Agronomic Performance of Sugarcane Varieties Derived from Tissue Culture (NovaCane®) and Conventional Seedcane under Rainfed Conditions

By

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DECLARATION

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2. This dissertation has not been submitted for any degree examination at any other university.

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As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Albert Thembinkosi Modi (Co-Supervisor)

GENERAL ABSTRACT

The use of tissue culture (TC) plants have been promising for the production of true-to-type, disease free planting material. However, TC plants have been shown to possess an altered phenotype (high tillering and thinner stalks) compared with conventionally propagated sugarcane from setts (hereafter referred to as conventional or Con). Limited information is available for the response of different varieties to the TC process. Additionally, the effects of any altered phenotype in subsequent stages has not been evaluated. Three field experiments were conducted under rainfed conditions at South African Sugarcane Research Institute (SASRI) Mount Edgecombe experiment station to gain insights into these factors. The aim of experiment 1 was to investigate growth and yield differences between the TC and the Con plants for different varieties. Here, a field trial was established as a randomised block design with four replications of four varieties (N12, N31, N41, and N48) planted using three methods: 1) TC derived plants (spaced at either 30 (TC30) or 50 (TC50) cm apart; 2) conventional hot water treated seedcane setts (Con); and 3) single-budded sett derived plants (speedlings) planted 50 cm apart (SP50).

The aim of experiment 2 was to investigate the effects of different *in vitro* procedures on several phenotypic and agronomic characteristics of TC plants of sugarcane. A field trial was established as a randomised block design with four replications consisted of two varieties (N41 and N48) derived through three variations of the in vitro NovaCane® procedure, namely i) the standard procedure, ii) plantlets exposed to $CoCl_2$ (NovaCane® ($CoCl_2$)) and iii) plantlets that underwent a secondary meristem excision process (NovaCane® (secondary)) from in vitro material. The plantlets from all three treatments were planted using two (30 and 50 cm) plant spacings.

The aim of experiment 3 was to compare the performance of seedcane obtained from TC (stage 1) and Con when planted at different planting rates. The seedcane for experiment 3 was derived from the corresponding treatments in experiment 1, which were: 1) stalks derived from TC50 and planted at a lower planting rate (TC50 low); 2) stalks derived from TC50 and planted at a higher planting rate (TC50 high); 3) stalks derived from TC30 and planted at a lower planting rate (TC30 low); 4) stalks derived from TC30 and planted at a higher planting rate (TC30 high); and 5) stalks derived from Con and planted at a normal planting rate. Yield and yield component measurements for these experiments were taken at harvest and data were analysed by ANOVA.

For experiment 1, there were no significant differences in cane yield, stalk height and stalk mass between propagation methods for all varieties in both crops harvested.

For varieties N12 and N31, both TC treatments produced significantly thinner stalks and higher stalk population compared with the Con treatment when averaged across crops. Variety N48 was insensitive to the TC process, indicating that the phenotype of this variety was maintained during the TC process. The TC30 and TC50 treatments did not differ significantly for any parameter in both crops for all varieties, showing that plant spacing did not affect growth. The SP50 treatment produced significantly thicker stalks compared with the TC50 for varieties N12, N31 and N41 in the plant crop.

For experiment 2, the plants produced through the NovaCane® ($CoCl_2$) procedure resembled those produced through NovaCane® for all phenotypic and agronomic characteristics in the plant and first ratoon crops. The plants produced through the NovaCane® and the NovaCane® (secondary) procedures differ significantly for stalk population only, with the NovaCane® treatment having significantly lower stalk population compared with the NovaCane® (secondary) treatment for variety N41. Planting at closer (30 cm) or wider (50 cm) spacings did not have an effect on plant growth and to the response of varieties to the in vitro treatments.

For experiment 3, crop derived from TC had a significantly higher mean cane yield and TERC compared with the crop derived from the Con. The crop derived from TC had a significantly higher mean stalk population compared with the crop derived from Con. This was observed for varieties N12 and N41 in particular. The crop derived from TC produced significantly thinner and taller stalks compared with the crop derived from Con. The effects of planting rates and TC source (TC30 vs TC50) were not significant for any parameter.

Varieties responded differently to the TC process (N48 did not show phenotypic variations). As a result, screening of varieties for phenotypic to TC is recommended to make grower aware of expected changes in the phenotype. This should mitigate the risks of possible poor adoption of varieties based on thin stalks. It is recommended that TC plants be propagated using wider (50 cm) plant spacings, as this is more economical. The lack of differences between *in vitro* procedures suggests that propagation of new genotypes through standard NovaCane® procedures for commercial release should continue. The seedcane derived from the TC at stage 1 can be used as planting materials for commercial production without any negative effects on productivity in subsequent propagation stages. This is despite persistence of the reduce stalk diameter, higher stalk population phenotype. Lack of differences between the higher and the lower planting rates of TC-derived crops suggests that lower planting rates should be used for economic reasons.

ACKNOWLEDGEMENTS

DEDICATION

This dissertation is dedicated to my late father (Muziwendoda P. Shezi) who has been my constant source of inspiration in my life even beyond the grave. Papa, this is for you.

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CHAPTER 1 : GENERAL INTRODUCTION

Sugarcane (*Saccharum spp.*) is known as a tropical perennial crop, belonging to a grass family called Poaceae (Besnard et al., 2002). This family includes crops like maize, wheat, sorghum, rice, and many other forage crops. Sugarcane is proving to be a versatile crop because while sugar is still being obtained from the stalk, numerous by-products are produced from it (Berding and Pendrigh, 2009). Sugarcane plays a crucial role in the economy of South Africa as sugar (sucrose) is the third most important largest valuable agricultural export. Sugarcane generates rural employment opportunities and it is known as an agricultural commodity that generates more income than other crops. In South Africa, there is 430 000 hectares currently under sugarcane production (SASA, 2010/2011). Of this total, KwaZulu-Natal is the leading sugarcane growing province followed by Mpumalanga, with Eastern Cape being the least sugarcane producing province. Of the total sugarcane production in South Africa, approximately 70% is produced under rainfed conditions and the rest of the production occurs under irrigation conditions.

Sugarcane is vegetatively propagated using a section of a mature cane stalk that contains buds (or "bud-eyes") called seedcane (Willcox et al., 2000; Salassi et al., 2004). This method of planting seedcane into furrows has been the traditional way of planting sugarcane. However, this propagation method has limitations as high quantities of seedcane is used for planting (Singh et al., 2014). For example, about 8-10 tons of seedcane is used to plant a hectare. This amount of seedcane that is used causes problem and expense in handling and transporting, and furthermore, this results in rapid deterioration thus reducing the viability of buds. Furthermore, this method is labour intensive as more labour are required to cut the seedcane into shorter pieces (3-budded setts) to avoid apical dominance (Chattha et al., 2007). Additionally, the method is limited by the length of time taken to produce enough stalk material to plant commercially (Snyman et al., 2009; Mishra et al., 2014). It normally takes about two propagation stages before sufficient material is available for commercial planting. Hence, farmers are facing a major problem of non-availability of quality seed material, especially of new varieties that are released for the industry. The successive use of seedcane from the same source as planting material can cause a general decline in cane yield and quality (Sood et al., 2006; Croft and Cox, 2013). This decrease may be associated with pests and diseases that accumulate over vegetative cycles thus leading to yield and quality decline over time.

Research has been done on finding other propagation methods that will reduce the quantity of seedcane used for planting sugarcane fields. According to Narasimha Rao and Satyanarayana (1974) under favourable growing conditions, cutting with only one bud did as well as conventional planting material (i.e. approximately 3-budded setts). These single-budded setts are planted in pots or trays filled with growing media and later transplanted in the field (Singh et al., 2014). Using the bud only (i.e. a single-budded sett – SBS) as the planting material is called speedlings/transplants. This method reduces quantity of planting material, for example, only 50-75 kg SBSs can be utilized to plant a hectare and the remaining canes (internodes) can be sent to the mill for sugar extraction (Jain et al., 2010). However, research carried out has shown that using transplants as a propagation method has constraints. This includes poor survival of SBSs plants under field conditions due to low food reserves (1.2-1.8 g sugar/bud) compared to conventional 3-budded setts (6.0-8.0 g sugar/bud). Additionally, Jain et al. (2011) reported a faster depletion of food reserves and moisture when using SBSs compared to 2 or 3-budded setts.

The other propagation method of sugarcane is production of plantlets through tissue culture technique (Devarumath et al., 2007; Mishra et al., 2014). In the tissue culture process, stage 1 is referred to the production of actual tissue culture plants under lab conditions, and their subsequent hardening off and planting into field nurseries. Stage 2 and stage 3 then progress in a similar manner to conventional methods (Snyman et al., 2009). Using tissue culture propagation methods have helped in producing large quantities of planting material within a short period of time (Fitch et al., 2001). Tissue culture plants have also helped in minimising yield loss in sugarcane production by providing clean planting material (i.e. free from pests and diseases) (Sandhu et al., 2009). However, previous local studies (unpublished) and other studies done elsewhere have shown that there is a yield depression encountered when using tissue culture plants compared with other propagation methods. Limited studies have been carried out to compare sugarcane propagation methods. Therefore, this project is aimed at evaluating the yield performance and agronomic differences between conventionally propagated, tissue culture propagated, and single-budded setts propagated sugarcane.

The objectives of this study were:

- To investigate growth and yield differences between the TC and the Con plants for different varieties.
- To investigate the effects of different *in vitro* procedures on several phenotypic and agronomic characteristics of TC plants of sugarcane.
- To compare the performance of seedcane obtained from TC (stage 1) and Con when planted at different planting rates.

Dissertation outline

This dissertation includes 6 chapters, chapter 1 is written as general introduction for the dissertation, while chapter 2 is a literature review. Chapters 3-5 are written in the form of discrete research papers, each following the format of a stand-alone research paper. Chapter 6 is a general discussion and conclusion. This is the dominant format adopted by the University of KwaZulu-Natal. As such there is some unavoidable repetition of references and some introductory information between chapters. The structure of the dissertation is outlined below:

Chapter	Title
1	General introduction
2	Literature review
3	Agronomic comparison of tissue culture (NovaCane®) and conventional propagated sugarcane under rainfed conditions
4	The effects of *in vitro* treatments on the growth and development of tissue culture-derived sugarcane plants
5	The effects of planting rates and tissue culture as a seedcane source on growth and development of sugarcane in the second propagation stage
6	General Discussion and Conclusion

1.1 References

Berding, N.I.L.S. and Pendrigh, R.S., 2009. Breeding implications of diversifying end uses of sugarcane. *International Sugar Journal* 111, 24-38.

Besnard, G., Offmann, B., Robert, C., Rouch, C. and Cadet, F., 2002. Assessment of the C 4 phosphoenolpyruvate carboxylase gene diversity in grasses (Poaceae). *Theoretical and Applied Genetics* 105, 404-412.

Chattha, M.U., Ali, A. and Bilal, M., 2007. Influence of planting techniques on growth and yield of spring planted sugarcane (*Saccharum officinarum* L.). *Pakistan Journal of Agricultural Sciences* 44, 452-456.

Croft, B.J. and Cox, M.C., 2013. Procedures for the establishment and operation of approved-seed plots. SRA manual. 4th Edition.

Devarumath, R.M., Doule, R.B., Kawar, P.G., Naikebawane, S.B. and Nerkar, Y.S., 2007. Field performance and RAPD analysis to evaluate genetic fidelity of tissue culture raised plants vis-à-vis conventional setts derived plants of sugarcane. *Sugar Tech* 9, 17-22.

Fitch, M.M.M., Lehrer, A.T., Komor, E. and Moore, P.H., 2001. Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology* 50, 676-680.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2010. Sugarcane bud chips: A promising seed material. *Sugar Tech* 12, 67-69.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2011. Effect of ethephon and calcium chloride on growth and biochemical attributes of sugarcane bud chips. *Acta Physiologiae Plantarum* 33, 905-910.

Mishra, S., Nailwal, T.K. and Pant, R.C., 2014. In vitro study of role of ethylene during tillering in sugarcane. *Sugar Tech* 16, 255-263.

Narasimha Rao, G. and Satyanarayan, Y., 1974. Studies in control of sett borne infection of red rot of sugarcane. *Journal of Research Andhra Pradesh Agricultural University* 1, 83-86.

Salassi, M.E., Breaux, J.B. and Hoy, J.W., 2004. Estimated cost differences between whole-stalk and billet sugarcane planting methods in Louisiana. *Journal of American Society Sugar Cane Technologists* 24, 250-257.

Sandhu, S.K., Gosal, S.S., Thind, K.S., Uppal, S.K., Sharma, B., Meeta, M., Singh, K. and Cheema, G.S., 2009. Field performance of micropropagated plants and potential of seed cane for stalk yield and quality in sugarcane. *Sugar Tech* 11, 34-38.

Singh, R., Singh, W. and Choudhary, S., 2014. Nursery raising with High Density Sugarcane Transplanting for higher cane yield and profitability. *Indian Farming* 64, 31-34.

Snyman, S.J., Meyer, G.M., Banasiak, M., Nicholson, T.L., Antwerpen, T.V., Naidoo, P. and Erasmus, J.D., 2009. Micropropagation of sugarcane via NovaCane®: preliminary steps in commercial application. *Sugar Cane International* 27, 245-247.

Sood, N., Gupta, P.K., Srivastava, R.K. and Gosal, S.S., 2006. Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. *Plant Tissue Culture and Biotechnology* 16, 25-29.

South African Sugar Association (SASA), 2010/2011. The South African Sugar Industry Directory.

Willcox, T., Garside, A. and Braunack, M., 2000. The sugarcane cropping system. *Manual of cane growing", M Hogarth, P Allsopp, eds. Bureau of Sugar Experiment Stations, Indooroopilly, Australia*, 127-139.

CHAPTER 2 : LITERATURE REVIEW

2.1 Sugarcane as a crop

2.1.1 History and description of sugarcane

Sugarcane (*Saccharum officianum L.*) is a tall perennial true grass species that belongs to the genus Saccharum, tribe Andropogoneae, and is mainly used for sugar production (Berding and Roach, 1987; Julien et al., 2012). It is suggested that around 6000 BC sugarcane was first domesticated as a crop in New Guinea, and the plant was chewed for its sweet juice by farmers (Singh, 2003; Hunsigi, 2012). Sugarcane is regarded as one of the main staple sources of sweetening worldwide. Karkara, which is the Sanskrit word, is the word from which the names sugar and sugarcane were derived (Singh, 2003; Hunsigi, 2012). Saccharum was a generic name given to sugarcane by Linnaeus in 1753 (Irvine, 1999). Sugarcane originates in tropical South and Southeast Asia (Deressa et al., 2005; Fischer et al., 2008). Different sugarcane species are believed to have originated in dissimilar locations. For example, *Saccharum barberi* is indigenous to India while *S. officinarum* and *S. edule* originated in New Guinea (Fischer et al., 2008).

Sugarcane is a crop that belongs to the grass family called Poaceae which includes crops like rice, wheat, maize, and many forage crops (Bull, 2000; Hunsigi, 2012). It is known as the most efficient C4 photosynthesizer in the plant kingdom and is commercially grown utilizing stem cuttings (Sage et al., 2014). This crop is a clonally propagated plant species from which seed canes for next propagation are typically acquired from each planting (Verheye, 2010). At the base of the sugarcane plant, lateral shoots (tillers) are formed which produce multiple cane stalks that can be up to 3-4 metres high each with a diameter of approximately 5 cm (Devarumath et al., 2007). The cane stalk constitutes about 75 % of the whole plant when matured, and this stalk comprises of 12-16% soluble sugars, 11-16% fiber, 63-73% water, and 2-3% non-sugars (Bull, 2000).

2.1.2 Uses and importance of the crop

The production of sugar is the primary use of sugarcane, and the type of sugar produced is called sucrose and it is stored in the stalk (Fischer et al., 2008). Sugarcane is proving to be a versatile crop because of numerous by-products that can be produced from sugarcane while sugar is still being obtained from the stalk (Zucchi et al., 2002; Hunsigi, 2012). The juice that is produced from sugarcane is used for making jaggery, white sugar and numerous by-products such as molasses and bagasse (Fageria et al., 2013).

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The molasses can be utilized in distilleries for manufacturing of butyl alcohol, ethyl alcohol and citric acid (Goldemberg et al., 2008). This by-product can also be utilized as an additive to livestock feeds. The bagasse is mainly used as the fuel for fiber board, plastics, papers and furfural production (Fischer et al., 2008). The green tops of the cane are a good manure in the soil and they can also be utilized as the source of fodder for cattle. According to previous studies, it has been shown that sugarcane is high in polyphenols that are powerful phytonutrients containing antioxidants (Caderby et al., 2013). Sugarcane has been recommended for burning fat and building muscle because it's loaded with complete profile of essential amino acids like pipecolic acid, β-alanine, and methionine (Valli et al., 2012). It also contains vitamins and minerals such as calcium, magnesium, potassium, iron, and manganese which are essential for converting food into energy and repairing cellular damage in the body (Caderby et al., 2013). The sugarcane juice in its raw form is very essential in healing the body because it is alkaline and most of the human diseases are unable to survive in alkaline environment (Sahal et al., 2014).

Sugarcane plays a crucial role in the economy of developing countries. For example, in South Africa sucrose is the third most important largest valuable agricultural export product (Watt et al., 2009). The sugarcane industry also generates rural employment opportunities and also promotes education and co-operative movement (Hunsigi, 2012). According to Cockburn et al. (2014), sugarcane was regarded as the main livelihood resource, generating more income than any other single agricultural commodity. Sugarcane is amongst the crops that will play an important role in producing ethanol that is aimed to substitute crude oil, thereby meeting the crude oil needs (Fischer et al., 2008).

2.1.3 Production of sugarcane in South Africa

In South Africa, sugarcane is mostly grown in the KwaZulu-Natal province, followed by Mpumalanga, with the Eastern Cape being the province producing the least sugarcane (Mnisi and Dlamini, 2012). During the 2013/2014 season an average of 19 million tons of sugarcane was produced and crushed (Jones et al., 2015). There are six milling companies that are responsible for manufacturing of sugar with 14 sugar mills operating in the sugarcane growing areas (Figure 2.1). The South African sugarcane industry produces an average of 2.2 million tons of sugar per season (SASA, 2010/2011). There is a growing number of indigenous African growers that are entering the commercial sugarcane farming sector (Cockburn et al., 2014).

Figure 2.1. Sugarcane growing areas and the distribution of sugar mills in South Africa (SASA, 2010/2011).

2.2 Botany and agronomy

2.2.1   Plant morphology

There are three main parts of the sugarcane plant: the root system, the stalk and the leaf (Bull, 2000). Under certain photoperiod and soil moisture conditions the sugarcane plant may produce another part called the inflorescence or flower (Julien et al., 2012).

2.2.2   Root system

The root system is responsible for intake of water and nutrients, and serves to anchor the plant (Huang, 2000). The sugarcane plant roots are fibrous and are divided into two types (Figure 2.2). The first one are sett roots, which are those that are mostly temporary and which arise from the root band (dormant root zone) and are characterised by being thin and highly branched. The second type are shoot roots, which are permanent and originate from the base of the shoot after the buds have sprouted, and are thick, fleshy, less branched and white in colour (Bull, 2000).

The first shoot roots that are formed go downwards, while those formed later provide anchorage for the plant and are situated near the soil surface (Julien et al., 2012). The plant depends on the sett roots in the early stages (germination) for water and nutrients (Moore, 1987). The new tillers (shoots) – which develop from buds that are still underground produce their own roots from their root primordia (Verheye, 2010).
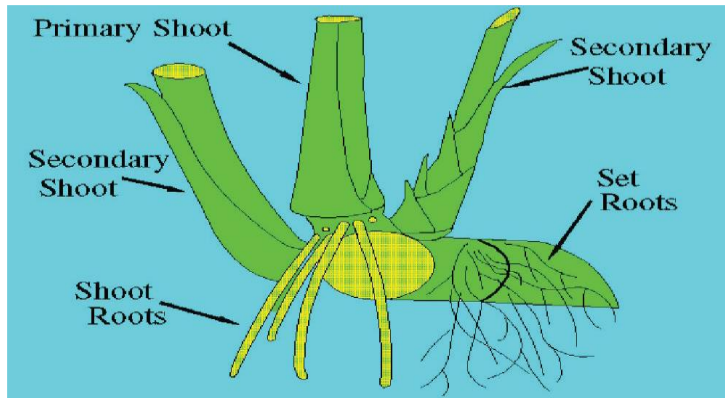
Figure 2.2. The root system of the sugarcane plant (Julien et al., 2012)

2.2.3   Stalk

The stalk (millable cane containing sucrose) develops from planted seed-cane buds. The buds of seed-cane produce a primary shoot which develops into a stalk (Julien et al., 2012). Other secondary or tertiary shoots then develop into secondary or tertiary stalks, respectively. The process of tillering results in a large number of stalks being produced from a single germinated bud (Meyer et al., 2011). The group of mature stalks formed from a single bud are referred to as a stool. The stalk itself is roughly cylindrical and it consists of many different nodes and internodes, bearing leaves and flowers (Julien et al., 2012). There is generally a variation in length and diameter of internodes, depending on varieties and growing conditions (Verheye, 2010). The leaf sheath that is folded tightly around the internode protects the buds that arise from the nodes. The raised portion just below the bud at which the leaf sheath attaches to the stalk is known as the leaf scar (Figure 2.3).
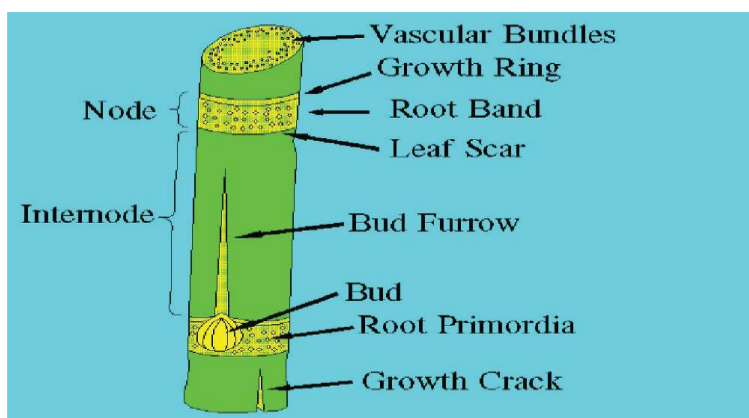
Figure 2.3. The parts of sugarcane stalk (Julien et al., 2012).

2.2.4   Leaf

The leaf of the sugarcane plant is generally like those of grasses. It is made up of two parts; leaf blade (lamina) and the leaf sheath (Figure 2.4). The sheath covers the stalk completely, extending over at least one complete internode (Moore, 1987). The sheath may possess some traits of certain varieties to differentiate between them; such traits may incorporate hair on the back of the sheath, the ligule and the auricle. The leaf blade is normally 1-1.2 m long and 5-7 cm wide (Verheye, 2010). When the sugarcane plant reaches maturity, the number of leaves that are green is approximately 10 per stalk and this can depend on the growing conditions and the variety grown (Bull, 2000).
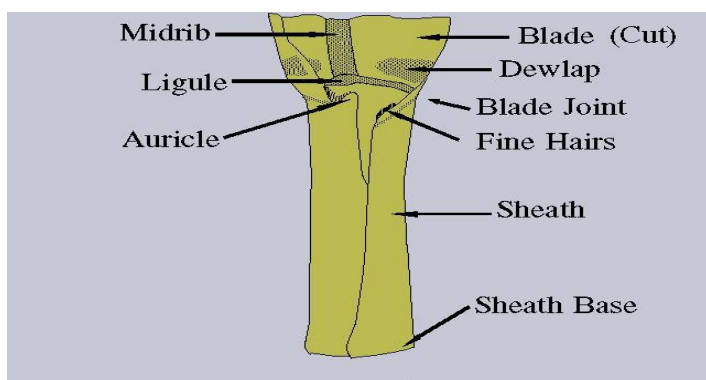
Figure 2.4. The sugarcane leaf parts (Bull, 2000).

The sugarcane plant changes from vegetative to a reproductive stage when it reaches a relatively mature phase of development under certain soil moisture conditions and photoperiod (Medeiros et al., 2016). At this stage the production of an inflorescence or a tassel (open-branched panicle) takes place while the growing point of the plant ceases forming leaf primordia. The inflorescence consists of thousands of very small flowers that are capable of producing one seed each. The tassel can be long (30 cm or more) and tapering (Moore, 1987).

The sugarcane produced from the seed is vital for sugarcane breeders but not desirable for farmers because the sugarcane plants produced from true seeds are not vigorous (Bull, 2000). Inflorescence development can be inhibited if there is water stress or temperatures that are too low.

2.3 Soil and climatic requirements

Sugarcane is one of the crops that do not require any specific type of soil to grow as it can be successfully grown under diverse soil types ranging from sandy soils to clay loams and heavy clays (Meyer, 2005). The best soils to grow sugarcane are those with good drainage, deep (>100 cm), high water retention and those that contain high contents of organic matter and plant nutrients. Moderate acidity and alkalinity can be tolerated by the sugarcane plant, however; for optimum production, sugarcane prefers soils with a pH of 6.0-8.0 (Pierre et al., 2015). Soils rich in Ca and P are advantageous because they produce cane with better juice quality and lodging resistance, while soils having deficiency of Ca and P retard development of the crop (Verma, 2004).

Sugarcane is regarded as a tropical plant, however; it can also be grown in sub-tropical regions. Adequate moisture, and long, warm growing seasons that have high incidences of solar radiation are ideal climates for production of maximum sugar in canes (Meyer et al., 2011). The sugarcane crop can continue to grow under warm humid conditions unless terminated by flowering. The growth of sugarcane is closely related to temperature, with temperature of 20 to 30 °C being the optimum for germination of stem cuttings. The optimum temperature for sugarcane to grow well is 25 to 34 °C, whereas temperatures above 35 °C reduces the growth (Singh et al., 2007). Bud sprouting of a ratoon crop can be inhibited under severe cold climates. Moderate relative humidity (45-65%) coupled with limited water supply are good conditions for the ripening phase, while high levels (80-85%) are favourable for cane elongation (Cardozo and Sentelhas, 2013). Sugarcane normally requires a total rainfall ranging from 1100 to 1500 mm (Deressa et al., 2005). The distribution of rainfall must be appropriate, with the months of vegetative growth requiring abundant rainfall as it encourages cane growth and internode formation. Minimum or less rainfall during the ripening period is favourable, as high rainfall leads to poor juice quality (Deressa et al., 2005).

2.4 Agronomy and husbandry

2.4.1 Land preparation

The non-selective herbicide Roundup® or other registered glyphosate products is applied to the field prior field preparation (Singh and Kaur, 2003). After 3-4 weeks, a subsoil ripper maybe used to destroy deep compaction layers of the soil. The field is then prepared for planting by breaking up dirt clods and levelling the surface of the field using a disc harrow. A week prior to planting, a ridger is used to open furrows and fertiliser is applied in furrows according to recommendations from soil analysis (Braunack et al., 1999). It is important to prepare a good seedbed before planting sugarcane. A good seedbed provides good tilth which is essential for good bud germination and subsequent root development of the plant.

2.4.2 Planting

It is essential to select good quality seed cane that is disease free in order to obtain optimum sugarcane production (Verma, 2004). Such seedcane can be obtained from registered nurseries where plant crops and first ratoons are the only seed used/sold as planting material. There is a variation in planting dates of sugarcane due to the diverse local climatic conditions where this crop can be grown in (Meyer et al., 2011). For example, under dryland conditions, spring is considered as the best time to plant cane. Setts are planted in furrows, and a light layer of soil is added to cover the seed cane. The sugarcane can be planted using two methods, namely manual and mechanical (Chattha et al., 2007). Sugarcane is mechanically planted by means of special equipment that simultaneously opens the furrows, plants the setts and applies fertilizer, whereas manually it can be planted by placing cane setts end to end together with fertilizer in the furrows. Planting sugarcane by hand is labour intensive and can takes more time to plant the same piece of land than it can take when using special equipment (Chattha et al., 2007). For weed control, pre-emergence and post-emergence herbicides are applied to the field. Hand weeding is also done after 4 weeks of post-emergence application for any surviving weeds.

2.4.3 Harvesting

Depending on the variety and environmental conditions, sugarcane can reach maturity over the span of 9 to 24 months (Gilbert et al., 2006). In the South African sugar industry, variation exist in sugarcane harvesting ages depending on whether the fields are plant or ratoon crops and the climatic conditions where the cane is grown (Meyer et al., 2011). The harvesting for ratoon crops occurs much earlier than for a plant crop. When the climatic conditions are unsuitable, the cane age at harvest is higher.

Under dryland conditions, the cane is harvested at approximately 12-24 months whereas with irrigated land, harvesting occurs annually at roughly 12 months (Ramburan, 2015). The green and dead (brown) leaves of the plant need to be removed before canes are harvested (Jalaja et al., 2008). This can be attained by burning the sugarcane field or manual stripping of any leaves on the stalks. Sugarcane can be harvested manually or mechanically (Pongpat et al., 2017). With manual harvesting, a cane-cutter cuts the standing stalks (cane) off at the ground level. Mechanical harvesting entails the cutting of the stalks off at the ground level utilizing a tractor that is mounted with rotating disk fitted with sharp blades.

2.5 Stages of growth and development

2.5.1   Germination

Sugarcane is propagated vegetatively using stalks cut up into shorter segments called setts (seed cane) that usually contain two or more nodes containing buds (Jalaja et al., 2008; Pierre et al., 2015). Germination of sugarcane is described as the initiation of growth from buds (Meyer et al., 2011). After the period of dormancy has passed, the buds start forming new shoots (Bull, 2000). The changes that occurs in the activity of growth regulating substances (hormones) and enzymes is a complex phenomenon responsible for the transition from the dormant into the active stage of buds (Willcox et al., 2000). This phase can start from 7 to 10 days under field conditions and it can last for approximately 30 to 35 days. In the early stages of germination, the flush of roots are produced by the root primordia around the nodes of the sett (Verheye, 2010). These roots are essential in maintaining the moisture in the sett. There are a number of closely spaced internodes and nodes on the primary shoot and each node is capable of developing a new bud and root primordia. The shoot roots that are responsible for supporting further plant growth are produced by germinating root primordia (Bull, 2000).

Many internal and external factors can influence germination of buds (Singh, 2015). The internal factors include sett nutrient status, sett moisture and bud health; while external factors are aeration, soil temperature and soil moisture. Rapid germination occurs when there is warm, moist soils and optimum temperatures of around 28-30 $^{\circ}$C. According to Verma, (2004), 3-bud setts have high germination percentage when contrasted with setts having more or less than 3-buds. The single-budded setts have very poor germination capacity due to moisture that is lost from cut ends on either side and furthermore they also lack vigour which results in low yield (Sriwongras et al., 2014).

These single-budded setts are therefore usually germinated individually in seedlings trays under good conditions, and then transplanted into the field once hardened off appropriately. The term used for this is "speedlings". Planting the whole cane stalk results in germination of the few buds at the top end and buds at lower end remain inactive due to apical dominance (Jain et al., 2010). This dominance is eliminated by cutting the cane stalk into pieces (setts). When the internal and external factors are optimal, they can result in maximum germination and shoot vigour.

2.5.2 Tillering process

Tillering or development of secondary shoots is the physiological process that occurs when there is repetition of underground branching from nodal joints of the primary shoot (Rossler et al., 2013). Under optimum conditions this physiological process starts from approximately 45 days after planting and usually occurs until about 120 days (Roodagi et al., 2000). Tillering is the most important stage in sugarcane production because it provides a plant with the total number of stalks (population) for a good yield (Matsuoka and Stolf, 2012). In addition, tillering also aids in weed control because it increases the rate of canopy closure (Bezuidenhout et al., 2003). The tillering capability is greatly influenced by genotype. For example, some varieties can produce many tillers that are thin in diameter while other varieties can produce few tillers with thicker diameter.

Many factors also play a role in tillering capability of sugarcane plant besides varieties (Bokhtiar and Sakurai, 2005). These factors includes moisture, temperature, nutrition, plant spacing and light. Amongst these factors, studies have shown that light is the most important factor (Bokhtiar and Sakurai, 2005; Matsuoka and Stolf, 2012). The tillers that are formed earlier produces stalks that are thicker and heavier while late formed tillers either remain short or die (Bezuidenhout et al., 2003). Tillering in a ratoon crop proceeds much earlier and is more profuse than in a plant crop due to the larger number of buds that are available underground (Meyer et al., 2011). In a plant crop, tillers are formed from one bud, whereas in a ratoon crop, tillers are formed from the underground buds of tillers from the previous crop.

2.5.3 Stalk elongation

Stalk elongation stage begins from 120 days after planting and may last up until 270 days (Bull, 2000). The tiller stabilization takes place during the early period of this stage, and only 40-50% of tillers produced survive by 150 days to form stalk (millable cane) (Rossler et al., 2013).

Stalk elongation is the most significant stage of sugarcane growth because at this stage there is actual sugarcane stalk formation and yield build up takes place (Bokhtiar and Sakurai, 2005). At this stage there is frequent and rapid leaf production with leaf area index (LAI) reaching approximately 6-7 m^2. When conditions are favourable, 4-5 internodes per month can be produced by the rapidly growing stalk (Verheye, 2010). This stage is very sensitive to soil moisture and temperature. Internode length can be reduced under moisture stress conditions while good growth is obtained with a temperature of about 30 °C and humidity of around 80% (Meyer et al., 2011).

Stalk elongation can be slowed or stopped when there is too much water in the soil after rain or irrigation due to waterlogging in the root zone (Jones et al., 2015). At this stage stalk lodging can occur when the climatic and soil conditions are wet, warm and windy. The stalk death and damage in a lodged crop are usually minimal in the cooler and drier areas thus leading to only minor yield losses under these conditions. Under high potential irrigation conditions, lodging may be more profuse, with increased difficulty when harvesting (Jones et al., 2015).

2.5.4 Maturation and ripening

Ripening is described as physiological senescence characterized by minimized or arrested stalk elongation and the storage of sucrose in the stalk (Bull, 2000; Julien et al., 2012). This stage is divided into two phases (Cardozo and Sentelhas, 2013). In the first phase, there is a separation of the leaf that is ageing from the subtending internode. In the second phase, there is accumulation of sugar in the stalk. The simple sugars (monosaccharides) are converted into the cane sugar (sucrose, a disaccharide) as the ripening stage advances (Hunsigi, 2012). The degree of ripening in sugarcane relies on the age of the plant (stalk) and other factors such as variety (Bull, 2000; Cardozo and Sentelhas, 2013).

For example, the sucrose content in young stalks are generally higher at the bottom of the stalk, and sugar content decreases towards the top of the stalk. Sucrose content is found more uniform throughout the stalk as the sugarcane plant matures except for the few top internodes. This stage can last for approximately three months beginning from 270-360 days after planting (Moore, 1987; Rossler et al., 2013). The ripening stage requires moisture content that is low, so that sugar that is accumulated is not of poor quality (Cardozo and Sentelhas, 2013). The conducive environmental conditions for ripening are dry weather, cool nights and warm days.

2.6 Sugarcane propagation methods

2.6.1   True seed

Sugarcane is capable of producing true seed through sexual reproduction, although these true seeds cannot be used for growing a commercial crop of sugarcane (Zhou, 2013; Pierre et al., 2014). This potential of sugarcane producing true seed was first discovered by J.W. Parris of Highland Plantation in late 1800s (Tai et al., 1999). When compared to other sugarcane planting materials, true seeds germinate very poorly and for them to develop into full-grown plants they require a much longer period of time (Pierre et al., 2014). In addition to this, the plants that are produced using true seed are usually inferior to the parent varieties and they are not true to type. However, the advantage of producing true seed is that this is an essential first step in the sugarcane variety improvement by plant breeders (Zhou, 2013). Sugarcane breeder's use true seed to achieve their goal of developing genetically improved varieties. Superior parents are manually crossed in artificial environments to produce true seed of new hybrids that enter into selection programs.

2.6.2   Conventional propagation for commercial production

2.6.2.1 Sett preparation

For raising a healthy sugarcane crop, setts used for planting should be free from diseases and pests (Benda and Ricaud, 1978). Harvesting of seedcane should take place when the crop is 5 to 9 months old for setts to have high moisture content (Cardozo and Sentelhas, 2013). It is essential to select good canes during setts preparation; such canes should be well developed, and with buds that are well formed. The stalks with buds that have started to sprout should not be used as seed cane, as these buds can easily be damaged during handling and transportation of canes. During harvesting of canes, trash can either be removed or left on the stalk. However, it is unnecessary to remove the trash from the stalks that will be treated before planting as lots of trash automatically falls off during harvesting and also when the canes receive hot water treatment (HWT) the trash remains very soft and therefore easily rots in the ground. Leaving trash on the canes also protects the buds from being damaged during transportation and planting of setts. The cane stalks are cut into appropriate lengths (usually 1m long) to fit into baskets designed specifically for HWT.

2.6.2.2 Hot water treatment of setts

The sugarcane crop is vulnerable to various diseases such as red rot (*Glomerella tucumanensis*), smut (*Ustilago scitaminea*), leaf scald (*Xanthomonas albillineans*) and yellow spot (*Mycovellosiella koepkei*) (Benda and Ricaud, 1978). The crop can also be attacked by insects and borers that can cause heavy damage to the newly planted crop. The use of hot water treatment (HWT) has assisted in getting rid of several diseases and pests (Croft and Cox, 2013). Curing certain diseases and insect pests of seeds and plants using HWT is not a new technique. According to Hol et al. (1992), the HWT has been used in the bulb industry for some time. Furthermore, this technique has recently been used to kill nematodes present in berry plants (Croft and Cox, 2013). After discovering that sugarcane was attacked by viral diseases, many studies were undertaken to determine the thermal death point of the viruses. Other studies were carried out on controlling these viral diseases in canes, and this was attained by subjecting the setts to a temperature that would cause no damage to the seed canes but killed the virus (Benda and Ricaud, 1978). According to the findings of Nyland and Goheen (1969), red rot and smut diseases of sugarcane was controlled by treating the setts using HWT at 52 °C for 18 minutes. The mealie bug and moth borer of sugarcane was also controlled by treating the setts using HWT at 50°C for 20-30 minutes.

The HWT technique is routinely utilized in Southern Africa. When using this method, water in the tank should be heated up to a temperature of 50 °C followed by dipping setts into the tank (Benda and Ricaud, 1978). Dipping setts in the tank causes the temperature of water in the tank to drop, so water should be heated up again to maintain the temperature of 50°C for two hours. The two-hour period begins when the temperature of water has risen back to 50 °C. It is important to control temperature of water and accurate timing is also essential. Diseases and insect pests can survive if there is a slight drop in temperature or when treatment time is shortened, whereas germination can be reduced if treatment is prolonged (Croft and Cox, 2013). Water inside the tank must be kept clean to avoid the build-up of contaminants that can inhibit germination. The setts should be completely immersed in a fungicide for 2-5 minutes immediately after a two-hour treatment because they are more prone to attack by pathogenic organisms after treatment. When seed canes are heat treated, the dominant effect of the apical bud is eliminated thus resulting in equal chances of development of lateral buds.

This means that if the canes are heat treated it is not necessary to cut them into smaller setts for good germination to occur.

2.6.2.3 Seedcane production

Seedcane production systems comprises of first stage nurseries (stage 1), second stage nurseries (stage 2) and commercial cane fields as detailed below.

2.6.2.3.1   Stage 1 nursery

During stage 1 of seedcane production, cane that is treated using HWT (described above) is planted into well-prepared fields (nursery), where sugarcane was not grown for at least six months (Croft and Cox, 2013). These fields need to be clear of any volunteers, weed free, and ideally have access to some irrigation. During planting, canes are placed in furrows and then manually chopped into setts (although this is not always necessary after HWT). Stage 1 nurseries need to be regularly inspected for off-types (i.e. variety mixes within the stand of cane) and routinely scouted for pest and disease levels. Only after the cane has been certified (true to type and below certain pest and disease thresholds), can the grower then harvest the seedcane. Stage 1 seedcane should be harvested at 7 to 10 months of age and used for planting secondary seed nurseries (stage 2) to increase the planting material. Alternatively, stage 1 seed can be planted out into commercial fields. It is conventional practice to use the plant crop from the stage 1 nursery to establish a stage 2 nursery, while the ratoon crop is planted out into commercial fields or used again to establish a stage 1 nursery.

2.6.2.3.2   Stage 2 nursery

Stage 2 seed production begins when the stalks that are produced from stage 1 are planted into larger farm nurseries to produce more planting material. Once again, this material is regularly inspected for quality before it can be harvested. The sugarcane crop at stage 2 seed production should be harvested at 7 to 10 months and planted out commercially using conventional methods. Only the plant crop from stage 2 may be used for direct commercial planting.

2.6.2.3.3   Commercial planting

The seedcane that is obtained from nurseries (either stage 1 plant or ratoon crops, or stage 2 plant crops) are used to plant commercial sugarcane fields. The planting rate of this seed is usually around 10 tons/ha. This seedcane is not hot water treated, and is planted directly into the ground, chopped into setts (usually three-budded setts) and covered using conventional practices. During distribution of seedcane, care should be taken to ensure that the buds are not damaged.

2.6.3   Propagation via tissue culture (NovaCane® process)

Sugarcane is known as a vegetatively propagated crop through stem cuttings for commercial planting, however this method has constraints as it is labour intensive and has a low seed multiplication rate (Pandey et al., 2011; Ibrahim et al., 2016). Hence, farmers are facing a major problem of non-availability of quality seed material, especially of new varieties that are released for industries. Furthermore, there is a general decrease in cane yield and quality due to the successive use of cane cuttings for planting. This decrease may be associated with pests and diseases that accumulate over vegetative cycles thus leading to yield and quality decline over time (Ramanand and Singh, 2005; Yadav and Ahmad, 2013). Many studies have been undertaken over the years with the aim of improving and refining this vegetative propagation method.

More than 40 years ago, *in vitro* studies of sugarcane were initiated with the intention of producing disease-free plantlets and enhancing multiplication rates (Heinz and Mee, 1969; Tiel et al., 2006; Ntoyi et al., 2007). Farmers and industries have successfully adopted a number of micropropagation techniques in sugarcane growing countries. These incorporate planting material produced either through somatic embryos from callus or directly from apical shoot material (Grisham and Bourg, 1989). Sugarcane Mosaic Virus free plants were obtained by Hendre et al. (1975) using apical meristem culture. Lee (1987) applied axillary bud culture successfully produced true to type clones in many sugarcane varieties.

In South Africa, a rapid propagation procedure called NovaCane® has been developed by the South African Sugarcane Research Institute (SASRI) (Snyman et al., 2009). Phase 1 of this technique is *in vitro* plantlet generation; while phase 2 is an *ex vitro* hardening off process; followed by phase 3 which is the multiplication of healthy seedcane by field planting. This technique is currently utilised to eliminate sugarcane pathogens such as Sugarcane Yellow Leaf Virus (SCYLV) (Meyer et al., 2010). According to Ramgareeb et al. (2010), this technique can be used to remove disease-causing agents when a less than 2 mm apical meristem is dissected out and utilised to initiate cultures. Furthermore, this technique increases multiplication rates, for example; an average of 150 plants from one apical meristem can be obtained after 11 weeks in culture (Fitch et al., 2001). Different routes (*in vitro* and *in vivo*) that can be used to produce sugarcane plants are summarized in Figure 2.5 below.
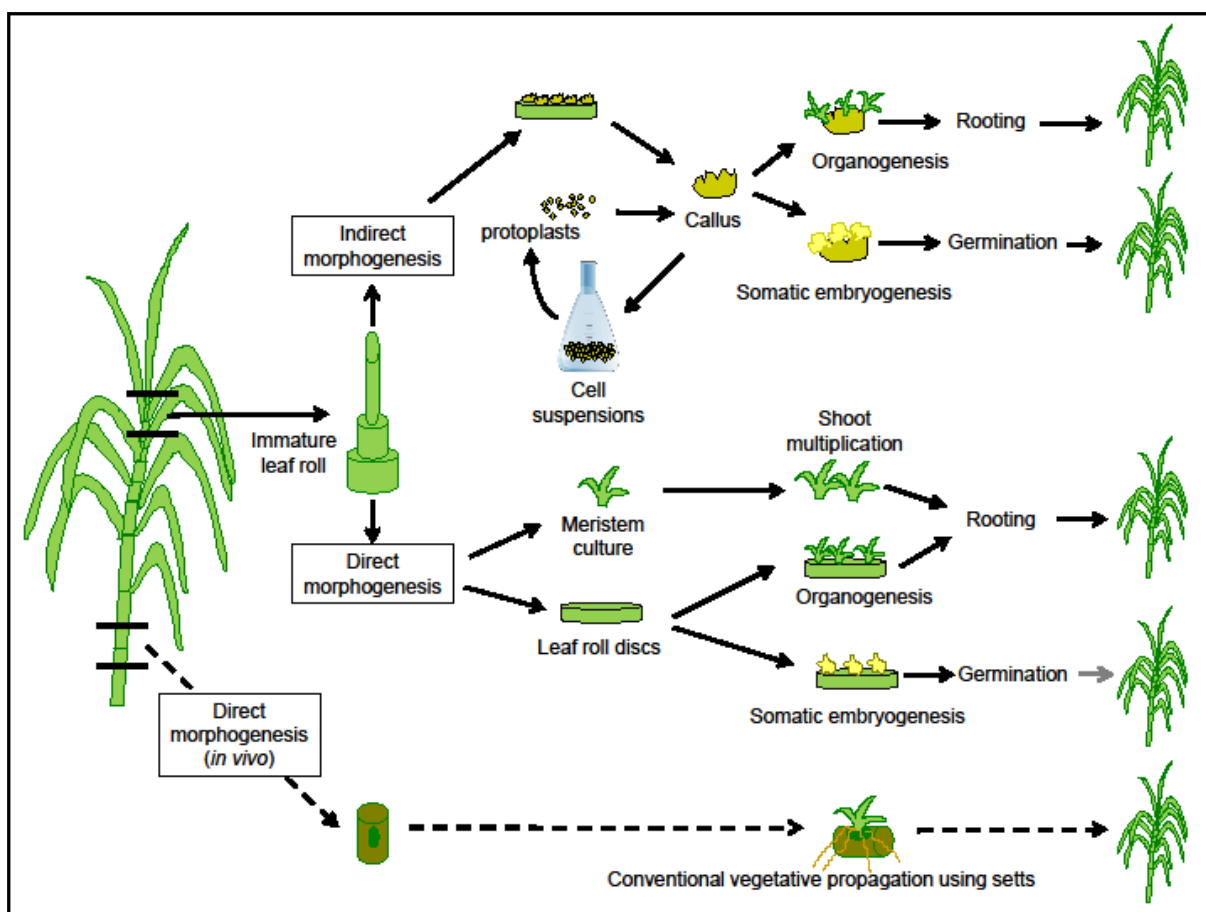
Figure 2.5. Flow chart of different routes by which sugarcane plants can be produced in vitro and *in vivo* (Snyman, 2004).

2.6.3.1 Production of plants by meristem/shoot tip culture

The apical meristem is located at the top of the stem in a growing sugarcane plant, and is surrounded by young, developing leaves and leaf sheaths (Zucchi et al., 2002). As long as the apical growing point is functional, meristems can also be found in axillary buds located down the stems that are dormant. For initiating meristem tip cultures, both the axillary and apical buds can be utilised (Jalaja et al., 2008; Cheong et al., 2012). During the vegetative growth, meristems remain in an active state and the cells of meristem are in a permanent juvenile young undifferentiated state. The plants produced from meristem cells are identical to the donor plants due to genetically stable cells; however, rare mutations can occur (Hendre et al., 1983; Screenivasan and Screenivasan, 1992).

2.6.3.2 Collection and decontamination of shoots

The first step in micropropagation is the collection and decontamination of shoots. To be able to verify varietal characteristics during stages of seed production, selected varieties should have accompanying morphological descriptions (Jalaja et al., 2008) and molecular fingerprinting (Joshi and Albertse, 2013). It is ideal to use shoots from nursery crops raised from HWT setts to avoid diseases such as Ratoon Stunting Disease. However, each stalk should be subjected to a disease-indexing procedure before being placed in culture for diseases mentioned above (Snyman et al., 2009). The material used for initiating the culture are only those certified and pathogen-free (Ramanand and Singh, 2005). Shoots tips for in vitro culture should be harvested 120 to 180 days after planting for best results (Saini et al., 2004).

2.6.3.3 *In vitro* micropropagation (Phase 1)

The explants (apical or axillary shoots) are excised aseptically, transferred to Petri dishes containing semi-solid culture medium and incubated in the dark room at 26 -28 °C for one week (Ramgareeb et al., 2010). Although the initial protocol for NovaCane® production used somatic embryogenesis for plant production (Snyman et al., 2009), the shoot multiplication technique from apical meristems as described by Ramgareeb et al. (2010) is more efficient and is now routinely used to produce NovaCane® plants (Snyman personal communication, 2016). In this protocol, once the shoot emerging from the meristem reaches a size of 1 cm, it is transferred to liquid medium containing the cytokinins kinetin and 6-benzylaminopurine for shoot multiplication (Ramgareeb et al., 2010). The shoots are continuously split and placed on fresh media for a period of 5 months. Thereafter a rooting agent, indole-3-acetic acid, is added for the last 2 weeks of culture.

2.6.3.4 *Ex vitro* acclimation (Phase 2)

Only plantlets with well-developed shoots and roots are removed from the culture vessels and planted into seedlings trays containing peat moss for hardening. Until the new leaves emerge (approximately 14 days), the plantlets must be kept under shade or mist chamber covered with polyethylene sheets to maintain humidity (Sandhu et al., 2009). During this period, irrigation is required, however; excess watering must be avoided. To boost plantlet growth, application of fertilizer is required once a week after plant establishment. The plants are ready for planting into commercial fields approximately 8-12 weeks later (Snyman et al., 2009).

2.6.3.5 Field planting and bulking (Phase 3)

After plants are hardened, they are planted in the field. To reduce soil-borne diseases, fields that are used for planting hardened plants should be kept fallow during the previous year (SASRI, 2015). For planting hardened NovaCane® plants, furrows that have 90-150 cm row-to-row spacings should be opened with 30-100 cm distance between pits (holes made for individual plants). Fertiliser can be broadcasted throughout the field or applied directly in the pits and mixed with the soil before planting. Immediately after planting, the field should be irrigated. Irrigating the field should be done weekly until successful establishment is achieved; this can be seen by new leaf growth. If proper maintenance and irrigation regimes are followed, 95 % establishment can be attained using tissue culture raised plants (Jalaja et al., 2008). It is important to note that the field planting of a tissue culture derived plant described above is an equivalent propagation stage to the planting of HWT cane stalks i.e. stage 1 propagation. Therefore, tissue culture plants are not conventionally used for commercial planting, but rather used as material for stage 1 nurseries.

2.6.3.6 Characteristics of plants derived from tissue culture

Some studies have been undertaken to compare agronomical performance of tissue culture (TC) raised plants against conventional propagated (Con) plants. Ibrahim et al. (2016) conducted a study comparing plants derived from the TC (stage 1) and the Con plants of two varieties (B52-298 and NCo-334) under irrigated conditions. The results showed that higher propagation rates of 1:44 and 1:40 for variety B52-298 and NCo-334 were obtained when using planting materials derived from the TC method compared with the propagation rates of 1:13 for both varieties (B52-298 and NCo-334) when using Con plants. Furthermore, the TC derived plants produced 235000 tillers per hectare compared with 11000 tillers per hectare of the Con plants. These results are in agreement with the findings of Sood et al. (2006), who indicated that the TC plants at stage 1 attained a greater number of tillers per hectare compared with the Con plants.

Sandhu et al. (2009) compared agronomic performance of single bud setts (SBSs), Con and TC (stage 1) plants in one trial and also compared performance of Con and TC (stage 2) plants in a second trial, under rainfed conditions. According to the first trial results, a higher number of tillers per hectare was recorded in the TC raised plants (163 000), followed by that from the SBSs (138 200) and the Con (126 200), respectively. Furthermore, thinner stalks were observed in the TC raised plants (1.75 cm) compared with the SBSs (2.48 cm) and the Con (2.42 cm) plants.

The second trial results showed that cane diameter (cm) and single cane weight (g) were statistically similar between the Con and the TC (stage 2) plants. However, the number of tillers per hectare at harvest was significantly higher in the TC raised plants (95 600) as compared with the Con plants (81 700). These results are in line with the findings of Ramanand and Singh (2005), who compared the TC (stage 2) and the Con plants of two varieties (CoJ 64 and CoJ 83) under rainfed conditions. They found that both varieties of the TC plants had a significantly higher number of tillers per hectare compared with the Con plants. Furthermore, both varieties of the TC plants were observed to have a significantly higher average sugarcane stalk height compared with the Con plants. Varieties CoJ 64 and CoJ 83 of the Con plants were 183.5 and 188.5cm in height as contrasted to the TC derived plants that were 208.7 and 203.6 cm, respectively. These results suggest that some of the effects of the TC propagation on plant morphology (e.g. stalk population) may persist into stage 2.

Flynn et al. (2005) compared the TC derived with the Con plants of four varieties (CP 72-2086, CP 80-1743, CP 84-1198 and CP 89-2143). The results from this study showed that the TC derived plants of all four varieties had a significantly greater stalk population compared with the Con plants in both plant-cane and second ratoons crop, but this was not observed in the first ratoon crop. Comstock and Miller (2004) compared yield parameters of five varieties (CP 72-1210, CP 80-1827, CP 84-1198, CP 85-1382 and CP 89-2143) grown from the TC and the Con plants under rainfed conditions. The results from this study showed that the number of stalks, stalk mass, and sucrose per plot was significantly higher for the TC plants compared with the Con plants for all five varieties. Furthermore, the number of stalks and weight per plot was higher for CP 72-1210, CP 84-1198 and CP 85-1382 in the first ratoon crop for the TC derived plots than in plots planted with Con plants. Results of CP 80-1827 in first ratoon crop were opposite to other varieties, with plots planted with the Con plants having higher number of stalks and weight per plot compared with plots planted with the TC derived plants. In contrast, Burner and Grisham (1995) found that the TC derived plants had significantly higher stalk population compared with the Con in the plant crop, however, differences had disappeared in subsequent ratoon crops. Furthermore, the study showed that the TC derived plants had low mean stalk diameter, stalk weight and stalk length compared with the Con plants in the plant crop, but these differences disappeared in the subsequent ratoon crops.

There have been some conflicting outcomes with regards to studies done on comparing cane and sugar yield between the TC and the Con plants, and such studies are limited. According to Sood et al. (2006), 13.2 % increase in cane yield and 11.03 % sugar recovery were obtained when using the TC raised plants at stage 1 compared with the Con plants. These result are in contradiction with the findings of Sandhu et al. (2009), who showed that the Con plants produced higher cane yield (76.3 t/ha) compared with the TC raised plants at stage 1 (49.1 t/ha), however, the TC raised plants at stage 2 produced higher yield (83.4 t/ha) than the Con plants (74.7 t/ha). These results are in line with the findings of Ramanand and Singh (2005), who indicated that all varieties (CoJ 64 and CoJ 83) of the TC raised plants (stage 2) produced an average yield of 206.15 t/ha as compared to 186.00 t/ha of the Con plants. According to Flynn et al. (2005), the TC had a significantly higher cane and sugar yield compared with the Con plants in the plant and second ratoon crop, but this was not observed in the first ratoon crop. These results are in contradiction with the findings of Hoy et al. (2003), who showed that there were no significant differences in cane yield between the TC and the Con plants up to the second ratoon crop. The conflicting results above may be due to differences in growing conditions and varieties, which suggests that work on local varieties under prevailing conditions in South Africa is necessary.

2.6.4   Transplants

Approximately 10 tons/ha seed cane is utilized as planting material to plant a hectare in conventional systems of sugarcane cultivation (Singh et al., 2014). The large mass of planting material creates a great problem in handling, transporting and storage of seed cane, and furthermore, this results in rapid deterioration thus reducing the viability of buds. Many studies have been carried out to find alternative ways of reducing the quantity of seed cane used for planting sugarcane fields. Dating back to the early 1950s, sugarcane researcher van Dillewijn was the first scientist to demonstrate that a small volume of tissue and a single root primordium adhering to the bud can be used as planting material (Jain et al., 2010; Ramaiah et al., 1977). Furthermore, Shanthy and Ramanjaneyulu (2016) also indicated that cutting with only one bud under favourable growing conditions did as well as conventional planting material (i.e. setts). This was in agreement with the findings of Narasimha Rao and Satyanarayana (1974) and Ramaiah et al. (1977) who showed that commercial sugarcane fields were established by eliminating the internode part of the seed canes and using only buds (nodes) as the planting material. The single-budded sett (SBS) planting material is called transplants or speedlings.

Using this method, only 50-75 kg SBSs can be utilized to plant a hectare of land and the canes (internodes) that remain can be sent for crushing (Jain et al., 2010). Studies carried out have shown that there are various limitations in transplants method (hereafter referred to as the SBS method). The limitations of using the SBSs are mainly due to low food reserves (1.2-1.8 g sugar/ bud) contrasted to conventional 3-budded setts (6.0-8.0 g sugar/ bud) which results in poor survival of plants under field conditions (Jain et al., 2011). Furthermore, the depletion of food reserves and moisture in the SBSs occurs at a faster rate when contrasted to 2 or 3-budded setts (Jain et al., 2010). This faster rate of depletion is reflected by their poor sprouting. Sprouting of bud setts, root growth and plant vigour can be promoted by treating them with calcium chloride (0.1 %) and ethephon at 100 mg per litre. According to Jain et al. (2011) this treatment works by reducing sugar contents and changing the key biochemical activities that are important for early growth of bud's setts and better establishment of the plants.

2.6.4.1 Bud selection and preparation

In the SBS method, nurseries are raised using single budded setts from canes that are healthy and which have been HWT. This method begins when canes that have good internode length (15 to 20 cm) are selected, and such canes should be 7 to 10 months old (Singh, 2003). When removing the dry leaves from the canes, care should be taken to avoid damaging the buds (Jain et al., 2010). Using knives to remove the leaves can cause damage to the buds, so it is preferable to use hands (Shanthy and Ramanjaneyulu, 2016). After removing the leaves, single budded setts are cut using various types of equipment ranging from a simple knife to custom built machines. To avoid infestation, single-budded setts (SBS) should be treated with various organic or chemical solutions before planting. The SBSs need to be dried for 2-3 hours under shade after being treated before used for nursery plantation.

2.6.4.2 Raising nurseries using single budded setts

Buds that are selected for germination are placed individually into biodegradable germination trays filled with pine bark (Shanthy and Ramanjaneyulu, 2016). Buds should be placed in the germination trays that are half-filled with pine bark in a slightly slanting or flat position. After placing the buds in the trays, trays should be completely covered with coco-pith. For high percentage germination in the nursery, coco-pith should be well decomposed. Pressing or pushing of buds hard in the trays should be avoided and buds must face upward.

The germination trays should then be placed on a fully covered structure that will provide shade. The structure should be covered in such a way that it creates favourable conditions (i.e. warm and wind free environment). Based on the agro-climatic conditions, within a week a high percentage of germination can be attained through this method (Meyer et al., 2011). Watering of trays using watering cans should be done in the evening depending on the moisture of pine bark inside the trays. The buds will begin emerging and leaves will start sprouting. Depending on the moisture level in the trays, application of water can be increased gradually after the appearance of two leaves. In some cases, special growth rooms are used to create ideal conditions that promote germination. Regularly and carefully inspection of diseases on the nurseries is required. Care should be taken to avoid any weed growth that can be a host of diseases and the soil inside the nursery should be soaked with insecticides to control termites. Following germination of the SBSs, the new seedlings are then hardened off in the nursery before being transferred into the field.

2.6.4.3 Transferring young seedlings/transplants to the field

Once young seedlings at the nursery are at the age of 25-35 days, they should be transplanted to the field or nurseries (Shanthy and Ramanjaneyulu, 2016). Care should be taken during transportation of seedlings to the fields or nurseries for bulking because damage during transportation can results in high mortality after transplantation (Lal et al., 2015). To loosen the seedlings in plugs, water should not be given to seedlings one day before transplanting. This assists in lifting of seedlings from the trays during transplanting. Furrows and pits should be opened for planting seedlings. The spacing between furrows differs depending on the grower requirements. Immediately after planting, the field should be irrigated. In the field where soil moisture is a severe limiting factor, irrigation should be applied until the plants establish for seedlings to grow well (Shanthy and Ramanjaneyulu, 2016). To ensure more number of tillers and millable canes per plant, the mother plants are usually cut (5-10 cm above the ground) after the establishment of plants on the field.

2.7 Requirements for handling different sources of seedcane

2.7.1   Watering regime

Plants raised from the TC (stage 1) or those raised from the SBSs requires more water in the early stages of growth than those raised from the Con (Saini et al., 2004). To grow seedlings plants from either the TC or the SBSs irrigation is required to maintain growth of these plants especially in fields where soil moisture is a severe limiting factor. For example, 6.5 L of water per tray per day is required to maintain seedlings when they are still in the nursery (Snyman et al., 2009). Unlike plants raised from the Con, after being planted in the field they can thrive even when soil moisture is a limiting factor. If there are high temperatures after planting, there is a higher risk of poor establishment of plants raised from the TC and the SBSs compared to those raised from the conventional method. As the TC plants produce a significant number of stalks, this leads to competition for water amongst stalks, so watering the plants even after establishment might be required.

2.8 Plant spacing and planting density

Numerous studies have been undertaken to evaluate the correlation between stalk population and cane yield. According to James (1971), cane yield can be affected by stalk thickness, stalk length, and stalk density. However, all these components are influenced by stalk population which determine cane yield per unit area. The choice of variety, spacing of planting material and cultural practices can be utilised to modify stalk population per unit area (Khairwal and Babu, 1975). For maximum production of cane and sugar, spacings for different varieties must differ because each variety has optimum inter-row spacing (Ayele et al., 2012).

Since sugarcane became a commercial plantation crop, researchers have attempted to increase yields by varying spacings between plants. The studies that have been undertaken to evaluate the effect of plant spacing on sugarcane yield are voluminous and often conflicting. According to Ayele et al. (2014), soil fertility determines the most economical width of row, with fertile soils requiring wide spacings (91 – 213 cm) to enable freedom of growth. In South Africa, studies of Thompson and du Toit (1965) and Boyce (1968) have shown that if the soil moisture content is not a severe limiting factor, a persistent trend towards higher yield with closer row spacing occurs. The results from their studies indicated that for each 30 cm reduction in row spacing, approximately 5 per cent increase in yield was obtained and their row spacings ranged from 46-160 cm.

In South Africa, Bailey and Bechet (1989) compared performance of the TC raised plants with SBSs of three varieties (N12, NCo310 and NCo376) planted at the same plant spacing (50 cm). Trials were planted at two sites, one under irrigated conditions at Pongola and the other under rainfed conditions at La Mercy. The results indicated that at both sites, the TC plots of all three varieties produced higher stalk population compared with the SBSs plants; however, the differences were greatest in NCo376. At La Mercy site, stalk length was generally less in plots raised with the TC derived plants and NCo376 showed the greatest difference, whereas at Pongola stalk length was slightly less in the TC raised plots of all varieties and the differences were not significant. At La Mercy site, there was also a significant difference in stalk diameter with TC derived plants producing thinner stalk as compared to the SBSs plants, whereas at Pongola the differences in stalk diameter were small (not significant). Furthermore, the cane yield of all three varieties was significantly higher in the SBSs plants at both Pongola (+ 7.1 %) and La Mercy (+12.3 %).

Saini et al. (2004), compared field performance of the TC and the SBSs plants of two early maturing varieties (CoH92 and CoH99) planted at the same intra-row spacing (60 cm) under irrigated conditions. According to the results, the TC derived plants (stage 1) had relatively higher number of millable canes in both varieties, but they were thinner as compared to SBSs plants. However, the TC (stage 2) and the SBSs plants showed no significant difference in terms of number of millable canes and cane diameter in both varieties. According to the study done by Sandhu et al. (2009) comparing plant to plant spacing of 45 cm and 60 cm of the TC raised plants (stage 1), 142 900 tillers per hectare were obtained in a plant to plant spacing of 45 cm than 138 200 tillers per hectare in 60 cm plant to plant spacing. Furthermore, their results showed that there were no significant differences in cane yield, sucrose percentage, cane diameter and single cane weight between plant to plant spacing of 45 cm and 60 cm. The cane and sugar yield can be improved by high-density planting of sugarcane through promoting rapid canopy closure by increasing radiation interception earlier in the crop growth (Singels and Smit, 2002; Garside and Bell, 2009).

Studies comparing sugarcane planting rates have been undertaken, however such studies are limited under irrigated conditions. Rice (1981), compared three planting rates of one, two and three stalks under rainfed conditions. According to the plant crop results, there were no statistically differences in cane yield between planting rates, but as the planting rate was increased there was a slight numeric increase in cane yield.

The first-ratoon crop results showed that although there were no statistical differences in cane yield between the planting rates, the single-stalk planting rate yields were numerically greater than the two higher planting rates. The lower planting rate (one stalk) yielded significantly greater compared with the other planting rates in the second ratoon crop, however, three-stalk planting rate had the lowest cane yield of any treatment. The author concluded that a planting rate of one stalk is adequate for planting if proper care is given to the buds of sugarcane prior to planting. This is in contradiction with the findings of Singh et al. (2016), who showed that significantly greater yield in the plant cane was obtained when using planting rate of three stalks compared with two and single stalk planting rates under rainfed conditions.

Matherne (1971), compared two planting rates of one and the half (1 ½) and two stalks in the first trial, and also compared two and three-stalk planting rates in a second trial under rainfed conditions. The results from the first trial showed that planting rate of 2-stalks had a significantly higher stalk population and cane yield compared with 1 ½ stalk planting rate; however, there were no statistical differences in stalk length and single stalk weight between the treatments. The second trial results showed that there were no significant differences in stalk population between treatments; but cane yield of 3-stalk planting rate was significantly greater than that of 2-stalk planting. Orgeron et al. (2007), compared three planting rates of two, three and four stalks under rainfed conditions. According to the results from this study, planting rates of three and four stalks had significantly greater cane yield and stalk population compared with the two-stalk planting rate. However, the planting rate of two stalks had significantly greater single stalk weight and stalk diameter compared with the planting rates of three and four stalks. Studies reveal that cane yield and stalk population can be increased with increases in planting rate; however, stalk weight decreases with the increase in planting rate due to the competition among stalks.

2.9 Conclusion

There are many sugarcane propagation methods; however, there is a need to optimise these methods for improved sugarcane yields. Hence, it is important to understand the effects of these methods on yields, however, work done previously showed contrasting results. Therefore, there is a need for local experimentation to address current research questions.

2.10   References

Ayele, N., Getaneh, A., Negi, T. and Dechassa, N., 2012. Effect of Intra-row Sett Spacing on Yield and Yield Components of Sugarcane Varieties at Metahara Sugar Estate. *East African Journal of Science* 6, 117-124.

Ayele, N., Abiy, G. and Tadesse, N., 2014. Influence of Intra-row Row Setts Spacing on Yield and Yield Components of Some Sugarcane Varieties at Finchaa Sugar Estate. *ARPN Journal of Science and Technology* 4, 39-44.

Bailey, R.A. and Bechet, G.R., 1989. A comparison of seedcane derived from tissue culture with conventional seedcane. *Proceedings South African Sugar Technologists Association* 63, 125-129.

Benda, G.T.A. and Ricaud, C., 1978. The use of heat treatment for sugarcane disease control. *Proceedings of the International Society of Sugarcane Technologists* 16, 483-496.

Berding, N. and Roach, B.T., 1987. Germplasm collection, maintenance, and use. *Amsterdam*, 143-210.

Bezuidenhout, C.N., O'Leary, G.J., Singels, A. and Bajic, V.B., 2003. A process-based model to simulate changes in tiller density and light interception of sugarcane crops. *Agricultural Systems* 76, 589-599.

Bokhtiar, S.M. and Sakurai, K., 2005. Effects of organic manure and chemical fertilizer on soil fertility and productivity of plant and ratoon crops of sugarcane. *Archives of Agronomy and Soil Science* 51, 325-334.

Boyce, J.P., 1968. Plant crop results of row spacing experiments at Pongola. *Proceedings South African Sugar Technologists Association* 42, 136-141.

Braunack, M.V., McGarry, D., Crees, L.R. and Halpin, N.V., 1999. Strategic tillage for planting sugarcane. *Proceeding-Australian Society of Sugar Cane Technologists* 21, 101-107.

Bull, T., 2000. The sugarcane plant. *Manual of cane growing", M Hogarth, P Allsopp, eds. Bureau of Sugar Experimental Stations, Indooroopilly, Australia*, 71-83.

Burner, D.M. and Grisham, M.P., 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop Science* 35, 875-880.

Caderby, E., Baumberger, S., Hoareau, W., Fargues, C., Decloux, M. and Maillard, M.N., 2013. Sugar cane stillage: a potential source of natural antioxidants. *Journal of agricultural and food chemistry* 61, 11494-11501.

Cardozo, N.P. and Sentelhas, P.C., 2013. Climatic effects on sugarcane ripening under the influence of cultivars and crop age. *Scientia Agricola* 70, 449-456.

Chattha, M.U., Ali, A. and Bilal, M., 2007. Influence of planting techniques on growth and yield of spring planted sugarcane (*Saccharum officinarum* L.). *Pakistan Journal of Agricultural Sciences* 44, 452-456.

Cheong, E.J., Mock, R. and Li, R., 2012. Elimination of five viruses from sugarcane using in vitro culture of axillary buds and apical meristems. *Plant Cell, Tissue and Organ Culture* 109, 439-445.

Cockburn, J., Coetzee, H., Van den Berg, J. and Conlong, D., 2014. Large-scale sugarcane farmers' knowledge and perceptions of Eldana saccharina Walker (Lepidoptera: Pyralidae), push–pull and integrated pest management. *Crop Protection* 56, 1-9.

Comstock, J.C. and Miller, J.D., 2004. Yield comparisons: disease-free tissue culture versus bud-propagated sugarcane plants and healthy versus yellow leaf infected plants. *Journal of the American Society of Sugar Cane Technologists* 24, 31-40.

Croft, B.J. and Cox, M.C., 2013. Procedures for the establishment and operation of approved-seed plots. SRA manual. 4th Edition.

Deressa, T., Hassan, R. and Poonyth, D., 2005. Measuring the impact of climate change on South African agriculture: the case of sugarcane growing regions. *Agrekon* 44, 524-542.

Devarumath, R.M., Doule, R.B., Kawar, P.G., Naikebawane, S.B. and Nerkar, Y.S., 2007. Field performance and RAPD analysis to evaluate genetic fidelity of tissue culture raised plants vis-à-vis conventional setts derived plants of sugarcane. *Sugar Tech* 9, 17-22.

Fageria, N.K., Moreira, A., Moraes, L.A.C., Hale, A.L. and Viator, R.P., 2013. Sugarcane and energycane. *Biofuel Crops: Production, Physiology and Genetics*, 151-171.

Fischer, G., Teixeira, E., Hizsnyik, E.T. and Velthuizen, H.V., 2008. Land use dynamics and sugarcane production. In: Zuurbier, P. and De Vooren, J., eds. *Sugarcane ethanol*, 29-62.

Fitch, M.M.M., Lehrer, A.T., Komor, E. and Moore, P.H., 2001. Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology* 50, 676-680.

Flynn, J., Powell, G., Perdomo, R., Montes, G., Quebedeaux, K. and Comstock, J., 2005. Comparison of sugarcane disease incidence and yield of field-run, heat-treated, and tissue-culture based seedcane. *Journal of American Society of Sugar Cane Technologists* 25, 88-100.

Garside, A.L. and Bell, M.J., 2009. Row spacing and planting density effects on the growth and yield of sugarcane. 3. Responses with different cultivars. *Crop and Pasture Science* 60, 555-565.

Gilbert, R.A., Shine, J.M., Miller, J.D., Rice, R.W. and Rainbolt, C.R., 2006. The effect of genotype, environment and time of harvest on sugarcane yields in Florida, USA. *Field Crops Research* 95, 156-170.

Goldemberg, J., Coelho, S.T. and Guardabassi, P., 2008. The sustainability of ethanol production from sugarcane. *Energy Policy* 36, 2086-2097.

Grisham, M.P. and Bourg, D., 1989. Efficiency of in vitro propagation of sugarcane plants by direct regeneration from leaf tissue and by shoot-tip culture. *Journal of American Society of Sugar Cane Technologists* 9, 97-102.

Heinz, D.J. and Mee, G.W., 1969. Plant differentiation from callus tissue of Saccharum species. *Crop Science* 9, 346-348.

Hendre, R.R., Mascarenhas, A.F., Nadgir, A.L., Pathak, M. and Jagannathan, V., 1975. Growth of mosaic virus-free sugarcane plants from apical meristems. *Indian Phytopathology* 28, 175-178.

Hendre, R.R., Iyyar, R.S, Katwal, M., Khuspe, S.S., and Mascarenhas, A.F., 1983. Rapid multiplication of sugarcane through tissue culture. *Sugarcane* 1, 5-7.

Hol, G.M.G.M. and Van Der Linde, P.C.G., 1992. Reduction of contamination in bulb-explant cultures of Narcissus by a hot-water treatment of parent bulbs. *Plant Cell, Tissue and Organ Culture* 31, 75-79.

Hoy, J.W., Bischoff, K.P., Milligan, S.B. and Gravois, K.A., 2003. Effect of tissue culture explant source on sugarcane yield components. *Euphytica* 129, 237-240.

Huang, B., 2000. Role of root morphological and physiological characteristics in drought resistance of plants. *Plant–environment interactions. Marcel Dekker Inc., New York*, 39-64.

Hunsigi, G., 2012. Production of sugarcane: theory and practice. *Springer Science and Business Media* 21, 12-24.

Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., Bekele, I. and Negi, T., 2016. Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture* 3, 1-4.

Irvine, J.E., 1999. Saccharum species as horticultural classes. *Theoretical and Applied Genetics* 98, 186-194.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2010. Sugarcane bud chips: A promising seed material. *Sugar Tech* 12, 67-69.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2011. Effect of ethephon and calcium chloride on growth and biochemical attributes of sugarcane bud chips. *Acta Physiologiae Plantarum* 33, 905-910.

Jalaja, N.C., Neelamathi, D., Sreenivasan, T.V., 2008. Micropropagation for quality seed production in sugarcane in Asia and the Pacific. Food and Agriculture Organization of the United Nations, Rome; Asia–Pacific Consortium on Agricultural Biotechnology, New Delhi; Asia-Pacific Association of Agricultural Research Institutions, Bangkok, p. i-x + 46.

James, N.I., 1971. Yield components in random and selected sugarcane populations. *Crop Science* 11, 906-908.

Jones, M.R., Singels, A. and Ruane, A.C., 2015. Simulated impacts of climate change on water use and yield of irrigated sugarcane in South Africa. *Agricultural Systems* 139, 260-270.

Joshi, S.V, and Albertse, E.H. (2013). Development of a DNA fingerprinting database and cultivar identification in sugarcane using a genetic analyser. *Proceedings of South African Sugar Technologists Association* 86, 200 – 212

Julien, M.H.R., Irvine, J.E. and Benda, G.T.A., 2012. Sugarcane anatomy, morphology and physiology. *Diseases of Sugarcane-Major Diseases* 2, 1-19.

Khairwal, I. S., and Babu, C. N. (1975). Path coefficient analysis of cane yield in sugarcane. *Sugarcane Breeders' Newsletter* 36, 58-61.

Lal, M., Tiwari, A.K. and Gupta, G.N., 2015. Commercial Scale Micropropagation of Sugarcane: Constraints and Remedies. *Sugar Tech* 17, 339-347.

Lee, T.S.G., 1987. Micropropagation of sugarcane (Saccharum spp.). *Plant Cell, Tissue and Organ Culture* 10, 47-55.

Matherne, R.J., 1971. Influence of inter-row spacing and planting rate on stalk population and cane yield in Louisiana. *Proceedings of the International Society of Sugar Cane Technologists* 14, 640-645.

Matsuoka, S. and Stolf, R., 2012. Sugarcane tillering and ratooning: key factors for a profitable cropping. *Sugarcane: Production, Cultivation and Uses*, 137-157.

Medeiros, A.L., Furtado, C.M., Leite, F.S., Souto, V.S., de Setta, N., Van Sluys, M.A., Kitajima, J.P., Costa, A.P.P., Benedito, V.A. and Scortecci, K.C., 2016. Molecular Genetic Dissection of Sugarcane Flowering under Equatorial Field Conditions. *Tropical Plant Biology* 9, 252-266.

Meyer, E., 2005. Machinery systems for sugarcane production in South Africa. MSc Eng Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Meyer, G.M., Banasiak, M., Keeping, N., Pillay, N., Parfitt, R. and Snyman, S.J., 2010. NovaCane® as a tool for rapid propagation of material for the SASRI Plant Breeding Programme. *Proceedings of the Annual Congress-South African Sugar Technologists' Association* 83, 117-121.

Meyer, J., Rein, P., Turner, P. and Mathias, K., 2011. Good Management Practices manual for the Cane Sugar Industry (Final). *PGBI Sugar and Bio Energy, Johannesburg, South Africa*, 334-366.

Mnisi, M. S. and Dlamini, C.S., 2012. The concept of sustainable sugarcane production: Global, African and South African perceptions. *African Journal of Agricultural Research* 7, 4337-4343.

Moore, P.H., 1987. Anatomy and morphology. *Sugarcane Improvement Through breeding*, 85-142.

Narasimha Rao, G. and Satyanarayan, Y., 1974. Studies in control of sett borne infection of red rot of sugarcane. *Journal of Research Andhra Pradesh Agricultural University* 1, 83-86.

Ntoyi, T.T., Nicholson, T.L., Snyman, S.J., Meyer, G.M. and Banasiak, M., 2007, September. Sugarcane plants from temporary immersion culture: acclimating for commercial production. In *III International Symposium on Acclimatization and Establishment of Micropropagated Plants* 812, 323-328.

Nyland, G. and Goheen, A.C., 1969. Heat therapy of virus diseases of perennial plants. *Annual Review of Phytopathology* 7, 331-354.

Orgeron, A.J., Kenneth, G.A. and Keith, B.P., 2007. Planting rate effects on sugarcane yield trials. *Journal of American Society of Sugar Cane Technologists* 27, 23-34.

Pandey, R.N., Rastogi, J., Sharma, M.L. and Singh, R.K., 2011. Technologies for cost reduction in sugarcane micropropagation. *African Journal of Biotechnology* 10, 7814-7819.

Pierre, J.S., Rae, A.L. and Bonnett, G.D., 2014. Abiotic limits for germination of sugarcane seed in relation to environmental spread. *Tropical Plant Biology* 7, 100-110.

Pierre, J.S., Perroux, J., Whan, A., Rae, A.L. and Bonnett, G.D., 2015. Poor fertility, short longevity, and low abundance in the soil seed bank limit volunteer sugarcane from seed. *Frontiers in Bioengineering and Biotechnology* 3, 1-10.

Pongpat, P., Gheewala, S.H. and Silalertruksa, T., 2017. An assessment of harvesting practices of sugarcane in the central region of Thailand. *Journal of Cleaner Production* 142, 1138-1147.

Ramaiah, B.B., Narasimha Rao, G. and Prasad, G.H., 1977. Elimination of internodes in sugarcane seed piece. *Proceedings of International Society for Sugar Cane Technologists*, 1509-1513.

Ramanand, M.L. and Singh, S.B., 2005. Comparative performance of micropropagated and conventionally raised crops of sugarcane. *Sugar Tech* 7, 93-95.

Ramburan, S., 2015. Interactions affecting the optimal harvest age of sugarcane in rainfed regions of South Africa. *Field Crops Research* 183, 276-281.

Ramgareeb, S., Snyman, S.J., Van Antwerpen, T. and Rutherford, R.S., 2010. Elimination of virus and rapid propagation of disease-free sugarcane (Saccharum spp. cultivar NCo376) using apical meristem culture. *Plant Cell, Tissue and Organ Culture* 100, 175-181.

Rice, E.R., 1981, June. Effects of planting rates on yields of sugarcane in Florida. *Proceedings. New series-American Society of Sugar Cane Technologists* 8, 53-56.

Roodagi, L.I., Itnal, C.J. and Khandagave, R.B., 2000. Influence of planting system and intercrops on sugarcane tillering and yield. *Indian Sugar* 50, 605-609.

Rossler, R.L., Singels, A., Olivier, F.C. and Steyn, J.M., 2013. Growth and yield of a sugarcane plant crop under water stress imposed through deficit drip irrigation. *Proceedings South African Sugar Technologists Association* 86, 170-183.

Sage, R.F., Peixoto, M.M. and Sage, T.L., 2014. Photosynthesis in sugarcane. In: Moore, P.H. and Botha, F.C., eds. *Physiology, Biochemistry, and Functional Biology* 1, 121–154.

Sahal, A., Hoque, A., Mallick, S.K. and Panda, S., 2014. Medicinal uses of grasses by the tribal people in West Bengal: An overview. *International Journal of Basic and Applied Sciences* 3, 63-70.

Saini, N., Saini, M.L. and Jain, R.K., 2004. Large-scale production, field performance and RAPD analysis of micropropagated sugarcane plants. *The Indian Journal of Genetics and Plant Breeding* 64, 102-107.

Sandhu, S.K., Gosal, S.S., Thind, K.S., Uppal, S.K., Sharma, B., Meeta, M., Singh, K. and Cheema, G.S., 2009. Field performance of micropropagated plants and potential of seed cane for stalk yield and quality in sugarcane. *Sugar Tech* 11, 34-38.

Shanthy, T.R. and Ramanjaneyulu, S., 2016. Socio-economic performance analysis of sugarcane cultivation under sustainable sugarcane initiative method. *Indian Research Journal of Extension Education* 14, 93-98.

Singels, A. and Smit, M.A., 2002. The effect of row spacing on an irrigated plant crop of sugarcane variety NCo376. *Proceedings South African Sugar Technologists Association* 76, 94-105.

Singh, A. and Kaur, C., 2003. Evaluation of herbicides for the control of weeds in spring planted sugarcane. *Sugar Tech* 5, 317-318.

Singh, K., Singh, K. and Kumar, S., 2016. Effects of seed rate, sett size and seed treatment on spring planted sugarcane. *Journal of Sugarcane Research* 3, 78-82.

Singh, K.P., Suman, A., Singh, P.N. and Srivastava, T.K., 2007. Improving quality of sugarcane-growing soils by organic amendments under subtropical climatic conditions of India. *Biology and Fertility of Soils* 44, 367-376.

Singh, R., 2003. Tissue Culture Studies of Sugarcane. An M. *Sc* (Doctoral dissertation, Thesis submitted to Thapar Institute of Engineering and Technology, Patiala, India.

Singh, R., Singh, W. and Choudhary, S., 2014. Nursery raising with high density sugarcane transplanting for higher cane yield and profitability. *Indian Farming* 64, 31-34.

Singh, S., 2015. My experiences on physiological research on sugarcane in India. *Sugar Tech* 17, 227-234.

Snyman, S.J., Meyer, G.M., Banasiak, M., Nicholson, T.L., Antwerpen, T.V., Naidoo, P. and Erasmus, J.D., 2009. Micropropagation of sugarcane via NovaCane®: preliminary steps in commercial application. *Sugar Cane International* 27, 245-247.

Snyman, S.J., 2004. Transformation of sugarcane. In: Curtis, I.S. ed. *Transgenic Crops of the World-Essential Protocols. Kluwer*, 103–114.

South African Sugar Association (SASA), 2010/2011. The South African Sugar Industry Directory.

Sreenivasan, T.V. and Sreenivasan, J., 1992. Micropropagation of sugarcane varieties for increasing cane yield. *SISSTA Sugar Journal* 18, 61-64.

Sriwongras, P., Sayasoonthorn, S., Saitthit, B. and Dostál, P., 2014. Comparison of sugarcane growth established from bud chips, single-bud setts and three-bud setts for development the new sugarcane planter. In *The 8th Thailand-Taiwan Bilateral Conference*.

Tai, P.Y.P., Miller, J.D. and Legendre, B.L., 1999. Preservation of Saccharum spontaneum germplasm in the world collection of sugarcane and related grasses through storage of true seed. *Sugar Cane 3*, 4-10.

Thompson, G.D., and du Toit, J.L. (1965). The effects of row spacing on sugarcane crops in Natal. *Proceedings of International Society of Sugar Cane Technologists* 12, 103-111.

Tiel, K., Enríquez, G.A., Ceballo, Y., Soto, N., Fuentes, A.D., Ferreira, A., Coll, Y. and Pujol, M., 2006. Development of a system for rapid plant regeneration from in vitro sugarcane (Saccharum officinarum L.) meristematic tissue. *Biotecnología Aplicada* 23, 22-24.

Tiwari, A., Bharti, Y., Tripathi, S., Mishra, N., Lal, M., Rao, G., Sharma, P. and Sharma, M., 2010. Review Article: Biotechnological approaches to improve sugarcane crop with special reference to disease resistance. *Acta Phytopathologica Entomologica Hungarica* 45, 235-249.

Valli, V., Gómez-Caravaca, A.M., Di Nunzio, M., Danesi, F., Caboni, M.F. and Bordoni, A., 2012. Sugar cane and sugar beet molasses, antioxidant-rich alternatives to refined sugar. *Journal of agricultural and food chemistry* 60, 12508-12515.

Verheye, W., 2010. Growth and production of sugarcane. *Soils, Plant Growth and Crop Production 2*, 1-23.

Verma, R. S., 2004. Sugarcane Production Technology in India. International Book Distributing Co, 228-267.

Watt, M.P., Banasiak, M., Reddy, D., Albertse, E.H. and Snyman, S.J., 2009. In vitro minimal growth storage of Saccharum spp. hybrid (genotype 88H0019) at two stages of direct somatic embryogenic regeneration. *Plant Cell, Tissue and Organ Culture* 96, 263-271.

Willcox, T., Garside, A. and Braunack, M., 2000. The sugarcane cropping system. *Manual of cane growing", M Hogarth, P Allsopp, eds. Bureau of Sugar Experiment Stations, Indooroopilly, Australia*, 127-139.

Yadav, S. and Ahmad, A., 2013. Standardization of callus culture techniques for efficient sugarcane micropropagation. *Cibtech Journal of Bio-Protocols* 2, 29-32.

Zhou, M., 2013. Conventional sugarcane breeding in South Africa: progress and future prospects. *American Journal of Plant Sciences* 4, 189–197

Zucchi, M.I., Arizono, H., Morais, V.A., Fungaro, M.H.P. and Vieira, M.L.C., 2002. Genetic instability of sugarcane plants derived from meristem cultures. *Genetics and Molecular Biology* 25, 91-96.

CHAPTER 3 : AGRONOMIC PERFORMANCE OF TISSUE CULTURE AND CONVENTIONAL PROPAGATED SUGARCANE VARIETIES UNDER RAINFED CONDITIONS IN STAGE 1

Abstract

Conventionally propagated (Con) sugarcane has limitations as it uses high quantities of seedcane and has a low seed multiplication rate. The recent use of tissue cultured (TC) plants as a means of propagation have been promising, and results in true-to-type, disease free planting material. However, plants produced through TC have been shown to possess an altered phenotype (high tillering and thinner stalks) compared with Con derived plants. The effects of this on yields under rainfed conditions, and the effect of varieties in South Africa are unclear. Therefore, the objective of this study was to investigate growth and yield differences between TC and Con propagated material under rainfed conditions for four varieties. The study also aimed to determine if any altered phenotype could be mitigated through manipulation of plant spacing.

A field trial was established under rainfed conditions at Mount Edgecombe and harvested over two crop-years. A completely randomised block experiment with four replications consisted of four varieties (N12, N31, N41, and N48) planted using three different methods: 1) TC derived plants (spaced at either 30 (TC30) or 50 (TC50) cm apart; 2) conventional hot water treated seedcane (Con); and 3) speedlings planted 50 cm apart (SP50). In the SP50 method, plantlets are raised using single budded setts from canes that are healthy and which have been hot water treated. Yield and yield component measurements were taken at harvest and data were analysed by ANOVA using an unbalanced factorial investigating the effects of propagation method, variety, and crop-year.

Both TC treatments significantly reduced stalk diameter compared with the Con plants for varieties N12 and N31 when averaged across crops (plant and first ratoon). In the first ratoon crop, both TC treatments produced significantly higher stalk population compared with the Con plants for varieties N12 and N31, but this was not observed in the plant crop. There were no significant differences in cane yield, stalk height and stalk mass between the TC and the Con plants for all varieties in the plant and first ratoon crops. Plants propagated through the TC and the Con for variety N48 were generally stable for any parameter in the plant and first ratoon crops, indicating that the phenotype of variety N48 was maintained during the TC process.

The TC30 and TC50 treatments did not differ significantly for all parameters in the plant and first ratoon crops for all varieties, showing that plant spacing did not affect growth. Based on these results, it is recommended to plant TC plants at 50 cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics. The SP50 treatment produced a significantly greater number of stalks that were thicker compared with the TC50 for selected varieties and crops. This means that there were some secondary effects of the TC process on plant phenotype. We concluded that the TC process alters the phenotype of selected varieties by increasing stalk populations and reducing stalk diameter in plant and first ratoon crops. These effects, however, do not result in significant differences in productivity/yield between TC and Con propagation methods. Varieties responded differently to the tissue culture process, Therefore, varieties that show an altered phenotype due to the tissue culture process should be identified routinely and given low priority for TC resources

Keywords: variety, phenotype, plant spacing, propagation methods, tissue culture

3.1 Introduction

Sugarcane is regarded as a warm-temperate and sub-tropical to tropical crop that grows well under moist, warm and sunny climatic conditions and requires well aerated soils that are deep and fertile (Mishra et al., 2014). Most sugarcane production occurs under rainfed conditions in South Africa, and the crop is typically harvested between April and December. Depending on factors such as climatic conditions, cultivar maturity rate, pest pressure (particularly African sugarcane borer (*Eldana saccharina L.*)) and management practices sugarcane grown under rainfed conditions can be harvested between the age of 12 and 24 months (Ramburan et al., 2009). Climatic conditions influence sugarcane growth. Moisture and heat favours development, while dry sunny conditions and low temperatures at night are suitable for maturation and accumulation of sugar (Bull, 2000). The economic value of this crop lies mainly in the stalks produced and the quantity of sucrose that can be extracted after crushing. Sugarcane contributes more than half of the sugar that is consumed worldwide (Snyman et al., 2011). After planting, the first vegetative growth is called plant cane and the stumps or shoots that are produced after first harvest are called ratoon crops (Verheye, 2010). Sugarcane is therefore a perennial crop whereby multiple harvests are possible from a single planting.

Sugarcane is conventionally propagated using stalks cut up into shorter segments called setts or whole stalk (seedcane) that usually contain two or more nodes with buds (Willcox et al., 2000). The planted stalks contain two to three buds and under favourable growing conditions these buds sprout to give rise to a primary stalk and tillers (Verma, 2004). Slow rates of propagation are one of the characteristic features of sugarcane production when using conventional propagation methods (Snyman et al., 2009; Mishra et al., 2014). In South Africa, approximately 8-10 tons of seedcane are needed to plant one hectare using conventional methods. Additionally, there is accumulation of pests and diseases that reduces quality and yield of sugarcane when using this method (Sood et al., 2006).

Studies have been done on finding alternative methods to overcome the problems associated with using conventional methods. Propagation rates of x100 have been obtained by using transplants or speedlings (SP) method (hereafter referred to as the SBS method) (Jain et al., 2010). In the SBS method, nurseries raise single budded setts from canes that are healthy and which have been hot water treated to get rid of diseases and pests (Singh et al., 2014). Buds that are selected to produce generate seedlings are placed individually into biodegradable germination trays filled with pine bark.

Based on the agro-climatic conditions, within a week a high percentage of germination can be attained through this method (Jain et al., 2011). Once young seedlings at the nursery are at the age of 25-35 days, they are transplanted to the field. In South Africa, this method is increasingly used for the rapid propagation of seedcane of new varieties. This method ensures higher propagation rates compared with the conventional method as there is a greater chance of survival of each individual bud, however, the method is also more resource intensive (Singh et al., 2014). For this reason, SBSs are usually only used to establish smaller nurseries from which cane is harvested to be planted out into larger commercial fields.

Another propagation method that has been found to produce extremely high rates of propagation is through using the tissue culture (TC) procedure (Devarumath et al., 2007; Mishra et al., 2014). With the TC process, stage 1 is referred to as the production of actual TC plants under laboratory conditions, and their subsequent hardening off and planting into field nurseries. Stages 2 and 3 then progress in a similar manner to conventional methods (Snyman et al., 2009). The recent use of the tissue cultured plants as a means of propagation have been promising, and results in true-to-type, disease free planting material (Sandhu et al., 2009). Furthermore, this technique increases multiplication rates. For example, an average of 150 plants from one apical meristem can be obtained after 11 weeks in culture (Fitch et al., 2001). However, previous work has shown that there may be yield depression encountered when using the tissue culture plants compared with the conventionally propagated plants, and this may negatively affect production of sugarcane commercially (Sandhu et al., 2009). There are many reports of altered phenotype of the TC plants compared with the conventionally propagated plants, and this altered phenotype (higher population of thinner, taller stalks) may be the reason for observed yield differences between the two methods. The altered phenotype of the TC plants (and subsequent effects on yields) may also be variety dependant, however, this hypothesis has not been tested under rainfed conditions in South Africa to date.

Since sugarcane became a commercial plantation crop, researchers have attempted to increase yields by varying spacings between plants. The studies that have been undertaken to evaluate the effect of plant spacing on sugarcane yield are voluminous and often conflicting. Saini et al. (2004), compared field performance of the TC and the SBSs plants of two early maturing varieties (CoH92 and CoH99) planted at the same intra-row spacing (60 cm) under irrigated conditions.

According to the results, the TC derived plants (stage 1) had relatively higher number of millable canes in both varieties, but they were thinner as compared with the SBSs plants. However, the TC (stage 2) and the SBSs plants showed no significant differences in terms of number of millable canes and cane diameter in both varieties.

Sandhu et al. (2009) compared plant to plant spacings of 45 and 60 cm for the TC raised plants (stage 1), and founded no significant differences in cane yield, sucrose percentage, cane diameter and single cane weight. This study is in contradiction with the study done by Garside and Bell (2009) who compared plant to plant spacing of 30 and 60 cm of the TC raised plants at stage 1. They showed that closer (30 cm) spacing resulted in significantly higher yield compared with 60 cm spacings. However, 30 cm spacings produced thinner stalks that were taller compared with those planted at 60 cm spacings. Cane yield can be affected by stalk thickness, stalk length, and stalk density. However, all these components are influenced by stalk population which determine cane yield per unit area. The choice of variety, spacing of planting material and cultural practices may interact to modify stalk population per unit area.

This study aimed to compare field performance of rainfed sugarcane propagated from conventional methods, transplants (which are referred as speedlings (SP)) and tissue culture (NovaCane®) (stage 1). The objective was to determine if varieties differed in the phenotypic response to the tissue culture production and whether any altered phenotype could be mitigated through manipulation of plant spacing.

3.2 Materials and methods

3.2.1   Experimental design and treatment application

A field trial was established in September 2015 under rainfed conditions at the South African Sugarcane Research Institute (SASRI) Mount Edgecombe experiment station which is located 29° 42' 24S, 31° 1' 30E and 108 m above sea level. This site represented the coastal growing region of the South African sugar industry. The plant crop was harvested in September 2016, while the first ratoon was harvested in September 2017. The trial was established as a factorial experiment designed as a randomised block design with four replications (Figure 3.1). In this experiment, four varieties were used, which were:

- N12 (high stalk population, average diameter, moderate canopy),
- N31 (very high population, average diameter, wide canopy),
- N41 (low population, thin diameter, sparse canopy),
- N48 (very low population, very thick diameter, dense canopy)

The varieties were chosen based on their popularity in the sugarcane industry and their contrasting growth characteristics. Each of the above varieties were planted using four planting methods:

a. Conventional HWT setts (Con),
b. Tissue culture plants planted at 30 cm plant spacing (TC30),
c. Tissue culture plants planted at 50 cm plant spacing (TC50),
d. Speedlings (single-budded sett derived plants) planted at 50 cm spacing (SP50).

Therefore, the treatment combinations consisted of four sugarcane varieties (N12, N31, N41 and N48), four propagation methods (Con, SP50, TC50 and TC30) and two crops (plant and first ratoon). The trial was never intended to be a balanced factorial, as some of the treatment combinations were simply not practical. For example, it is not practically feasible to plant conventional sugarcane setts at specific plant spacing's. The space between buds on seedcane (i.e. the internode length) vary dramatically with variety and growing conditions under which the seedcane was produced. Furthermore, not all buds on conventional seedcane will germinate to produce a plant. The SP50 treatment was included as a comparison to the TC50 treatment to investigate possible secondary effects of the TC process on plant phenotype. Therefore, in the analysis, propagation method (Con, SP50, TC30 and TC50) was treated as a factor, and the effects of plant spacing was inferred by comparing individual treatment combinations.

Each experimental plot consisted of six rows, that were 10 m long (nett plots of four rows) at inter-row spacings of 1.2m.

57 N48 TC50	58 N12 Con	59 N41 TC50	60 N31 Con	61 N31 TC50	62 N48 TC30	63 N31 SP50	64 N12 TC50
56 N41 TC30	55 N48 Con	54 N12 TC30	53 N12 SP50	52 N48 SP50	51 N41 SP50	50 N31 TC30	49 N41 Con
41 N12 TC50	42 N41 Con	43 N48 Con	44 N12 TC30	45 N31 SP50	46 N41 TC50	47 N12 Con	48 N41 TC30
40 N48 TC30	39 N31 TC30	38 N31 TC50	37 N48 SP50	36 N41 SP50	35 N31 SP50	34 N31 Con	33 N48 TC50

CONTOUR ROAD

25 N48 TC50	26 N12 TC30	27 N12 Con	28 N41 SP50	29 N41 TC50	30 N41 TC30	31 N48 TC30	32 N12 TC50
24 N31 TC30	23 N48 SP50	22 N31 SP50	21 N12 Con	20 N12 SP50	19 N41 Con	18 N31 TC50	17 N48 Con
9 N12 SP50	10 N48 TC30	11 N12 Con	12 N48 Con	13 N31 Con	14 N48 TC50	15 N31 TC30	16 N41 TC50
8 N31 SP50	7 N41 Con	6 N12 TC30	5 N48 SP50	4 N41 SP50	3 N41 TC30	2 N31 TC50	1 N12 TC50

Row direction

Figure 3.1 Trial plan, showing the different treatment combinations and their randomisation. Different shaded areas represent replicates (4).

3.2.2 Description of propagation methods

3.2.2.1 Propagation method for TC plants

Field grown varieties N12, N31, N41 and N48 (6-12 months old) were selected for use from the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal. The tissue culture plants (NovaCane®) were produced at the Biotechnology laboratory at SASRI using the shoot multiplication protocol as described by Ramgareeb et al. (2010). The technique is summarized in Figure 3.2 below.

Single budded setts from disease-indexed stalks were planted in plastic trays containing peat moss: vermiculite (1:1 ratio) and germinated for 2 weeks at 40 ºC.

⇩

The apical meristem (2 - 4 mm in size) was excised from the primary shoot. The explant was placed upside down onto semi-solid medium in a Petri-dish and subcultured on to fresh medium every 2 days for 1 week; incubation was in the dark at 28 ºC.

Medium: MS [(Murashige and Skoog, 1962); Highveld Biological, South Africa; MS salts and vitamins HP09] containing sucrose (20 g/L), agar (8 g/L), benzyl amino purine (BAP; 0.1 mg/L), kinetin (0.015 mg/L), methylene blue (1 ml/L from a 1 g/L stock solution) and activated charcoal (3.5 g/L), pH 4.5.

⇩

Meristems were subcultured onto the above medium without activated charcoal in a low light intensity for 1 week; followed by 1 week in the photoperiod (16 h light and 8 h dark) growth room.

⇩

When the green shoot that emerged from the meristem was approximately 1 cm in size, it was transferred to liquid medium in a pill vial (3 ml medium; medium as above, but pH 5.3 for all further culturing) for 1 week.

⇩

Shoots were transferred to glass jars (15 ml medium) for 2 weeks.

⇩

Shoots were transferred to Magenta jars (60 ml medium) and subcultured every 2 weeks. When the clump contained 8-10 shoots, it was split into 2-3 clumps.

⇩

After 3-5 months, single shoots were separated for rooting. No plant growth regulators were present in the medium for 2 weeks. Thereafter, rooting was done in Magenta jars, each containing approximately 20 shoots [indole-3-butyric acid (IBA; 1 mg/L)] for 2 weeks.

⇩

When plants were 10 cm, they were hardened off by planting in multi-cell seedling trays (22.2 cm^2) in ambient conditions. The planting medium comprised peat moss: vermiculite (1:1 ratio). Plantlets were fertilized with N: P:K (5:1:3) granules (Grovida) every 2 weeks and leaves were trimmed monthly. Plants were transferred to the field after 3 months.

Figure 3.2 Flow diagram of production of tissue culture (NovaCane®) plants through a shoot multiplication procedure using the apical meristem as the excised explant (modified protocol of Ramgareeb et al., 2010).

3.2.2.2 Speedlings

The speedlings produced from single-budded setts was obtained from the Sezela speedling nursery which is located 30° 24' 02S, 30° 40' 19E and 74 m above sea level. The buds were taken from stalks that had been planted with hot water treated (HWT) seedcane. The single-eyed setts were removed from the stalks and pre-germinated in biodegradable germination trays filled with peat moss under special growth rooms that create ideal conditions for germination. Following germination of the SBSs, the plantlets were hardened off under ambient conditions in the nursery before being transferred into the field.

3.2.2.3 Conventional three-budded setts seedcane

The seedcane used for planting the trial was collected from a seedcane nursery at the SASRI Kearsney selection farm which is located 29° 17' 45S, 31° 16' 23E and 223 m above sea level. The seedcane from this nursery was previously HWT before planting and was harvested at an approximate age of 10 months. After collecting the harvested seedcane from the nursery, it was first HWT before being planted in the trial, using conventional planting methods i.e. furrow planted, double-stick (two sticks side-by-side) cut into approximately 3-budded setts.

3.2.3   Measurements during crop growth

3.2.2.1.1   Stalk population and heights

The stalk population and heights measurements were done once a month. The stalk height measurement was done by selecting 20 stalks randomly per plot on the centre rows (row 3 and 4) which are 10m long with a 1.2m row spacing in size. A tape measure was used to measure the heights of 20 selected plants from the soil surface to the top visible dewlap (TVD), which is the collar of the uppermost, fully expanded leaf. The stalk population was measured per plot by manually counting all the number of stalks per 10 m line length on the centre rows (row 3 and 4), and thereafter expressed on a per hectare basis.

3.2.3.2 Stalk diameter

The measurements of stalk diameter were done once a month. In each plot 10 randomly selected stalks of the nett rows (row 2, 3, 4 and 5) were used to measure stalk diameter using a vernier caliper (ABS Digital Caliper DCA 150; Oxford Precision, UK). The caliper was placed perpendicular to the middle of the stalks before readings were taken.

3.2.3.3 Leaf length and width

The leaf length and width measurements were taken on a monthly basis. These measurements were done on the TVD leaf of 10 selected stalks of the nett rows (row 2, 3, 4 and 5). The leaf length was measured from the stalk (where the leaf is attached) to the tip of the leaf using a tape measure. The leaf width was measured at the middle of the TVD leaf.

3.6.3.1 Measurements at harvest

At harvest, cane yield was measured by cutting and measuring the weight of the stalks (bundles) of the nett rows (row 2, 3, 4 and 5) in each plots using a scale attached to a hydraulic weighing boom (Figure 3.3). An African sugar-cane borer (*Eldana saccharina L.*) survey was done on 20 randomly selected stalks per plot. This was achieved by first counting the number of internodes of each stalk before splitting it along its length. The number of internodes that had been bored were counted for each stalk. Eldana damage was then calculated as the percentage of bored internodes.

A sample of 12 stalks per plot was taken from the nett rows (row 2, 3, 4 and 5) and submitted to the SASRI mill room to determine the estimated recoverable crystal percent (ERC %) which was used to estimate the quantity of sucrose in cane that was recovered as sugar. ERC % gives an indication of the cane constituents which reduce sugar recovery and it was calculated using the following formula:

$$ERC \% = aS - bN - cF$$

Where S is sucrose % cane, N is non-sucrose % cane, F is fibre % cane, a is the undetermined loss of sucrose from sugar production, including filter loss, b is the loss of sucrose from sugar production per unit of N and c is the loss of sucrose from sugar production per unit of F.

Figure 3.3 A weighing bakkie with a scale attached to a hydraulic weighing boom

3.2.5   Statistical analysis

All data collected was statistically analysed using GenStat 18th edition and Microsoft Office Excel was used to process collected data form the trial. Growth and yield variables were subjected to analysis of variance (ANOVA) to establish main (variety, propagation methods and ratoon) and interaction effects. Comparison of means was performed using Fisher's protected LSD test at 5% significance difference. Significant differences between treatments at selected dates for in-season growth were represented by LSD bars (5%).

3.3 Results

3.3.1   Yield and quality traits

Propagation method had a significant effect on TERC, and no significant effect on any other trait (Table 3.1). Variety had a highly significant (p≤0.001) effect on ERC %, and TERC, however, no significant effect on any other trait. The crop had a highly significant effect on ERC % and fibre % (FIB). The variety x propagation method interaction was not significant for any traits, showing that varieties responded the same to propagation methods. The propagation method x crop, variety x crop and the three-way propagation method x variety x crop interaction was not significant for any trait. The responses of each trait are discussed in more detail in figures 3.4 -3.7 below.

Table 3.1 ANOVA for yield and quality traits (cane yield, estimated recoverable crystal (ERC %), ERC yield (TERC) and fibre content (FIB)).

		Cane yield		ERC %		TERC		FIB	
Source of variation	D.F	M.S	F-value	M.S	F-value	M.S	F-value	M.S	F-value
Propagation method (PM)	3	1260.60	2.57^{NS}	1.50	1.49^{NS}	16.97	3.33^{*}	0.40	0.65^{NS}
Variety (V)	3	774.70	1.58^{NS}	9.87	9.83^{**}	30.56	6.01^{**}	0.61	1.11^{NS}
Crop (Year)	1	188.30	0.38^{NS}	13.90	13.8^{**}	16.22	3.19^{NS}	30.13	49.63^{**}
PM. V	9	473.50	0.96^{NS}	0.94	1.27^{NS}	5.04	0.99^{NS}	1.29	2.12^{NS}
PM. Crop	3	179.70	0.37^{NS}	2.87	2.85^{NS}	0.80	0.16^{NS}	0.65	1.06^{NS}
V. Crop	3	572.80	1.17^{NS}	2.40	2.39^{NS}	12.70	2.50^{NS}	2.54	4.18^{NS}
PM. V. Crop	9	83.40	0.17^{NS}	0.58	0.57^{NS}	1.54	0.30^{NS}	0.68	1.11^{NS}
Replications	3	168.90	0.34	0.94	0.94	1.67	0.33	0.31	0.51
Error	93	490.9		1.00		5.09		0.61	

^{NS} not significant (p>0.05) *significant (p<0.05) **highly significant (p≤0.001)
Squares indicate significant F-values, for easy reference

Cane yield: There were no significant differences in cane yield between propagation methods for all varieties in the plant and first ratoon crops (Figure 3.4). The TC30 had a greater (not significant) cane yield compared with the other propagation methods for varieties N31 and N48 in both crops (plant and first ratoon).

Figure 3.4 Cane yield of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

ERC %: Varieties N12, N31 and N41 showed a significant difference in ERC % between the propagation methods in the plant crop, but this was not observed in the first ratoon crop (Figure 3.5). The main reason for the significant effect of propagation methods on ERC% in the plant crop was because the SP50 was significantly lower than TC30 (N31 and N41) and Con (N12 and N41) treatments. For variety N48, there were no significant differences in ERC % between the propagation methods in the plant and the first ratoon crops.
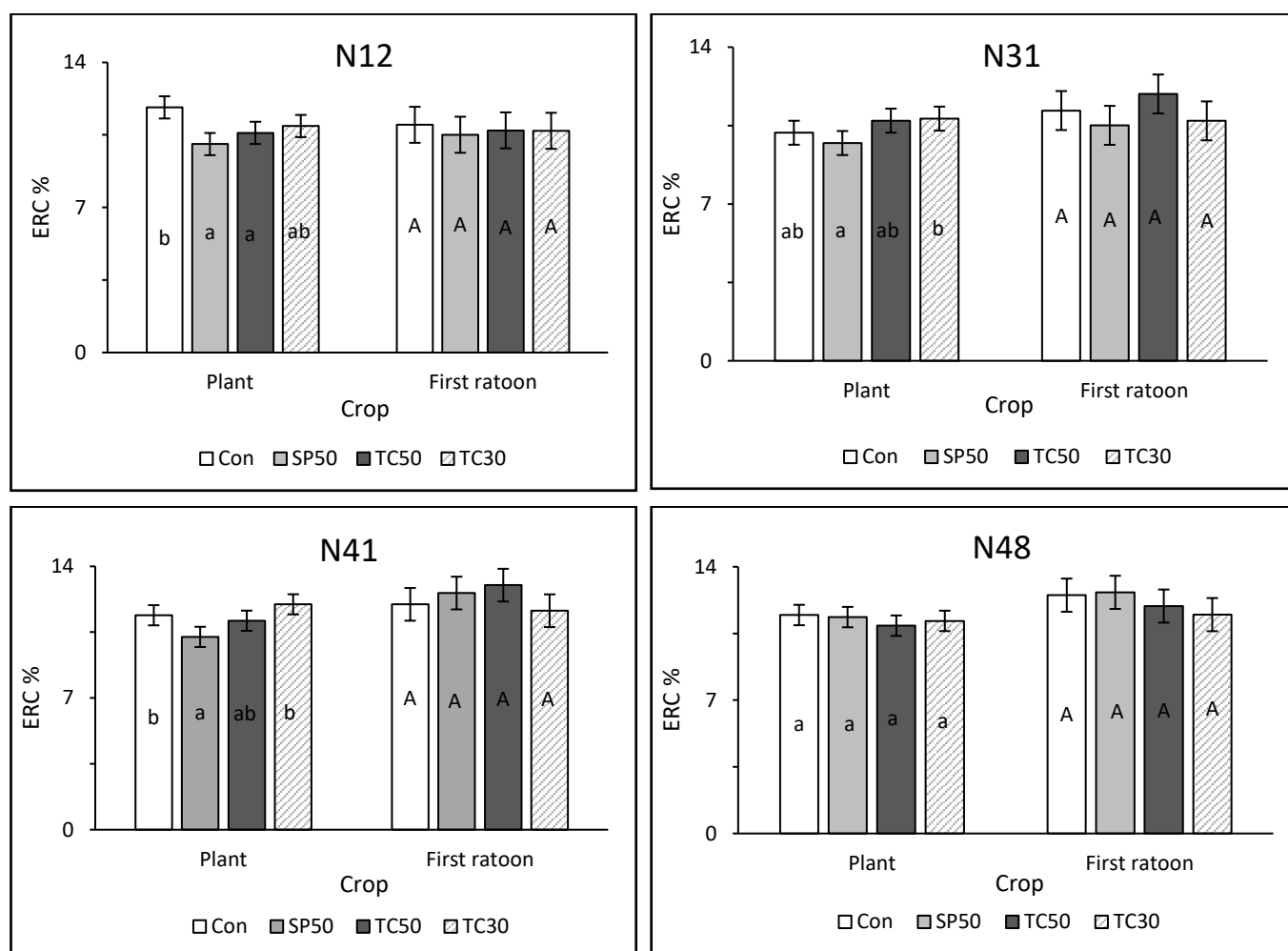
Figure 3.5 Estimated recoverable crystal percentage (ERC) of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

TERC: There were no significant differences in TERC between the propagation methods for all varieties in the plant crop (Figure 3.6). Varieties N12 and N31 showed a significant difference in TERC between propagation methods in the first ratoon crop, with the SP50 treatment having significantly lower TERC compared with the TC30. For varieties N41 and N48, there were no significant differences in TERC between propagation methods in both crops.
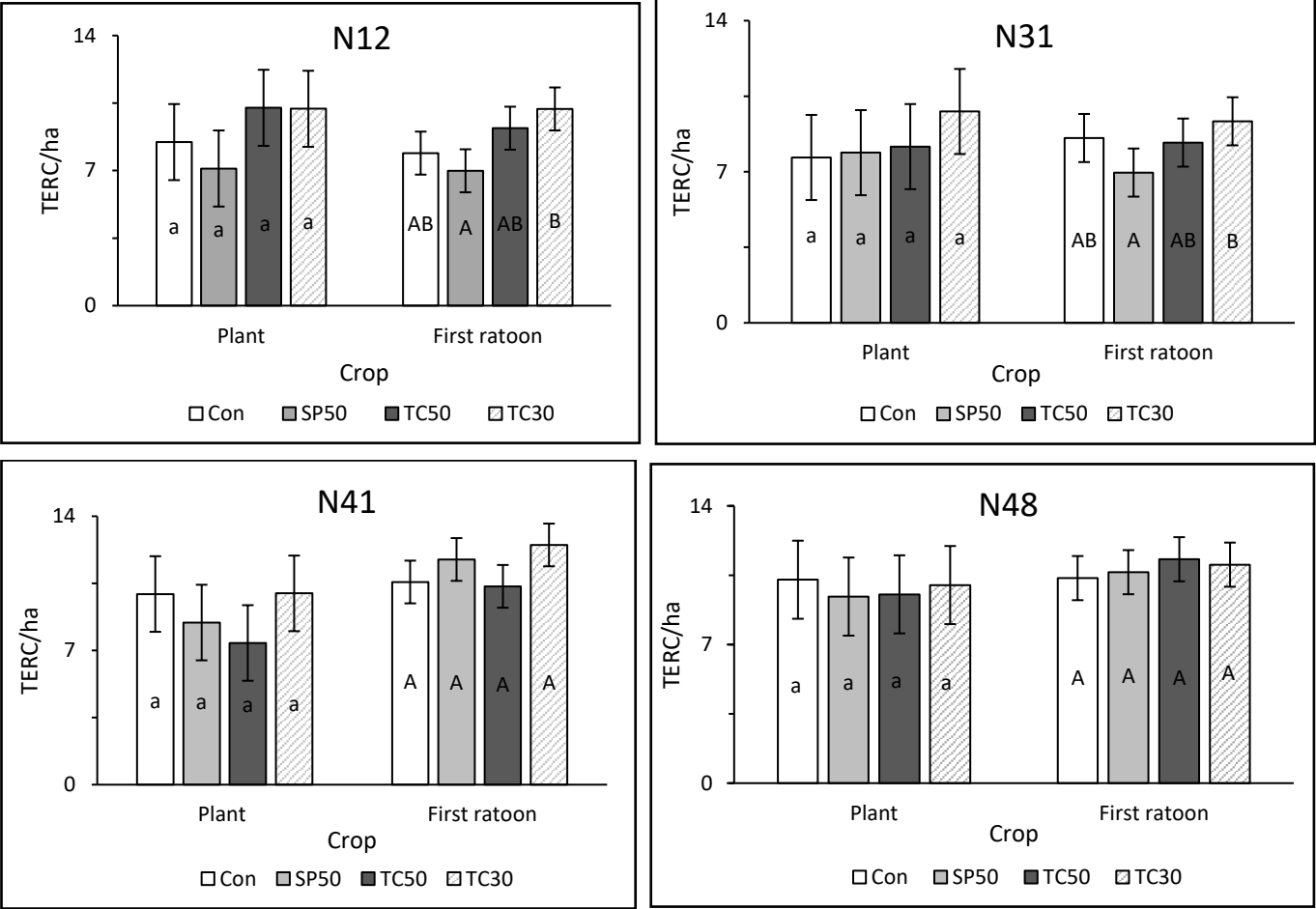
Figure 3.6 Estimated recoverable crystal yields (TERC) of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Fibre content: There were no significant differences in fibre content between the propagation methods for varieties N12, N31 and N48 in both crops (plant and first ratoon) (Figure 3.7). In the first ratoon crop, the Con had a significantly lower fibre content compared with the other propagation methods for variety N41, representing the only statistically significant result.

Figure 3.7 Fibre content of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

3.3.2 Yield components

Propagation method (PM) had a highly significant (p<0.001) effect on stalk population and stalk diameter, however, no significant effect on stalk height and stalk mass (Table 3.2). Variety (V) had a highly significant (p<0.001) effect on stalk population, stalk diameter, stalk mass and significant (p<0.049) effect on stalk height. The variety x propagation method interaction was significant for stalk population and stalk diameter, showing that varieties responded differently to propagation methods. The PM x crop, V x crop and the three-way PM x V x crop interaction was not significant for any trait. The responses of each trait are discussed in more detail in figures 3.8 -3.16 below.

Table 3.2 ANOVA table for yield components (stalk population, stalk diameter, stalk height and stalk mass) at harvest.

Source of variation	D.F	Stalk population (x1000)		Stalk diameter		Stalk height		Stalk mass	
		M.S	F-value	M.S	F-value	M.S	F-value	M.S	F-value
Propagation method (PM)	3	3.60	7.30^{**}	23.30	15.20^{**}	1869.00	2.40^{NS}	26272	1.80^{NS}
Variety (V)	3	6.60	13.40^{**}	87.50	57.20^{**}	5939.00	7.60^{*}	306400	21.00^{**}
Crop (year)	1	2.50	5.10^{*}	20.40	13.40^{**}	60.50	0.10^{NS}	51936	3.60^{NS}
PM. V	9	1.10	2.20^{*}	4.20	2.80^{*}	362.70	0.50^{NS}	16232	1.10^{NS}
PM. Crop	3	2.20	0.50^{NS}	1.40	0.90^{NS}	136.00	0.20^{NS}	8828	0.60^{NS}
V. Crop	3	3.30	0.70^{NS}	0.50	0.40^{NS}	520.10	0.70^{NS}	12936	0.90^{NS}
PM. V. Crop	1	1.30	0.30^{NS}	1.40	0.90^{NS}	193.60	0.30^{NS}	7983	0.60^{NS}
Replications	3	4.85	0.99	13.77	9.00	1248.20	1.59	53364	3.65
Error	93	4.92		1.53		782.80		14613	

^{NS} not significant (p>0.05) *significant (p<0.05) **highly significant (p≤0.001)

Squares indicate significant F-values, for easy reference

Stalk population: In the plant crop, only variety N31 showed a significant difference in stalk population between propagation methods, with the TC30 treatment producing significantly higher stalk population compared with the Con (Figure 3.8) in the first ratoon, both the TC treatments produced significantly higher stalk population compared with the Con for varieties N12 and N31. The TC50 treatment produced significantly higher stalk population compared with the SP50 for varieties N12 and N31 in the first ratoon crop. For varieties N41 and N48, there were no significant differences observed in stalk population between propagation methods in both crops.
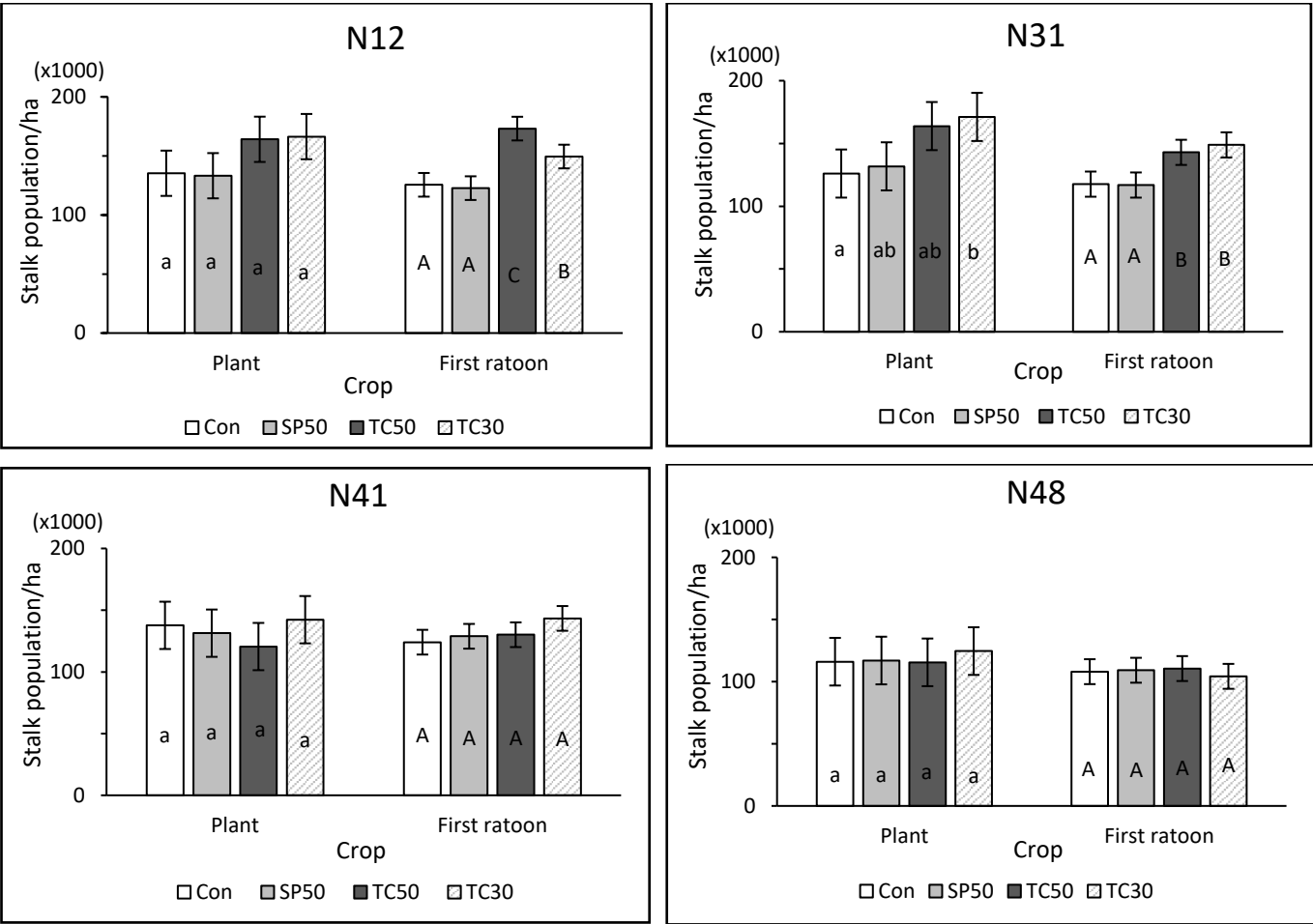
Figure 3.8 Stalk population of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD (5 %).

Stalk diameter: Varieties N12 and N31 showed the same trends in stalk diameter with both the TC treatments significantly reducing stalk diameter compared with the Con in both crops (Figure 3.9). The Con had a significantly thicker stalks compared with the SP50 for variety N12 in the first ratoon crop, but this was not observed in the plant crop. For variety N41, both the TC treatments significantly reduced stalk diameter compared with the SP50 in the plant crop, while reduction in the ratoon crop was not significant. There were no significant differences in stalk diameter between propagation methods for variety N48 in both crops.
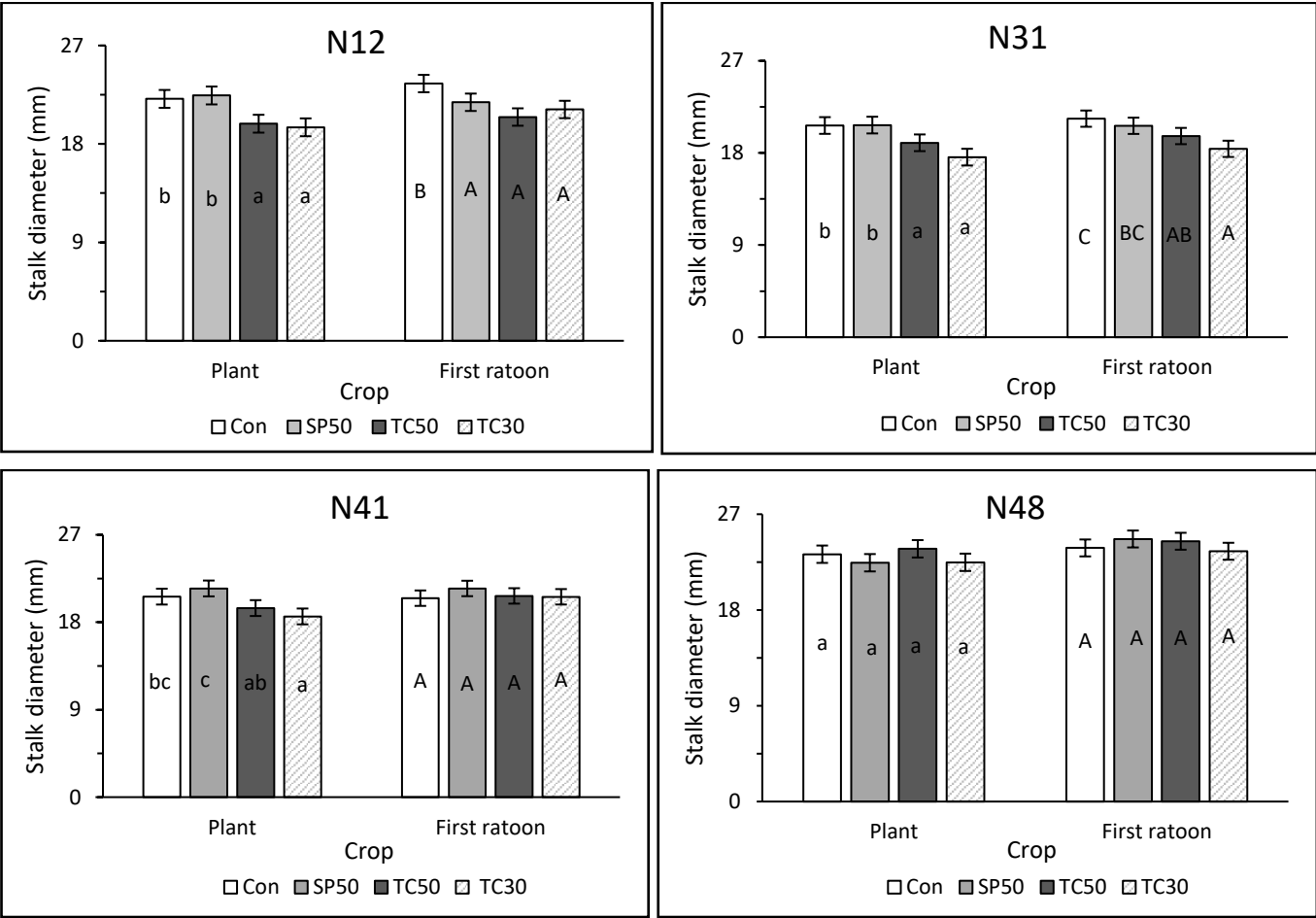
Figure 3.9 Stalk diameter of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Stalk height: All varieties showed no significant differences in stalk height between propagation methods in the plant and first ratoon crops (Figure 3.10). The TC30 tended to have taller stalks compared with the other propagation methods for both N31 and N41 in both crops. For variety N12, the SP50 tended to have shorter stalks than the other propagation methods in both plant and in the first ratoon crops.
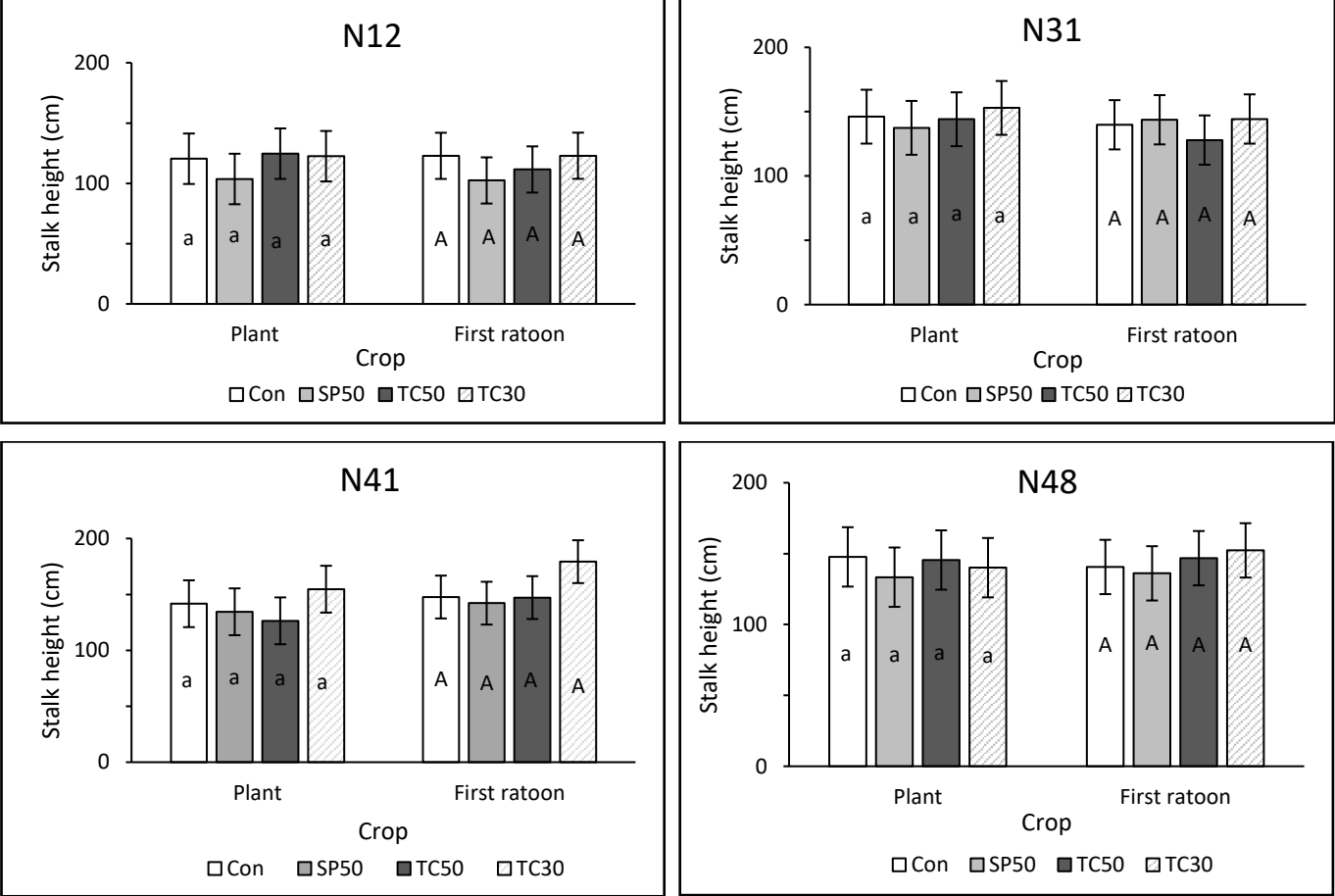
Figure 3.10 Stalk height of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Stalk mass: There were no significant differences in stalk mass between the propagation methods for all varieties in both crops (Figure 3.11). For varieties N12, N31 and N41, both the TC treatments tended to produce lighter stalks compared with the SP50 and the Con in the plant crop. For variety N48, the TC50 treatment had a greater (not significant) stalk mass compared with the SP50 in both crops.
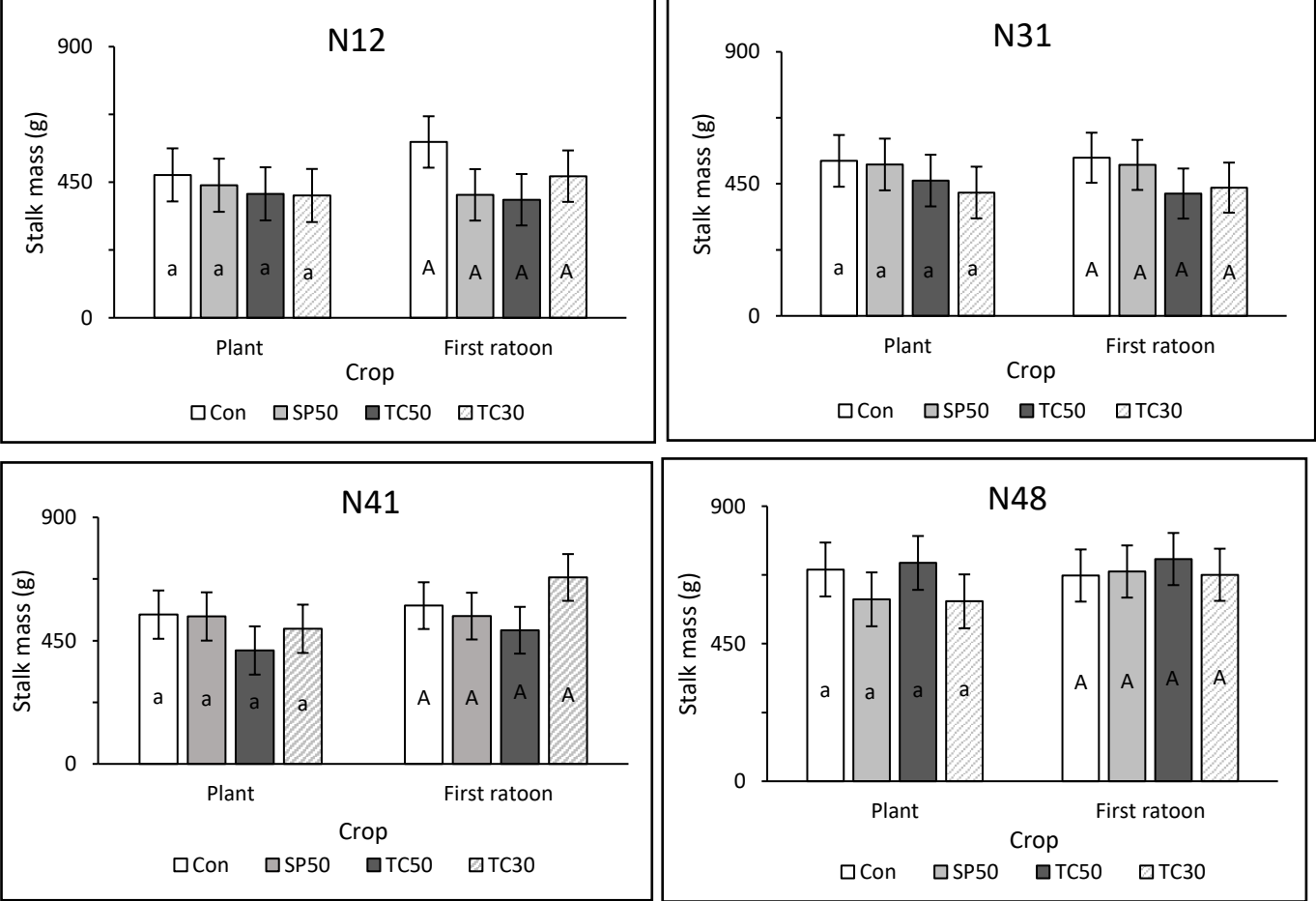
Figure 3.11 Stalk mass of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and ratoon crops. Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

3.3.3 In-season growth

Stalk population: For varieties N12 and N31, both the TC treatments produced more stalks compared with the Con and the SP50 throughout the growing season in both crops (Figure 3.12). The differences in stalk population between the propagation methods throughout the season was much smaller for varieties N41 and N48 in both crops.
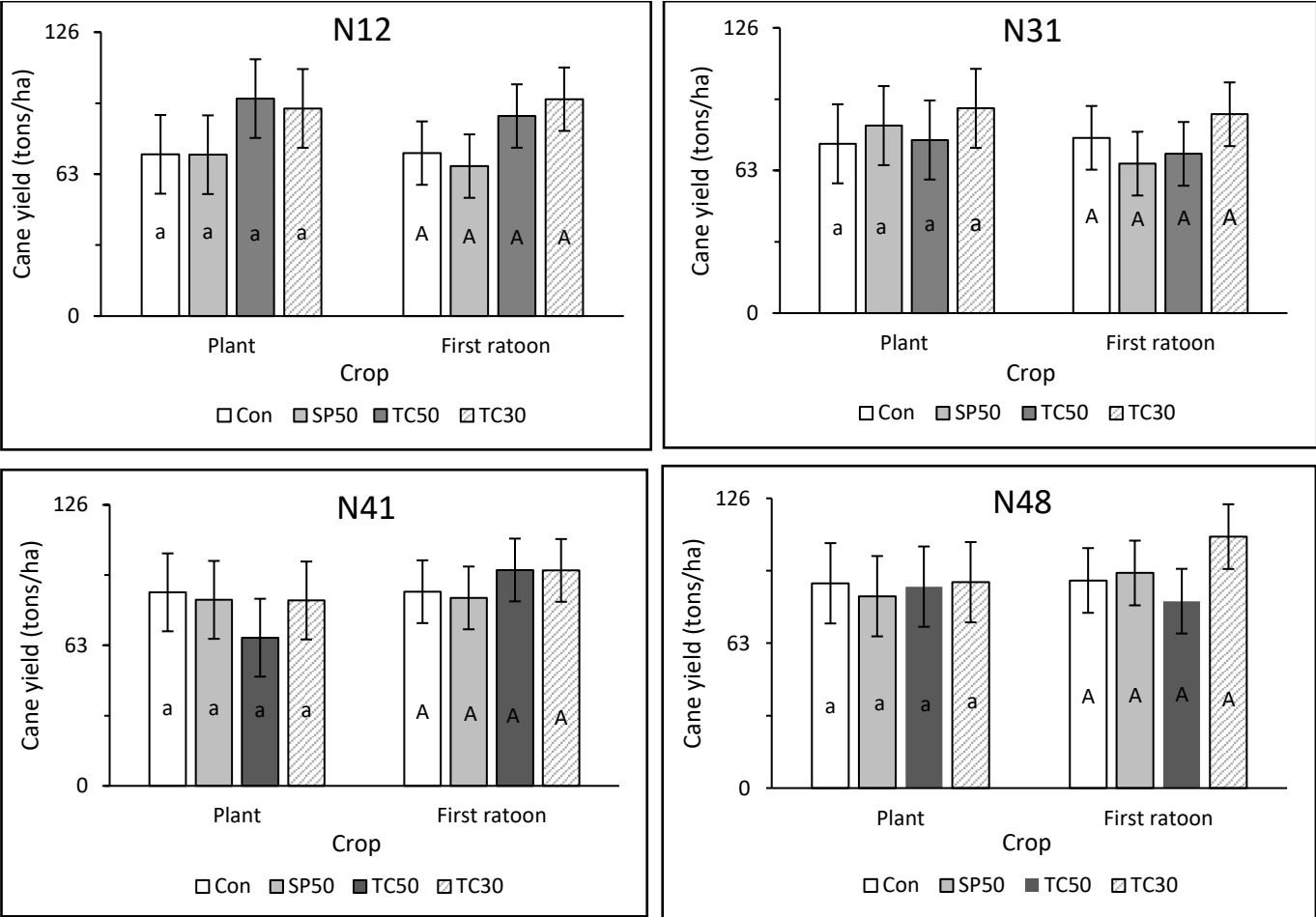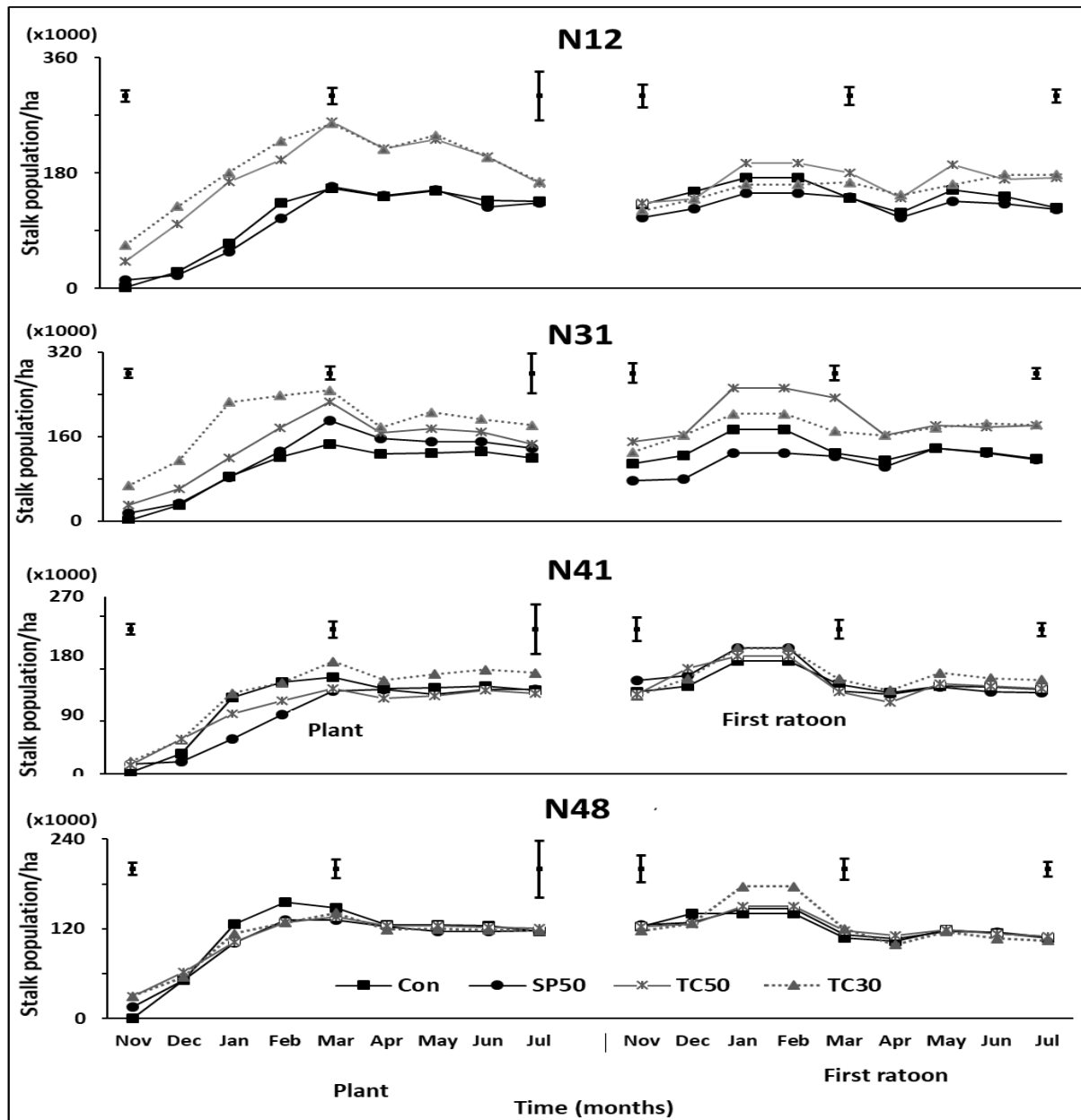

Figure 3.12 Stalk population of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crop. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk diameter: For varieties N12 and N31, both TC treatments had a tendency of producing thinner stalks compared with the Con in the plant and first ratoon crops (Figure 3.13). In both crops, the differences in stalk diameter between the propagation methods throughout the season was much smaller for varieties N41 and N48.
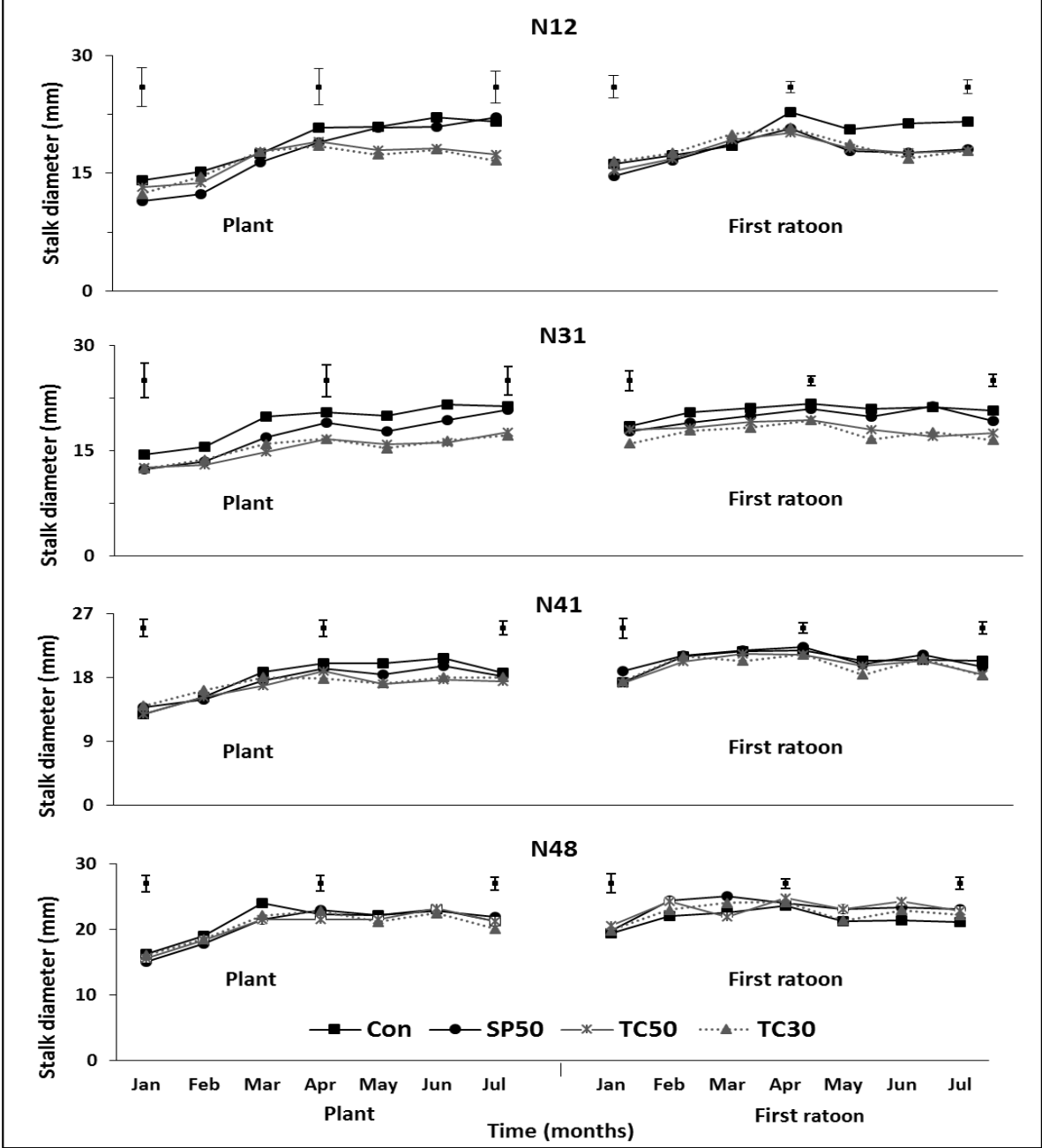
Figure 3.13 Stalk diameter of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crop. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk height: Throughout the growing season, no significant differences were detected in stalk height between propagation methods for all varieties in both crops (Figure 3.14). The TC30 treatment tend to produced stalks that are taller compared with the other propagation methods for all varieties in both crops.
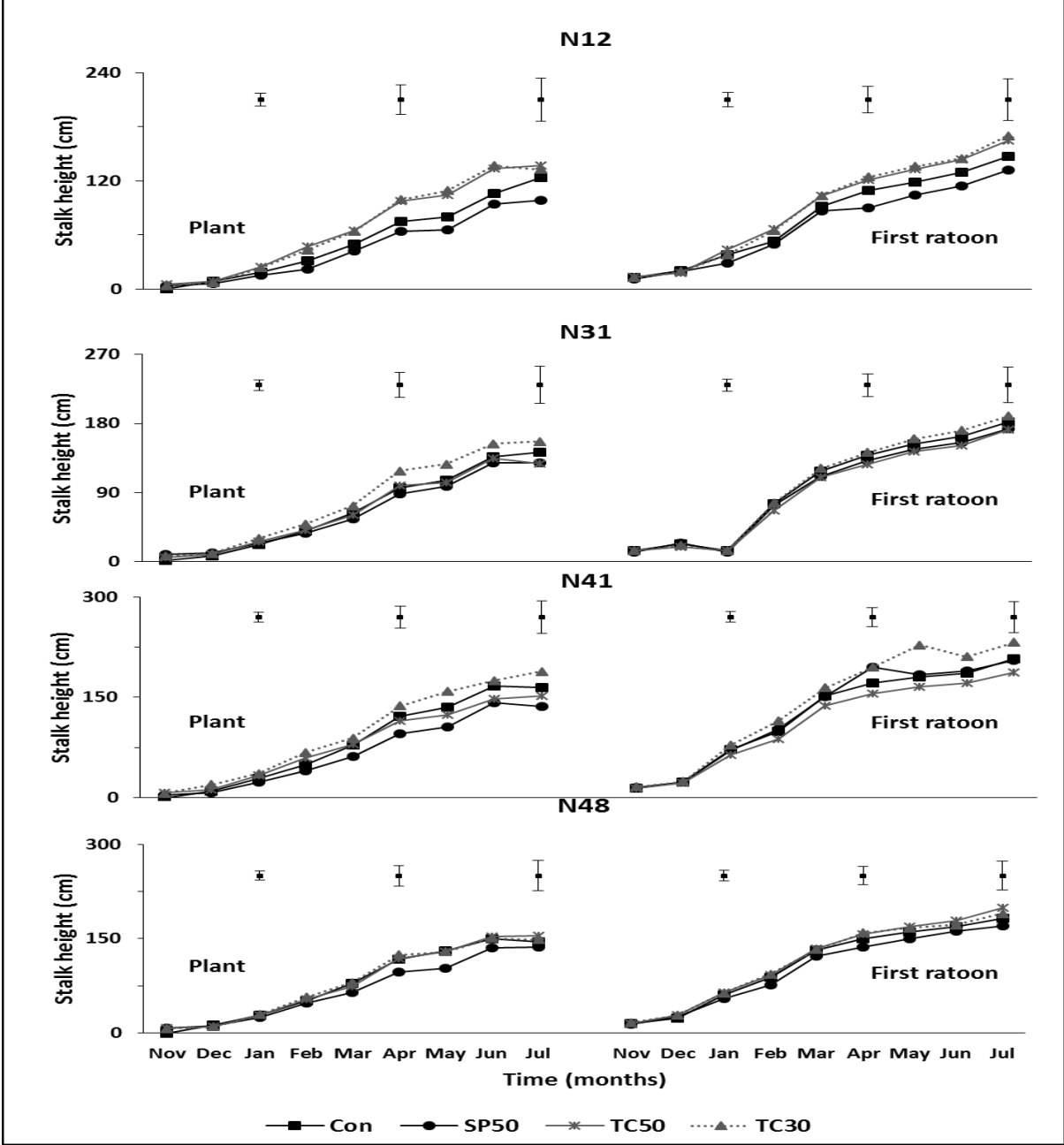
Figure 3.14 Stalk height of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crop. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Leaf length: Both the TC treatments had a tendency of having shorter leaves compared with the Con throughout the growing season for both N31 and N41 in the plant crop (Figure 3.15). In the first ratoon crop, the differences in leaf length between the propagation methods throughout the season was much smaller for all varieties.
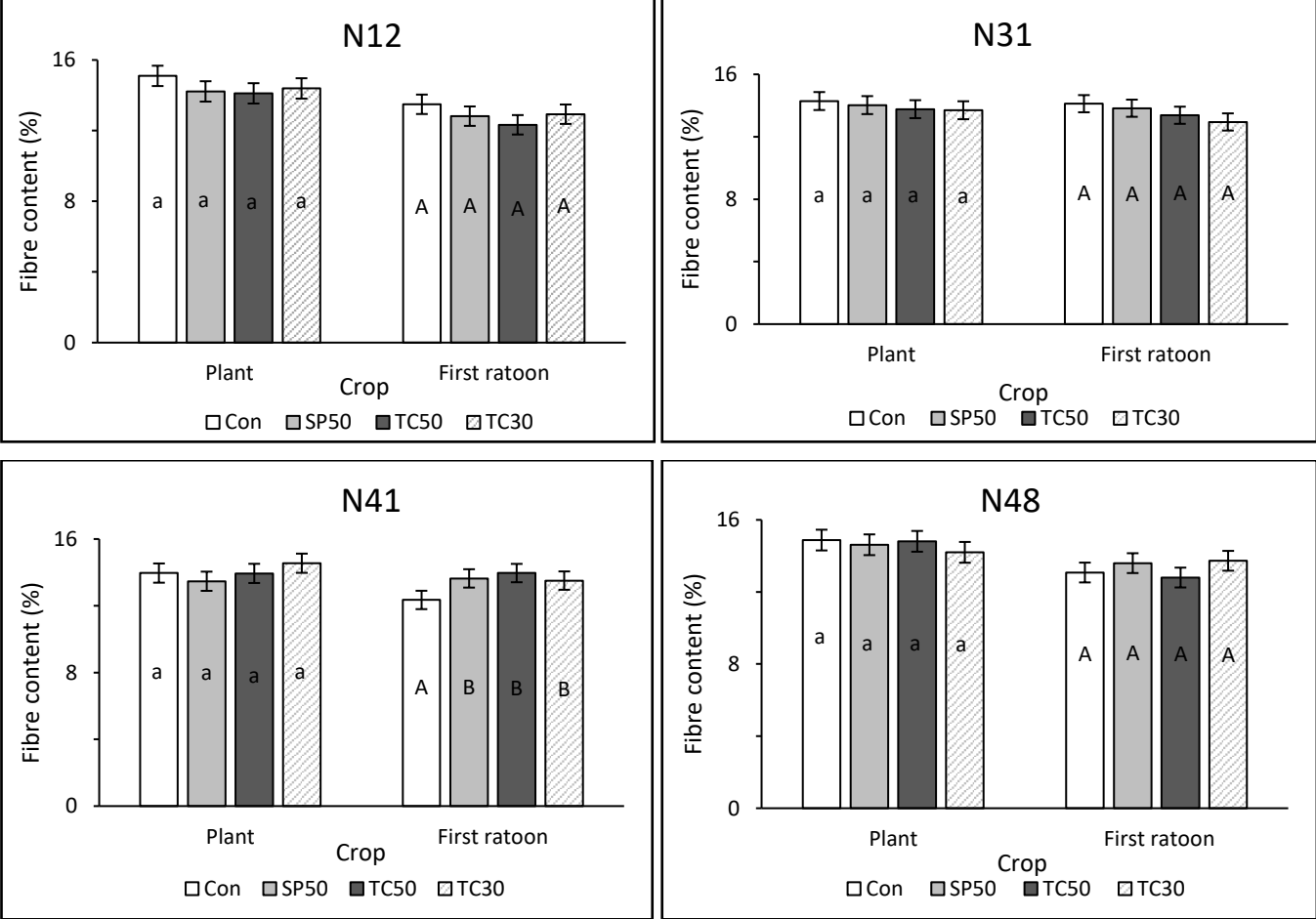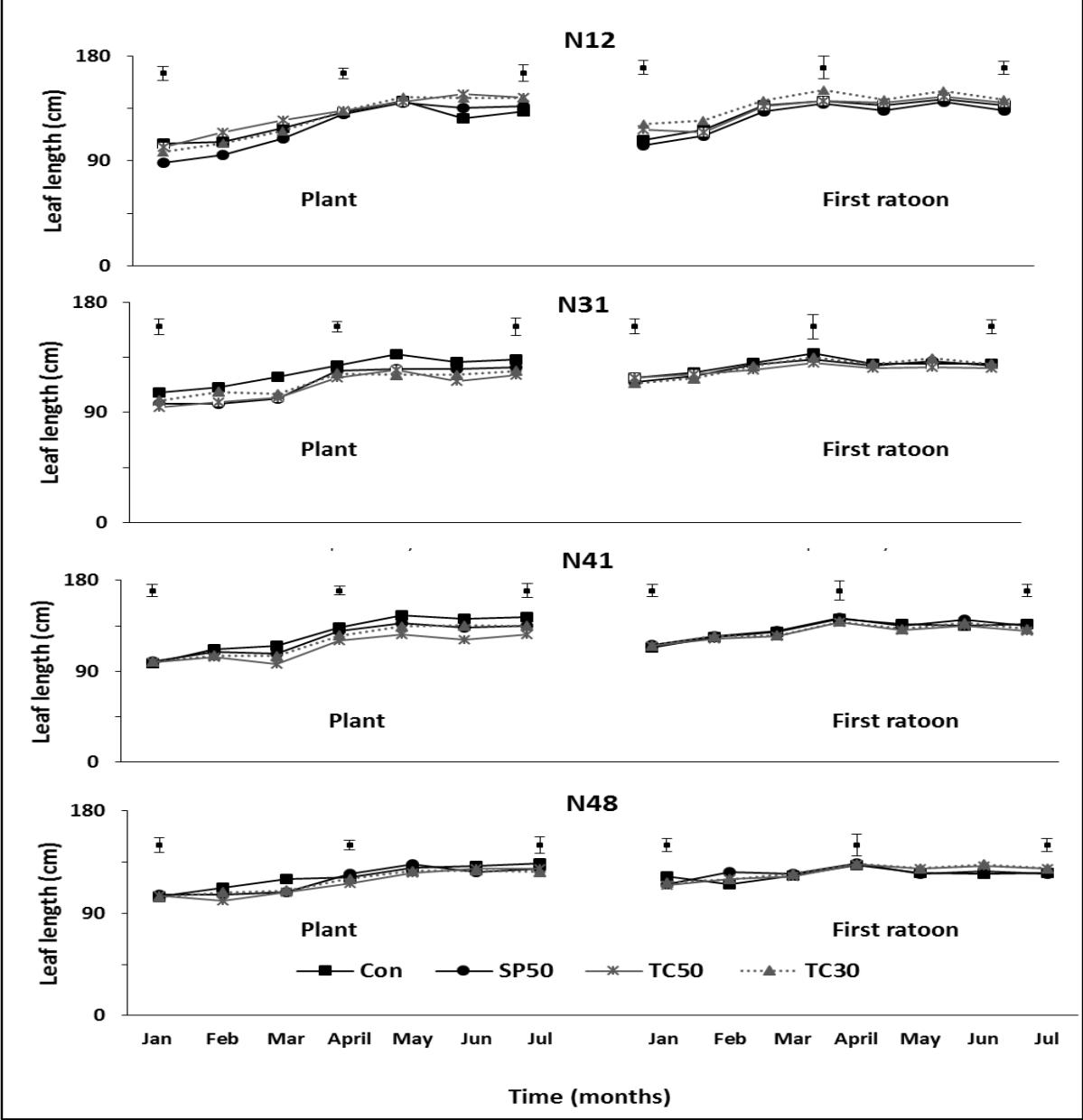

Figure 3.15. Leaf length of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crop. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Leaf width: The Con propagation method had wider leaves compared with both the TC treatments for varieties N12, N31 and 41 in the plant and first ratoon crops (Figure 3.16). For both crops, the differences in leaf width between the propagation methods throughout the season was much smaller for variety N48.
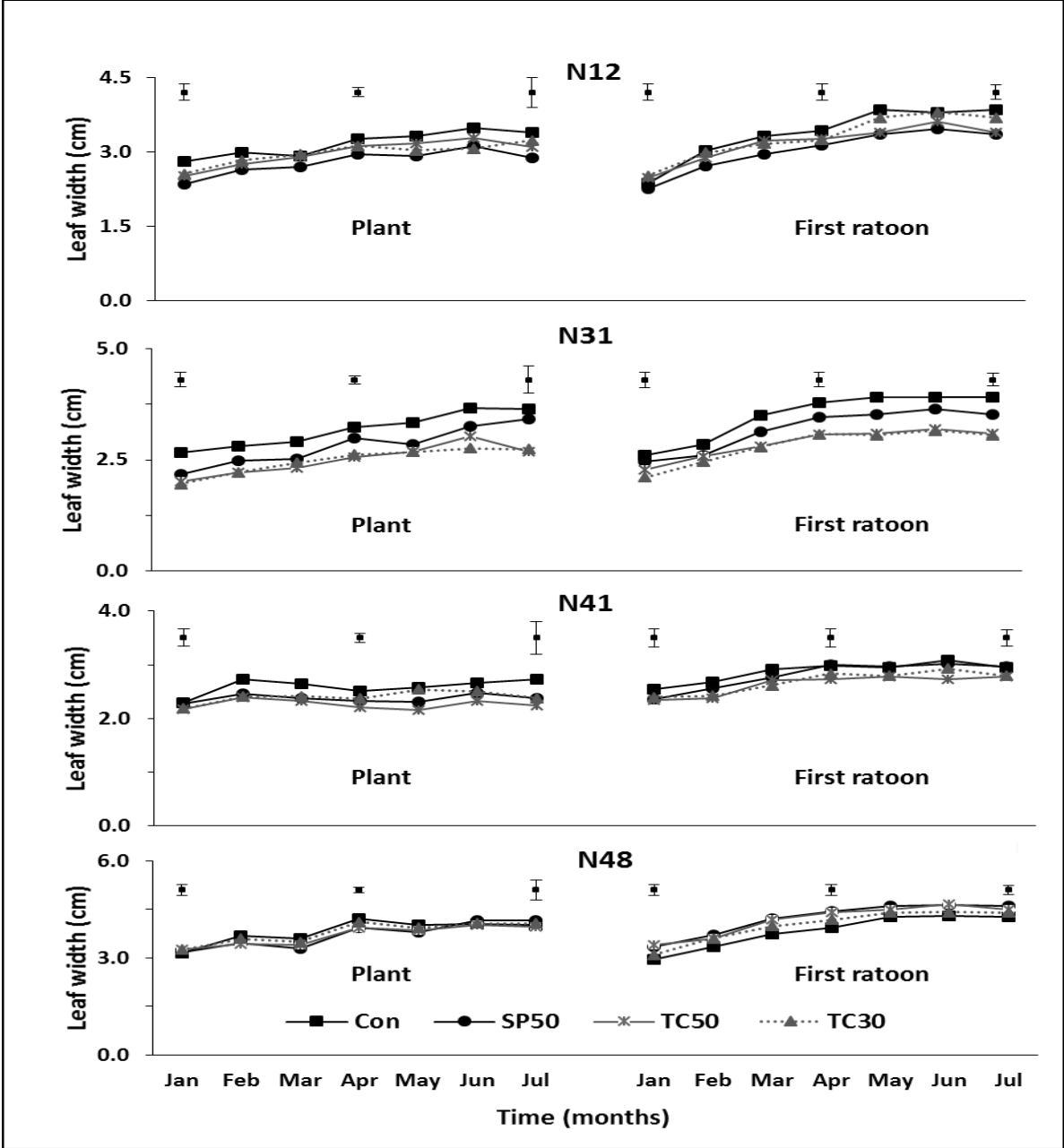
Figure 3.16. Leaf width of four varieties (N12, N31, N41 and N48) when propagated conventionally (Con), through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and through speedlings (speedlings planted at 50 cm plant spacing (SP50) in the plant and first ratoon crop. Significant differences between treatments at selected dates are represented by LSD bars (5%).

3.4 Discussion

Both TC treatments had significantly thinner stalks compared with the Con propagation method for varieties N12 and N31 when averaged across crops (plant and first ratoon). A tendency towards uniform reduction in stalk diameter in the TC plants have been reported by Burner and Grisham (1995) and Sandhu et al. (2009). However, these results are in contradiction with the findings of Geetha and Padmanabhan (2002), who showed that there were no significant differences for cane diameter between the TC and the Con plants. Furthermore, Burner and Grisham (1995), showed that the variation in stalk diameter between the TC and the Con plants disappears in the following ratoon crops. According to De Sousa-Vieira (2005), stalks that are thinner in diameter are known to be less vigorous. Therefore, the thinner stalks produced by the TC plants will affect adoption of newly produced varieties by growers. Based on the results from the present study, it is recommended that small plots should be planted to test the effect of the TC process on newly produced varieties. Growers can then be made aware of expected changes in the phenotype of the varieties due to the tissue culture process before planting the varieties commercially.

Higher stalk populations were recorded in sugarcane crop raised through the TC process compared with the Con derived plants for varieties N12 and N31 in the plant crop, although the differences were only statistically significant for variety N31. These results are in agreement with the findings of Sood et al. (2006) and Ibrahim et al. (2016), who indicated that the TC plants attained a greater number of millable stalks per hectare compared with the Con plants. High tillering in the TC plants might have resulted in production of thinner canes because thickness of cane is inversely related the number of tillers per clump (Sood et al., 2006).

In the first ratoon crop, both TC treatments produced significantly higher stalk population compared with the Con plants for varieties N12 and N31. The TC plants were expected to have high stalk population in the first ratoon crop as there were larger number of buds that were available underground from the previous crop compared with the Con plants. The results from the present study are in contradiction with the findings of Burner and Grisham (1995) and Flynn et al. (2005) who showed that the variation in stalk population between the TC and the Con plants disappeared in the following ratoon crops. According to Julien et al. (1980), resources are wasted due to high tillering.

As the tissue culture plants produce high stalk numbers, this leads to competition for water and nutrients amongst stalks, and subsequent death of tillers. This means more resources are used without reflecting in yield at harvest. To minimise the loss of inputs (water and fertiliser), varieties that show an altered phenotype (high tillering) due to the tissue culture process should be identified routinely and given low priority for TC resources.

The higher number of millable cane that was recorded in the TC plants in both crops for varieties N12, N31 and N41 was not reflected in the yields, as there were no significant differences in cane and ERC yields between the TC and the Con plants. The results from this study are in line with the findings of Hoy et al. (2003), who showed that there were no significant differences in cane and ERC yields between the TC and the Con plants. However, these results conflict with the results of Sood et al. (2006), who reported that increase in cane yield was obtained when using the TC plants compared with the Con plants. In contrast, Sandhu et al. (2009) showed that the Con derived plants produced higher cane yield compared with the TC plants. The results from the present study suggest that the TC plants does not compromise the commercial yields when compared with Con plants under rainfed conditions in both crops. Therefore, based on these results, it is recommended to use the TC derived plants in commercial fields and this will have low risks of yield loss.

Plants propagated through the TC and the Con for variety N48 were generally stable for all parameters in both crops, indicating that the phenotype of variety N48 was maintained during the TC process. According to Sreenivasan and Sreenivasan (1992), the differences between plants raised through the TC process and the Con might be affected by cultivar. The results from Comstock and Miller (2004), also showed a different responses of varieties to the tissue culture process. The results from the present study suggest that the effect of the tissue culture process must be examined on a variety-by-variety basis. Therefore, growers can then be made aware of expected changes in the phenotype of the varieties due to the tissue culture process before planting the varieties commercially. Variety N48 is a thick-stalked, low population variety. These characteristics may have contributed to the stability of this variety when going through the TC process, and this phenomenon should be further investigated.

The TC30 and TC50 treatments did not differ significantly for any parameter in the plant and first ratoon crops for all varieties. However, the TC30 treatment produced a higher number of millable stalks compared with the TC50 treatment.

The closer plant spacing was obviously expected to give higher stalk population. This was significant early on for variety N31 (Figure 3.12), but not later at harvest. These results are in agreement with the findings of Sandhu et al. (2009), who showed that there were no significant differences in cane yield, stalk diameter and single cane weight between the TC plants planted at 45 and 65 cm spacings. However, their results showed that plant to plant spacing of 45 cm produced significantly higher stalk population compared with the 60 cm spacings. The closer plant spacing resulted in a very strong competition among the main shoots, which in turn, reduced the number of millable cane at harvest. Furthermore, planting at closer spacing means more plants should be planted per hectare which imposes higher costs. The present study has shown that planting at closer spacings does not give any advantage compared with planting at wider spacings. Therefore, based on these results, it is recommended that TC plants be propagated using wider (50 cm) plant spacings, as this is more economical.

The TC50 treatment resembled SP50 for stalk height, stalk mass, fibre content, cane yield and TERC for all varieties in both crops (plant and first ratoon). For varieties N12 and N31, the TC50 treatment produced significantly more stalks compared with the SP50 in the first ratoon crop, but this was not observed in the plant crop. These results are in line with the findings of Saini et al. (2004), who indicated that the TC plants produced higher stalk population compared with the single budded setts (SBSs) plants. The higher number of millable cane was also reported in the TC plants compared with the SBSs plants by Bailey and Bechet (1989). In contrast, the SBSs plants produced significantly higher cane yield compared with the TC plants (Sandhu et al., 2009).

The SP50 produced stalks that were significantly thicker compared with the TC50 for varieties N12, N31 and N41 in the plant crop, but this was not observed in the first ratoon crop. These results are in agreement with the findings of Bailey and Bechet (1989), who reported that the TC plants produced significantly thinner stalks compared with the SBSs plants. Saini et al. (2004) and Sandhu et al. (2009) also showed that stalks produced by the TC plants were significantly thinner compared with the SBSs plants. Based on the results the present study, it is clearly shown that the altered phenotype (high tillering) of the TC plants is not due to environmental factors. There are possible secondary effects of the tissue culture process on the plant phenotype. Some of these effects are investigated in the following Chapter.

3.5 Conclusion

The tissue culture and the conventional plants did not differ significantly in cane yield, ERC yield, stalk height and stalk mass for all varieties. The tissue cultured process produced plants with significantly reduced stalk diameter compared with the conventionally propagated plants for varieties N12 and N31 when averaged across crops (plant and first ratoon). However, the effect of thinner stalks was more of a visual effect than a true effect and did not reduced yield in this study. In the first ratoon crop, the tissue culture plants produced significantly higher stalk population compared with the conventionally propagated plants for varieties N12 and N31, but this was not observed in the plant crop. There were no significant differences in cane yield, stalk height and stalk mass between propagation methods for all varieties in both crops. Plants propagated through the TC and the Con for variety N48 were generally stable for any parameter in the plant and first ratoon crops, indicating that the phenotype of variety N48 was maintained during the TC process. For variety N41, the differences between the Con and the TC plants were only observed in stalk diameter and fibre content.

The TC30 and TC50 treatments did not differ significantly for any parameter in the plant and first ratoon crops for all varieties, showing that plant spacing did not affect growth. Based on these results, it is recommended to plant TC plants at 50 cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics. The TC50 treatment resembled the SP50 propagation method for stalk height, stalk mass, fibre content and cane yield for all varieties in the plant and first ratoon crops. However, the SP50 propagation method produced stalks that were significantly thicker compared with the TC50 treatment for varieties N12, N31 and N41 in the plant crop, but this was not observed in the first ratoon crop. Furthermore, the TC50 treatment produced significantly more stalks compared with the SP50 for varieties N12 and N31 in the first ratoon crop, but this was not observed in the plant crop. Based on the results from the present study, it is clearly shown that the altered phenotype of the TC is not due to environmental factors. There are possible secondary effects of the tissue culture process on the plant phenotype. In summary, the tissue culture plants did not compromise the commercial yields when compared with the conventionally propagated plants under rainfed conditions in both crops. However, it is recommended that wider plant spacings should be used for commercial planting of tissue culture plants. Varieties responded differently to the tissue culture process, Therefore, varieties that show an altered phenotype due to the tissue culture process should be identified routinely and given low priority for TC resources.

3.6 References

Bailey, R. A., and Bechet, G. R., 1989. A comparison of seedcane derived from tissue culture with conventional seedcane. *Proceedings South African Sugar Technologists Association* 63, 125-129.

Bull, T., 2000. The sugarcane plant. *Manual of cane growing", M Hogarth, P Allsopp, eds. Bureau of Sugar Experimental Stations, Indooroopilly, Australia*, 71-83.

Burner, D. M., and Grisham, M. P., 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop Science* 35, 875-880.

Comstock, J.C. and Miller, J.D., 2004. Yield comparisons: disease-free tissue culture versus bud-propagated sugarcane plants and healthy versus yellow leaf infected plants. *Journal of the American Society of Sugar Cane Technologists* 24, 31-40.

De Sousa-Vieira, O., 2005. Interrelationships of cane yield components and their utility in sugarcane family selection: Path coefficient analysis. *Interciencia* 30, 93-96.

Devarumath, R.M., Doule, R.B., Kawar, P.G., Naikebawane, S.B. and Nerkar, Y.S., 2007. Field performance and RAPD analysis to evaluate genetic fidelity of tissue culture raised plants vis-à-vis conventional setts derived plants of sugarcane. *Sugar Tech* 9, 17-22.

Fitch, M.M.M., Lehrer, A.T., Komor, E. and Moore, P.H., 2001. Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology* 50, 676-680.

Flynn, J., Powell, G., Perdomo, R., Montes, G., Quebedeaux, K. and Comstock, J., 2005. Comparison of sugarcane disease incidence and yield of field-run, heat-treated, and tissue-culture based seedcane. *Journal of American Society of Sugar Cane Technologists* 25, 88-100.

Garside, A.L. and Bell, M.J., 2009. Row spacing and planting density effects on the growth and yield of sugarcane. 3. Responses with different cultivars. *Crop and Pasture Science* 60, 555-565.

Geetha, S. and Padmanabhan, D., 2002. Evaluation of tissue culture raised sugarcane for yield and quality. *Sugar Tech* 4, 179-180.

Gosal, S.S., Thind, K.S. and Dhaliwal, H.S., 1998. Micropropagation of sugarcane-an efficient protocol for commercial plant production. *Crop Improvement* 25, 1-5.

Hoy, J.W., Bischoff, K.P., Milligan, S.B. and Gravois, K.A., 2003. Effect of tissue culture explant source on sugarcane yield components. *Euphytica* 129, 237-240.

Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., Bekele, I. and Negi, T., 2016. Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture* 3, 1-4.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2010. Sugarcane bud chips: A promising seed material. *Sugar Tech* 12, 67-69.

Jain, R., Solomon, S., Shrivastava, A.K. and Chandra, A., 2011. Effect of ethephon and calcium chloride on growth and biochemical attributes of sugarcane bud chips. *Acta Physiologiae Plantarum* 33, 905-910.

Julien, M.H.R., Soopramanien, G.C., Martine, J.F. and Medan, H., 1980. The role of side shoots in flowered stalks of sugar cane. *In Seventeenth Congress of the International Society of Sugar Cane Technologists* 1, 571-582.

Mishra, S., Nailwal, T.K. and Pant, R.C., 2014. In vitro study of role of ethylene during tillering in sugarcane. *Sugar Tech* 16, 255-263.

Ramburan, S., Sewpersad, C. and McElligott, D., 2009. Effects of variety, harvest age and eldana on coastal sugarcane production in South Africa. *Proceedings South African Sugar Technologists Association* 82, 580-588.

Ramgareeb, S., Snyman, S.J., Van Antwerpen, T. and Rutherford, R.S., 2010. Elimination of virus and rapid propagation of disease-free sugarcane (Saccharum spp. cultivar NCo376) using apical meristem culture. *Plant Cell, Tissue and Organ Culture* 100, 175-181.

Saini, N., Saini, M. L., and Jain, R. K., 2004. Large-scale production, field performance and RAPD analysis of micropropagated sugarcane plants. *The Indian Journal of Genetics and Plant Breeding* 64, 102-107.

Sandhu, S. K., Gosal, S. S., Thind, K. S., Uppal, S. K., Sharma, B., Meeta, M., and Cheema, G. S., 2009. Field performance of micropropagated plants and potential of seed cane for stalk yield and quality in sugarcane. *Sugar Tech* 11, 34-38.

Singh, R., Singh, W. and Choudhary, S., 2014. Nursery raising with High Density Sugarcane Transplanting for higher cane yield and profitability. *Indian Farming* 64, 31-34.

Snyman, S.J., Meyer, G.M., Banasiak, M., Nicholson, T.L., Antwerpen, T.V., Naidoo, P. and Erasmus, J.D., 2009. Micropropagation of sugarcane via NovaCane®: preliminary steps in commercial application. *Sugar Cane International* 27, 245-247.

Snyman, S.J., Meyer, G.M., Koch, A.C., Banasiak, M. and Watt, M.P., 2011. Applications of in vitro culture systems for commercial sugarcane production and improvement. *In Vitro Cellular and Developmental Biology-Plant* 47, 234-249.

Sood, N., Gupta, P. K., Srivastava, R. K., and Gosal, S. S., 2006. Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. *Plant Tissue Culture and Biotechnology* 16, 25-29.

Sreenivasan, T. V., and Sreenivasan, J., 1992. Micropropagation of sugarcane varieties for increasing cane yield. *SISSTA Sugar Journal* 18, 61-64.

Verheye, W., 2010. Growth and production of sugarcane. *Soils, Plant Growth and Crop Production 2*, 1-23.

Verma, R. S., 2004. Sugarcane Production Technology in India. International Book Distributing Co, 228-267.

Willcox, T., Garside, A. and Braunack, M., 2000. The sugarcane cropping system. *Manual of cane growing", M Hogarth, P Allsopp, eds. Bureau of Sugar Experiment Stations, Indooroopilly, Australia*, 127-139.

CHAPTER 4 : THE EFFECTS OF *IN VITRO* TREATMENTS ON THE GROWTH AND DEVELOPMENT OF TISSUE CULTURE DERIVED SUGARCANE VARIETIES

Abstract

Tissue culture-derived plants have been shown to possess an altered phenotype (high tillering) compared with conventionally propagated plants. Several factors in the *in vitro* culture process may be the cause of this altered phenotype. Therefore, the aim of this study was to investigate the effects of different *in vitro* procedures on several phenotypic and agronomic characteristics of tissue culture-derived plants of sugarcane. A randomised complete block design was used with for replications involving the following treatments: two varieties (N41 and N48), three levels of *in vitro* treatments (standard NovaCane® procedure, NovaCane® ($CoCl_2$)) and NovaCane® (secondary)), two spacing (30 and 50 cm) and two crop years (plant and first ratoon). The plantlets from all three treatments were planted using two (30 and 50 cm) plant spacings. Yield and yield component measurements were taken at harvest and data were analysed by ANOVA.

The plants produced through the NovaCane® ($CoCl_2$) procedure resembled those produced through NovaCane® for all phenotypic and agronomic characteristics in the plant and first ratoon crops. The plants that underwent the NovaCane® and NovaCane® (secondary) procedures did not differ significantly for cane yield, ERC %, TERC, fibre content, stalk diameter, stalk mass and stalk height in the plant and first ratoon crops for both varieties. However, the plants produced through NovaCane® had a significantly lower stalk population compared with the plants produced through NovaCane® (secondary) for variety N41 in the first ratoon crop. Planting at closer (30 cm) or wider (50 cm) spacings did not have an effect on plant growth and to the response of varieties to the *in vitro* treatments.

The increased in tillering of the *in vitro* cultured sugarcane plants were not as a result of the build-up of the ethylene in the culture vessels, as the $CoCl_2$ treatment (aimed at reducing ethylene build-up) resembled standard treatment. Hence, other factors in the *in vitro* protocol might play a role in this increased tillering. In summary, it is recommended to continue using the standard NovaCane® procedure for the production of sugarcane tissue cultured plants for commercial plantings as the other *in vitro* variations did not results in any differences in agronomic characteristics. It is also recommended to plant NovaCane® plants at 50 cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics.

Keywords: NovaCane® procedure, plant spacing, variety, ethylene

4.1 Introduction

Sugarcane (*Saccharum officianum L.*) is a tall perennial true grass species that belongs to the genus Saccharum and tribe Andropogoneae (Yadav et al., 2012). The production of sugar is the primary use of sugarcane, and the type of sugar produced is called sucrose (Waclawovsky et al., 2010). After sugarcane emergence, there is a repetition of the vegetative development of secondary shoots from nodes of the primary shoot and this phenomenon is called tillering (Roodagi et al., 2000). Under optimum conditions this growth phase starts from approximately 45 days after planting and usually occurs until about 120 days (Meyer et al., 2011). Many factors play a role in the tillering capacity of sugarcane plants besides variety. These factors include moisture, temperature, nutrition, plant spacing and light. Amongst these factors, studies have shown that light is the most important factor (Matsuoka and Stolf, 2012). From the growth of the tillers, stalks are formed and these are the main sinks of the product of photosynthesis (sucrose) (McCormick et al., 2006). Therefore, the profitability of the crop depends largely on the quantity of tillers produced and the number of those tillers that become harvestable stalks. According to Roach (1976), sugarcane traits most highly correlated with yield was stalk population at harvest followed by stalk diameter and stalk height, respectively. However, the stalk diameter correlated negatively with stalk population.

The conventional sugarcane propagation method (the planting of stalk material into furrows and ridges) has been the traditional way of planting sugarcane (Geijskes et al., 2003). Using this propagation method has limitations. Diseases like ratoon stunting (*Leifsonia xyli subsp. xyl*), red rot (*Glomerella tucumanensis*) and leaf scald (*Xanthomonas albillineans*) are easily carried to succeeding crops through infected seeds (Hoy et al., 2003). Thus, heavy financial losses occur annually on account of reduction in sucrose recovery and cane yield. Moreover, lack of rapid multiplication procedures has long been a serious problem in sugarcane breeding programmes as it takes 10 -15 years of work to complete a selection (Zhou, 2013). Growers require a reliable supply of good quality seedcane to be able to rapidly expand the area planted to new varieties. The continuous supply of adequate quantity of good quality seedcane assists in sustaining high production of sugarcane per unit area. Good quality seedcane must be free from diseases and pests and be genetically pure (Sawant et al., 2014).

The recent use of tissue cultured plants as a means of propagation have been promising, and results in production of disease-free material that is true-to-type (Ramgareeb et al., 2010; Lal et al., 2015; Ibrahim et al., 2016).

There is, however, some evidence in the literature that the tissue culture-derived material possesses an altered phenotype such as high tillering and thinner stalks compared with the conventionally propagated plants (Bailey and Bechet, 1989; Hoy et al., 2003; Jalaja et al., 2008; Lal et al., 2015). These traits could result in a yield penalty and this may negatively affect production of sugarcane commercially. According to Lourens and Martin (1987), these altered phenotypes appeared to be transient as they disappear in the first ratoon crop. These effects, and their persistence into the subsequent ratoon crop was shown in the preceding chapter of this thesis.

Several factors in the *in vitro* culture process may be the cause of an altered phenotype of tissue culture-derived plants. The addition of plant growth regulators (PGR) to promote shoot production in *in vitro* may have a lasting effect. Jalaja et al. (2008) recommended the removal of PGR in the last stage of *in vitro* culture to mitigate this effect. In addition, there could be a build-up of ethylene in the culture vessels which causes increased tillering. According to Harrison and Kaufman (1982), tillering and tiller growth was stimulated by ethylene in oat (*Avena sativa*). If the build-up of ethylene can be reduced by adding an ethylene inhibitor such as cobalt chloride ($CoCl_2$) to the medium, then tillering will be reduced (Mishra et al., 2014).

Furthermore, the production of virus free plantlets was one of the main reasons why *in vitro* techniques e.g. meristem cultures were implemented. However, *in vitro* methods did not result in production of 100 % virus-free plants, irrespective of the procedure used (Goncalves et al., 2012; Ramgareeb et al., 2010; Snyman et al., 2012). This has negatively affected the exchange of sugarcane germplasm between countries. Consequently, a second meristem excision from *in vitro* plants that were derived from the initial field-derived meristem produces virus-free plantlets from infected plant material (Banasiak personal communication, 2016). However, there is a concern that secondary meristem excision would exacerbate any negative phenotypic effect due to the culturing process. Moreover, the phenotype of the resultant plants would not have been evaluated previously, as no hardening or acclimation period occurs in this approach.

Therefore, the aim of the study was to compare several phenotypic and agronomic characteristics of *in vitro* produced NovaCane® plants with two *in vitro* treatments, namely those exposed to $CoCl_2$ and those that underwent a secondary meristem excision process from *in vitro* material. These two treatments were compared with the conventional NovaCane protocols described in earlier chapters for two sugarcane varieties planted at two plant spacings.

4.2 Materials and method

4.2.1   Trial site characteristics and plantlets production

A field trial was established in September 2015 under rainfed conditions at the South African Sugarcane Research Institute (SASRI) Mount Edgecombe experiment station which is located 29° 42' 24S, 31° 1' 30E and 108 m above sea level. This site represented the coastal growing region of the South African sugar industry. The plant crop was harvested on 26 September 2016, while the first ratoon was harvested on 18 September 2017. The tissue culture plants (NovaCane®) were produced at the Biotechnology laboratory at SASRI using the shoot multiplication protocol as described by Ramgareeb et al. (2010). The tissue culture plantlets were either untreated (NovaCane® (standard)) or were treated with two *in vitro* treatments aimed at manipulating their tillering patterns, which were:

- NovaCane® (CoCl$_2$)
- NovaCane® (secondary)

A portion of the NovaCane® plantlets were treated with an *in vitro* anti-tillering treatment referred to as 'reduced tillering treatment' (NovaCane® (CoCl$_2$)). For this treatment, cobalt (II) chloride (CoCl$_2$; 5 mg/L) was added to the MS culture medium for 2 weeks prior to the rooting stage. Plants from another treatment, 'secondary meristem excision' (NovaCane® (secondary)), were included in the comparative study, where apical meristems were excised from *in vitro* plantlets and underwent a second round of shoot multiplication as per Figure 3.2. The standard NovaCane® plants were the control treatment for this study and those plants were produced as per Figure 3.2 detailed in Chapter 3. The treatments combination for this experiment two varieties (N41 and N48), three levels of *in vitro* treatments (standard NovaCane® procedure, NovaCane® (CoCl$_2$)) and NovaCane® (secondary)), two spacing (30 and 50 cm) and two crop years (plant and first ratoon). The plantlets from all three treatments were planted using two (30 and 50 cm) plant spacings.

4.2.2   Measurements during crop growth

4.2.2.1 Stalk population and heights

The stalk population and heights measurements were done once a month. The stalk height measurement was done by selecting 20 stalks randomly per plot on the centre rows (row 3 and 4) which are 10m long with a 1.2m row spacing in size. A tape measure was used to measure the heights of 20 selected plants from the soil surface to the top visible dewlap (TVD), which is the collar of the uppermost, fully expanded leaf.

The stalk population was measured per plot by manually counting all the number of stalks per 10 m line length on the centre rows (row 3 and 4), and thereafter expressed on a per hectare basis.

4.2.2.2 Stalk diameter

The measurements of stalk diameter were done once a month. In each plot 10 randomly selected stalks of the nett rows (row 2, 3, 4 and 5) were used to measure stalk diameter using a vernier caliper (ABS Digital Caliper DCA 150; Oxford Precision, UK). The caliper was placed perpendicular to the middle of the stalks before readings were taken.

4.2.2.3 Leaf length and width

The leaf length and width measurements were taken on a monthly basis. These measurements were done on the TVD leaf of 10 selected stalks of the nett rows (row 2, 3, 4 and 5). The leaf length was measured from the stalk (where the leaf is attached) to the tip of the leaf using a tape measure. The leaf width was measured at the middle of the TVD leaf.

4.2.3 Measurements at harvest

At harvest, cane yield was measured by cutting and measuring the weight of the stalks (bundles) of the nett rows (row 2, 3, 4 and 5) in each plots using a scale attached to a hydraulic weighing boom. An African sugar-cane borer (*Eldana saccharina L.*) survey was done on 20 randomly selected stalks per plot and the procedure is described in chapter 3. A sample of 12 stalks per plot was taken from the nett rows (row 2, 3, 4 and 5) and submitted to the SASRI mill room to determine the estimated recoverable crystal percent (ERC %) which was used to estimate the quantity of sucrose in cane that was recovered as sugar.

4.2.4 Statistical analysis

All data collected was statistically analysed using GenStat 14th edition and Microsoft Office Excel was used to process collected data from the trial. Growth and yield variables were subjected to analysis of variance (ANOVA) to establish main (variety, *in vitro* treatments, plant spacings and ratoon) and interaction effects. Comparison of means was performed using Fisher's protected LSD test at 5% significance difference. Significant differences between treatments at selected dates for in-season growth were represented by LSD bars (5%).

4.3 Results

4.3.1 Yield and quality

Variety (V) had a significant (p<0.05) effect on ERC % (Table 4.1). *In vitro* treatment (IVT) and plant spacing (PS) was not significant for any trait. The crop (plant and first ratoon) had a highly significant (p≤0.001) effect on cane yield, ERC %, TERC and fibre content. The PS x crop had a significant (p<0.05) effect on ERC %. The V x IVT, V x PS, V x IVT x PS, and four-way V x PS x IVT x crop was not significant for any trait. The responses of each trait are discussed in more detail in figures 4.1 – 4.4 below.

Table 4.1 A summary of ANOVA for yield and quality traits (cane yield, estimated recoverable crystal (ERC %), ERC yield (TERC) and fibre content (FIB)).

Source of variation	D.F	Cane yield M.S	Cane yield F-value	ERC % M.S	ERC % F-value	TERC M.S	TERC F-value	FIB M.S	FIB F-value
Variety (V)	1	2076.30	3.81^{NS}	7.04	8.41^{*}	4.35	0.78^{NS}	0.35	0.63^{NS}
Plant spacing (PS)	1	1083.40	1.99^{NS}	0.14	0.18^{NS}	6.76	1.21^{NS}	1.57	2.80^{NS}
In vitro treatment (IVT)	2	156.60	0.29^{NS}	1.10	1.32^{NS}	3.18	0.57^{NS}	0.45	0.80^{NS}
Crop (year)	1	4747.20	8.72^{**}	20.70	24.8^{**}	104.6	18.70^{**}	13.70	24.40^{**}
V.PS	1	699.20	1.28^{NS}	0.10	0.02^{NS}	5.30	0.95^{NS}	0.00	0.01^{NS}
V.IVT	2	48.70	0.09^{NS}	1.19	1.42^{NS}	0.23	0.04^{NS}	0.04	0.07^{NS}
PS.IVT	2	269.60	0.49^{NS}	0.74	0.88^{NS}	5.10	0.91^{NS}	0.76	1.36^{NS}
V. Crop	1	883.60	1.62^{NS}	0.53	0.63^{NS}	9.54	1.71^{NS}	2.03	3.63^{NS}
PS. Crop	1	332.10	0.61^{NS}	4.00	4.78^{*}	8.01	1.43^{NS}	0.12	0.03^{NS}
IVT. Crop	2	45.70	0.08^{NS}	0.33	0.39^{NS}	1.38	0.25^{NS}	0.71	1.27^{NS}
V.PS.IVT	2	712.30	1.31^{NS}	0.03	0.03^{NS}	16.4	2.93^{NS}	0.33	0.58^{NS}
V.PS. Crop	1	394.90	0.73^{NS}	0.69	0.82^{NS}	7.39	1.32^{NS}	0.02	0.03^{NS}
V.IVT. Crop	2	208.90	0.38^{NS}	0.22	0.27^{NS}	1.83	0.33^{NS}	0.12	0.22^{NS}
PS.IVT. Crop	2	354.60	0.65^{NS}	0.62	0.75^{NS}	1.20	0.21^{NS}	0.60	1.07^{NS}
V.PS.IVT. Crop	2	3.40	0.01^{NS}	1.20	1.43^{NS}	3.14	0.56^{NS}	2.40	4.28^{NS}
Replications	3	1377.20	2.53	3.68	4.39	9.30	1.66	0.22	0.39
Error	69	544.60		0.84		5.59		0.56	

^{NS} not significant (p>0.05) *significant (p<0.05) **highly significant (p≤0.001)

Squares indicate significant F-values, for easy reference

Cane yield: There were no significant differences in cane yield between the treatments in the plant and first ratoon crops for both varieties (Figure 4.1). For both varieties, the NovaCane® treatment had a higher (not significant) cane yield compared with the other treatments for 30 cm spacings in the plant and first ratoon crop.

Figure 4.1 Cane yield of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.

ERC %: There were no significant differences in ERC % between the treatments for both varieties in the plant and first ratoon crop (Figure 4.2).

Figure 4.2 Estimated recoverable crystal percentage (ERC %)of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.

TERC: In general, there were no significant differences in TERC between the treatments for both varieties in the plant and first ratoon crops (Figure 4.3). In the first ratoon crop, the NovaCane® treatment had a higher (not significant) TERC compared with the other treatments for both varieties spaced at 30 cm apart.
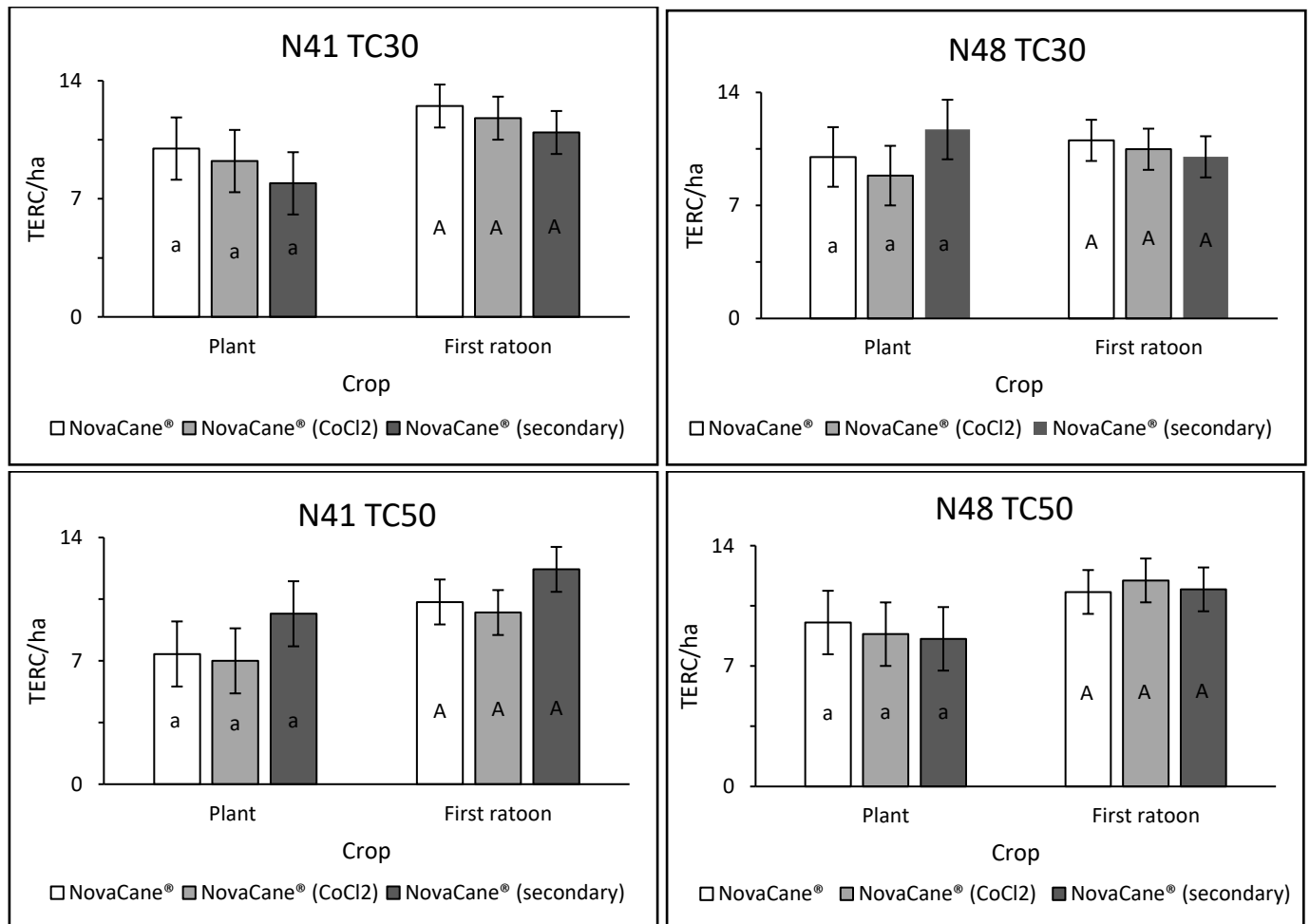
Figure 4.3 Estimated recoverable crystal yields (TERC) of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl$_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.

Fibre content: There were no significant differences in fibre content between the treatments for both varieties in the plant and first ratoon crop (Figure 4.4).

Figure 4.4 Fibre content of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl$_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.

4.3.2 Yield components

Variety had a highly significant (p≤0.001) effect on stalk population, stalk diameter and stalk mass (Table 4.2). *In vitro* treatment had a significant (p<0.05) effect on stalk diameter and stalk mass. Plant spacing was not significant for any trait. The crop had a highly significant (p≤0.001) effect on stalk diameter, stalk mass and significant (p<0.05) effect on stalk height. The V x PS had a significant effect on stalk diameter. The V x IVT, V x PS, V x IVT x PS, and four-way V x PS x IVT x crop was not significant for any trait. The response of each yield component are discussed in more details in figures 4.5 - 4.13 below.

Table 4.2 A summary of ANOVA for yield components (stalk population, stalk diameter, stalk height and stalk mass) at harvest.

Source of variation	D.F	Stalk population M.S	F-value	Stalk diameter M.S	F-value	Stalk height M.S	F-value	Stalk mass M.S	F-value
Variety (V)	1	8.40^{x09}	26.30^{**}	439.00	254.00^{**}	422.00	0.49^{NS}	676850	36.4^{**}
Plant spacing (PS)	1	9.40^{x08}	2.97^{NS}	2.89	1.67^{NS}	1298.00	1.49^{NS}	439	0.02^{NS}
In vitro treatment (IVT)	2	2.60^{x07}	0.04^{NS}	6.37	3.68^{*}	1545.00	1.77^{NS}	77577	4.17^{*}
Crop (year)	1	3.80^{x07}	0.12^{NS}	28.00	16.20^{**}	3564.00	4.09^{*}	217469	11.6^{**}
V.PS	1	5.10^{x08}	1.60^{NS}	10.50	6.06^{*}	2365.00	2.72^{NS}	120677	6.48^{*}
V.IVT	2	3.90^{x07}	0.06^{NS}	2.70	1.56^{NS}	29.40	0.03^{NS}	7731	0.42^{NS}
PS.IVT	2	2.05^{x08}	0.40^{NS}	1.17	0.67^{NS}	704.80	0.81^{NS}	4675	0.25^{NS}
V. Crop	1	9.80^{x08}	3.10^{NS}	0.47	0.27^{NS}	549.70	0.63^{NS}	42819	2.30^{NS}
PS. Crop	1	1.20^{x08}	0.37^{NS}	1.15	0.66^{NS}	58.50	0.07^{NS}	521	0.03^{NS}
IVT. Crop	2	7.00^{x08}	1.10^{NS}	1.64	0.95^{NS}	124.30	0.14^{NS}	10850	0.58^{NS}
V.PS.IVT	2	6.10^{x08}	0.96^{NS}	2.86	1.65^{NS}	823.30	0.95^{NS}	52240	2.81^{NS}
V.PS. Crop	1	1.80^{x08}	0.55^{NS}	0.86	0.50^{NS}	23.40	0.03^{NS}	2447	0.13^{NS}
V.IVT. Crop	2	8.10^{x07}	0.13^{NS}	0.88	0.51^{NS}	88.30	0.10^{NS}	4772	0.26^{NS}
PS.IVT. Crop	2	9.20^{x08}	1.45^{NS}	1.21	0.70^{NS}	315.40	0.36^{NS}	16415	0.88^{NS}
V.PS.IVT. Crop	2	2.50^{x08}	0.39^{NS}	0.69	0.40^{NS}	39.20	0.05^{NS}	58	0.00^{NS}
Replications	3	2.16^{x09}	2.27	11.84	6.84	4213.50	4.84	124866	6.71
Error	69	3.17^{x08}		1.73		870.80		18623	

^{NS} not significant (p>0.05) * significant (p<0.05) ** highly significant (p≤0.001)

Squares indicate significant F-values, for easy reference

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Stalk population: There were no significant differences in stalk population between the treatments for both varieties in the plant crop (Figure 4.5). Variety N41 spaced at 50 cm apart showed a significant difference in stalk population between the treatments, with the NovaCane® (secondary) treatment significantly having higher stalk population compared with the other treatments in the first ratoon crop.

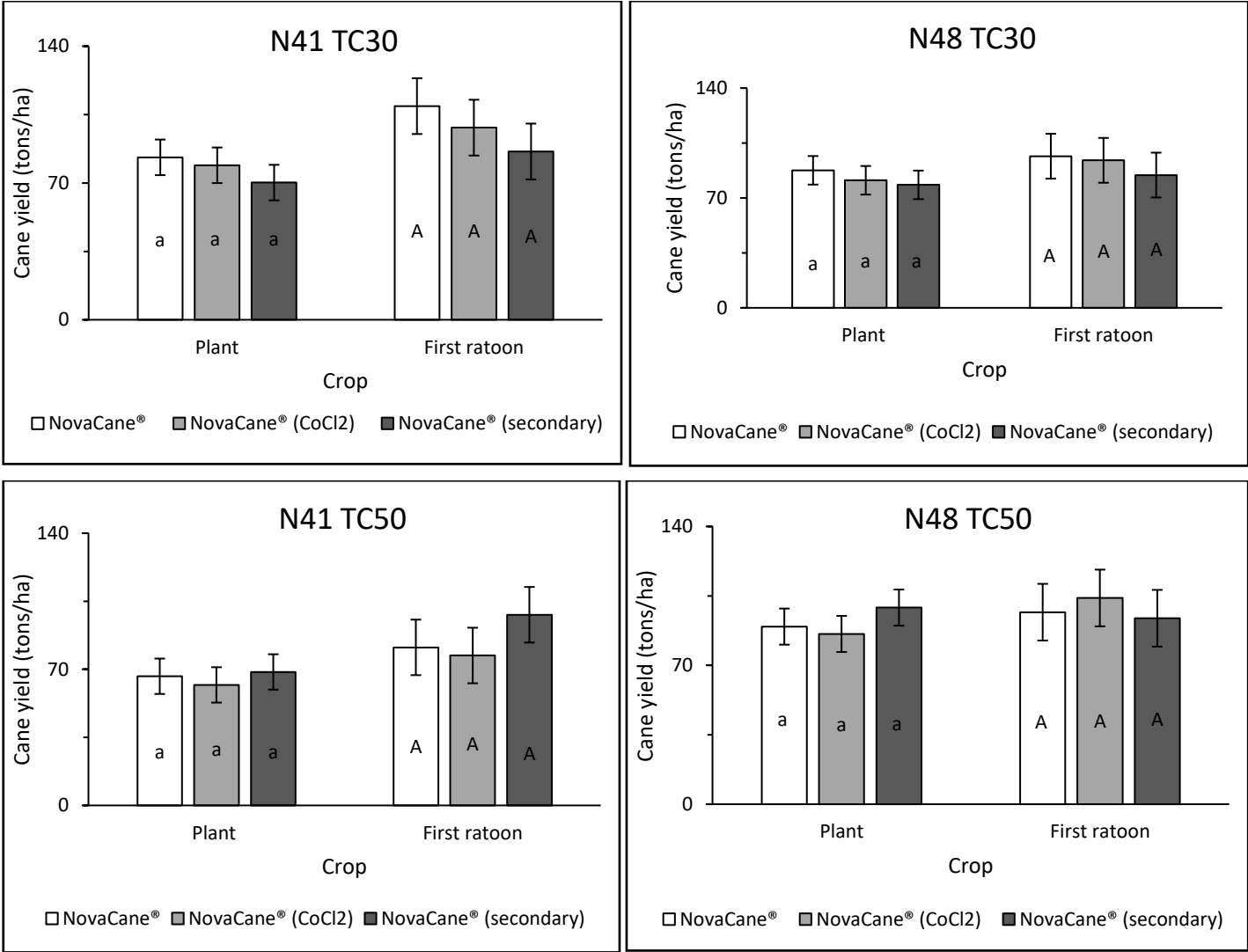**Figure 4.5 Stalk population (x1000) of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl$_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.**

Stalk diameter: The NovaCane® (CoCl$_2$) treatment had a significantly thicker stalks compared with the NovaCane® (secondary) treatment for N41 spaced at 30 cm spacings and N48 spaced at 50 cm spacings in the plant crop (Figure 4.6). There were no significant differences in stalk diameter between the treatments for both varieties in the first ratoon crop.

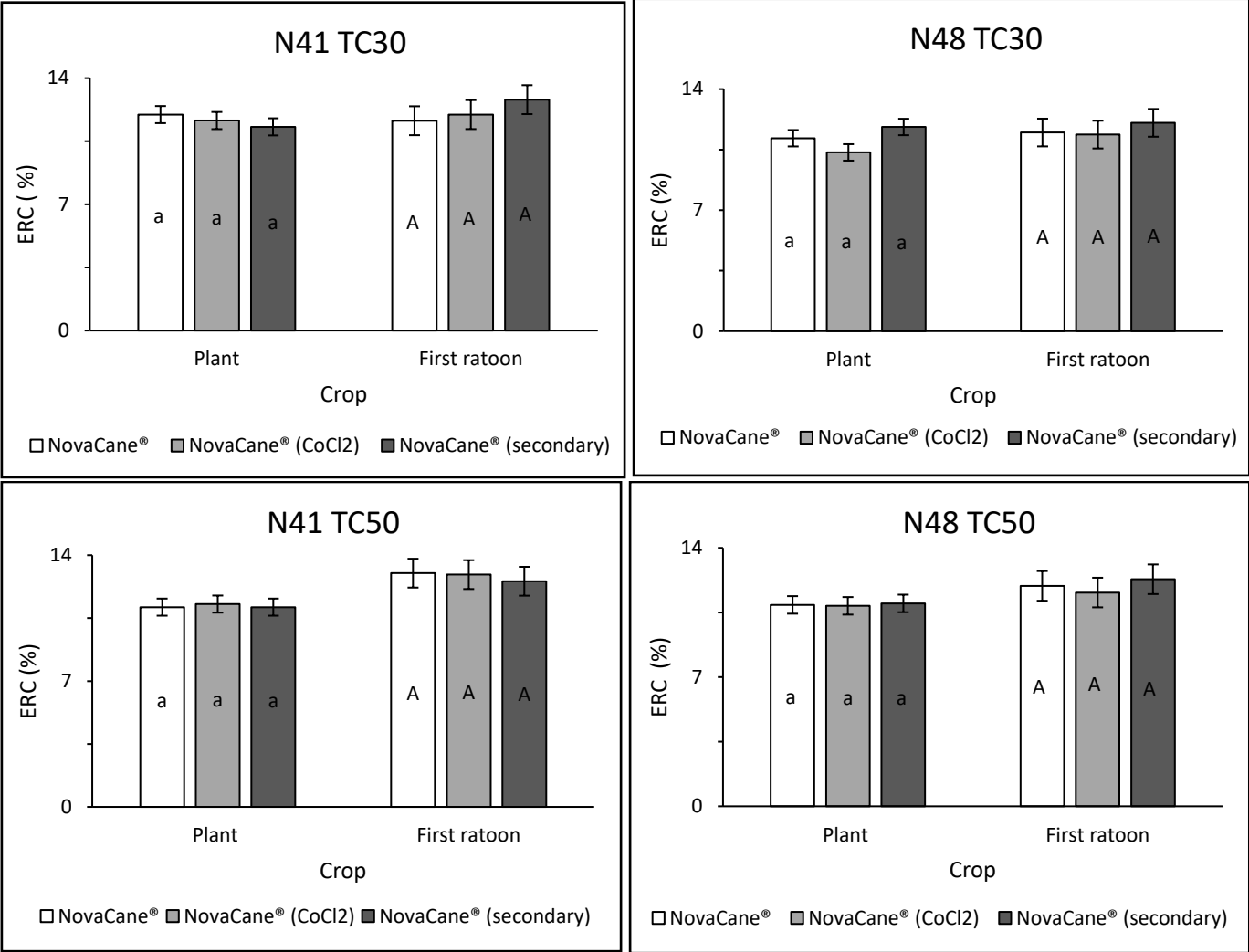**Figure 4.6 Stalk diameter of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl$_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.**

Stalk height: For both varieties, no significant differences were observed in stalk height between the treatments in the plant and first ratoon crops (Figure 4.7). The NovaCane® (secondary) treatment produced shorter (not significant) stalks compared with the other treatments in the plant and first ratoon crops for both varieties spaced at 30 cm apart.
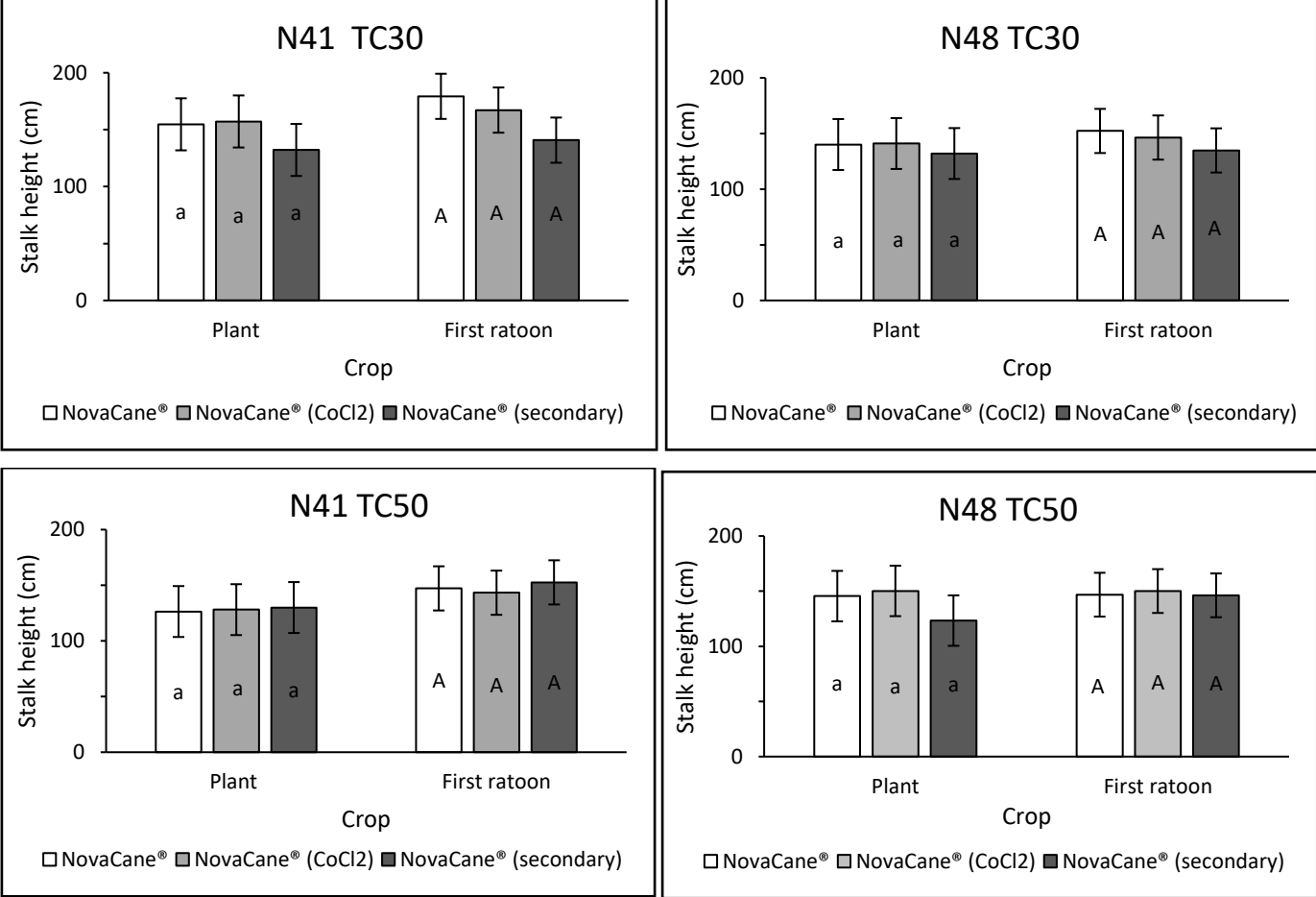
Figure 4.7 Stalk height of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.

Stalk mass: The NovaCane® (secondary) produced significantly lighter stalks compared with the NovaCane® ($CoCl_2$) treatment for variety N48 spaced at 50 cm apart in the plant crop (Figure 4.8). There were no significant differences in stalk mass between the treatments for both varieties in the first ratoon crop.
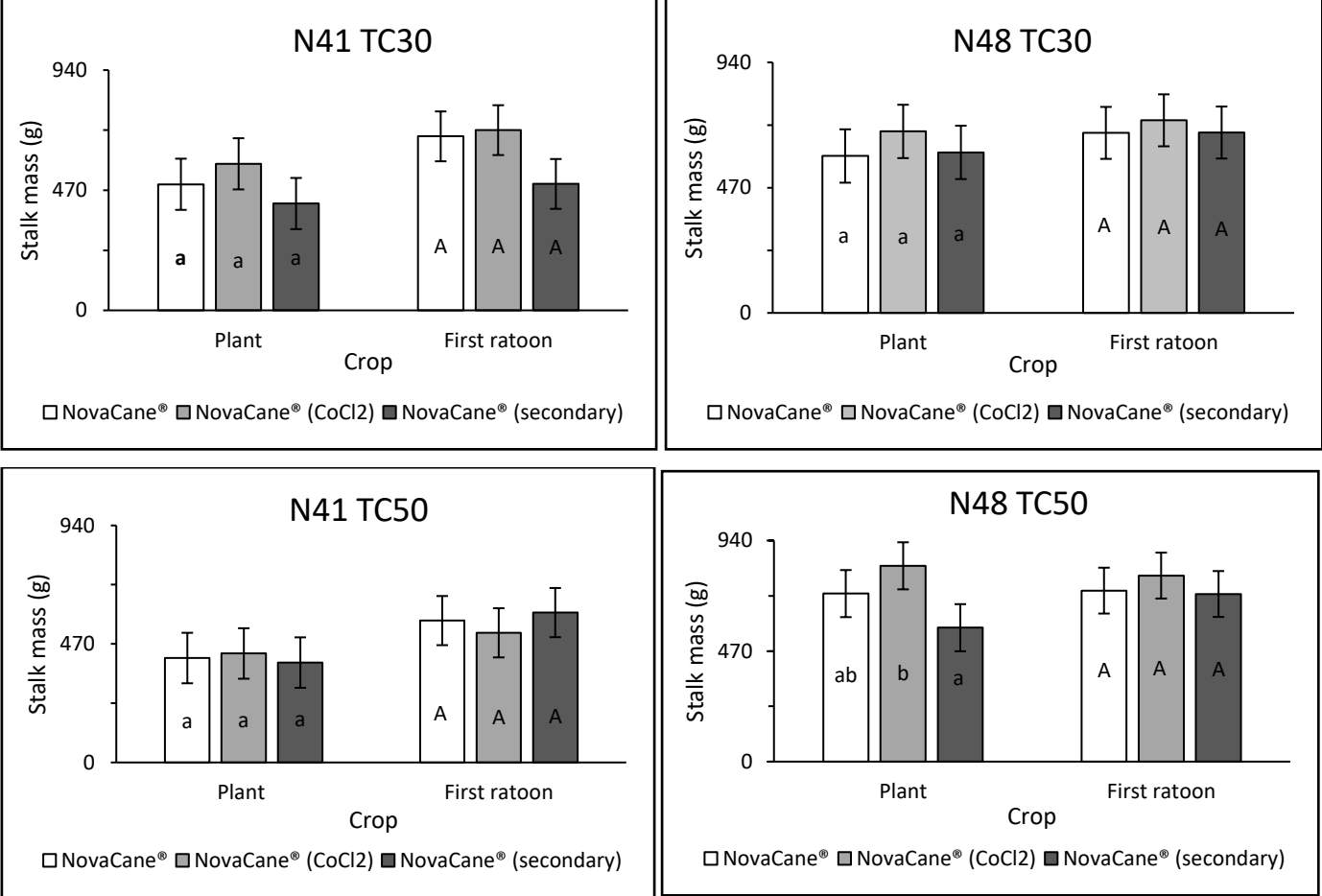
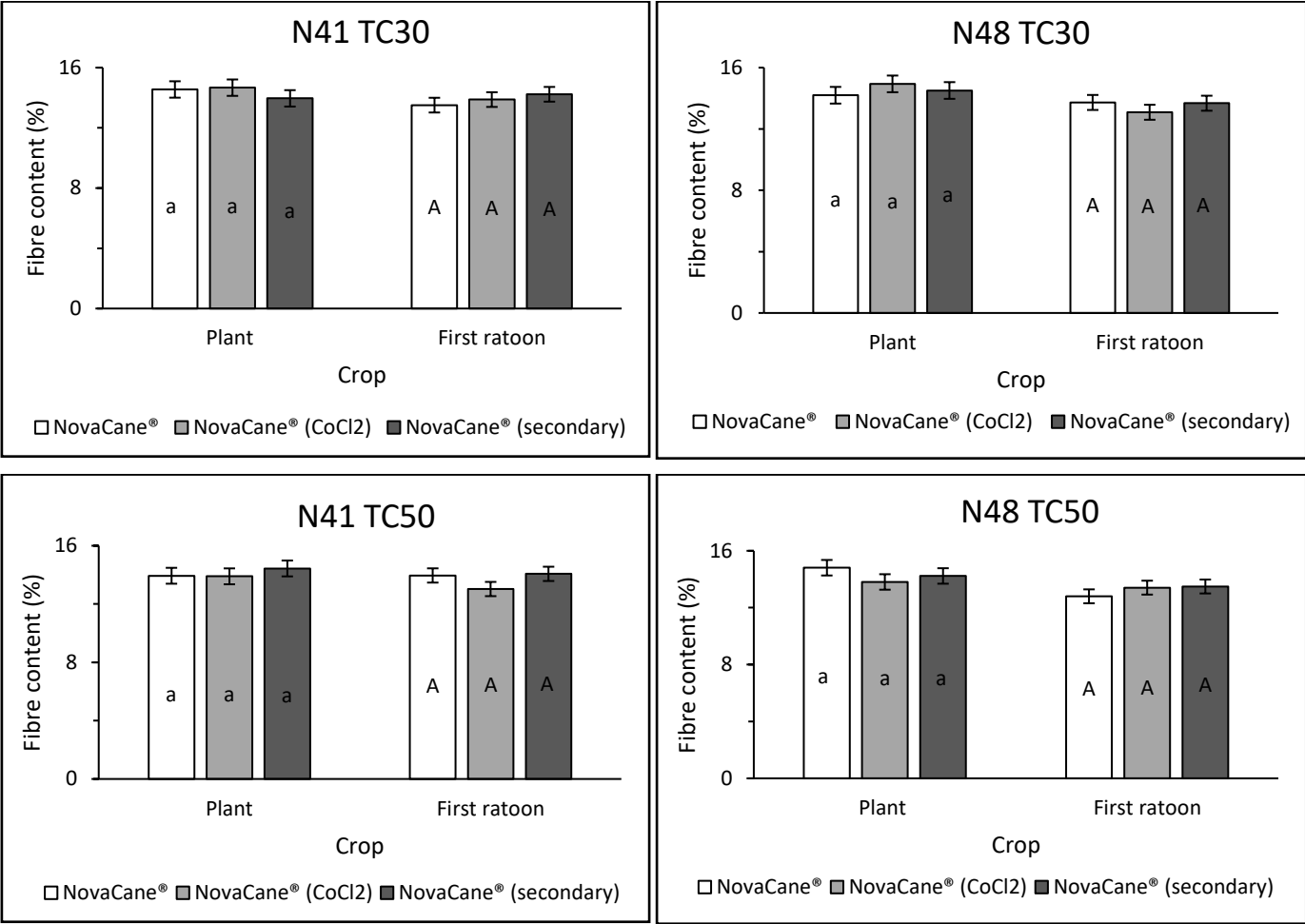**Figure 4.8 Stalk mass of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® ($CoCl_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences across all treatment combinations are reflected by differing letters.**

4.3.3 In season measurements

Stalk population: Throughout the growing season, no significant differences were detected in stalk population between the treatments for both varieties in the plant crop (Figure 4.9). The differences in stalk population between the propagation methods throughout the season was much smaller for both varieties spaced at 30 cm apart in the first ratoon crop.
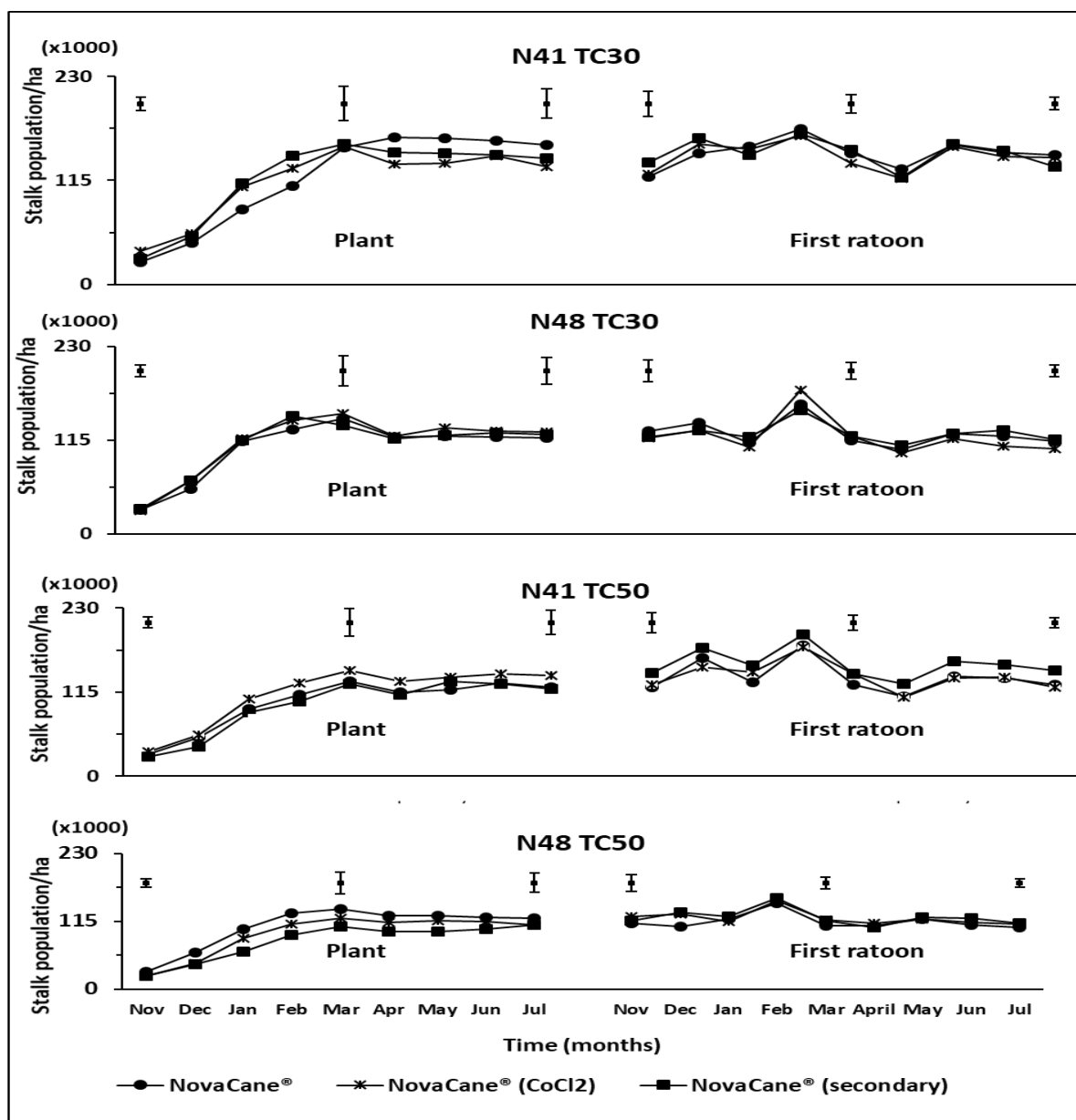
Figure 4.9 Stalk population (x1000) of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk diameter: The NovaCane® (secondary) had a tendency of having thinner stalks throughout the growing season compared with the other treatments in the plant crop for both varieties (Figure 4.10). In the first ratoon crop, the differences in stalk diameter between the propagation methods throughout the season was much smaller for both varieties.
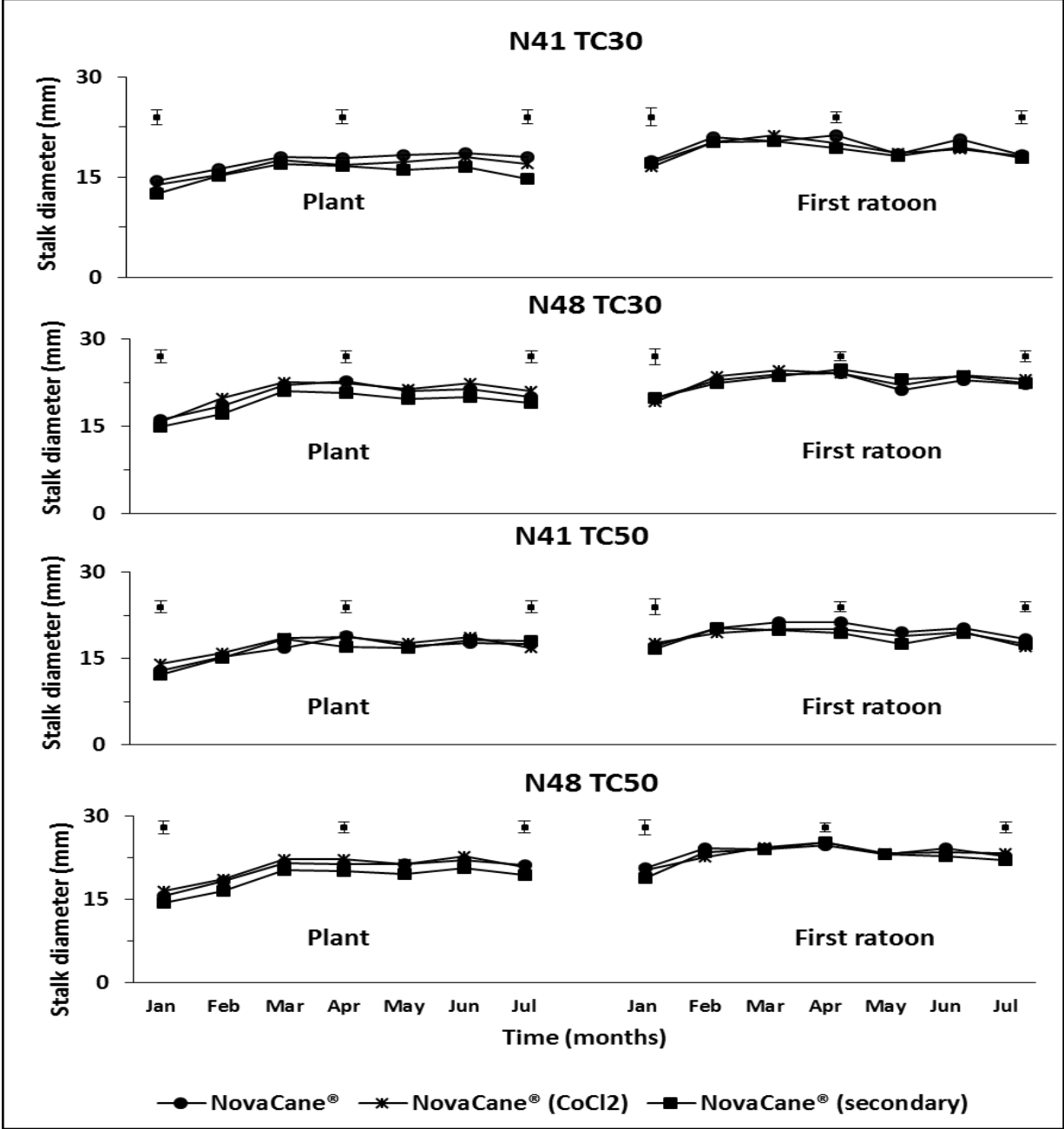
Figure 4.10 Stalk diameter of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk height: Both varieties showed no significant differences in stalk height between the treatments throughout the growing season in the plant and first ratoon crop (Figure 4.11). The NovaCane® treatment had a tendency of having taller stalks compared with the NovaCane® (secondary) treatment throughout the growing season for both varieties in the plant crop.
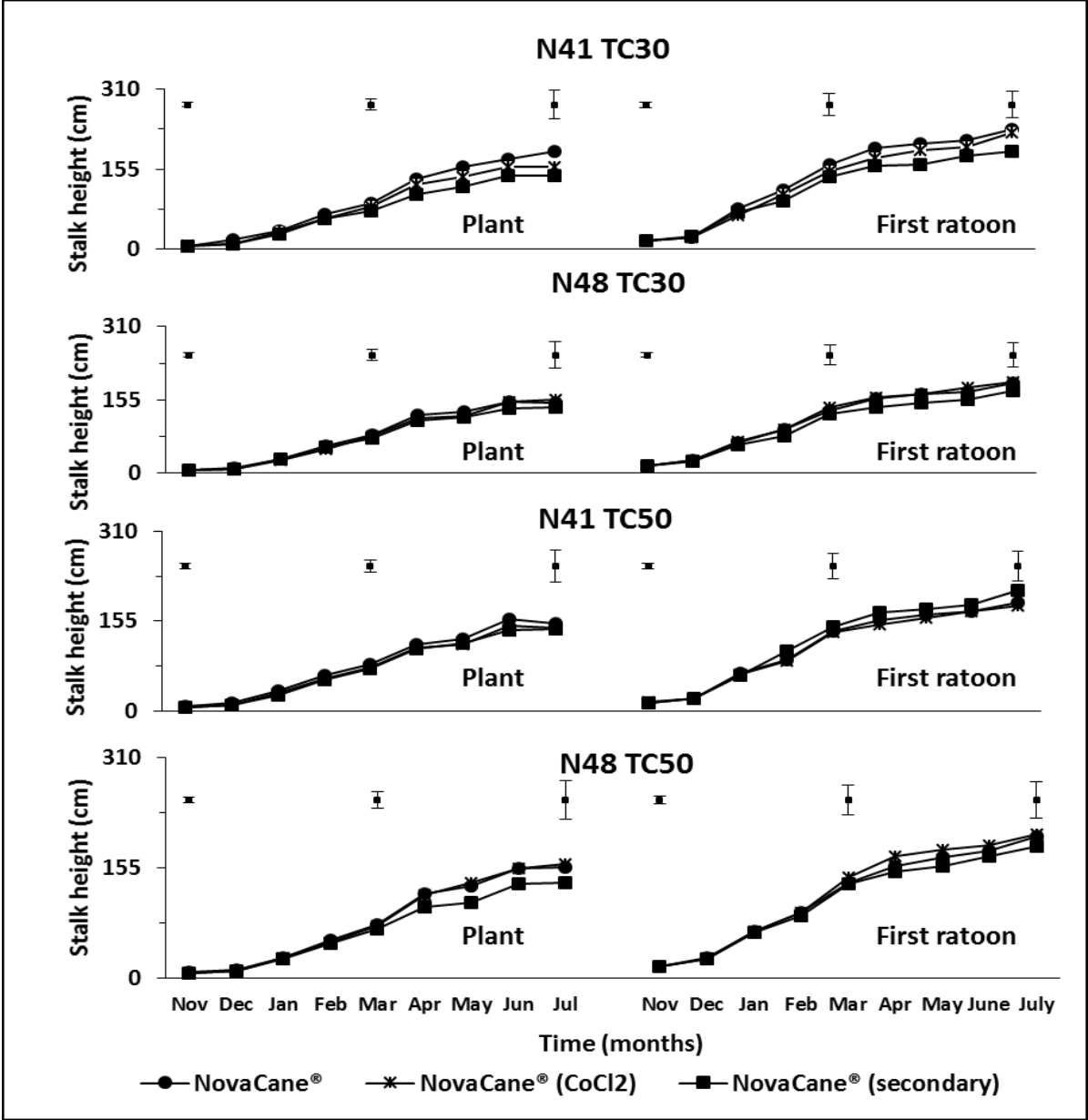
Figure 4.11 Stalk height of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences between treatments at selected dates are represented by LSD bars (5%).

Leaf length: In the plant and first ratoon crops, the differences in leaf length between the propagation methods throughout the season was much smaller for both varieties (Figure 4.12).
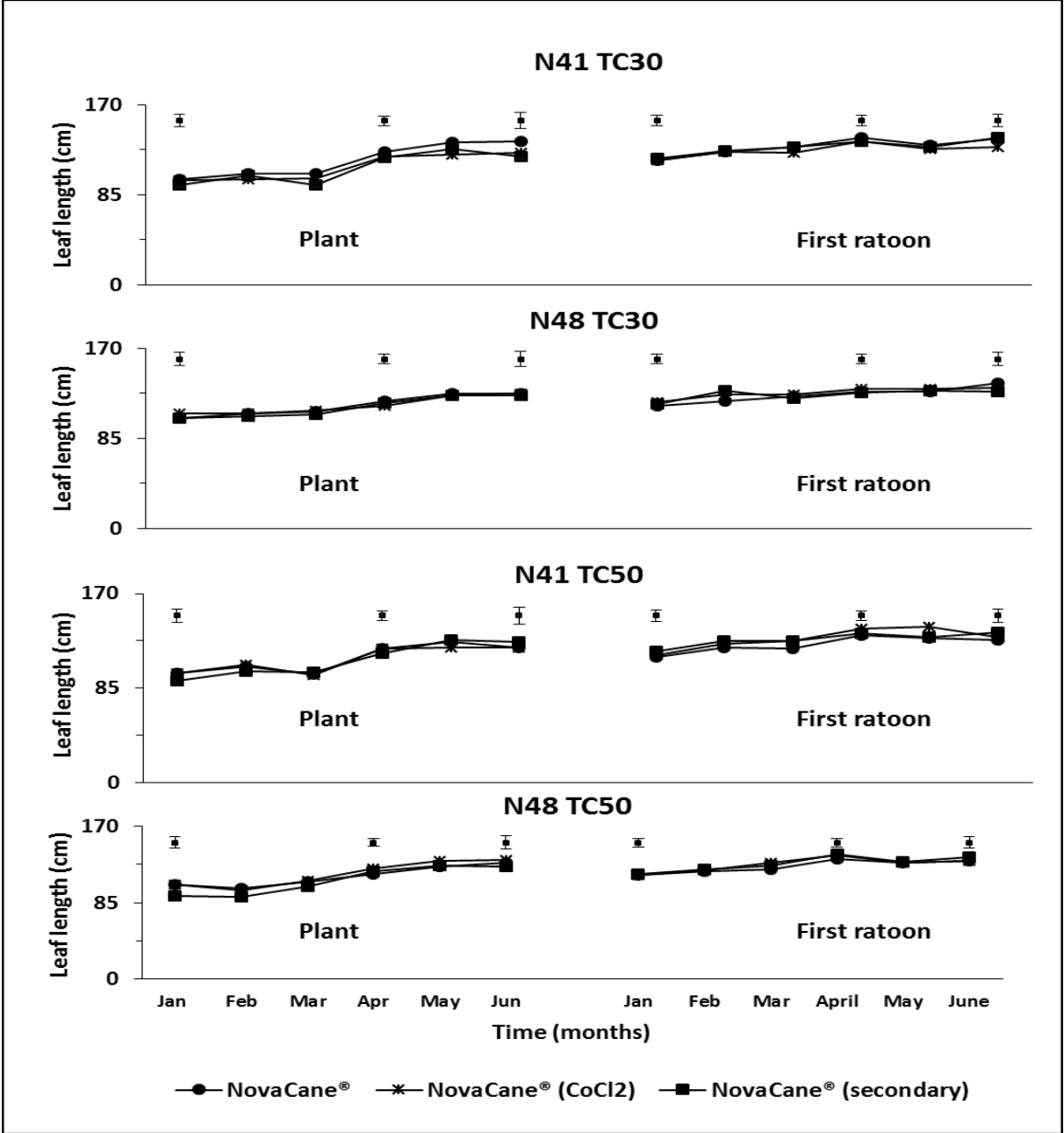
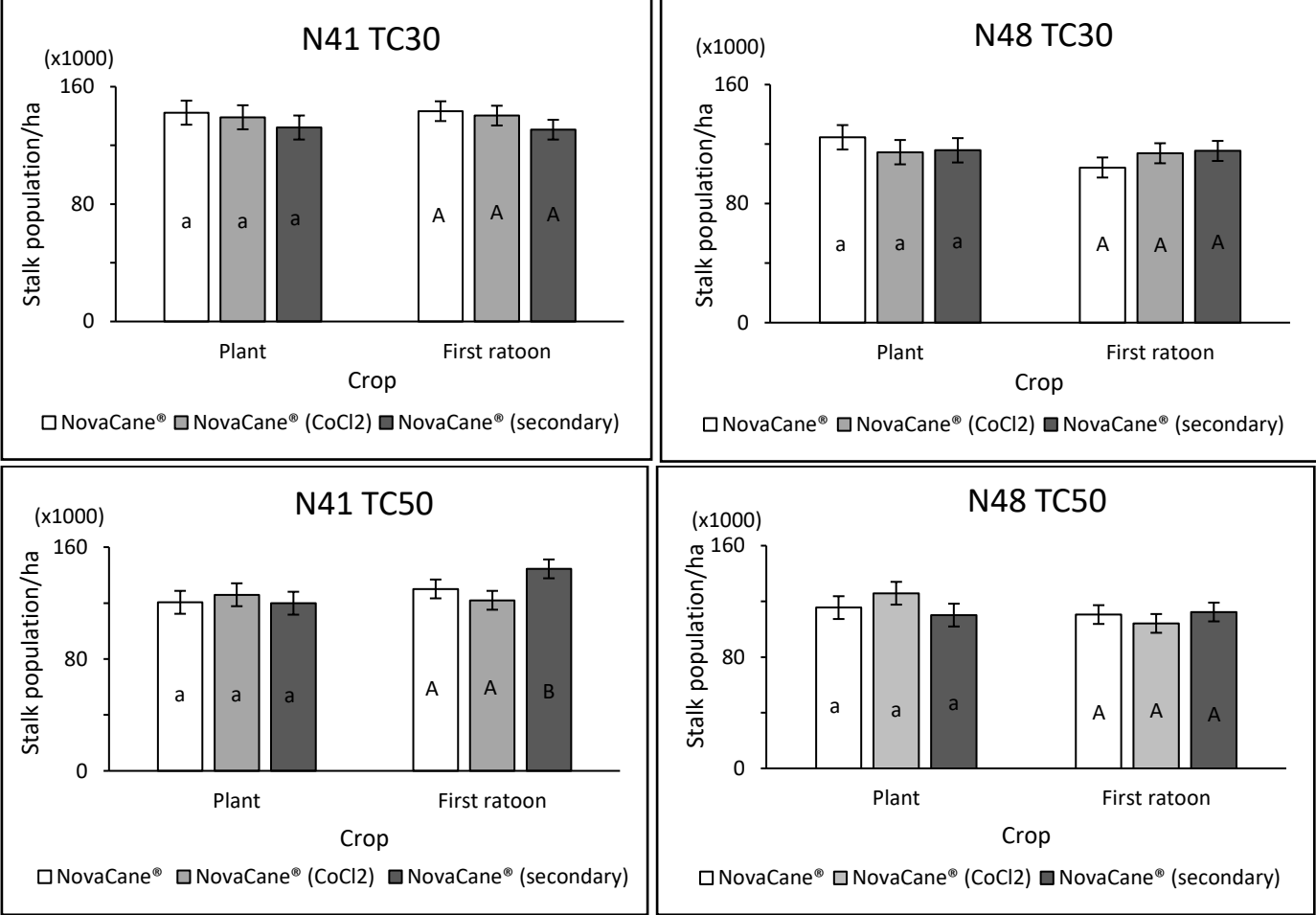**Figure 4.12 Leaf length of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl$_2$) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences between treatments at selected dates are represented by LSD bars (5%).**

Leaf width: In the plant crop, the NovaCane® (secondary) had a tendency of having narrow leaves throughout the season compared with the other treatments for variety N48 spaced at 30 and 50 cm apart (Figure 4.13). In the first ratoon crop, the differences in leaf width between the propagation methods throughout the season was much smaller for both varieties.
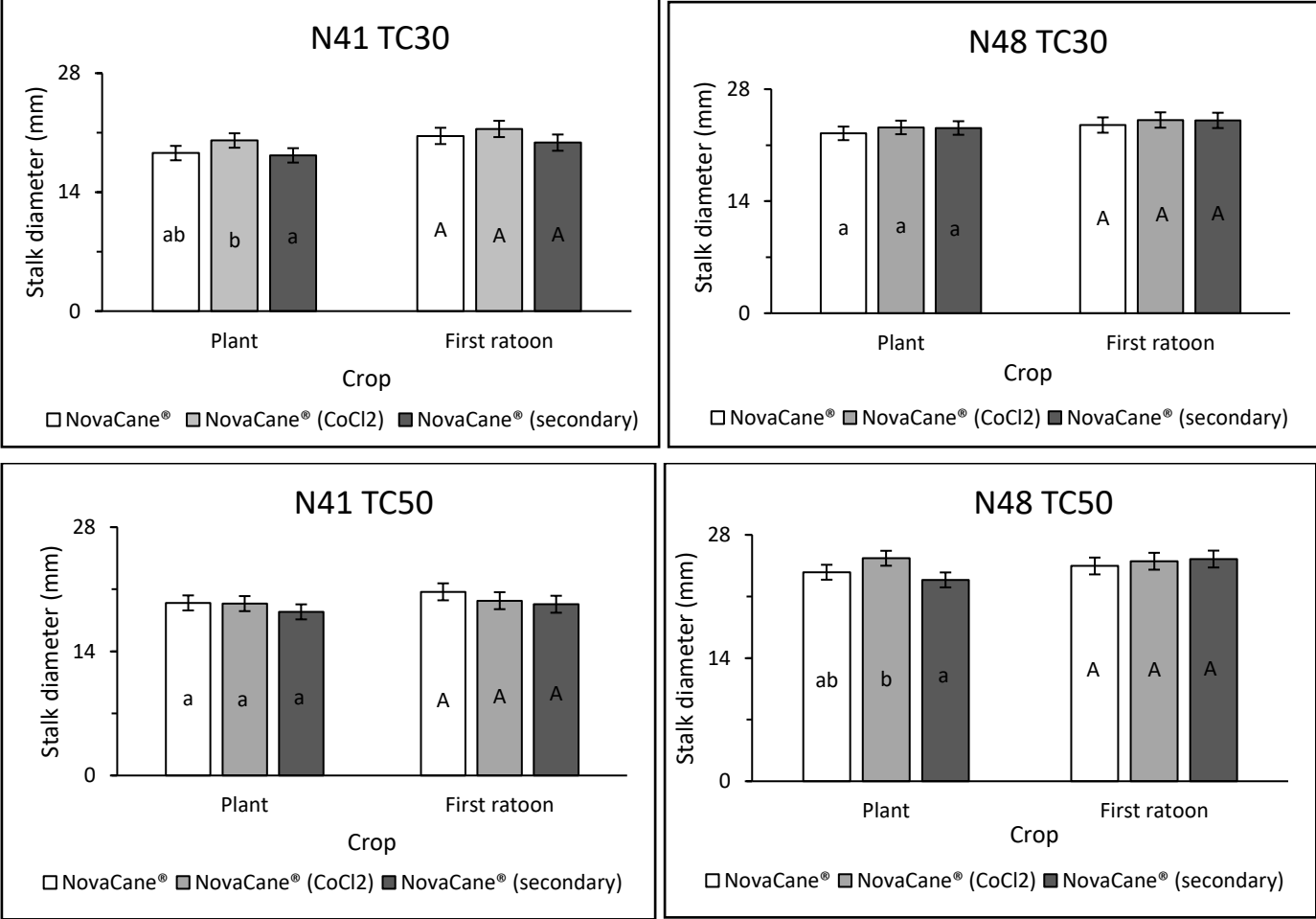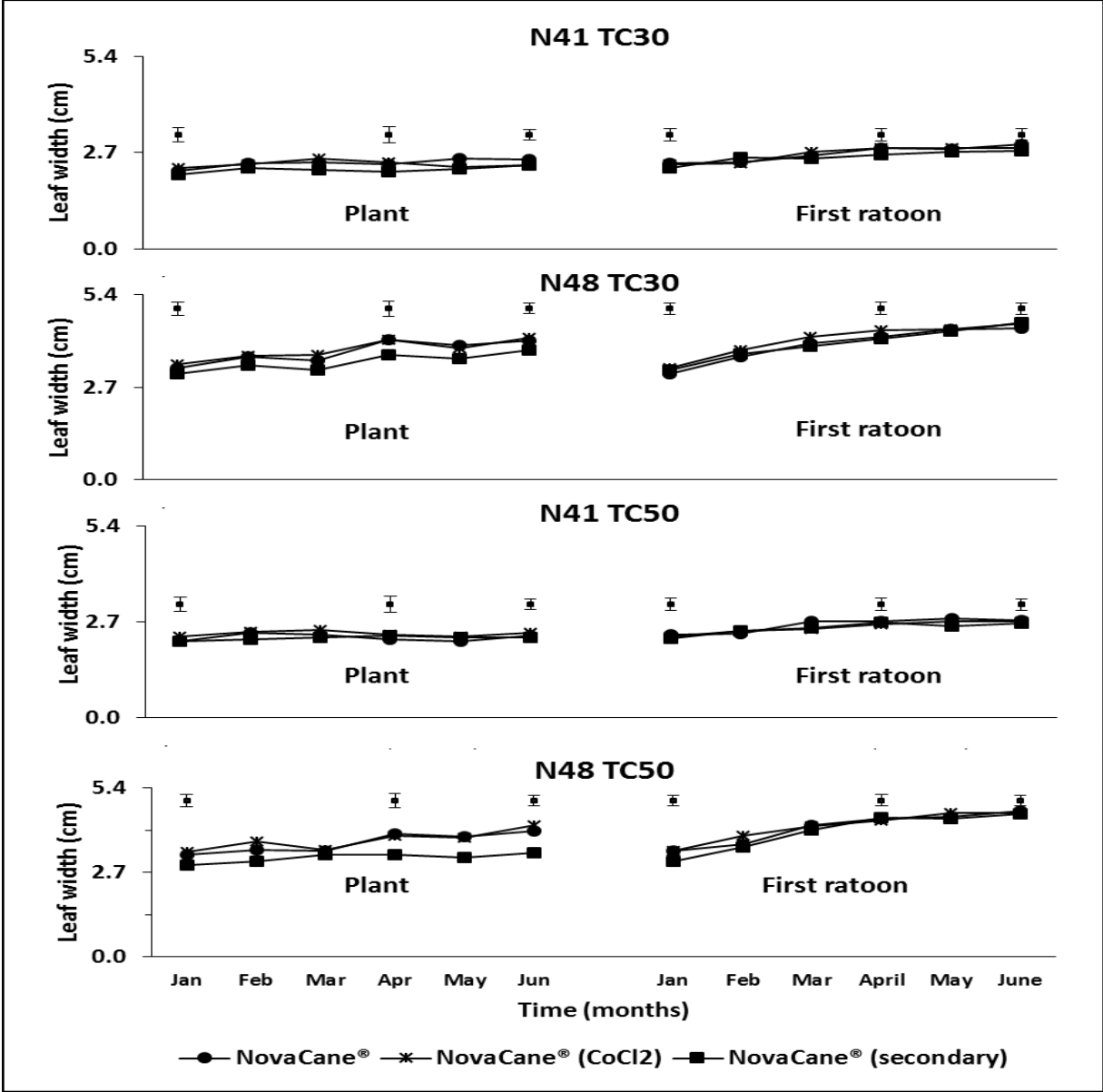

Figure 4.13 Leaf width of two varieties (N41 and N48) when propagated through tissue culture (tissue culture plants planted at 30 cm plant spacing (TC30) or tissue culture plants planted at 50 cm plant spacing TC50) and treated with three in vitro treatments (NovaCane®, NovaCane® (CoCl₂) and NovaCane® (secondary)) in the plant and first ratoon crops. Significant differences between treatments at selected dates are represented by LSD bars (5%).

4.4 Discussion and Conclusion

For both varieties, the NovaCane® (CoCl$_2$) treatment resembled the NovaCane® for all phenotypic and agronomic characteristics in both crops (plant and first ratoon). These results suggest that there may have been no excessive build-up of ethylene in the culture vessels because addition of the cobalt chloride (CoCl$_2$) to the medium had no effect. Consequently, the stalks from the NovaCane® and the NovaCane® (CoCl$_2$) treatments behaved the same and there were no significant differences. The results of the present study are in line with the findings of Brar (1999), who showed that no significant effect was observed on the regeneration capacity of cowpea cotyledon explants in response to the addition of CoC1$_2$. Latche (1991), showed that 10 or 20 ppm of CoCl$_2$ did not modify the growth rate of the sunflower callus, but adding 40 or 80 ppm did affect growth. However, this contradicts the findings of Mishra et al. (2014, who showed that 10 ppm of CoCl$_2$ was found effective in reducing ethylene concentration inside the culture bottles in *in vitro* cultured sugarcane plants. Furthermore, using 20 ppm CoCl$_2$ was the best concentration to inhibit ethylene formation in the culture vessels and induce potato plant growth (Taghizadeh and Ehsanpour, 2013). In addition, Ishida (2000) also reported significant inhibition of ethylene production at 10 ppm CoCl$_2$ treatments in *in vitro* cultured tomato plants. These results suggest that the concentration of CoCl$_2$ required to inhibit the build-up of ethylene vary for different crops.

Based on the results from the present study, there was no benefit of adding CoCl$_2$ in *in vitro* cultured sugarcane plants. These results suggest that the increased tillering of the *in vitro* cultured sugarcane plants might not be as a result of the build-up of the ethylene in the culture vessels. Hence, this altered phenotype might be as a result of other factors in the *in vitro* culture process. However, the in-effectiveness of CoCl$_2$ on inhibiting ethylene build-up on culture vessels might also be as a result of low or high concentration of CoCl$_2$. This suggest that different concentration of CoCl$_2$ should be tested in *in vitro* cultured sugarcane plants to determine which concentration would be effective at reducing ethylene and subsequently reducing excessive tillering. One of the limitations of this study was that the actual concentrations of ethylene in the culture vessels were not monitored to confirm effectiveness of the CoCl$_2$. Another possibility is the hormonal stimulation of shoot production might be prolonged after splitting plants. Further work could reduce the strength of these products to avoid excess tillering after splitting the plants. The NovaCane® and the NovaCane® (secondary) treatments did not differ significantly for Cane yield, ERC %, TERC, fibre content, stalk diameter, stalk mass and stalk height in both crops for both varieties.

Variety N41 spaced at 50 cm apart showed a significant difference in stalk population between treatments, with the NovaCane® treatment significantly having lower stalk population compared with the NovaCane® (secondary) treatment in the first ratoon crop. These differences in stalk population between the NovaCane® and the NovaCane® (secondary) treatments in the first ratoon crop were not expected, as no differences were observed in the plant crop. These results suggest that varieties responded differently to the *in vitro* process. The phenotype of the NovaCane® (secondary) plants has not been evaluated previously. Consequently, it was necessary to evaluate plants derived from this process to check that this practice did not exacerbate any negative effects of the *in vitro* protocol. Based on the results from this study, it was observed that the NovaCane® (secondary) plants did not exacerbate any negative effects. Therefore, it is recommended to use this *in vitro* protocol. However, this *in vitro* protocol should only be used to produce virus free material for international germplasm exchange programs as it is a lengthy process because of the second meristem excision from *in vitro* plants.

The standard plant spacing used in commercial fields for planting seedlings is 50 cm. The results from the current study, showed that planting at closer (30 cm) or wider (50 cm) spacings did not have an effect on plant growth and to the response of varieties to the *in vitro* treatments. However, planting at closer spacing in commercial field's means more plants should be planted per hectare which imposes high costs. Therefore, it is recommended that TC plants continue to be propagated using wider (50 cm) plant spacings, as this is more economical.

4.5 References

Bailey, R.A. and Bechet, G.R., 1989. A comparison of seedcane derived from tissue culture with conventional seedcane. *Proceedings South African Sugar Technologists Association* 63, 125-129.

Brar, M.S., Moore, M.J., Al-Khayri, J.M., Morelock, T.E. and Anderson, E.J., 1999. Ethylene inhibitors promote *in vitro* regeneration of cowpea (Vigna unguiculata L.). *In Vitro Cellular and Developmental Biology-Plant* 35, 222-225.

Geijskes, R.J., Wang, L.I.F.A.N.G., Lakshmanan, P.R.A.K.A.S.H., McKeon, M.G., Berding, N., Swain, R.S., Elliott, A.R., Grof, C.P.L., Jackson, J.A., Smith, G.R. and Hogarth, D.M., 2003. SmartSett™ seedlings: tissue cultured seed plants for the Australian sugar industry. *Proceedings-Australian Society of Sugar Cane Technologists* 9, 13-17.

Goncalves, M.C., Pinto, L.R., Souza, S.C. and Landell, M.G.A., 2012. Virus diseases of sugarcane. A constant challenge to sugarcane breeding in Brazil. *Functional Plant Science and Biotechnology* 6, 108-116.

Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., Bekele, I. and Negi, T., 2016. Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture* 3, 1-4.

Ishida, B.K., 2000. Inhibitor-resistant early ethylene production during tomato fruit development. *Plant Physiology and Biochemistry* 38, 325-331.

Jalaja, N.C., Neelamathi, D. and Sreenivasan, T.V., 2008. Micropropagation for quality seed production in sugarcane in Asia and the Pacific. Food and Agriculture Organization of the United Nations, Rome; Asia–Pacific Consortium on Agricultural Biotechnology, New Delhi; Asia-Pacific Association of Agricultural Research Institutions, Bangkok, p. i-x + 46.

Lal, M., Tiwari, A.K. and Gupta, G.N., 2015. Commercial Scale Micropropagation of Sugarcane: Constraints and Remedies. *Sugar Tech* 17, .339-347.

Latche, A., Roustan, J.P. and Fallot, J., 1991. Stimulation of shoot regeneration from cotyledons of Helianthus annuus by the ethylene inhibitors, silver and cobalt. *Plant Cell Reports* 10, 204-207.

Lourens, A.G. and Martin, F.A., 1987. Evaluation of in vitro propagated sugarcane hybrids for somaclonal variation. *Crop Science* 27, 793-796.

Matsuoka, S. and Stolf, R., 2012. Sugarcane tillering and ratooning: key factors for a profitable cropping. *Sugarcane: Production, Cultivation and Uses* 5, 137-157.

McCormick, A.J., Cramer, M. and Watt, D.A., 2006. Sink strength regulates photosynthesis in sugarcane. *New Phytologist* 171, 759-770.

Meyer, J., Rein, P., Turner, P. and Mathias, K., 2011. Good Management Practices manual for the Cane Sugar Industry (Final). *PGBI Sugar and Bio Energy, Johannesburg, South Africa*, 334-366.

Mishra, S., Nailwal, T.K. and Pant, R.C., 2014. *In vitro* study of role of ethylene during tillering in sugarcane. *Sugar Tech* 16, 255-263.

Ramgareeb, S., Snyman, S.J., Van Antwerpen, T. and Rutherford, R.S., 2010. Elimination of virus and rapid propagation of disease-free sugarcane (Saccharum spp. cultivar NCo376) using apical meristem culture. *Plant Cell, Tissue and Organ Culture* 100, 175-181.

Roach, B.T., 1976. Observations on the effect of row spacing on sugar cane yield. *Proceedings Qd Society Sugar Cane Technology* 43, 95-101.

Roodagi, L.I., Itnal, C.J. and Khandagave, R.B., 2000. Influence of planting system and intercrops on sugarcane tillering and yield. *Indian Sugar* 50, 605-609.

Sawant, R.A., Tawar, P.N., Meti, N.T. and Ranjekar, P.K., 2014. Role of Sugarcane Micropropagation for Production of Quality Seed. *International Journal of Recent Biotechnology* 2, 34-41.

Snyman, S., van Antwerpen, T., Ramgareeb, S. and McFarlane, S., 2012. Sugarcane pathogens: detection and elimination through *in vitro* culture. *Functional Plant Science and Biotechnology* 6, 12-18.

Taghizadeh, M. and Ehsanpour, A., 2013. The *in vitro* effects of $CoCl_2$ as ethylene synthesis inhibitor on PI based protein pattern of potato plant (Solanum tuberosum L.). *Journal of Cell and Molecular Research* 5, 42-46.

Waclawovsky, A.J., Sato, P.M., Lembke, C.G., Moore, P.H. and Souza, G.M., 2010. Sugarcane for bioenergy production: an assessment of yield and regulation of sucrose content. *Plant Biotechnology* 8, 263-276.

Yadav, S., Ahmad, A., Rastogi, J. and Lal, M., 2012. Tissue culture strategies in sugarcane (Saccharum officinarum L.). *International Journal of Pharmaceutical and Biological Sciences* 3, 427-441.

Zhou, M., 2013. Conventional sugarcane breeding in South Africa: Progress and future prospects. *American Journal of Plant Sciences* 4, 189- 196.

CHAPTER 5 : AGRONOMIC COMPARISONS OF SUGARCANE VARIETIES BASED ON PROPAGATION SOURCES AND PLANTING RATES UNDER RAINFED CONDITIONS IN STAGE 11

Abstract

Tissue culture (TC) plants (stage 1) are known to possess an altered phenotype (high tillering and thin stalks) compared with conventionally (Con) derived plants. At stage 2, when stalks derived from TC are used for planting commercial fields, it is commonly assumed that the TC plants will resemble conventional plants for all traits. However, studies comparing the growth and yield performance of stage 2 TC plants with Con are limited. Therefore, this study aimed at comparing the performance of stage 2 TC-derived plants with Con derived plants at different planting rates.

A field trial was established under rainfed conditions at the South African Sugarcane Research Institute (SASRI's) Mount Edgecombe experiment station. The randomised block experiment with five replications consisted of the three most contrasting varieties (N12, N41 and N48) from the first experiment. The seedcane for the present experiment was derived from the corresponding treatments in the first experiment, which were: 1) stalks derived from TC50 and planted at lower planting rate (TC_{50} L); 2) stalks derived from TC50 and planted at higher planting rate (TC_{50} H); 3) stalks derived from TC30 and planted at lower planting rate (TC_{30} L); 4) stalks derived from TC30 and planted at higher planting rate (TC_{30} H); and 5) stalks derived from conventional and planted at normal planting rate (Con). The lower planting rate was a one and the half stalk, the normal planting rate was a double stalk, and the higher planting rate was triple stalk. Yield and yield component measurements were taken at harvest and data were analysed by ANOVA.

The crop derived from TC significantly improved cane yield and TERC compared with Con when averaged over varieties and planting rates. The $TC_{50}H$ and $TC_{30}L$ treatments had a significantly greater TERC compared with the Con treatment for variety N41. The treatments derived from TC had a significantly higher mean stalk population compared with the Con treatment. This was observed for varieties N12 and N41 in particular. The stalks grown from TC had a significantly lower mean stalk diameter compared with those from Con. The $TC_{50}H$ treatment produced stalks that were significantly thinner compared with the Con treatment for variety N12. The TC treatments had a significantly higher mean stalk height compared with the Con treatment.

The lower vs higher planting rates and TC30 vs TC50 seed source did not differ significantly for all parameters. The seedcane derived from the TC at stage 1 can be used as planting material for commercial production without any negative effects on productivity. This is despite persistence of the phenotype with reduced stalk diameter and higher stalk population. The lack of differences between lower and higher planting rates in this study suggests that lower planting rates may be more economical, without negative effects on growth and productivity.

5.1 Introduction

Germination of sugarcane is described as the vegetative initiation of growth from buds found on stalks. Each viable bud of seedcane produces a primary shoot which develops into a stalk. After the period of dormancy has passed, the buds start forming new shoots. There are numerous internal and external factors that can influence germination of buds (Singh, 2015). The internal factors include sett nutrient status, sett moisture and bud health, while external factors are aeration, soil temperature and soil moisture (Singh et al., 2007). If any of these factors are limiting, poor stalk emergence in the field occurs which results in yield loss. This yield loss is associated with the limited number of stalks that are produced due to poor emergence. Numerous studies have been undertaken to evaluate the correlation between stalk population and cane yield. According to James (1971), cane yield can be affected by stalk thickness, stalk length, and stalk density. However, all these components are influenced by stalk population which determines cane yield per unit area (Ayele et al., 2012). The choice of variety, cultural practices and planting rate can be used to modify stalk population per unit area (Kanwar and Sharma, 1974).

Studies have been done on increasing stalk population by increasing planting rates, however such studies are limited. According to Orgeron et al. (2007), using 3 or 4 stalks planting rates resulted in achieving maximum sugar yield, cane yield and stalk population compared with the 2 stalks planting rate. However, the 2-stalk planting rate produced highest mean stalk weight indicating a compensatory correlation between stalk population and stalk weight. Matherne (1971), compared two planting rates of one and a half (1 ½) and two stalks under rainfed conditions. The results from this study showed that planting rate of 2 stalks had significantly higher stalk population and cane yield compared with the 1 ½ stalk planting rate. However, there were no statistical differences between stalk length and single stalk weight between the treatments. The studies that have been undertaken to evaluate the effect of planting rates on sugarcane yield are voluminous and often conflicting.

More than 40 years ago, *in vitro* studies of sugarcane were initiated with the intention of producing diseases-free plantlets and enhancing multiplication rates (Heinz and Mee, 1969; Tiel et al., 2006; Ntoyi et al., 2007). The recent use of tissue cultured (TC) plants as a means of propagation have been promising. Large quantities of planting material have been produced within a short period of time when using the TC technique (Fitch et al., 2001). With the TC method, stage 1 is referred to as the production of actual TC plants under laboratory conditions, and their subsequent hardening off and planting into field nurseries.

Stage 2 and stage 3 then progress in a similar manner to conventional method (Snyman et al., 2009; Ramgareeb et al., 2010). This method has been commonly used in many industries. A handful of studies have compared growth and development of the TC plants to plants derived from conventional (Con) methods. According to Watt et al. (2009), stage 1 TC plants are generally known to produce thinner stalks and higher stalk population compared with the Con plants. Some studies suggest that the differences in stalk population and stalk diameter between the TC and the Con plants fall away in subsequent ratoon crops (Burner and Grisham, 1995). At stage 2, when the TC derived stalks are used for establishment, it is commonly assumed that there would be no agronomic differences from the Con plants. Information on the growth and yield performance of stage 2 TC stalk is important, as such stalks are routinely planted out commercially. However, studies on the growth characteristics and cane yield performance of stage 2 TC are limited. Therefore, the present study is aimed at comparing the performance of seedcane obtained from stage 1 tissue culture plants and from conventional setts. The study is also aimed at determining the effects of different planting rates on performance of TC-derived plants under rainfed conditions.

5.2 Materials and Methods

5.2.1   Experimental design and treatments

A field trial was established under rainfed conditions at the South African Sugarcane Research Institute (SASRI's) Mount Edgecombe experiment station which is located 29° 42' 24S, 31° 1' 30E and 108 m above sea level. This site represented the coastal growing region of the South African sugar industry. The field trial was established in 2016 as a randomised block design with five replications (Figure 5.2). Of the four varieties tested in the first experiment, three most contrasting varieties (in term of population and stalk characteristics) were carried forward to plant this experiment. These varieties were:

- N12 (high stalk population, average diameter, moderate canopy),
- N41 (low population, thin diameter, sparse canopy),
- N48 (very low population, very thick diameter, dense canopy)

The varieties were chosen based on their contrasting growth characteristics ranging from high stalk population to low stalk population. The varieties were also chosen based on their stalk diameter characteristics ranging from thin to thick stalks. The seedcane for this second experiment was derived from the corresponding treatments in the first experiment. The SP50 treatment was excluded. The treatments for the second experiment were:

- $TC_{50}L$ – stick derived from TC_{50} and planted at lower planting rates
- $TC_{50}H$ – Stick derived from TC_{50} and planted at Higher planting rates
- $TC_{30}L$ – stick derived from TC_{30} and planted at lower planting rates
- $TC_{30}H$ – stick derived from TC_{30} and planted at Higher planting rates
- Con – Stick derived from conventional and planted at Normal planting rates

The Lower (L) planting rates was a one and the half stick, Normal (Con) planting rates was a double stick, and Higher (H) planting rates was a triple stick (Figure 5.1).
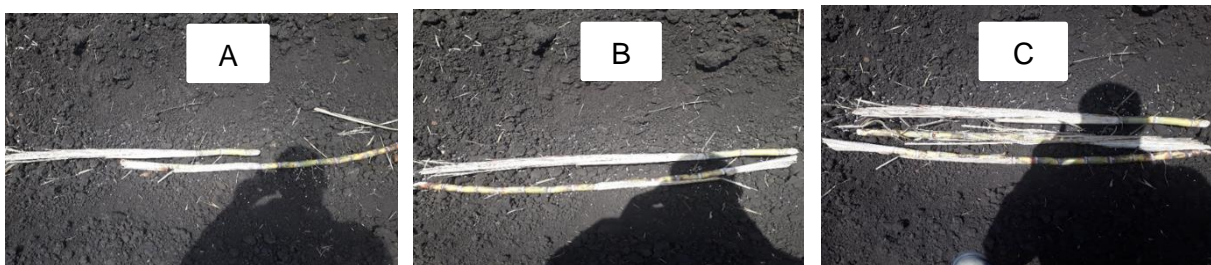
Figure 5.1 The low (A), normal (B) and high (C) planting rates used in this experiment.

The above resulted in a total of 15 treatment combinations, which are shown in Table 5.1. Each experimental plot consisted of six rows, 8 m long (nett plots of four rows) at inter-row spacings of 1.2m.

Table 5.1 The 15 treatment combination

Conventional	Tissue culture (TC)			
	TC30		TC50	
Normal (Con)	Lower (L)	Higher (H)	Lower (L)	Higher (H)
N12 Con	N12 TC30 L	N12 TC30 H	N12 TC50 L	N12 TC50 H
N41 Con	N41 TC30 L	N41 TC30 H	N41 TC50 L	N41 TC50 H
N48 Con	N48 TC30 L	N48 TC30 H	N48 TC50 L	N48 TC50 H

1 N12 TC50 L	2 N48 TC30 L	3 N41 TC50 H	4 N12 Con	5 N48 TC50 H	6 N41 TC30 L	7 N12 TC30 H
14 N48 TC50 L	13 N12 TC30 L	12 N41 Con	11 N48 Con	10 N12 TC50 H	9 N41 TC50 L	8 N48 TC30 H
15 N41 TC30 H	16 N41 TC50 L	17 N12 TC50 H	18 N48 TC30 L	19 N41 Con	20 N12 TC30 L	21 N48 TC50 L
28 N41 TC50 H	27 N48 Con	26 N12 TC30 H	25 N41 TC30 L	24 N48 TC30 H	23 N12 Con	22 N41 TC30 H
29 N12 TC50 L	30 N48 TC50 H	31 N12 TC50 H	32 N41 Con	33 N48 TC30 L	34 N48 TC50 H	35 N12 TC50 L
42 N41 TC30 L	41 N12 Con	40 N48 TC50 L	39 N41 TC50 L	38 N12 TC30 H	37 N41 TC30 H	36 N48 TC30 H
43 N48 Con	44 N12 TC30 L	45 N41 TC50 H	46 N48 Con	47 N41 Con	48 N12 Con	49 N48 TC50 L
56 N12 TC50 L	55 N48 TC50 H	54 N41 TC50 H	53 N12 TC30 H	52 N48 TC30 H	51 N41 TC50 L	50 N12 TC50 H
57 N41 TC30 H	58 N12 TC30 L	59 N48 TC30 L	60 N41 TC30 L	61 N41 TC30 H	62 N12 TC30 L	63 N48 TC50 L
70 N41 TC50 L	69 N48 TC30 H	68 N12 TC30 H	67 N41 TC30 L	66 N48 Con	65 N12 TC50 H	64 N41 Con
71 N12 Con	72 N48 TC50 H	73 N41 TC50 H	74 N12 TC50 L	75 N48 TC30 L	EXTRA PLOT N41 TC30 L	EXTRA PLOT N41 TC 30 L

→ Row direction →

Figure 5.2 Trial plan, showing the different treatment combinations and their randomisation. Different shaded areas represent replicates (5).

5.2.2　Measurement during crop growth

5.2.2.1 Stalk population and heights

The stalk population and heights measurements were done once a month. The stalk height measurement was done by selecting 20 stalks randomly per plot on the centre rows (row 3 and 4) which are 10 m by 1.2 m spacing in size. A tape measure was used to measure the heights of 20 selected plants from the soil surface to the top visible dewlap (TVD), which is the collar of the uppermost, fully expanded leaf. The stalk population was measured per plot by manually counting all the number of stalks per 10 m length on the centre rows (row 3 and 4), and thereafter expressed on a per hectare basis.

5.2.2.2 Stalk diameter

The measurements of stalk diameter were done once a month. In each plot 10 randomly selected stalks of the nett rows (row 2, 3, 4 and 5) were used to measure stalk diameter using a vernier caliper (ABS Digital Caliper DCA 150; Oxford Precision, UK). The caliper was placed perpendicular to the middle of the stalks before readings were taken.

5.2.2.3 Leaf length and width

The leaf length and width measurements were taken on a monthly basis. These measurements were done on the TVD leaf of 10 selected stalks of the nett rows (row 2, 3, 4 and 5). The leaf length was measured from the stalk (where the leaf is attached) to the tip of the leaf using a tape measure. The leaf width was measured at the middle of the TVD leaf.

5.2.3　Measurements at harvest

At harvest, cane yield was measured by cutting and measuring the weight of the stalks (bundles) of the nett rows (row 2, 3, 4 and 5) in each plots using a scale attached to a hydraulic weighing boom. An African sugar-cane borer (*Eldana saccharina L.*) survey was done on 20 randomly selected stalks per plot and the procedure is described in chapter 3. A sample of 12 stalks per plot was taken from the nett rows (row 2, 3, 4 and 5) and submitted to the SASRI mill room to determine the estimated recoverable crystal percent (ERC %) which was used to estimate the quantity of sucrose in cane that was recovered as sugar.

5.2.4 Statistical analysis

All data collected was statistically analysed using GenStat 18th edition and Microsoft Office Excel was used to process collected data from the trial. Growth and yield variables were subjected to analysis of variance (ANOVA) to establish main (propagation source, variety, planting rate and TC source) and interaction effects. The factors variety (N12, N41 and N48), planting rate (Low vs. High), and TC source (TC30 vs. TC50) were "nested" within the factor called propagation source (TC derived planting material vs. conventional stalk-derived planting material). This treatment structure was chosen to account for the imbalance in the design (the Con treatment could only be planted at one planting rate), and to investigate the overall main effect of the seedcane source (TC vs stalk-derived). Comparison of means was performed using Fisher's protected LSD test at 5% significance difference. Significant differences between treatments at selected dates for in-season growth were represented by LSD bars (5%).

5.3 Results

5.3.1 Yield and quality traits

Propagation source (Prop) had a highly significant ($p \leq 0.001$) effect on cane yield and TERC (Table 5.2). Variety (V) had a highly significant ($p \leq 0.001$) effect on ERC %. The TC source (S) and planting rate (PR) was not significant for any trait. The V x S, V x PR, S x PR and the three-way V x S x PR interaction was not significant for any trait. The responses of each trait are discussed in more detail in figures 5.3 – 5.7 below.

Table 5.2 ANOVA for yield and quality traits (cane yield, estimated recoverable crystal (ERC %), ERC yield (TERC) and fibre content (FIB)).

Source of variations	D.F	Cane yield M.S	Cane yield F-value	ERC % M.S	ERC % F-value	TERC M.S	TERC F-value	FIB M.S	FIB F-value
Propagation source (Prop)	1	1340.90	7.91^{**}	0.57	0.94^{NS}	29.01	10.89^{**}	0.03	0.02^{NS}
Variety (V) (within Prop)	4	49.60	0.29^{NS}	10.85	17.91^{**}	6.14	2.30^{NS}	2.81	2.40^{NS}
TC Source (S) (within Prop)	1	1.70	0.01^{NS}	0.17	0.27^{NS}	0.04	0.01^{NS}	0.42	0.36^{NS}
Planting rate (PR) (within Prop)	1	125.00	0.74^{NS}	0.07	0.12^{NS}	2.92	1.10^{NS}	0.05	0.04^{NS}
V. S (within Prop)	2	18.50	0.11^{NS}	0.43	0.71^{NS}	0.26	0.44^{NS}	2.47	2.11^{NS}
V. PR (within Prop)	2	56.10	0.33^{NS}	0.47	0.77^{NS}	0.26	0.10^{NS}	0.01	0.01^{NS}
S. PR (within Prop)	1	2.60	0.02^{NS}	0.36	0.60^{NS}	0.03	0.01^{NS}	0.82	0.70^{NS}
V.S.PR (within Prop)	2	72.00	0.42^{NS}	0.05	0.09^{NS}	1.68	0.63^{NS}	0.20	0.17^{NS}
Replications	4	528.60	3.12	0.46	0.77	9.68	3.63	1.57	1.34
Error	56	169.50		0.61		2.67		1.17	

^{NS} not significant ($p > 0.05$) *significant ($p < 0.05$) ** highly significant ($p \leq 0.001$)

Squares indicate significant F-values, for easy reference

In general, treatments derived from TC in stage 1 (averaged over TC source and planting rate) significantly improved cane yields and TERC compared with conventional stick-derived material (Figure 5.3). There were no significant differences in mean ERC % and fibre content between Con and TC treatments (not shown).
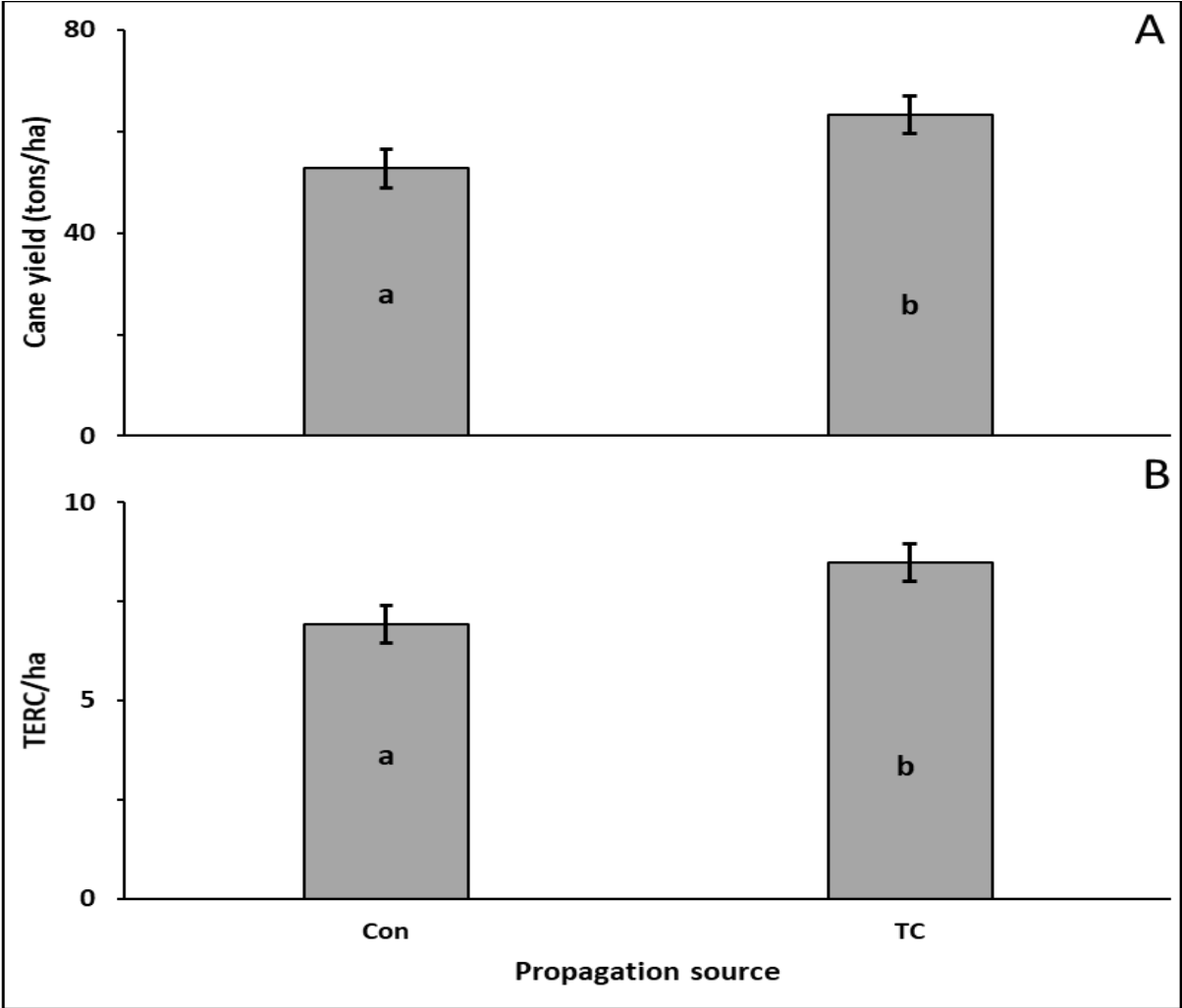
Figure 5.3 Mean cane yield (A) and TERC (B) of three varieties (N12, N41 and N48) when propagated using stick derived conventionally (Con) or from tissue culture (TC). Significant differences are reflected by differing letters and LSD bars (5 %).

Cane yield: For all varieties, there were no significant differences in cane yield between the individual treatment combinations (Figure 5.4). However, the Con treatment produced lower (not significant) cane yield compared with the other treatments in general. As a group, the TC treatments did significantly improve cane yield over the Con treatment (Figure 5.3A)
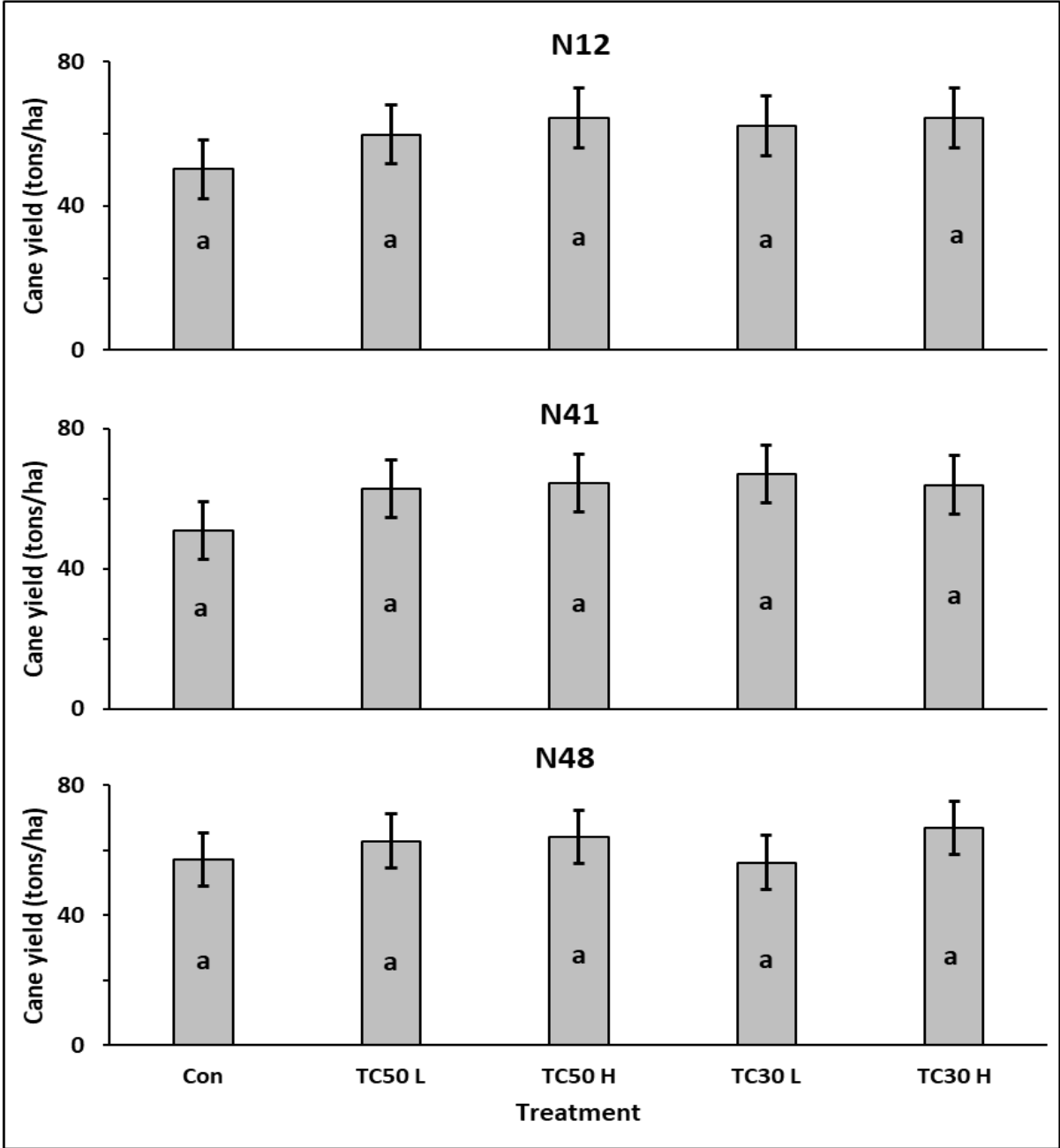
Figure 5.4 Cane yield of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Estimated recoverable crystal percentage (ERC %): There were no significant differences in ERC % between the treatments for all varieties (Figure 5.5). The $TC_{50}H$ treatment had a greater (not significant) ERC % compared with the other treatments for variety N41.
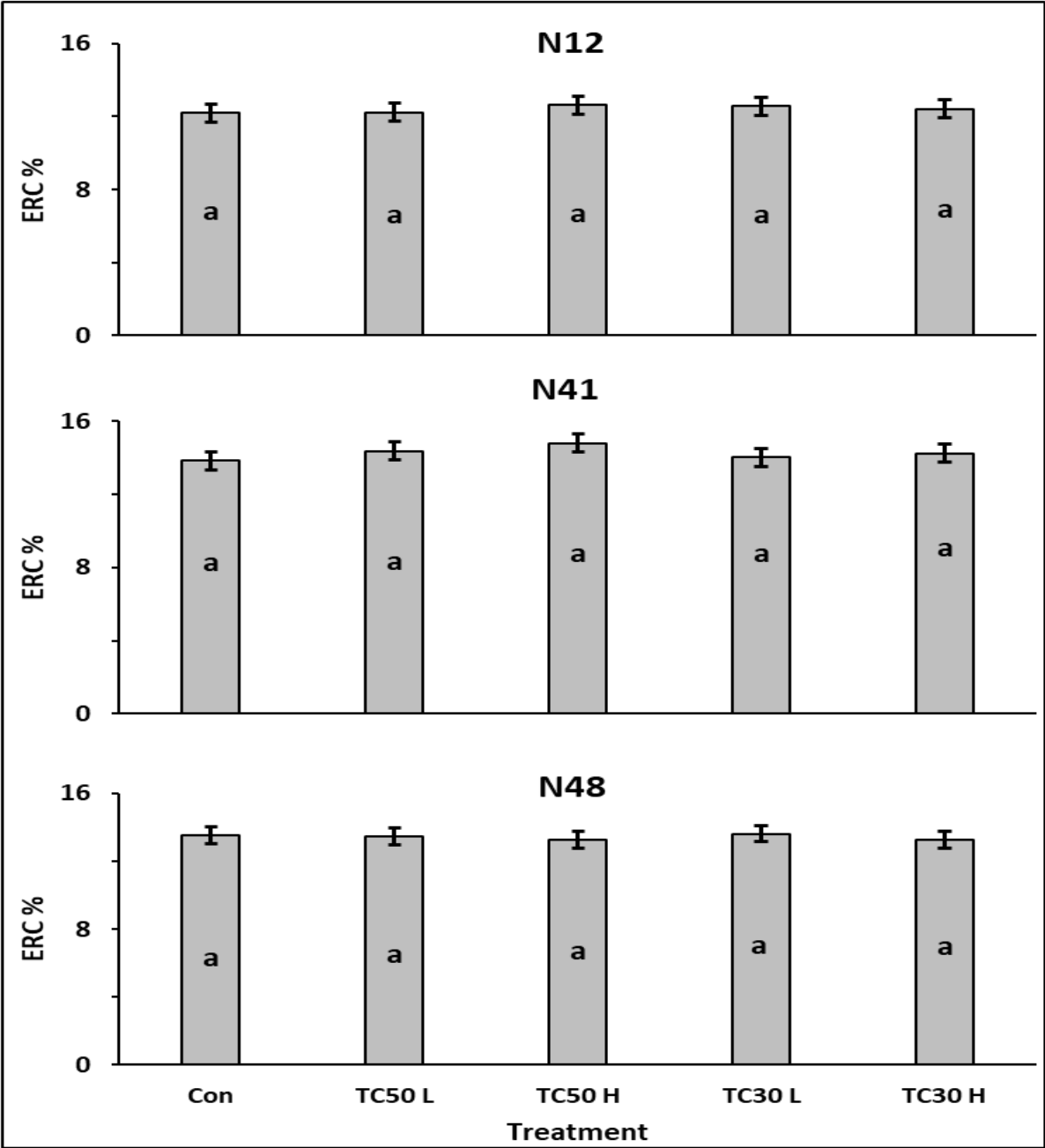
Figure 5.5 Estimated recoverable crystal percentage (ERC %) of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

ERC yields (TERC): The Con treatment had a significantly lower TERC compared with TC$_{50}$H and TC$_{30}$L treatments for variety N41 (Figure 5.6). For varieties N12 and N48, there were no significant differences in TERC between the treatments.
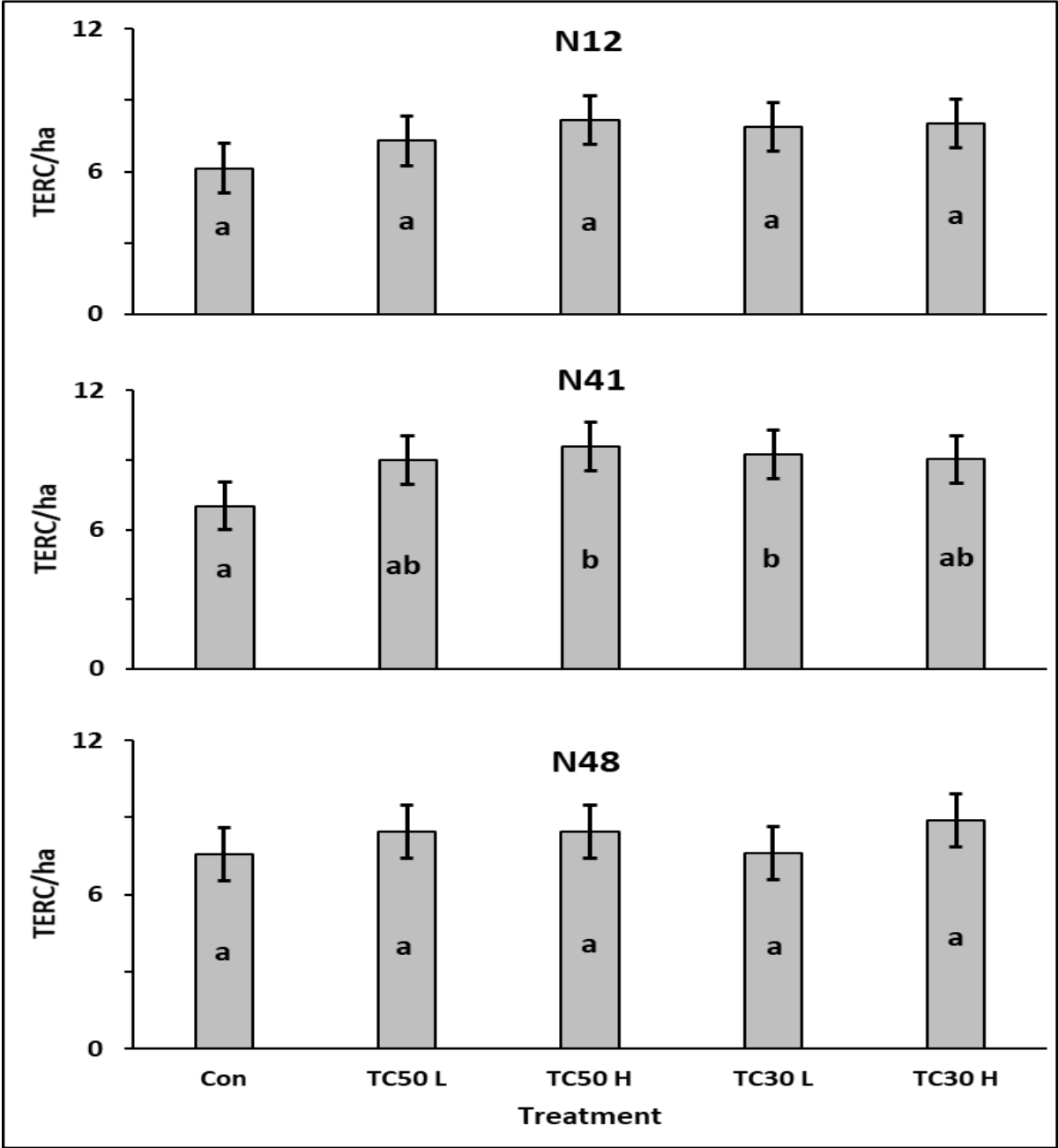
Figure 5.6 Estimated recoverable crystal yields (TERC) of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Fibre content: For all varieties, there were no significant differences in fibre content between the treatments (Figure 5.7). The Con treatment produced higher (not significant) fibre content compared with $TC_{50}L$ and $TC_{50}H$ treatments for variety N12.
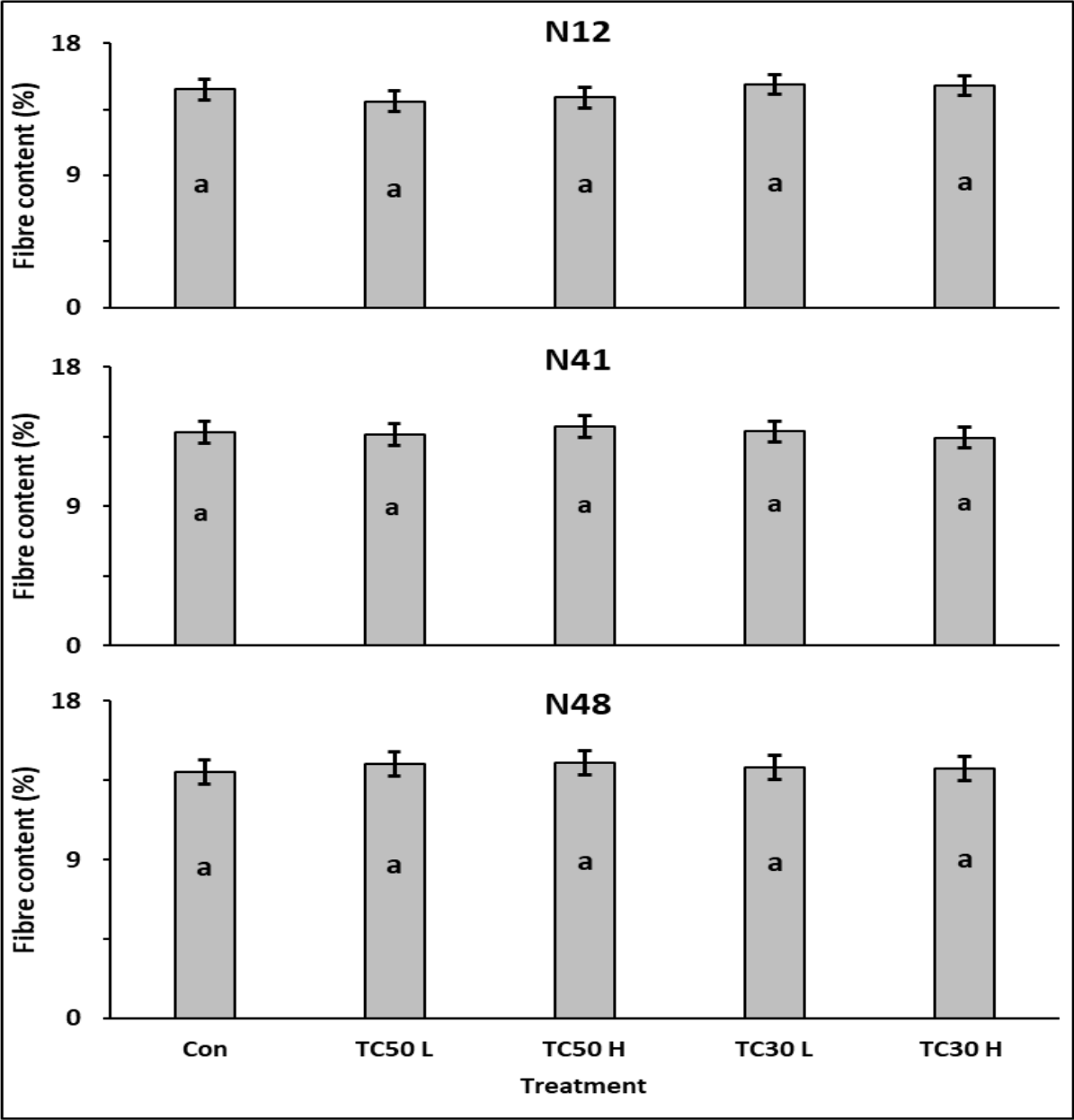
Figure 5.7 Fibre content of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

5.3.2 Yield components

Propagation source (Prop) had a highly significant ($p\leq0.001$) effect on stalk population and a significant ($p<0.05$) effect on stalk diameter and stalk height (Table 5.3). Variety (V) had a highly significant effect on stalk population, stalk diameter, stalk height and stalk mass. The TC source (S) and planting rate (PR) was not significant for any trait. The V x S, V x PR, S x PR and the three-way V x S x PR interactions were also not significant for any trait. The responses of each trait are discussed in more detail in figures 5.8 – 5.17 below.

Table 5.3 ANOVA table for yield components (stalk population, stalk diameter, stalk height and stalk mass) at harvest.

Treatments	D.F	Stalk population		Stalk diameter		Stalk height		Stalk mass	
		M.S	F-value	M.S	F-value	M.S	F-value	M.S	F-value
Propagation source (Prop)	1	2.89^{x09}	21.23^{**}	10.67	5.89^{*}	1296.20	4.27^{*}	1151	0.14^{NS}
Variety (within Prop)	4	1.00^{X10}	73.62^{**}	45.75	25.23^{**}	2455.10	8.08^{**}	123860	14.56^{**}
TC source (S) (within Prop)	1	1.09^{x08}	0.80^{NS}	0.92	0.51^{NS}	35.90	0.12^{NS}	2210	0.26^{NS}
Planting rate (PR) (within Prop)	1	2.33^{x08}	1.71^{NS}	0.88	0.48^{NS}	36.40	0.12^{NS}	4	0.00^{NS}
V. S (within Prop)	2	3.26^{x06}	0.02^{NS}	2.04	1.12^{NS}	121.20	0.40^{NS}	12032	0.41^{NS}
V. PR (within Prop)	2	8.39^{x07}	0.62^{NS}	0.35	0.19^{NS}	11.20	0.04^{NS}	727	0.09^{NS}
S. PR (within Prop)	1	9.25^{x07}	0.68^{NS}	0.13	0.07^{NS}	79.20	0.26^{NS}	52	0.01^{NS}
V.S.PR (within Prop)	2	4.77^{x07}	0.35^{NS}	8.94	4.93^{NS}	244.8	0.80^{NS}	189	0.02^{NS}
Replications	4	4.01^{x08}	2.94	0.26	0.14	781.30	2.57	15647	1.84
Error	56	1.36^{x08}		1.81		303.90		8507	

^{NS} not significant ($p>0.05$) *significant ($p<0.05$) **highly significant ($p\leq0.001$)

Squares indicate significant F-values, for easy reference

The Con had a significantly lower mean stalk population and stalk height compared with TC as a group (Figure 5.8). The Con had a significantly greater mean stalk diameter compared with the TC. There were no significant differences in mean stalk mass between the Con and TC treatments (not shown).
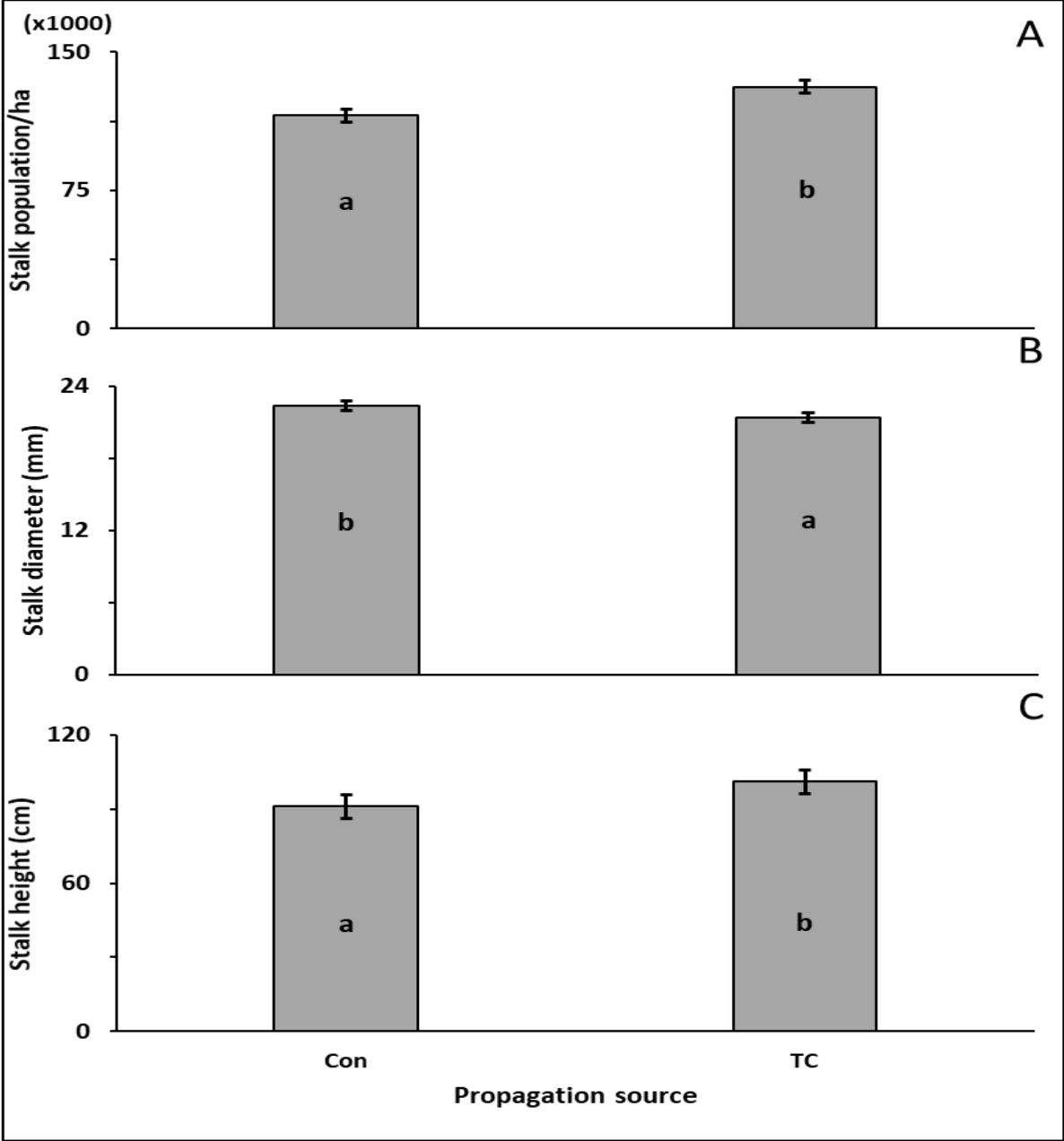
Figure 5.8 Mean stalk population (x1000) (A), stalk diameter (B) and stalk height (C) of three varieties (N12, N41 and N48) when propagated using stick derived conventionally (Con) or from tissue culture (TC). Significant differences are reflected by differing letters and LSD bars (5 %).

Stalk population: The Con treatment produced significantly fewer stalks compared with the other treatments for variety N12 (Figure 5.9). The Con treatment produced significantly fewer stalks compared with TC_{30} H treatment for variety N41. For variety N48, there were no significant differences in stalk population between the treatments.

Figure 5.9 Stalk population (x1000) of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Stalk diameter: The Con treatment produced stalks that were significantly thicker compared with TC_{50} H treatment for variety N12 (Figure 5.10). For varieties N41 and N48, there were no significant differences in stalk diameter between the treatments.

Figure 5.10 Stalk diameter of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Stalk height: There were no significant differences in stalk height between the treatments for all varieties (Figure 5.11). The Con treatment produced shorter (not significant) stalks compared with the other treatments for varieties N12 and N41.

Figure 5.11 Stalk height of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

Stalk mass: There were no significant differences in stalk mass between the treatments for all varieties (Figure 5.12). The Con treatment produced stalks that were lighter (not significant) compared with TC_{50} L and TC_{50} H treatments for variety N48.

Figure 5.12 Stalk mass of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences across all treatment combinations are reflected by differing letters and LSD bars (5 %).

5.3.3 In season growth measurements

Stalk population: The TC$_{30}$ H and TC$_{50}$ H treatments had a higher number of stalks compared with the other treatments at the beginning of the season for all varieties (Figure 5.13). At the end of the season, the differences in stalk population between the treatments was much smaller for varieties N41 and N48. The Con treatment had a tendency of producing fewer stalks compared than the other treatments throughout the season for varieties N12 and N48.
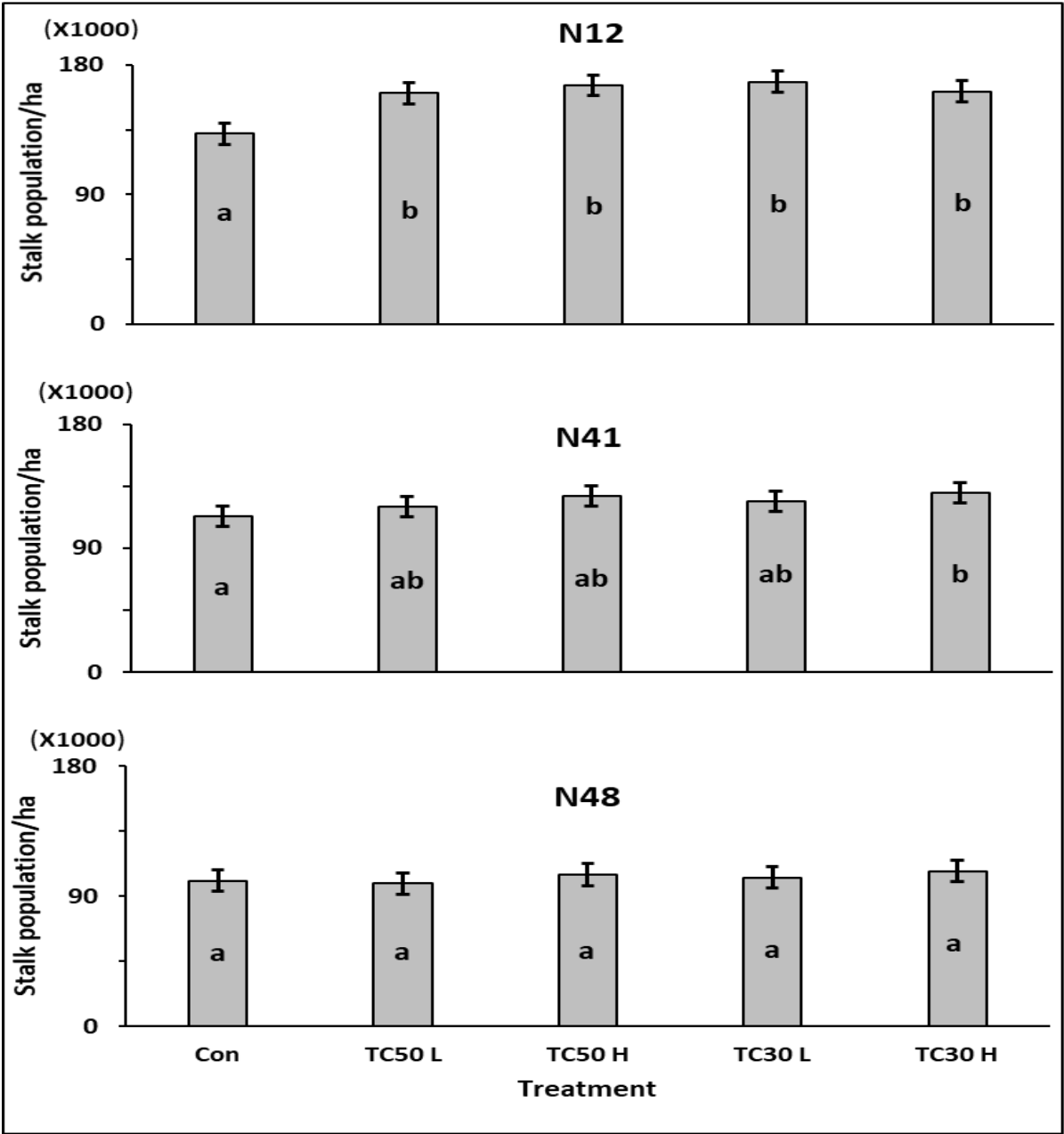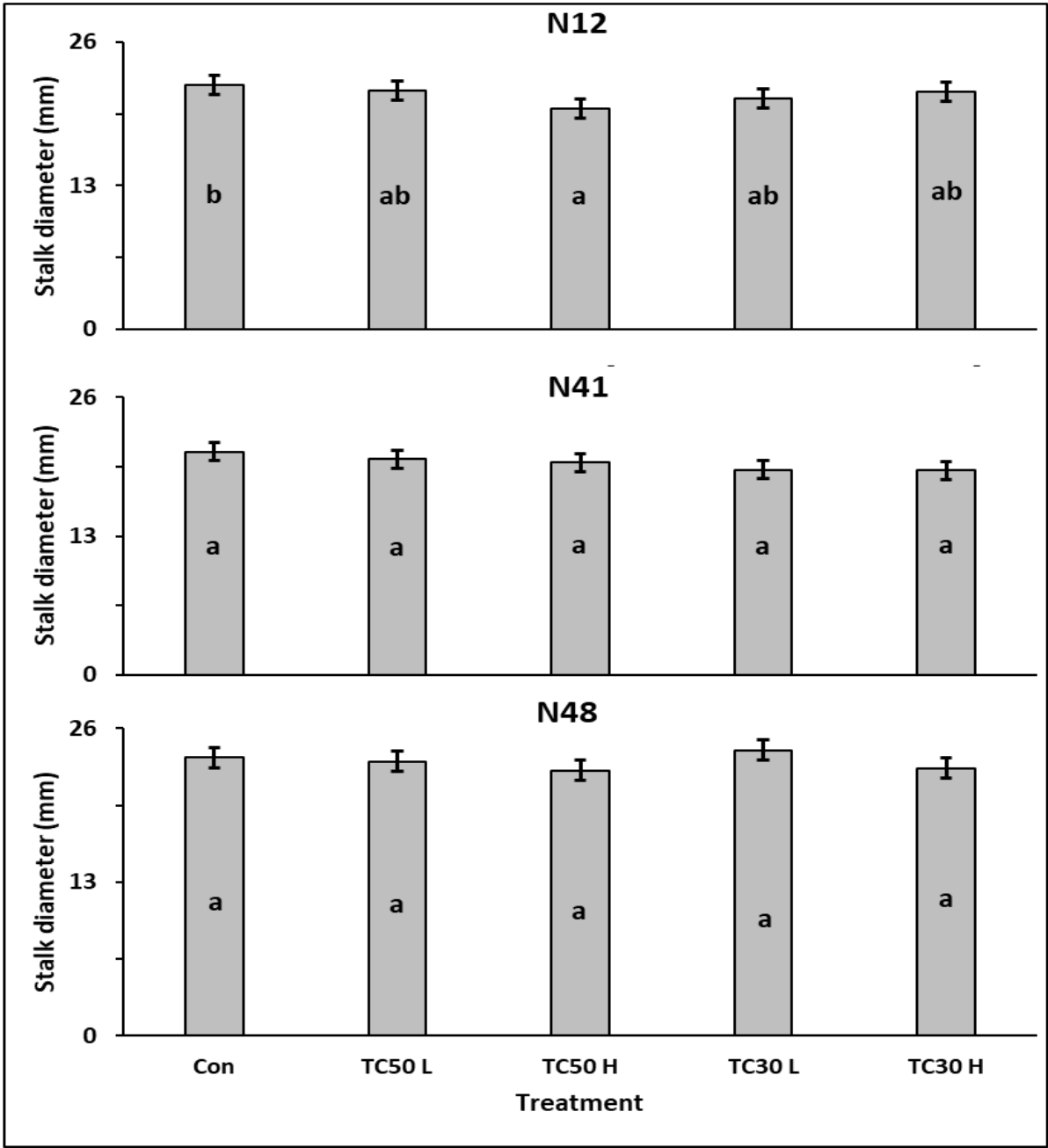

Figure 5.13 Stalk population of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk diameter: The Con treatment had a tendency of producing thicker stalks compared with the other treatments throughout the season for varieties N12 and N41 (Figure 5.14). The differences in stalk diameter between the seedcane obtained from the TC (TC_{30} and TC_{50}) plants was much smaller at the end of the season for all varieties.
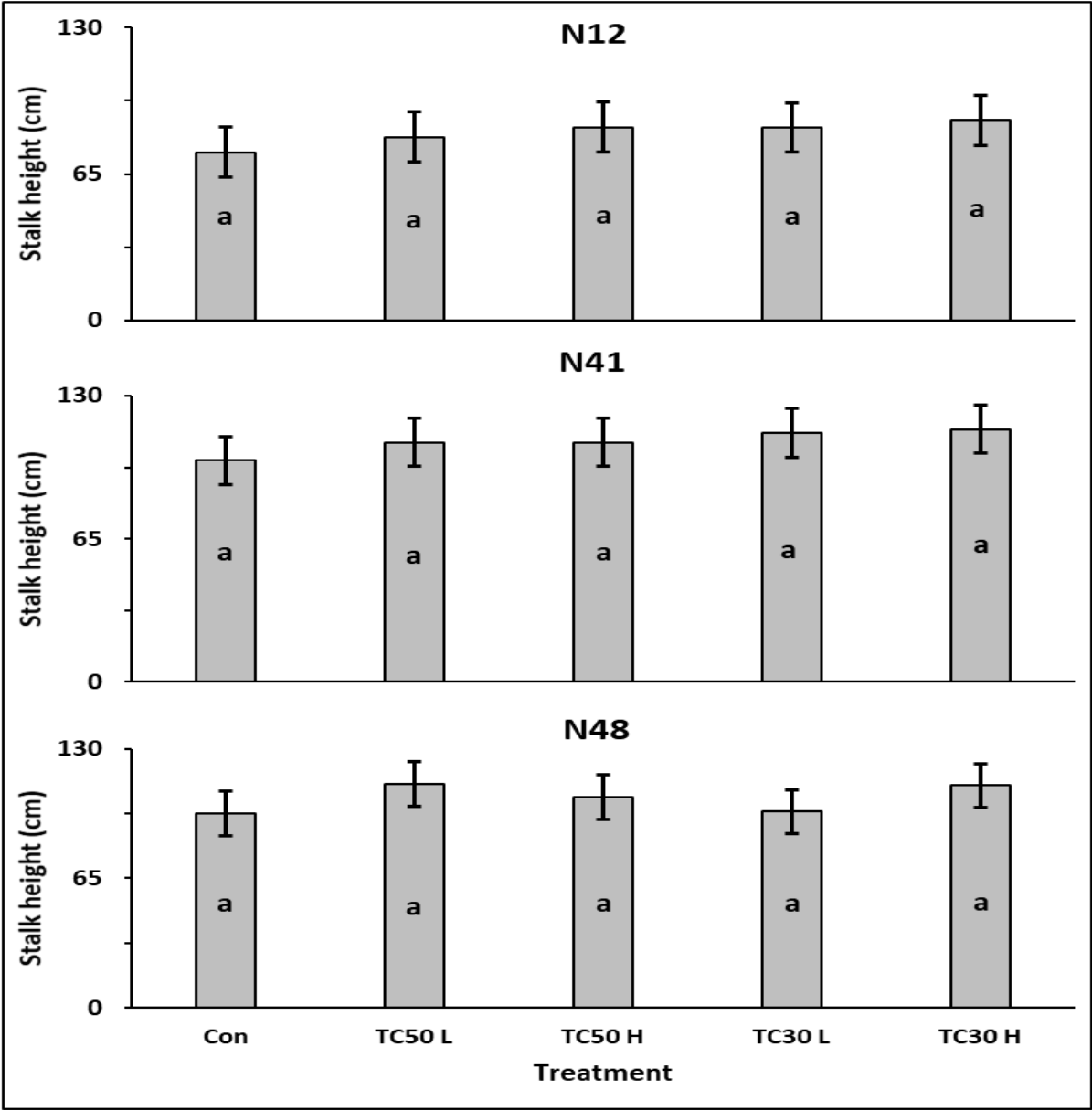

Figure 5.14 Stalk diameter of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences between treatments at selected dates are represented by LSD bars (5%).

Stalk height: The Con treatment had a tendency of having shorter stalks compared with the other treatments throughout the season for all varieties (Figure 5.15). At the end of the season, the TC_{30} H treatment had taller stalks (not significant) compared with the other treatments for varieties N12 and N48.

Figure 5.15 Stalk height of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences between treatments at selected dates are represented by LSD bars (5%).

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Leaf length: There were no significant differences in leaf length between the treatments throughout the season for all varieties (Figure 5.16). The Con treatment had a tendency of having shorter leaves compared with the other treatments at the end of the season for variety N12.
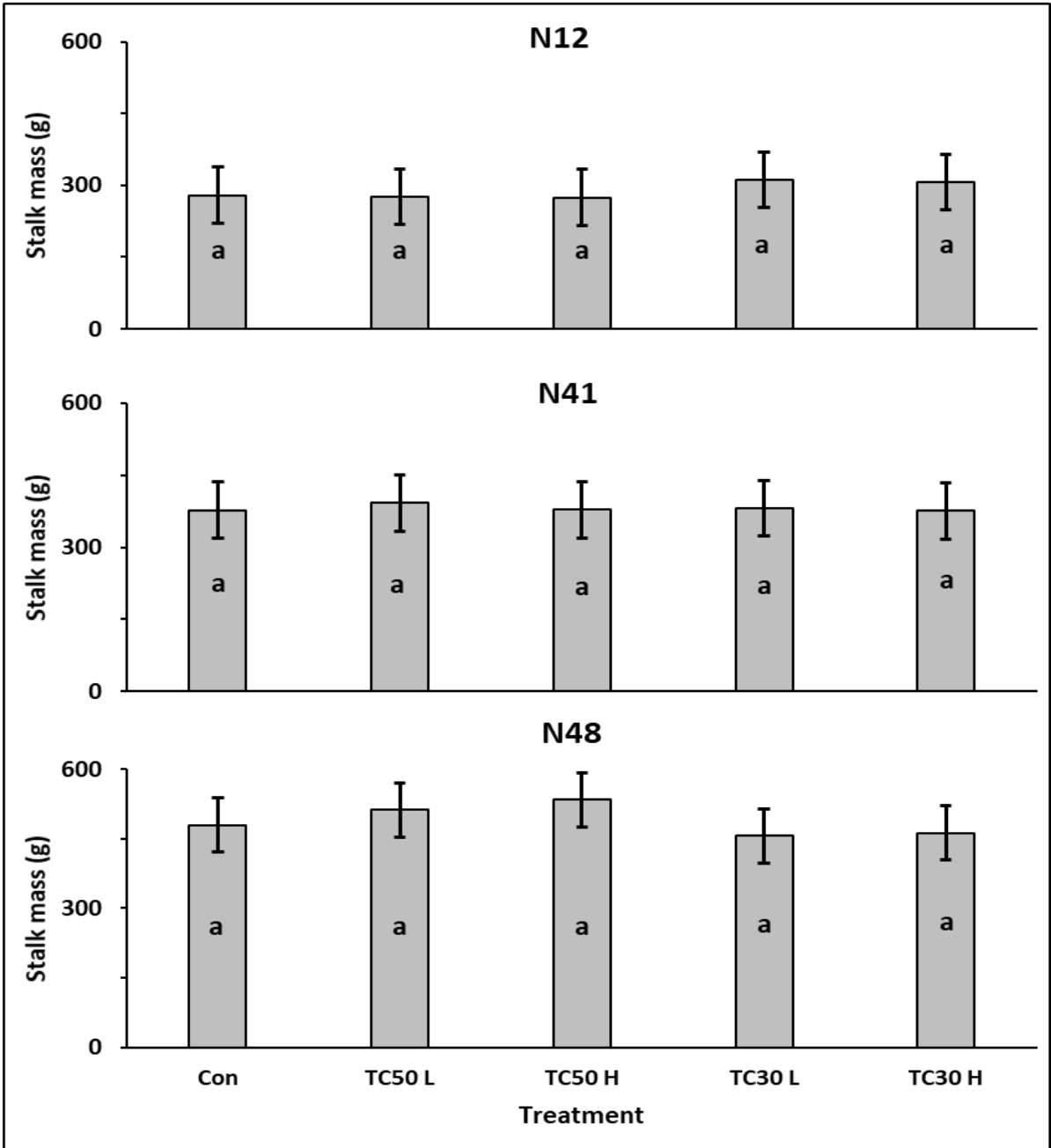

Figure 5.16 Leaf length of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences between treatments at selected dates are represented by LSD bars (5%).

Leaf width: The Con treatment had wider leaves (not significant) compared with the other treatments at the end of the season for varieties N41 and N48 (Figure 5.17).
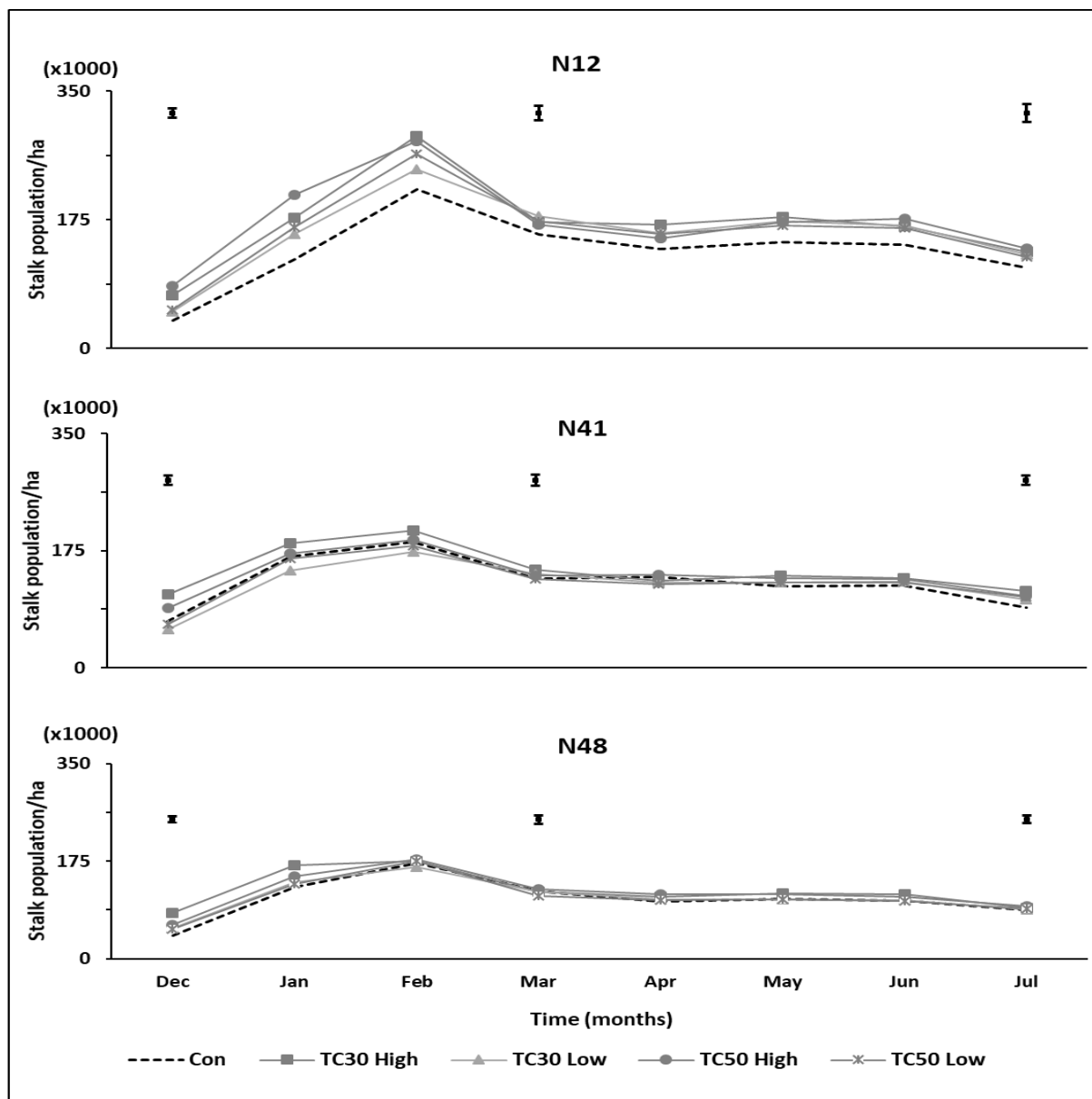

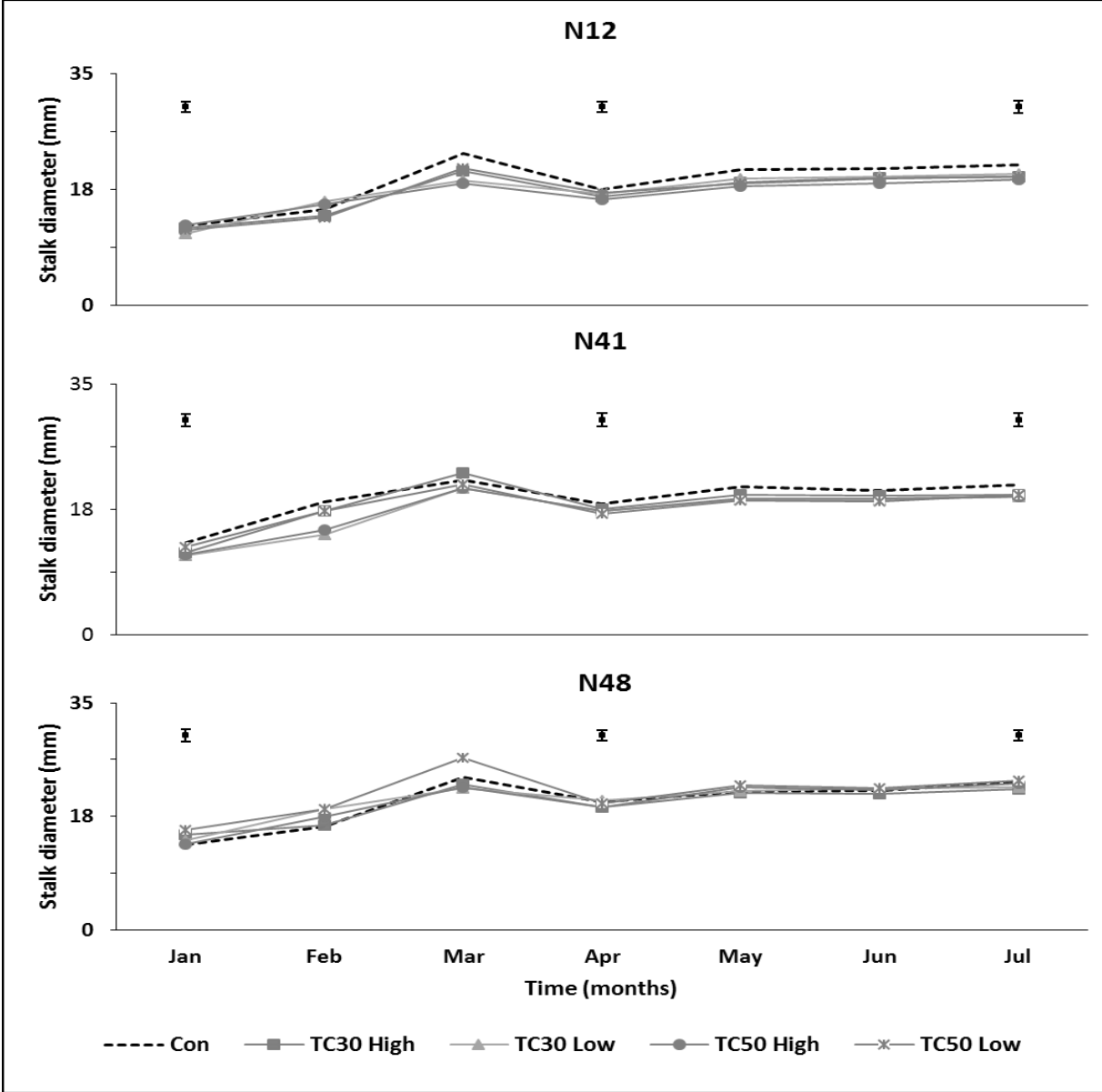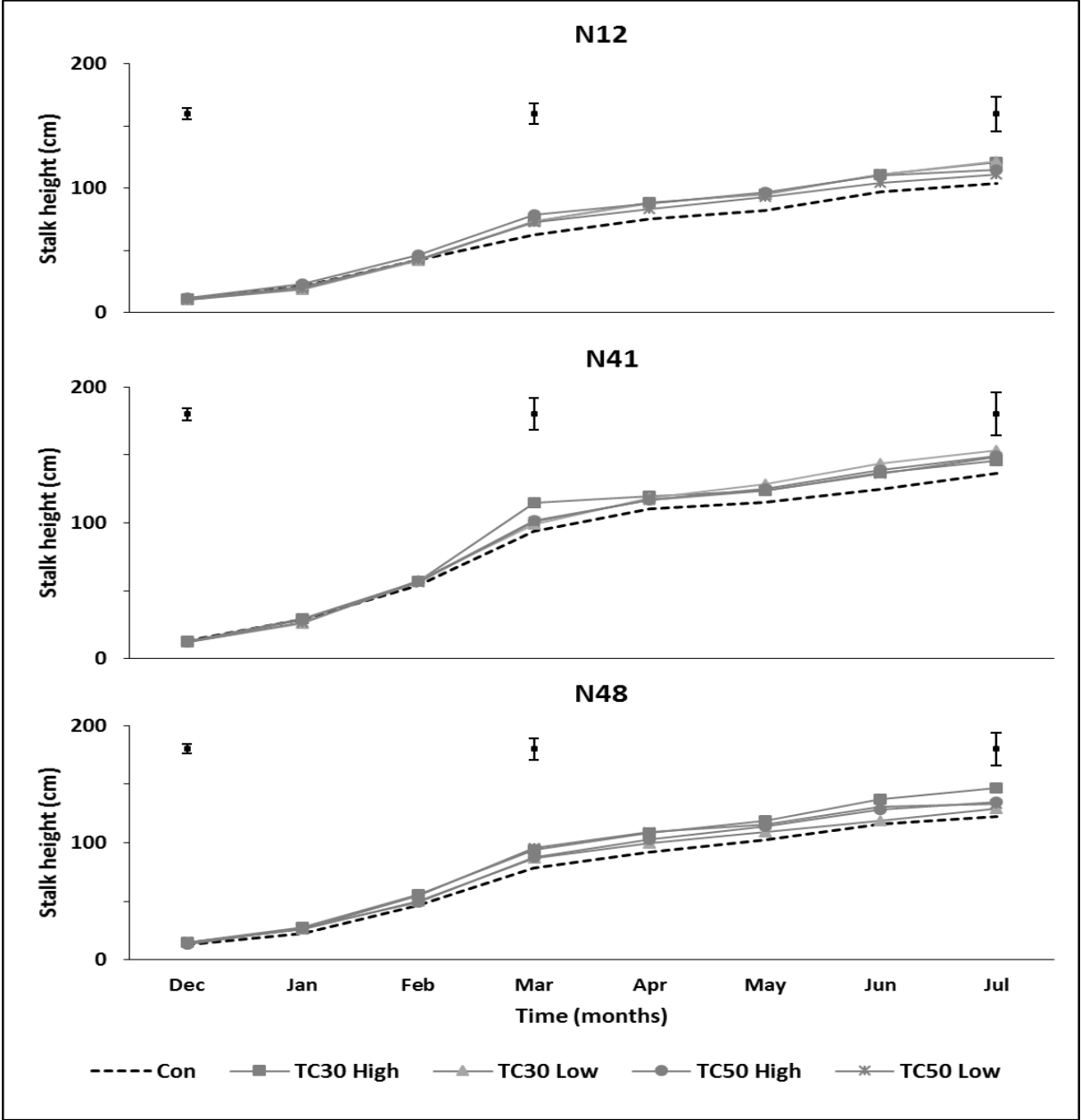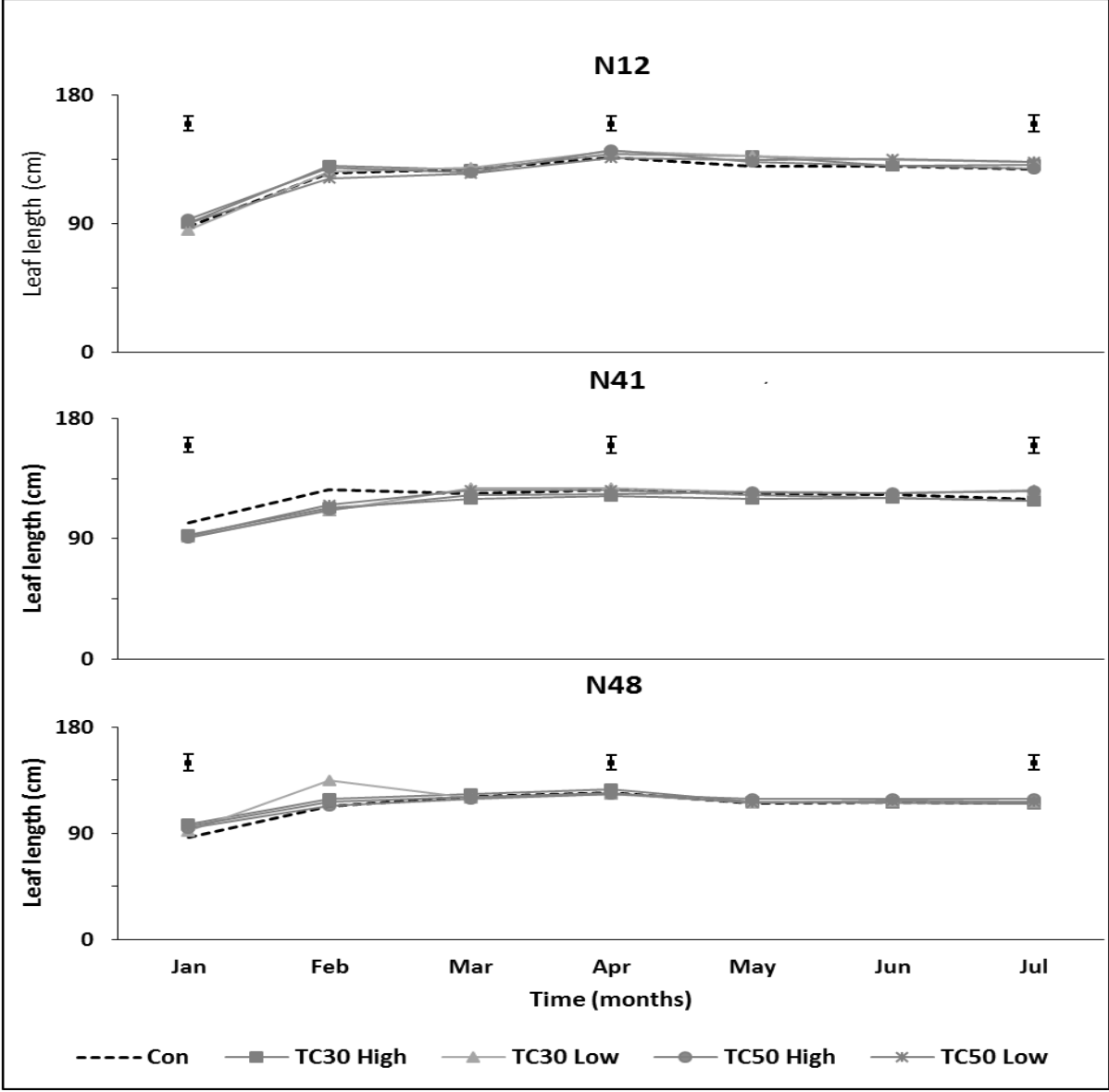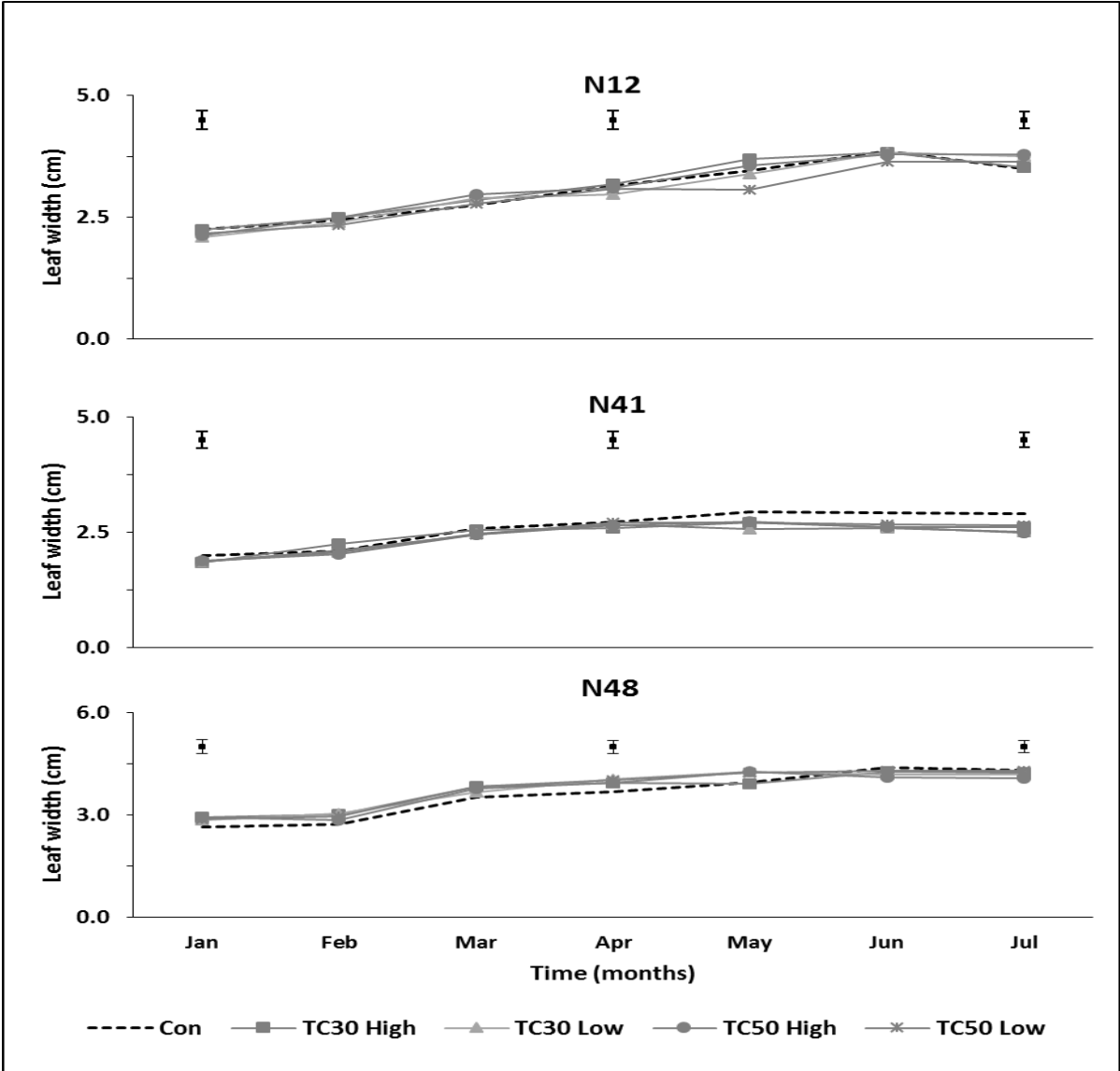**Figure 5.17. Leaf width of three varieties (N12, N41 and N48) planted at three planting rates (one and the half stick (low), double stick (normal) and triple stick (high)) using seedcane derived from: 1) conventional (Con); 2) tissue culture planted at 30 cm apart (TC30); and 3) tissue culture planted at 50 cm apart (TC50). Significant differences between treatments at selected dates are represented by LSD bars (5%).**

5.4 Discussion and Conclusion

The crop derived from tissue culture (TC) had a significantly higher mean stalk population compared with the crop derived conventionally (Con). This was observed for varieties N12 and N41 in particular. These results are in line with the findings of Ibrahim et al. (2016), who showed that the number of millable canes at harvest was significantly higher in TC (stage 2) derived plants as compared to conventionally derived plants. Furthermore, significantly higher stalk population was also observed in TC (stage 2) derived plants compared with conventionally derived plants (Ramanand and Singh, 2005; Sandhu et al., 2009). As the TC derived plants produces high stalk population, this means canopy is covered quickly. This assists in controlling weeds, as they compete with the crops for environmental resources such as water, nutrients, space and light. Therefore, growers establishing sugarcane derived from TC material may be able to reduce weed control costs and frequency of herbicide use.

The crop derived from TC had a significantly lower mean stalk diameter compared with the Con. For variety N12, the $TC_{50}H$ treatment produced stalks that were significantly thinner compared with the Con treatment. These results are in line with the findings of Ibrahim et al. (2016), who showed that the conventionally derived plants produced significantly thicker stalks compared with the tissue culture derived plants (stage 2). However, Sandhu et al. (2009), showed that the cane diameter was statistically similar between the conventionally and the tissue cultured (stage 2) derived plants. Thinner stalks in TC derived plants for the present study might have resulted in production of higher stalk population because stalk diameter correlates negatively with stalk population (Sood et al., 2006). Stalks that are thinner in diameter are known to be less vigorous (De Sousa-Vieira, 2005). This visual appearance of thinner stalks may affect the adoption of newly produced varieties that are bulked through TC methods, as growers may perceive them as less vigorous. To mitigate this, the newly produced varieties should be planted in small plots to investigate the effect of the TC process. Growers can then be made aware of expected changes in the phenotype of the varieties due to the TC process, while finding yields are not reduced, before planting the varieties commercially.

The crop derived from TC had a significantly higher mean stalk height compared with the crop derived from Con. These results are in agreement with the findings of Ramanand and Singh (2005), who showed that cane height was recorded to be significantly higher in tissue culture (stage 2) raised plants than in conventional plants.

The taller stalks produced by the TC plants might be as a results of thinner stalks and higher stalk population produced by the TC plants. Taller stalks are known to be susceptible to lodging, hence this might affect adoption of newly produced varieties. Varieties that show an altered phenotype (high tillering, thinner stalks and taller stalks) due to the tissue culture process should be identified routinely, and further manipulation of TC protocols might mitigate these phenotypes.

The crop derived from TC had a significantly higher mean cane yield and TERC compared with the crop derived from Con. The $TC_{50}H$ and $TC_{30}L$ treatments produced significantly higher TERC compared with the Con treatment for variety N41. These results are in line with the findings of Ramanand and Singh (2005), who showed that tissue culture (stage 2) raised plants produced significantly higher cane yield compared with conventional plants. Furthermore, Ibrahim et al. (2016) showed that the TC derived stick produced 48.14 tons more cane per hectare compared with the Con derived stick for variety N14. However, they also reported that the cane yield obtained from crop derived from TC of B52-298 and NCO-334 was inferior to the Con derived stick. This difference in responses of varieties was also observed in the current study, where N48 was not affected by the TC process in any way. The significantly higher cane yield in the TC derived plants in the present study might be as a result of higher stalk population and taller stalks produced by the TC plants. Based on the results from this study, the TC plants (stage 2) can be used as planting material for commercial production. Of all the varieties tested so far, N48 is the best candidate for this technology in South African industry.

The lower and higher planting rates did not differ significantly for all parameters. However, the higher planting rate gave higher stalk population compared with the lower planting rate. This was significant early on for all varieties (Figure 5.13), but not later at harvest. Similar results have been reported by Rice (1981), who showed no significant differences in all traits for planting rates of one stick, double stick and triple stick. However, these results contradict the findings of Singh et al. (2016), who showed that significantly greater cane and ERC yields were obtained when using planting rate of three stalk compared with two stalk and single stalk planting rates in the plant crop under rainfed conditions. Furthermore, Matherne (1971) showed that planting rate of two stalks had a significantly higher stalk population and cane yield compared with one and the half stalk planting rate. The higher planting rate results in a very strong competition for water and nutrients among the main shoots, which in turn, reduced the number of millable cane at harvest. This means more resources are used without reflecting in yield at harvest.

Furthermore, a higher planting rate means more seedcane (stalks) should be planted per hectare which imposes high costs and results in shortage of planting materials to cover annually planted commercial fields. Replanting 10 % of a farm per annum is the recommendation, and this would not be possible with high planting rates.

The lack of differences between lower and higher planting rates in this study suggests that lower planting rates may be more economical, without negative effects on growth and productivity. The TC source (TC30 vs.TC50) did not differ significantly for all parameters. Based on these results, the closer plant spacing (30 cm) of the TC plants in stage 1 did not changed the nature of the seedcane used for this study. However, planting the TC derived plants (stage 1) using closer spacing (30 cm) is not recommended for commercial production as it did not give any advantage over the wider (50 cm) spacing.

In summary, the seedcane derived from the TC at stage 1 can be used as planting materials for commercial production without any negative effects on productivity. This is despite persistence of the reduced stalk diameter, higher stalk population phenotype. There was a difference in responses of varieties, with N48 not affected by the TC process in any way. The reasons for the differential variety responses to the TC process are unknown, but could be related to the inherent cultivar traits e.g. N48 is a very low population, thick-stalked cultivar while the other varieties were characterised by higher populations of thinner stalks in general. Further research into this is therefore warranted. Furthermore, it is recommended that the effect of the TC process must be examined on a variety-by-variety basis. Lack of differences between TC source suggests that closer plant spacing (30 cm) of TC plants in stage 1 did not change the nature of the seedcane used in stage 2. However, it is recommended to plant TC plants in stage 1 at 50 cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics.

5.5 References

Ayele, N., Getaneh, A., Negi, T. and Dechassa, N., 2012. Effect of intra-row sett spacing on yield and yield components of sugarcane varieties at metahara sugar estate. *East African Journal of Science* 6, 117-124.

Burner, D.M. and Grisham, M.P., 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop Science* 35, 875-880.

De Sousa-Vieira, O., 2005. Interrelationships of cane yield components and their utility in sugarcane family selection: Path coefficient analysis. *Interciencia* 30, 93-96.

Fitch, M.M.M., Lehrer, A.T., Komor, E. and Moore, P.H., 2001. Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology* 50, 676-680.

Heinz, D.J. and Mee, G.W., 1969. Plant differentiation from callus tissue of Saccharum species. *Crop Science* 9, 346-348.

Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., Bekele, I. and Negi, T., 2016. Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture* 3, 1-4.

James, N.I., 1971. Yield components in random and selected sugarcane populations. *Crop Science* 11, 906-908.

Kanwar, R.S. and Sharma, K.K., 1974. Effect of interrow spacing on tiller mortality, stalk population and yield of sugarcane. *Proceedings of International Society of Sugar Cane Technologists* 2, 751-755.

Matherne, R.J., 1971. Influence of inter-row spacing and planting rate on stalk population and cane yield in Louisiana. *Proceedings of the International Society of Sugar Cane Technologists* 14, 640-645.

Ntoyi, T.T., Nicholson, T.L., Snyman, S.J., Meyer, G.M. and Banasiak, M., 2007, September. Sugarcane plants from temporary immersion culture: acclimating for commercial production. In *III International Symposium on Acclimatization and Establishment of Micropropagated Plants* 812, 323-328.

Orgeron, A.J., Kenneth, G.A. and Keith, B.P., 2007. Planting rate effects on sugarcane yield trials. *Journal American Society Sugar Cane Technologists* 36, 23-34.

Ramanand, M.L. and Singh, S.B., 2005. Comparative performance of micropropagated and conventionally raised crops of sugarcane. *Sugar Tech* 7, 93-95.

Ramgareeb, S., Snyman, S.J., Van Antwerpen, T. and Rutherford, R.S., 2010. Elimination of virus and rapid propagation of disease-free sugarcane (Saccharum spp. cultivar NCo376) using apical meristem culture. *Plant Cell, Tissue and Organ Culture* 100, 175-181.

Rice, E.R., 1981, June. Effects of planting rates on yields of sugarcane in Florida. *Proceedings. New series-American Society of Sugar Cane Technologists* 8, 53-56.

Sandhu, S.K., Gosal, S.S., Thind, K.S., Uppal, S.K., Sharma, B., Meeta, M., Singh, K. and Cheema, G.S., 2009. Field performance of micropropagated plants and potential of seed cane for stalk yield and quality in sugarcane. *Sugar Tech* 11, 34-38.

Singh, K., Singh, K. and Kumar, S., 2016. Effects of seed rate, sett size and seed treatment on spring planted sugarcane. *Journal of Sugarcane Research* 3, 78-82.

Singh, K.P., Suman, A., Singh, P.N. and Srivastava, T.K., 2007. Improving quality of sugarcane-growing soils by organic amendments under subtropical climatic conditions of India. *Biology and Fertility of Soils* 44, 367-376.

Singh, S., 2015. My experiences on physiological research on sugarcane in India. *Sugar Tech* 17, 227-234.

Snyman, S.J., Meyer, G.M., Banasiak, M., Nicholson, T.L., Antwerpen, T.V., Naidoo, P. and Erasmus, J.D., 2009. Micropropagation of sugarcane via NovaCane®: preliminary steps in commercial application. *Sugar Cane International* 27, 245-247.

Sood, N., Gupta, P. K., Srivastava, R. K., and Gosal, S. S., 2006. Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. *Plant Tissue Culture and Biotechnology* 16, 25-29.

Tiel, K., Enríquez, G.A., Ceballo, Y., Soto, N., Fuentes, A.D., Ferreira, A., Coll, Y. and Pujol, M., 2006. Development of a system for rapid plant regeneration from *in vitro* sugarcane (Saccharum officinarum L.) meristematic tissue. *Biotecnología Aplicada* 23, 22-24.

Watt, M.P., Banasiak, M., Reddy, D., Albertse, E.H. and Snyman, S.J., 2009. *In vitro* minimal growth storage of Saccharum spp. hybrid (genotype 88H0019) at two stages of direct somatic embryogenic regeneration. *Plant Cell, Tissue and Organ Culture* 96, 263-271.

CHAPTER 6 : GENERAL DISCUSSION AND CONCLUSION

Sugarcane propagation through tissue culture (TC) is commonly used in many industries to rapidly produce true-to-type, disease free material for planting. With the TC process, stage 1 is referred to as the production of actual TC plants under lab conditions, and their subsequent hardening off and planting into field nurseries. Stages 2 and 3 then progress in a similar manner to conventional methods. A handful of studies have compared growth and development of TC plants to plants derived from conventional setts. Previous work has shown that there may be yield depression encountered when using TC plants compared with conventionally (Con) propagated plants, and this may negatively affect production of sugarcane commercially.

There are many reports of altered phenotype of TC plants compared with Con propagated plants, and this altered phenotype (higher population of thinner, taller stalks) may be the reason for observed yield differences between the two methods. The altered phenotype of the TC plants (and subsequent effects on yields) may also be variety dependant, however, this hypothesis has not been tested under rainfed conditions in South Africa to date. Therefore, three field experiments were conducted under rainfed conditions at SASRI's Mount Edgecombe experiment station to gain insights into these factors. The aim of experiment 1 was to investigate growth and yield differences between TC and Con plants for different varieties while experiment 2 aimed at investigating the effects of different *in vitro* procedures on several phenotypic and agronomic characteristics of TC-derived plants of sugarcane and experiment 3 aimed at comparing the performance of seedcane obtained from the TC (stage 1) and the Con when planted at different planting rates, respectively. The information generated in this current study will provide recommendations to bulking co-operators and commercial growers regarding the expected agronomic performance of TC plants and TC derived sugarcane.

In experiment 1, there were no significant differences in cane yield, stalk height and stalk mass between the TC and the Con plants for all varieties in the plant and first ratoon crops. The results from this study are in line with the findings of Hoy et al. (2003), who showed that there were no significant differences in cane and ERC yields between TC and Con plants. However, this conflicts with the results of Sood et al. (2006), who reported that significantly higher cane yield was observed in TC plants compared with Con plants. Furthermore, Sandhu et al. (2009) showed that Con plants produced higher cane yield compared with TC plants.

The results from the present study suggest that the TC plants does not compromise the commercial yields when compared with the Con plants when averaged across crops under rainfed conditions. Therefore, it is recommended to use the TC plants at stage 1 for commercial fields. Although this is not currently a conventional practice due to the high cost of TC plants and large areas needed to be planted commercially, this information will be valuable in future, when the costs per plant are expected to be lower.

Both TC treatments produced significantly thinner stalks compared with the Con plants for varieties N12 and N31 when averaged across crops. A tendency towards uniform reduction in stalk diameter in the TC plants have been reported by Burner and Grisham (1995) and Sandhu et al. (2009). However, these results are in contradiction with the findings of Geetha and Padmanabhan (2002), who observed no significant differences in cane diameter between TC and Con plants. Thinner stalks are known to be less vigorous (De Sousa-Vieira, 2005). This visual appearance of thinner stalks may affect the adoption of newly produced varieties that are bulked through TC methods, as growers may perceive them as being less vigorous. To mitigate this, the newly produced varieties should be planted (screened) in small plots alongside conventionally grown plants to investigate the effect of the TC process. Growers can then be made aware of expected changes in the phenotype of the varieties due to the TC process before planting the varieties commercially.

Both TC treatments produced significantly higher stalk population compared with the Con plants for varieties N12 and N31 in the first ratoon crop, but this was not observed in the plant crop. The results from the present study are in contradiction with the findings of Burner and Grisham (1995) and Flynn et al. (2005) who showed that the variation in stalk population between TC and Con plants disappeared in the following ratoon crops. According to Julien et al. (1980), resources are wasted due to high tillering. As the tissue culture plants produce high number of stalks, this leads to competition for water and nutrients amongst stalks, and subsequent death of tillers. This means more resources are used without reflecting in yield at harvest. To minimise the loss of inputs (water and fertiliser), varieties that show an altered phenotype (high tillering) due to the tissue culture process should be identified routinely.

Plants propagated through the TC and the Con for variety N48 were generally stable for any parameter in the plant and first ratoon crops, indicating that the phenotype of variety N48 was maintained during the TC process. Variety N48 is the highest priority of the tested varieties for TC resources. According to Sreenivasan and Sreenivasan (1992), the differences between plants raised through the TC process and the Con might be affected by cultivar.

The results from Comstock and Miller (2004), also showed a different responses of varieties to the tissue culture process. The results from the present study suggest that the effect of the TC process must be examined on a variety-by-variety basis. The reasons for the differential variety responses to the TC process are unknown, but could be related to the inherent variety traits e.g. N48 is a very low population, thick-stalked variety while the other varieties were characterised by higher populations of thinner stalks in general. Further research into this is therefore warranted. Additional varieties with such traits would have high priority for research direction.

The TC30 and TC50 treatments did not differ significantly for all parameters in the plant and first ratoon crops for all varieties, showing that plant spacing did not affect growth. These results are in agreement with the findings of Sandhu et al. (2009), who showed that there were no significant differences in all parameters between the TC plants planted at 45 and 65 cm spacings. Based on these results, it is also recommended to plant TC plants at 50 cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics. Therefore, when propagating TC plants in stage 1 to increase the amount of planting material available in stage 2, it might not be worthwhile reducing the plant spacing.

The SP50 treatment produced stalks that were significantly thicker compared with the TC50 for varieties N12, N31 and N41 in the plant crop, but this was not observed in the first ratoon crop. For varieties N12 and N31, the TC50 treatment produced significantly higher stalk population compared with the SP50 in the first ratoon crop, but this was not observed in the plant crop. Based on the results from this study, it is clearly shown that the altered phenotype of the TC is not due to environmental factors. There are possible secondary effects of the tissue culture process on the plant phenotype. Some of these secondary effects were subsequently investigated and reported on in experiment 2 of this thesis.

In experiment 2, the plants produced through the NovaCane® ($CoCl_2$) procedure resembled those produced through NovaCane® for all parameters when averaged across crops. These results suggest that there may have been no excessive build-up of ethylene in the culture vessels because addition of the cobalt chloride ($CoCl_2$) to the medium had no effect. The results of the present study are in line with the findings of Brar (1999), who showed that no significant effect was observed on the regeneration capacity of cowpea cotyledon explants in response to the addition of $CoCl_2$. However; Latche (1991), showed that 10 or 20 ppm of $CoCl_2$ did not modify the growth rate of the sunflower callus, but adding 40 or 80 ppm did affect growth.

However, this contradict with the findings of Mishra et al. (2014), who showed that 10 ppm of $CoCl_2$ was found effective in reducing ethylene concentration inside the culture bottles in *in vitro* cultured sugarcane plants. Furthermore, using 20 ppm $CoCl_2$ was the best concentration to inhibit ethylene formation in the culture vessels and induce potato plant growth (Taghizadeh and Ehsanpour, 2013). These results suggest that the concentration of $CoCl_2$ required to inhibit the build-up of ethylene vary for different crops.

There was no benefit of adding $CoCl_2$ to *in vitro* cultured sugarcane plants in the present study. These results suggest that the increased tillering of the *in vitro* cultured sugarcane plants might be not as a result of the build-up of the ethylene in the culture vessels. Hence, this altered phenotype might be as a result of other factors in the *in vitro* culture process. Alternatively, the in-effectiveness of $CoCl_2$ on inhibiting ethylene build-up on culture vessels might also be as a result of incorrect concentrations of $CoCl_2$. This suggests that different concentration of $CoCl_2$ should be tested in *in vitro* cultured sugarcane plants to determine which concentration would be effective.

The NovaCane® and the NovaCane® (secondary) treatments did not differ significantly for Cane yield, ERC %, TERC, fibre content, stalk diameter, stalk mass and stalk height in both crops for both varieties. The NovaCane® treatment produced significantly lower stalk population compared with the NovaCane® (secondary) treatment for variety N41 spaced at 50 cm apart in the first ratoon crop, but this was not observed in the plant crop. These differences in stalk population between the NovaCane® and the NovaCane® (secondary) treatments in the first ratoon crop were not expected. These results suggest that varieties responded differently to the *in vitro* process. The NovaCane® (secondary) plants did not exacerbate any negative effects. It is therefore recommended to continue using this *in vitro* protocol particularly to produce virus free material for international germplasm exchange programs as it is a lengthy process. The general growth and vigour of the plants are not compromised by the secondary excision, and it produces higher stalk populations, combined with the assurance of being virus free.

In experiment 3, the crop derived from TC significantly improved cane yield and TERC compared with Con when averaged over varieties and planting rates. These results are in line with the findings of Ramanand and Singh (2005), who showed that tissue culture (stage 2) raised plants produced significantly higher cane yield compared with conventional plants. Furthermore, Ibrahim et al. (2016) showed that the TC derived stick produced 48.14 tons more

cane per hectare compared with the Con derived stick for variety N14. However, they also reported that the cane yield obtained from stick derived through TC of B52-298 and NCO-334 was inferior to the Con derived stick. This difference in responses of varieties was also observed in the current study, where N48 was not affected by the TC process in any way. The significantly higher cane yield in the TC derived plants in the present study might be as a result of higher stalk population and taller stalks produced by the TC plants. Based on the results from this study, stalks derived from TC plants in stage 1 can be used as planting material for commercial production without risks of yield depression. In fact, this work has shown that yields may be improved for some varieties when using stalks originating from TC compared with conventional methods

The crop derived from tissue culture (TC) had a significantly higher mean stalk population compared with the crop derived conventionally (Con). This was observed for varieties N12 and N41 in particular. Significantly higher stalk population was also observed in TC (stage 2) derived plants compared with conventionally derived plants (Ramanand and Singh, 2005; Sandhu et al., 2009; Ibrahim et al., 2016). As the TC derived plants produces high stalk population, this means canopy is covered quickly. This assists in controlling weeds, as they compete with the crops for environmental resources such as water, nutrients, space and light. Therefore, growers establishing sugarcane derived from TC material may be able to reduce weed control costs and herbicide use. This could be verified in small demonstration plots that are already recommended to introduce growers to altered phenotype (see below).

The crop derived from TC had a significantly lower mean stalk diameter compared with the crop derived from Con. These results are in line with the findings of Ibrahim et al. (2016), who showed that the Con derived plants produced significantly thicker stalks compared with TC derived plants (stage 2). However, Sandhu et al. (2009), showed that the cane diameter was statistically similar between Con and TC (stage 2) derived plants. This visual appearance of thinner stalks in the present study may affect the adoption of newly produced varieties that are bulked through TC methods, as growers may perceive them as being less vigorous. To mitigate this, the newly produced varieties should be planted in small plots to investigate the effect of the TC process. Growers can then be made aware of expected changes in the phenotype of the varieties due to the TC process before planting the varieties commercially.

The lower and higher planting rates did not differ significantly for all parameters. Similar results have been reported by Rice (1981), who showed no significant differences in all traits for planting rates of one stick, double stick and triple stick. However, these results contradict with the findings of Singh et al. (2016), who showed that significantly greater cane and ERC yields were obtained when using planting rate of three stalk compared with two stalk and single stalk planting rate in the plant crop under rainfed conditions. The higher planting rate results in a very strong competition for water and nutrients among the main shoots, which in turn, reduced the number of millable cane at harvest.

This means more resources are used without reflecting in yield at harvest. The lack of differences between lower and higher planting rates in this study suggests that lower planting rates may be more economical, without negative effects on growth and productivity. The TC source (TC30 vs.TC50) did not differ significantly for all parameters. Based on these results, the closer plant spacing (30 cm) of the TC plants in stage 1 did not change the nature of the seedcane and subsequent growth characteristics observed in stage 2. However, it is recommended that TC plants be propagated using wider (50 cm) plant spacings, as this is more economical.

In summary, TC plants did not compromise the commercial yields when compared with Con plants under rainfed conditions. However, the effect of the TC process must be examined on a variety-by-variety basis as varieties responded differently to the TC process. Growers should then be made aware of expected changes in the phenotype of varieties prior to propagation, as this will mitigate possible poor adoption of varieties based on thin stalks. The significantly thinner stalks and higher stalk population produced by TC50 treatment compared with the SP50, suggests that there are some secondary effects of the TC process on plant phenotype. The lack of differences between *in vitro* procedures suggests that propagation of new genotypes through standard NovaCane® procedures for commercial release should continue. It is recommended to plant TC plants at wider (50) cm spacings, as a closer (30 cm) spacing will results in higher costs (more plants per hectare) without any significant improvements in agronomic characteristics. The seedcane derived from the TC at stage 1 can be used as planting materials for commercial production without any negative effects on productivity. This is despite persistence of the reduce stalk diameter, higher stalk population phenotype. Lack of differences between the higher and the lower planting rates suggests that lower planting rates should be used for economic reasons.

6.1 References

Brar, M.S., Moore, M.J., Al-Khayri, J.M., Morelock, T.E. and Anderson, E.J., 1999. Ethylene inhibitors promote in vitro regeneration of cowpea (Vigna unguiculata L.). *In Vitro Cellular and Developmental Biology-Plant* 35, 222-225.

Burner, D. M., and Grisham, M. P., 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop Science* 35, 875-880.

Comstock, J.C. and Miller, J.D., 2004. Yield comparisons: disease-free tissue culture versus bud-propagated sugarcane plants and healthy versus yellow leaf infected plants. *Journal of the American Society of Sugar Cane Technologists* 24, 31-40.

De Sousa-Vieira, O., 2005. Interrelationships of cane yield components and their utility in sugarcane family selection: Path coefficient analysis. *Interciencia* 30, 93-96.

Flynn, J., Powell, G., Perdomo, R., Montes, G., Quebedeaux, K. and Comstock, J., 2005. Comparison of sugarcane disease incidence and yield of field-run, heat-treated, and tissue-culture based seedcane. *Journal of American Society of Sugar Cane Technologists* 25, 88-100.

Geetha, S. and Padmanabhan, D., 2002. Evaluation of tissue culture raised sugarcane for yield and quality. *Sugar Tech* 4, 179-180.

Hoy, J.W., Bischoff, K.P., Milligan, S.B. and Gravois, K.A., 2003. Effect of tissue culture explant source on sugarcane yield components. *Euphytica* 129, 237-240.

Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., Bekele, I. and Negi, T., 2016. Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture* 3, 1-4.

Julien, M.H.R., Soopramanien, G.C., Martine, J.F. and Medan, H., 1980. The role of side shoots in flowered stalks of sugar cane. *In Seventeenth Congress of the International Society of Sugar Cane Technologists* 1, 571-582.

Latche, A., Roustan, J.P. and Fallot, J., 1991. Stimulation of shoot regeneration from cotyledons of Helianthus annuus by the ethylene inhibitors, silver and cobalt. *Plant Cell Reports* 10, 204-207.

Mishra, S., Nailwal, T.K. and Pant, R.C., 2014. In vitro study of role of ethylene during tillering in sugarcane. *Sugar Tech* 16, 255-263.

Ramanand, M.L. and Singh, S.B., 2005. Comparative performance of micropropagated and conventionally raised crops of sugarcane. *Sugar Tech* 7, 93-95.

Rice, E.R., 1981, June. Effects of planting rates on yields of sugarcane in Florida. *Proceedings. New series-American Society of Sugar Cane Technologists* 8, 53-56.

Sandhu, S. K., Gosal, S. S., Thind, K. S., Uppal, S. K., Sharma, B., Meeta, M., and Cheema, G. S., 2009. Field performance of micropropagated plants and potential of seed cane for stalk yield and quality in sugarcane. *Sugar Tech* 11, 34-38.

Singh, K., Singh, K. and Kumar, S., 2016. Effects of seed rate, sett size and seed treatment on spring planted sugarcane. *Journal of Sugarcane Research* 3, 78-82

Sood, N., Gupta, P. K., Srivastava, R. K., and Gosal, S. S., 2006. Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. *Plant Tissue Culture and Biotechnology* 16, 25-29.

Sreenivasan, T. V., and Sreenivasan, J., 1992. Micropropagation of sugarcane varieties for increasing cane yield. *SISSTA Sugar Journal* 18, 61-64.

Taghizadeh, M. and Ehsanpour, A., 2013. The in vitro effects of $CoCl_2$ as ethylene synthesis inhibitor on PI based protein pattern of potato plant (Solanum tuberosum L.). *Journal of Cell and Molecular Research* 5, 42-46.