect N uptake of plants. For example, crop residue application at a rate of 10 tons ha^{-1} significantly reduced N uptake in corn plants (Nguyen et al., 2016). Therefore, different organic amendments differentially affected N accumulation in plants dependent on the types and application rates.

The accumulation of N in plants is related to N levels supplied in soil. In the PM66 treatment, uric acid in the amendment metabolised rapidly to NH_4^+ and so higher concentration of NH_4^+ was found in soil. As a result, higher N accumulation was observed in this treatment as increasing N supply led to higher N accumulation in plants (Pederson et al., 2002). Nitrogen stimulated leaf growth through the synthesis of proteins involved in cell expansion and cytoskeleton synthesis (Bassi et al., 2018). The content of N in leave affected photosynthesis (Allison et al., 1997) as it is partly related to photosynthetic enzymes, pigment content and the size, number and composition of chloroplast. Sufficient N supply leads to easy assimilation which need to be balance by more carbon (Lawlor, 2002). Nitrogen is assimilated in roots but the larger part is translocated to leaves which are a sink for N during vegetative stage (Xu et al., 2012). It is also related to allocation. In general, when soil N level is high, plants do not need many roots to absorb resources from soil. In this case, biomass mainly allocate to shoots, leading to high shoot to root ratio, as well as a balance between photosynthates produced by leaves and nutrients absorbed by root. Moreover, some plant hormones such as cytokinin, auxin are associated to N levels (Matsumoto-Kitano et al., 2008; Samuelson et al, 1992; Kiba et al., 2011). These phytohormones stimulate cell growth and plant growth. Therefore, the accumulation and allocation of N were also related to the plant physiological processes such as photosynthesis and plant hormone functions.

5.4.6. Agronomic indication of organic soil amendment application for sweetpotato

Results from this study suggested that the initiation of vascular cambium was strongly affected by organic soil amendment applications. Under our experimental conditions, SCT amendment promoted the formation of vascular cambium and AC in sweetpotato ARs, and thereby more SRs were formed in the SCT10 and SCT30 treatments. However, PM application from 22 to 66 tons ha^{-1} reduced the formation and development of cambium in ARs as well as promoted lignification of stele cells. This led to a reduced rate of SRs and increased the percentage of lignified roots in the PM22 and PM66 treatments. The different effects of those two amendments on SR initiation may be

due to their impacts on soil N availability. Generally, the effects of amendments on SR formation in this study are well in line with the relationship of SR formation to N fertilisation observed in Chapter 3 and Chapter 4 of this thesis (concave pattern, with an optimal value). In our experiment, the optimal soil available N for SR initiation occurs at around 70 mg kg^{-1}, and this needs to be tested in other soil types and other growth conditions.

In this study, we used high rates of PM for the crop, and thereby its effects adversely impacted on the initiation of SRs due to the high rate of N released into soil after amending. Therefore, a low rate of PM may be used as an amendment for sweetpotato as, in some previous studies, the addition of PM into soil resulted in higher SR yield. Growing this crop under high levels of this amendment during the SR formation could lead to fewer and larger SRs that may result in less income for growers. Also, the ammonium peak in the PM treatments occurred early and offset soon, and so planting cuttings 2–3 weeks after amendment may reduce the impact. Therefore, the rate and application timing of PM should be considered to maintain optimum N availability in soil for the formation of SRs.

Sugarcane trash application at a low rate (10 tons ha^{-1}) is generally beneficial for SR initiation. A high rate of this amendment is better for improving soil bulk density, but it has problems with the application approach and may seriously immobilise N. Sugarcane trash can also be used to bring down soil available N in case the soil has excessive available N.

5.5. Conclusions

Results from this study indicated that the application of soil amendments had significant effects on SR initiation of sweetpotato due to their effects on N availability in soil. Both PM treatments increased N availability in soil, which resulted in suppression of SR formation and promotion of lignified roots. The PM66 treatment promoted shoot growth but inhibited root growth, resulting in the highest shoot NSC and N accumulation in plants, but the lowest root NSC and N accumulation. However, SCT application reduced available N in soil, which is associated with increasing vascular cambium development and SR initiation. Generally, both low and high rates of SCT treatments promoted the formation of SRs as indicated by a significantly higher rate of SRs at all sampling times compared to other treatments. In addition, those treatments maintained

shoot growth and enhanced the growth of roots as suggested by the highest NSC in roots and moderate N accumulation in plants. Our results suggested that only a low rate of PM could be applied to sweetpotato and planting should be delayed from 2–3 weeks after amending to avoid the detrimental effect on SR initiation. A low rate of SCT could be used to promote SR initiation but a high rate of this C rich amendment could lead to N deficiency, probably due to N immobilisation.

Chapter 6. Thesis summary, conclusions and future perspectives

6.1. General summary and conclusions

6.1.1. General summary

Chapter 1 of this thesis provided a general introduction about plant root systems, the sweetpotato root system, the anatomical structures of sweetpotato ARs and the effects of N and organic amendments on plant root system development and crop productivity. This Chapter also introduced the research questions, aims and objectives of the project. Chapter 2 presented some preliminary experiments that examined the formation of sweetpotato SRs in different growth substrates. This investigation aimed to identify a suitable growing method that would permit observation of the root system with minimal physical damage in order to study root development of sweetpotato. Chapter 3 discussed the effects of N supply on the anatomical features of roots during the SR formation process. Chapter 4 evaluated the effect of N fertilisation timing on the initiation of SRs. Chapter 5 investigated the SR initiation of sweetpotato as affected by two organic amendments, PM and SCT, with treatments imposed to vary available N in amended soils. In Chapters 3-5, the accumulation of NSC, including soluble sugar and starch, and N acquisition within plant tissue, were also assessed. This series of experiments delivered a detailed examination of the relationship between SR initiation and chemical changes within the plants.

A preliminary experiment using a nutrient film technique hydroponic system (Chapter 2) indicated that sweetpotato could be successfully grown in this soilless system as some SRs were formed during the study period. However, these SRs developed below, not above the nutrient mat, so the system was not suitable for our non-destructive root observations. A fine sand culture pot experiment in Chapter 2 showed a prolonged delay of SR initiation in this substrate, so this method was not suitable for studies on SR initiation either. The maximum water holding capacity of fine sand was 18.8% with around 65% of particles being smaller than 0.1 mm. In addition, most of ARs would further develop into PRs and lignified roots but not SRs. A possible reason for this could be a lack of oxygen in the root zone as the fine sand was compacted after a several irrigation cycles and water failed to completely drain from the from bottom of the pots. By contrast, the initiation of SRs in river sand culture (coarse sand) was similar to that in a recent study using cultivar ‘Beauregard’ (Villordon et al., 2009) with SRs observed at 21 DAT and nearly 50% of roots developing into SRs under the experimental conditions.

Most of particles in the coarse sand were from 0.25-0.5mm and the maximum water holding capacity was 12.3%.

A pot trial was conducted to examine the effect of N supply levels on the initiation of SRs (Chapter 3). Cutting of 'Orleans' were planted in coarse river sand culture and supplied with modified Hoagland nutrient solution at different N rates. The influence of N application rates on SR initiation, NSC, N accumulation in plants and NRE were compared to the control without N supply. Plants were sampled at five different times, 10, 21, 35, 49 and 56 DAT. All ARs from two subterranean nodes were observed for anatomical features. The application of N at 50 and 100 mg/L in solution significantly improved the formation of vascular cambium in roots in the early stage of root development, and enhanced the initiation of SRs, resulting in significantly higher rate of SRs forming. Excessive N supply in the early stage of root development (N200) inhibited the formation of cambium and delayed the initiation of AC. However, this N level promoted the growth of SRs after the initial period of delayed initiation, as indicated by the highest values for SR size and weight among all treatments in the final harvest at 56 DAT. Deficiency of N (N0) also reduced SR initiation and promoted lignification in roots. According to the results in this experiment, moderate N supply should be supplied for sweetpotato during SR initiation, but additional applications would be required during SR development to promote faster growth, higher NSC, higher N acquisition and highest N recovery efficiency in the N200 treatment.

The glasshouse experiment in Chapter 4 was established to assess the influence of timings of N fertilisation on the formation of SRs, NSC accumulation, N acquisition and NRE in plants. N fertilisation in the form of a nutrient solution applied at the rate of 100mg/L, which was demonstrated to promote SR initiation in Chapter 3, was used to supply N to plants at five different times. Both none or delayed N application until 14 DAT led to inhibition of SR formation and increased lignification in ARs, and therefore reduced the percentage of SR in plants. By contrast, earlier N supply for sweetpotato (within one week after planting) promoted SR initiation and reduced lignification. In addition, earlier N application enhanced shoot and root growth as higher dried biomass, higher NSC, higher N acquisition and higher NRE were recorded in these plants. Generally, the earlier N was applied, the better SRs formed. Therefore, both none and delayed application had a negative effect on the initiation of SRs. In combination with

results from Chapter 3, moderate N should be available in soil before or at planting to stimulate SR formation.

Organic amendments have been demonstrated to improve soil properties to provide a better environment for root growth. Some studies have found a positive effect of these products on SR development and SR yield of sweetpotato. However, none of them have examined the association between organic amendments and SR initiation. A glasshouse experiment in Chapter 5 was set up to evaluate the effect of two locally available organic soil amendments, PM and SCT, on soil available N and total N, root anatomical changes, NSC and N acquisition in plants during SR formation. These amendments were added into soil to achieve two levels of C concentrations in the mixture at 2.16% and 2.55%, as a major goal of soil amendment is to improve organic C. Two unamended treatments, one with chemical fertiliser (AF) and one without (A0) were also included in the experiment. Macronutrients in available form were manipulated to the same level with chemical fertilisers for all treatments except A0. After adding to the soil, PM applications increased available N and total N in soil compared to other amended treatments. Application of SCT at both rates improved the C:N ratio in soil. SCT amended into soil at the rate of either, 10 or 30 tons ha^{-1}, promoted the formation of vascular cambium and AC which resulted in a higher number of SR. However, PM application at rate of 22 to 66 tons ha^{-1} inhibited the formation of SR and promoted the lignification of roots. The influence of these organic amendments on SR formation was associated with the change of available N in soil. The SR formation rate vs. soil available N relationship is in line with the finding in Chapter 3. Further, while PM enhanced shoot growth and reduced root growth, SCT addition maintained shoot growth and promoted root growth, leading to significantly higher SR weight in those treatments for the final harvest at 49 DAT. The unamended treatment with chemical fertiliser (AF) resulted in a lower percentage of SR compared to SCT treatments but higher than that of the PM treatments. The available N in soil, and N accumulation in plants for SCT treatments, generally were lower than that of AF treatment due to the decomposition of the amendment. However, PM treatments had significantly higher soil available N. During the SR initiation, the highest NSC in roots and moderate N accumulations in plants were recorded in the SCT treatments (SCT10 and SCT30) while the highest vine NSC and the lowest NSC were observed in the PM66 treatment. The findings suggested that SCT from 10 to 30 tons ha^{-1} can be used to improve SR formation. However, high rate of PM from

22 to 66 tons ha^{-1} should not be applied for sweetpotato as it promoted vine growth and reduced SR initiation which led to a lower plant yield.

6.1.2. General discussions

Results from this study suggest that N availability does not appear to affect the number of ARs and protoxylem elements. Different levels of N supply (Chapter 3), N fertilisation timing (Chapter 4) or different concentration of available N in the soil (Chapter 5) showed no significant effects on AR number and protoxylem number. Similarly, a previous study recorded that nitrate levels from 1-50mM had no effect on the root number of sweetpotato growing in mixture of sand and vermiculite (Kim et al., 2002). In contrast, a study of Villordon et al. (2013) in field conditions found a significant increase in the number of ARs when N application increased from 50 to 100 kg ha^{-1}. Possible reason for the difference could be that the experimental conditions and N levels are only one factor contributing to the formation of ARs and so other factors such as the source and condition of the planting material may have varied between the studies. Further, the formation of pencil roots was not affected by available N levels in our experiment conditions.

However, N levels had a significant effect on the formation of SRs, the growth of plants and the accumulation of non-structural carbohydrates and N in plants. When no N was supplied, the initiation of SRs was suppressed and the formation of the lignified roots was promoted in all experiments. Further, reduced growth of vines and roots, and lower accumulation of NSC and N in plants, were consistently recorded in the zero N supply treatments. This may be due to N deficiency limiting synthesis of the photosynthetic apparatus and other proteins in the plants. These results are in line with previous studies in which low N availability has been shown to reduce the number of SRs and SR yields (Okpara et al., 2009; Taranet et al., 2017), inhibit the growth of sweetpotato (Okpara et al., 2009; Osaki et al., 1995), lower NSC accumulation (Araya et al., 2010; Kim et al., 2002) and lower N acquisition (Taranet et al., 2017) due to the limited growth of both shoots and roots. Our results suggest that high N levels (200 mg/L of N in solution in Chapter 3 or high soil concentration in Chapter 5) delayed or inhibited the formation of SRs but enhanced the development of SRs. It was previously reported that high N applications delayed SR formation (Haynes et al, 1969, as cited in Villagarcia, 1996; Wilson, 1973), but resulted in more rapid bulking during later growth stages (Haynes et

al, 1969, as cited in Villagarcia, 1996). Another study indicated that excessive N suppressed the initiation of sweetpotato SRs (Wilson, 1973). Further, high N stimulated the growth of vines, and root growth was relatively suppressed, as indicated by the lower ratios of roots and vines at final samplings. Generally, both deficient and excess N supply had adverse effects on the formation of SRs.

While the nitrogen dose response curves were consistent with previous studies of sweetpotato and other crops, the anatomical changes by which N impacted the SR initiation process have not been previously described. A low rate of N is required to form and develop cambium which are related to the formation of SRs. The application of N affected SR initiation immediately after the cuttings are planted and the key time of the influence is the first few days (the first week in our experiment). Therefore, delayed N applications would inhibit SR initiation. This finding is contradictory to some previous field studies in which delayed N applications a few weeks after transplanting increased SR yields (Phillips et al., 2005; Villordon et al., 2009), possibly due to differences in growth rate under field compared to greenhouse conditions. The optimal available N level for SR root formation appeared to be lower than the optimal available N level for sweetpotato plant growth and SR growth. The optimum rates of N for SR initiation in our study were around 70 mg kg^{-1} soil or 100 mg/L N in nutrient solution. The recommended N levels to achieve maximal number of SRs were variable in different conditions. Previous studies revealed that the highest number of SRs growing in soil was observed at either none application (Duan et al., 2019; Guertal & Kemble, 1997) or the rate of 50 kg ha^{-1} (Mulkey et al., 1994). However, there was no information about available N in soil in these experiments. In a different study, the total N supply up to 240 kg ha^{-1} which was divided equally across weekly applications did not show any inhibition of SR formation (Villagarcia, 1996). Therefore, the optimum N level for SR initiation varied depending cultivars, experimental conditions, soil quality and other factors.

The effects of soil organic amendments on the SR formation were at least partially via their impact on available N in soil (Chapter 5). The application of PM from 22 to 66 tons ha^{-1} caused high concentration of N in soil in the first few weeks, so these rates of PM inhibited the SR formation and promoted the formation of lignified roots. Further, PM at rate of 66 tons ha^{-1} increased shoot growth but reduced root growth, as indicated by the highest shoot biomass, NSC and N accumulation in plants and the lowest root to shoot ratio, root NSC and N accumulation. As PM applications in previous studies resulted in

higher yield of sweetpotato, low rates of this amendment may be used as a soil organic amendment for sweetpotato. SCT application from 10 to 30 tons ha^{-1} for sweetpotato promoted the initiation of SRs cambium, AC and reduced the formation and development of cambium in ARs as well as promoted lignification of stele cells. This led to an increase in the formation of SRs and decreased the percentage of lignified roots in the SCT10 and SCT30 treatments.

6.1.2. Conclusion

The findings of this project suggested that N does not affect the number of ARs and protoxylem elements. There is an optimal level of available N for sweetpotato to form SRs. The optimal available N level for SR root formation appeared to be lower than the optimal available N level for sweetpotato plant growth and SR growth. Soil available N affects SR root immediately after the cuttings are planted and the key time of the influence is the first seven days after planting. The effect of available N on SR root formation has a permanent impact on the crop as the process of lignification cannot be reversed and lignified roots cannot develop into SRs at a later stage. Soil organic amendments can affect SR formation via their impact on soil available N and the pattern is generally in line with the observation in the sand medium model system as described in this thesis. The formation of pencil roots was not affected by available N level in the experimental conditions.

6.1.3. Limitation of the study

One potential limitation of this thesis is that the study was based on a model sand culture system and pot experiments in glasshouse conditions, so the pattern elicited in this study requires further investigation and validation under field conditions. During the experimental periods, the average maximum and minimum daily temperature varied from 14.3°C to 32.9°C, similar to that during the normal growth season in the Wide Bay Region of South East Queensland, Australia. However, the light and soil moisture levels may be different from field growth conditions (e.g. lower light and more consistent soil moisture). Therefore, the effect of N fertilisers and organic soil amendments on the initiation of the SR in our study may not be the same as what happens in the field and caution is required when extrapolation of the results to field crop conditions. Also, the experiments only focused on the effect of a single factor, N or amendments.

Climatic and agronomical factors have been recorded to influence the initiation and development of sweetpotato SRs. Both soil and air temperature were linked to SR formation (Ravi & Indira, 1999). Generally, the temperature between 20°C and 30°C favours the formation of SRs (Belehu & Hammes, 2004; Gajanayake et al., 2014; Spence & Humphries, 1972). The response of sweetpotato to soil moisture varied dependent on the cultivars (Gajanayake et al., 2013). The optimum soil moisture for the formation of SR was determined to be between 64-80% field capacity (Belehu & Hammes, 2004; Bok, 1998; Gajanayake et al., 2013) and under drought condition the initiation of SR was inhibited or delayed (Belehu & Hammes, 2004; Gajanayake et al., 2013). Thus, the experimental environment in this project reflects the formation of SR in a generally favourable condition.

Although no published information about the effects of other nutrients including P and K on the initiation of SRs could be found, various publications refer to the influence of P and K on the SR yield of the crop, which could be partially affected by their potential impacts on SR formation. The application of K resulted in positive effects on the SR yield (El-Baky et al., 2010; Liu et al., 2013). Similarly, previous studies revealed that P application also increased the yield of the crop (Abd-El-Fattah & Abd-El-Hamed, 1997; El-Sayed et al., 2011). Although it is important to understand the influence of nutrients on the initiation of SRs, only few related studies could be found. Therefore, this thesis would be one of the pioneer works on this topic and improves our understanding of SR formation, critical for enhancing the yield and improving the quality of the sweetpotato crop.

6.2. Future perspective

The findings from this project suggested that N at the rate of 100 mg/L in the nutrient solution would be optimum for sweetpotato SR initiation and the higher rate of N at 200mg/L was more suitable for SR development after forming. However, the experiment was conducted in sand culture and solution nutrient was applied for plants, so more study is required to determine the optimal available N level in field cropping system. Although our results indicate that non-optimal N level would reduce the number of SR formed, to what degree this would affect SR yield would be subject to many other factors such as temperature, moisture, P and K. Therefore, a future research direction would be to link the initiation and development of SRs with the marketable yield of the

crop as affected by N supply levels. As plant growth would be limited in pots, the efficacy of N on SR formation may be different in field conditions; however, findings from this pot experiment elicits the general pattern of N effects on SR formation, NSC and N accumulation as well as NRE during the formation of SRs.

Results from N fertilisation timing experiment produced general knowledge on N requirement time for SR initiation. Therefore, a field experiment needs to be conducted in the future to evaluate the influence of N fertilisation timings on the formation and development of SR and the final impact on commercial yield. In actual field conditions, available N in soil varies dependent on locations and soil types, it is necessary to test the effects of available N in soil on the initiation of SRs. Also, the effects of N supply rates and N fertilisation timings should be combined to examine the interactive effects of N application levels and timings.

The effects of organic soil amendments may be greater if trials were conducted for a longer time period to generate conclusions for marketable yield and the long-term effect of amendments. It was concluded that organic fertiliser released nutrients over time and residual effect could last several years (Hue & Silva, 2000). In the pot experiment our observation of anatomical features in roots and chemical changes in plants is limited to within the period of the SR initiation. However, the development of SR after forming is equally important as it affects the shape and size of SR at commercial harvesting. Also, the PM was amended into soil to achieve the same level of C in SCT treatments, high rates of PM were used in the experiment. Lower rates of PM should be evaluated in the future to understand the influence of PM on the formation of SR as it was demonstrated to increase the yield of sweetpotato in a previous study (Siose et al., 2018).

All experiments in this project were conducted in pots for a short term from 49 to 56 days, so do not represent real field growth conditions for plant developments. Therefore, future field experiments would be needed to get a comprehensive idea of the initiation and development of SRs and the marketable yield of the crop as affected by N levels and timing as well as by organic amendments.

There are other factors such as temperature, light, moisture, P and K that were reported to affect the initiation and development of SR (Bonsi et al., 1992; El-Baky et al., 2010; El-Sayed et al., 2011; Gajanayake et al., 2013; Ravi et al., 2009). Climatic factors such as temperature or light generally cannot be manipulated in a large scale field

production, but agronomical factors should be managed to achieve optimum outcome of the crop production. This project focused only on the impact of N on SR initiation. Therefore, the interaction of N and other factors, especially other nutrients, needs to be examined to achieve better practice management for the crop.

Further investigation is required to examine the SR development after forming to commercial harvest. The shape and size of sweetpotato determine the marketable yield of the crop as both too big or small sizes are rated as low quality and low value. Research is also needed to evaluate the effects of N on SR initiation, SR development and SR yield of other root and tuber crops to provide food security in the future. The interactive effects of N and other nutrient scenarios are required to test the influence of multiple nutrients on SR formation and development. Future research needs to focus on the model root traits affected by multiple nutrient stress scenarios in order to examine and predict root response to different stress.

Results from this thesis provide agronomical indicators that sweetpotato require a moderate N level after transplanting to promote adequate numbers of SR formation and then higher concentration of N is needed after SR is formed to boost SR growth. Regarding organic amendments, a low rate of SCT (e.g., ~10 tons ha^{-1} in our experiment) could be applied for sweetpotato to promote the formation of SR, but the higher rate of SCT application could lead to N immobilisation and crop N deficiency (as observed in 30 tons ha^{-1} SCT treatment). In addition, if a high rate of PM is used for soil amendment, it should be amended into the soil a few weeks before planting to offset the possible negative impact of high ammonium N release, or a lower rate of PM could be used for sweetpotato to reduce adverse effect of excessive soil available N on SR initiation. This conceptual fertilisation scheme proposal is based on current pot experiments and is to be tested in field conditions as the next step in the future to confirm its effectiveness. It is also notable that the results in the experiments are based on favourable conditions of soil moisture and other nutrients (P and K used at recommended rates), so these factors need to be considered when testing a proposed fertilisation scheme based on this thesis.

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Appendix 1: Half strength Optimum Growth nutrient solution ingredients

Nutrients	Concentration (mg/L)
Nitrate-Nitrogen	102.6
Ammonium-Nitrogen	2.48
Phosphorus	18
Potassium	135.4
Calcium	81
Magnesium	22.5
Sulfur	29.7
Iron	1.35
Manganese	0.39
Boron	0.1
Zinc	0.1
Copper	0.067
Molybdenum	0.022

Appendix 2: Hoagland Nutrient Solution Recipes

Composition of free N solution

Compounds	g/litter of distilled water	ml per litter of nutrient solution
0.5M K_2SO_4 (potassium sulfate)	87.13	5
M CaH_3PO_4 (calcium phosphate)	120.36	2
0.05M $CaSO_4$ (calcium sulfate)	11.70	10
0.01M $MgSO_4$ (magnesium sulfate)	1.36	200

Composition of micro-nutrients

Compounds	g/litter of distilled water	ml per litter of nutrient solution
H_3BO_4 (boric acid)	2.86	1
$MnCl_2.4H_2O$ (manganese chloride)	1.81	
$ZnSO_4.7H_2O$ (zinc sulfate)	0.22	
$CuSO_4.5H_2O$ (copper sulfate)	0.08	
$H_2MoO_4.H_2O$ (molybdic acid)	0.02	
Iron tartrate solution 0.5%		1ml L^{-1} twice a week

Appendix 3: Source of available N for growth substrates (mg pot-1)

Treatments	Soil		Amendment		Mineral fertiliser		Total	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
A0	36.20	77.03	0	0	0	0	36.20	77.03
AF	36.20	77.03	0	0	213.80	172.97	250	250
PM22	36.20	77.03	4.59	43.14	183.79	129.83	250	250
PM66	36.20	77.03	13.77	129.42	173.78	43.56	250	250
SCT10	36.20	77.03	0.04	3.35	188.76	169.62	250	250
SCT30	36.20	77.03	0.11	10.06	188.68	162.91	250	250

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