juice purity and pol % dry matter. Significant differences (p<0.05) were observed between varieties on fibre, moisture and juice purity. There were no significant (p>0.05) varietal differences on sucrose content and pol % dry matter. Like the first crop-year in Table 4, the interaction effects of propagation method and variety were not significant (p>0.05) for the five quality traits in the second crop-year.

Combined analysis

Tables 6 and 7 present combined data analysis of yield and quality traits for crop-years 1 and 2 (seedcane phase) and crop-years 3 and 4 (commercial phase). On yield traits, significant differences were observed only on cane (p<0.05) and sucrose (p<0.01) yields between the two propagation methods for the seedcane phase (Table 6). There were highly significant (p<0.01) varietal differences for all the yield traits. Highly significant (p<0.01) crop-year effects were observed for cane and sucrose yields, stalk mass and stalk dry mass. There were no significant (p>0.05) interaction effects of propagation method and variety for all yield traits for the seedcane phase. Significant (p<0.05) propagation method by crop-year effects were noted only on sucrose yield. Significant variety by crop-year interaction effects were recorded only on cane yield, stalk pol mass (p<0.05) and sucrose yield (p<0.01). The three way interaction of propagation method by variety by crop-year was not significant (p>0.05) for any of the yield traits for the seedcane phase.

There were no significant differences (p>0.05) between propagation methods on any of the quality traits for the seedcane phase (Table 6). Significant varietal differences were observed on fibre and moisture content (p<0.01), juice purity and pol % dry matter (p<0.05). Highly significant (p<0.01) crop-year effects were shown for sucrose content, juice purity and pol % dry matter. There were no significant (p<0.05) effects for any of the interactions (propagation by variety, propagation by crop-year, variety by crop-year and propagation method by variety by crop-year) on any of the quality traits except for variety x crop-year on sucrose content for the seedcane phase.

For the commercial phase (Table 7), significant differences (p<0.05) were observed only for the stalk mass and stalk pol mass. Similar to the results for seedcane, significant (p<0.01) varietal differences were observed for all the yield traits. Crop-year effects were significantly (p<0.01) different for cane and sucrose yield, stalk mass and stalk dry mass. There were no significant (p<0.05) interaction effect on any of the yield traits for the commercial phase. On the quality traits of the commercial cane, there were no significant differences (p>0.05) between conventional and tissue culture grown cane. Significant varietal differences were observed on sucrose content on a fresh and dry matter basis (p<0.01) and moisture content (p<0.05). Significant differences (p<0.01) between crop-years were observed for all of the quality traits. Interaction between treatment factors were not significant (p<0.05) for any quality traits for the commercial phase.

Discussion

The primary objectives of this study were to determine the performance of tissue culture planted cane under local field conditions, to ascertain the response of different sugarcane varieties to this biotechnology, and to establish the effect of pests and diseases on tissue culture cane. The results indicated that *Eldana* internode damage was insignificant across the four years of testing, irrespective of the propagation method or variety (results not shown). *Eldana* damages are known to be low on irrigated cane compared to dry land cane. The zero smut infected stools in the first year of the trial suggested that the spores had not yet established themselves on the crop, irrespective of the propagation method. The non-significant differences in the smut levels of cane grown either vegetatively or from tissue culture plantlets in the last three crop-years, indicated that smut effect on cane was not dependent on the propagation method. The highly significant varietal differences in smut infections confirm the general knowledge that the suite of varieties grown in the industry react differently to the pathogen's attack. For example, variety NCo376 was consistently the most infected, substantiating the common knowledge that the variety is the most susceptible amongst the industry varieties. The significant propagation method by variety interactions during the second crop-year, indicated that the level of smut infection on the different varieties was dependent on the propagation method in this particular year. However, this observation disappeared in crop-years three and four.

The vegetatively grown cane had significantly higher cane yield than the tissue culture cane in crop-year two, suggesting that seedcane estimates for tissue culture grown cane should be adjusted downward (by 9% relative to conventionally grown seed material). Similar findings were reported by Sandhu *et al*. (2009) and Hamza and Alebjo (2017). However, under rainfed conditions Shezi *et al*. (2017) reported that there were no significant differences between the two propagation methods in the first and second crop-years. For the commercial cane (crop-years three and four) the combined crop-years analysis of the cane yield was not statistically different between the two propagation methods suggesting that it may not be necessary to adjust yield estimates for cane grown from tissue culture plantlets post seedcane stage. The results showed that there were no significant propagation method by variety effects on cane yield allaying fears that some industry varieties may not respond favourably to the tissue culture technique. Significant varietal and crop-years effects on cane yield confirmed the variation in yield potential of the test varieties and the varying biotic and abiotic factors effect on cane yield across crop-years. The significant variety by crop-year effects for the seedcane years implied that the ranking of the varieties on cane yield was different cross the two crop-years. The non-significant propagation method by crop-year and propagation by variety by crop-year interactions across both seedcane and commercial phases, signalled that cane yield across crop-years was not dependent on the propagation method and the performance of varieties was not influenced by the combined effect of propagation method and crop-years.

Sucrose yield followed the same pattern as cane yield (with a few exceptions). The highly significant differences within propagation methods, varieties and crop-years during the seedcane phase indicated the existence of diversity within these three variance components on sucrose yield. The conventionally grown cane had significantly higher sucrose yield than the tissue culture cane during this phase. The second crop-year was significantly higher than the first crop-year on sucrose yield. The sucrose yield is not critical in the seedcane phase since the harvested material is purely used as seed. However, it does provide important information for any grower who for certain reasons may decide to mill the crop at these stages. For the commercial phase, the non-significant differences between propagation methods on sucrose yield intimated that monetary gains are not likely to be significantly different whether cane was grown from stalks or tissue culture plantlets. The non-significant interaction effects of propagation method by variety, propagation method by crop-year, variety by crop-year and propagation method by variety by crop-year suggested that the sucrose yield in the commercial years of the different varieties was not affected by propagation method, crop-years or the combined effect of both.

On stalk mass, the vegetatively grown cane, on average, was significantly higher than the tissue culture cane in the first crop-year. This is attributed to the relatively thicker stalks observed on conventional cane compared to tissue culture cane (results not shown). Sandhu *et al*. (2009) reported that cane crop derived from tissue culture plants was characterised by thinner stalks compared to conventional cane, which was associated with reduced stalk mass. Significant variety and crop-year effects observed on stalk mass in the seedcane phase indicated the existence of genetic diversity within the test varieties and differing environmental conditions between crop-years. The significant differences in stalk mass between the propagation methods in the commercial phase indicated that the trend observed in the first crop-year persisted to the latter crop-years. On dry matter basis, the non-significant differences between the propagation methods signalled that stalk dry mass was independent of the source of seed material used. The significantly higher stalk pol mass of vegetatively grown cane compared to tissue culture cane during the first crop-year is attributed to the higher stalk mass observed for the conventional method. The non-significant interactions of all the sources of variation on stalk mass, dry stalk mass and stalk pol mass in both seedcane and commercial phases showed that the ranking of varieties on these character traits was not influenced by the propagation method, crop-year or the combined effect of both.

The results showed that proportions of fibre, moisture and juice purity were not statistically different between the two propagation methods. However, vegetatively grown cane was significantly higher than tissue culture cane on sucrose content and pol % dry matter in the first and second crop-years, respectively. The study of Sandhu *et al*. (2009) also reported higher sucrose content on conventional cane compared to tissue culture cane. The significantly higher sucrose yield for the vegetatively planted cane at the seedcane phase is also attributed to the relatively higher sucrose content on this cane. On the other hand, the higher pol % dry matter on the vegetatively grown cane in the first crop-year can be linked to the higher stalk pol mass. The significant varietal and crop-year effects on most of the quality traits pointed to the existence of genetic variability within the varieties and the diverse environmental impact peculiar for each crop-year. This further explains the importance of testing sugarcane varieties across multiple crop-years prior to their release for commercial growing.

The combined data analysis of both seedcane and commercial cane showed no significant difference between the two propagation methods on all the five quality traits. The non-significant interaction effects of the possible combinations of propagation method, varieties and crop-years for the quality traits across all two phases suggested that variety performance was not influenced by propagation method and crop-years or the combined effect of these two sources of variation. The significant variety by crop-year effect on pol in the seedcane phase indicated a change in variety ranking on these traits across the first two crop-years.

Table 2. Yield character traits for the 1st crop-year for both the conventional (COM) and tissue culture (TCM) methods.

Variety	Cane yield (ton/ha)		Sucrose (ton/ha)		Stalk mass (grams/stalk)		Stalk dry mass (grams/stalk)		Stalk sucrose mass (grams/stalk)	
	COM	TCM	COM	TCM	COM	TCM	COM	TCM	COM	TCM
N19	137.5	120.1	22.0	19.1	962.5	1018.8	301.6	319.6	153.7	162.3
N23	132.5	141.5	20.7	21.4	941.3	860.0	282.7	274.6	146.8	129.8
N25	156.6	148.6	24.3	21.8	1091.3	1005.0	312.9	302.7	169.5	147.5
N36	133.7	127.2	21.4	20.7	1273.8	1068.8	386.7	333.6	203.8	175.2
N46	132.5	133.3	20.8	20.3	1017.5	835.0	290.7	247.7	160.1	127.6
NCo376	155.4	140.0	23.3	20.7	957.5	840.0	295.0	267.9	146.0	124.5
Average	141.4	135.1	22.1	20.7	1040.6	937.9	311.6	291.0	163.3	144.5
Propagation method (PM)	590.8^{ns}		29.904^{ns}		158235*		6360^{ns}		5323.4*	
Variety (Var)	950.7**		9.575^{ns}		113212**		11065**		3991.6**	
PM x Var	247.3^{ns}		5.261^{ns}		21524^{ns}		1676^{ns}		526.7^{ns}	
CV %	8.8		10		14.7		16.1		17.6	

* Significant at p<0.05; **Significant at p<0.01; ns – not significant

Table 3. Yield character traits for the 2nd crop-year for both the conventional (COM) and tissue culture (TCM) methods.

Variety	Cane yield (ton/ha)		Sucrose (ton/ha)		Stalk mass (grams/stalk)		Stalk dry mass (grams/stalk)		Stalk sucrose mass (grams/stalk)	
	COM	TCM	COM	TCM	COM	TCM	COM	TCM	COM	TCM
N19	131.1	111.7	22.4	19.2	906.3	848.8	288.2	265.8	152.6	145.9
N23	145.6	140.8	26.5	24.2	811.3	807.3	254.4	271.1	147.1	139.0
N25	152.0	137.0	26.6	22.7	857.5	782.5	254.5	227.4	150.0	129.2
N36	133.9	113.4	22.5	19.8	1035.0	861.8	331.4	277.0	175.5	150.0
N46	135.9	125.6	25.1	21.6	913.8	835.0	281.3	237.5	168.7	144.2
NCo376	135.3	130.1	23.7	22.0	868.8	850.0	266.7	255.1	152.1	143.5
Average	139.0	126.4	24.4	21.6	898.8	830.9	279.4	255.7	157.7	141.9
Propagation method (PM)	2356.8**		122.934**		69105^{ns}		8465^{ns}		3705.8^{ns}	
Variety (Var)	926.0*		33.563**		24720^{ns}		4542^{ns}		732.6^{ns}	
PM x Var	118^{ns}		1.66^{ns}		8937^{ns}		1573^{ns}		195.6^{ns}	
CV %	7.7		10.2		14.1		17.6		15.3	

* Significant at p<0.05; **Significant at p<0.01; ns – not significant

Table 4. Quality character traits for the 1st crop-year for both the conventional (COM) and tissue culture (TCM) methods.

Variety	Sucrose content		Fibre content		Moisture content		Juice purity		Pol % dry matter	
	COM	TCM	COM	TCM	COM	TCM	COM	TCM	COM	TCM
N19	16.00	15.96	12.86	13.05	68.70	68.70	92.12	92.91	51.19	51.00
N23	15.62	15.12	11.84	13.91	69.96	68.02	90.56	89.14	52.05	47.30
N25	15.52	14.68	11.05	12.95	71.30	69.88	92.52	90.99	54.16	48.73
N36	15.98	16.24	12.15	12.22	69.66	68.80	93.35	90.47	52.75	52.10
N46	15.72	15.34	10.77	11.30	71.40	70.50	92.81	88.80	54.98	52.18
NCo376	15.04	14.78	13.33	14.89	69.44	68.06	92.88	93.10	49.22	46.37
Average	15.65	15.35	12.00	13.05	70.08	68.99	92.37	90.90	52.39	49.61
Propagation method (PM)	1.2907^{ns}		16.622^{ns}		17.604^{ns}		32.341^{ns}		115.581*	
Variety (Var)	2.2568**		10.858**		9.637**		13.142^{ns}		41.836**	
PM x Var	0.3603^{ns}		1.99^{ns}		1.096^{ns}		8.202^{ns}		11.069^{ns}	
CV%	5.0		10.0		1.9		2.8		5.6	

*Significant at p<0.05; **Significant at p<0.01; ns – not significant

Table 5. Quality character traits for the 2nd crop-year for both the conventional (COM) and tissue culture (TCM) methods.

Variety	Sucrose content		Fibre content		Moisture content		Juice purity		Pol % dry matter	
	COM	TCM	COM	TCM	COM	TCM	COM	TCM	COM	TCM
N19	17.06	17.14	12.82	12.12	68.08	68.84	94.65	95.06	53.51	55.70
N23	18.16	17.24	10.79	14.28	68.70	65.90	93.52	92.93	58.60	51.75
N25	17.50	16.58	10.42	10.60	70.42	70.90	95.95	94.30	59.82	57.00
N36	16.92	17.42	13.38	12.76	67.94	68.02	96.48	96.25	52.83	55.34
N46	18.48	17.20	10.64	9.64	69.12	71.56	95.89	95.75	60.25	60.52
NCo376	17.46	16.88	11.46	11.12	69.32	69.90	95.69	93.61	57.64	56.60
Average	17.60	17.08	11.59	11.75	68.93	69.19	95.36	94.65	57.11	56.15
Propagation method (PM)	4.056*		0.423^{ns}		0.988^{ns}		7.619^{ns}		13.79^{ns}	
Variety (Var)	1.17^{ns}		14.361*		18.25*		11.692*		60.34^{ns}	
PM x Var	1.151^{ns}		7.002^{ns}		7.275^{ns}		2.286^{ns}		30.85^{ns}	
CV%	7.6		19.3		3.3		2.1		9.6	

*Significant at p<0.05; **Significant at p<0.01; ns – not significant

Table 6. Combined analysis of variance for yield and quality character traits for the seedcane phase for both the conventional (COM) and tissue culture (TCM) methods.

Source of variance	df	Yield traits					Quality traits				
		Cane yield (ton/ha)	Sucrose (ton/ha)	Stalk mass (grams/ stalk)	Stalk dry mass (grams/ stalk)	Stalk sucrose mass (grams/ stalk)	Sucrose content	Fibre content	Moisture content	Juice purity	Pol % dry matter
Propagation Method (PM)	1	2653.89*	137.05**	218240^{ns}	14750^{ns}	8956^{ns}	4.9613^{ns}	11.175^{ns}	5.125^{ns}	35.676^{ns}	104.61^{ns}
Variety (Var)	5	1626.46**	29.61**	103769**	12950**	3337**	1.6525^{ns}	17.525**	23.658**	16.982*	65.12*
Crop-year (CY)	1	923.76**	80.01**	464696**	34231**	506^{ns}	101.2003**	21.982^{ns}	6.816^{ns}	340.577**	949.6**
PM x Var	5	257.22^{ns}	4.02^{ns}	22142^{ns}	2569^{ns}	599^{ns}	1.2963^{ns}	7.113^{ns}	5.862^{ns}	4.312^{ns}	37.01^{ns}
PM x CY	1	293.79^{ns}	15.79*	9100^{ns}	75^{ns}	73^{ns}	0.3853^{ns}	5.87^{ns}	13.467^{ns}	4.283^{ns}	24.76^{ns}
Var x CY	5	250.29*	13.53**	34163^{ns}	2657^{ns}	1387*	1.7741*	7.694^{ns}	4.23^{ns}	7.851^{ns}	37.05^{ns}
PM x Var x CY	5	108.04^{ns}	2.90^{ns}	8319^{ns}	681^{ns}	123^{ns}	0.2147^{ns}	1.878^{ns}	2.509^{ns}	6.176^{ns}	4.91^{ns}
CV%		7.0	7.7	14.9	17.1	15.5	5.0	21.2	4.0	2.3	9.5

*Significant at p<0.05; **Significant at p<0.01; ns – not significant

Table 7. Combined analysis of variance for yield and quality character traits for the commercial phase for both the conventional (COM) and tissue culture (TCM) methods.

Source of variance	df	Yield traits					Quality traits				
		Cane yield (ton/ha)	Sucrose (ton/ha)	Stalk mass (grams/ stalk)	Stalk dry mass (grams/ stalk)	Stalk sucrose mass (grams/ stalk)	Sucrose content	Fibre content	Moisture content	Juice purity	Pol % dry matter
Propagation Method (PM)	1	183.1^{ns}	8.6^{ns}	95520*	5698^{ns}	3806.1*	0.5603^{ns}	11.011^{ns}	5.896^{ns}	0.69^{ns}	43.29^{ns}
Variety (Var)	5	1779.6**	24.2**	89798**	8741**	4671.1**	7.2183**	4.104^{ns}	5.606*	4.91^{ns}	53.11**
Crop-year (CY)	1	7373.9**	59.4**	191151**	39323**	499.5^{ns}	37.8563**	39.068**	41.067**	972.6**	935.45**
PM x Var	5	263.3^{ns}	8.8^{ns}	18000^{ns}	2116^{ns}	686^{ns}	0.0739^{ns}	1.352^{ns}	3.207^{ns}	6.5^{ns}	10.62^{ns}
PM x CY	1	497.4^{ns}	10.6^{ns}	16877^{ns}	1170^{ns}	341.7^{ns}	0.1763^{ns}	0.053^{ns}	0.616^{ns}	0.39^{ns}	0.01^{ns}
Var x CY	5	78.9^{ns}	6.7^{ns}	10377^{ns}	1430^{ns}	264.7^{ns}	0.4291^{ns}	0.259^{ns}	1.209^{ns}	6.04^{ns}	2.81^{ns}
PM x Var x CY	5	79.6^{ns}	7.8^{ns}	8321^{ns}	713^{ns}	373.3^{ns}	1.0231^{ns}	3.776^{ns}	0.771^{ns}	2.86^{ns}	18.65^{ns}
CV %		9.6	11.4	12.1	12.1	13.7	5.4	10.6	1.5	4	6.5

*Significant at p<0.05; **Significant at p<0.01; ns – not significant

Conclusions

The findings of this study indicate that cane yield derived from tissue culture propagated cane is lower than that of conventionally grown cane. This is at least partly attributed to lower stalk weight of *in vitro* propagated cane. However, this was only observed in the second crop-year of the seedcane phase. It is therefore pertinent that cane grown from the tissue culture technology in the first two crops is strictly used to establish secondary nurseries on grower farms. The tissue culture method should be viewed as a biotechnique for exclusively multiplying seedcane and not for commercial cultivation due to the associated yield penalty observed during the early crop-years. The performance of the different sugarcane varieties used in this investigation indicated that varietal yield was not dependent on the source of seed material (tissue culture or conventional). Cane and sucrose yield obtained from tissue culture plantlets at the commercial phase were not statistically different from those sourced from vegetatively grown crop. The effect of *Eldana* and smut were not dependent on propagation method, so their field management should not be determined by seed source.

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