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'Collar propagation' as an alternative propagation method for rhizomatous miscanthus

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Abstract

The demand for perennial nonfood crops, such as miscanthus, is increasing steadily, as fossil resources are replaced by biomass. However, as the establishment of miscanthus is very expensive, its cultivation area in Europe is still small. The most common propagation method for miscanthus is via rhizomes, the harvesting of which is very labour-intensive. Seed propagation is promising, but not suitable for sterile genotypes. In this study, a new vegetative propagation method, 'collar propagation', was tested in field and controlled environment studies. Collars are built at the junction between rhizome and stem. They can be harvested in a less destructive way than rhizomes by pulling out the stems from winter-dormant miscanthus plants. One genotype of each of the species *M. sacchariflorus*, *M.* × *giganteus*, *M. sinensis* in combination with three fragment types (collars, rhizomes, collars + rhizomes) were tested for establishment success and plant performance. The performance (e.g. dry matter yield) of collar-propagated plants was either better than or not significantly different from rhizome-propagated plants. Pregrown plantlets transplanted into the field showed no significant differences in establishment success between the fragments within a genotype. When directly planted into the field however, the fragment 'rhizome+collar' had a significantly better establishment success than the other two. The winter survival rate of the fragment 'rhizome+collar' was 70% for *M. sacchariflorus* and 75% for *M.* × *giganteus*. Emergence success from collar-derived plants was not affected by harvest date (harvested monthly from November to February). This study showed that miscanthus propagation via collars is feasible and a promising alternative to rhizome propagation, as the multiplication rate of collars is comparable to that of rhizome propagation. Collar propagation is the more suitable method for the tested genotypes of the species *M. sacchariflorus* and *M.* × *giganteus*, but not for *M. sinensis* genotypes, which may be better propagated by seeds.

Keywords: collar, establishment, miscanthus, overwintering, propagation, rhizome

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Introduction

As more and more fossil resources are being replaced by biomass for various energetic and material utilization options, the demand for biomass is increasing steadily. To satisfy the goals of a growing bioeconomy, this biomass needs to be produced sustainably and conflicts between food security and bioenergy avoided. For this purpose, perennial nonfood crops, such as miscanthus, offer a viable option thanks to their generally low-input requirements and high yield potential, also under conditions marginal for the production of food crops (McCalmont *et al*., 2017).

Miscanthus is a perennial rhizomatous C_4 grass originating from South-East Asia. Typical yields of the most commonly grown, and so far only commercially available, genotype *Miscanthus* × *giganteus* range between 15 and 25 Mg dry matter ha^{-1} yr^{-1} in temperate climates (Lewandowski *et al*., 2000; Heaton *et al*., 2004; Lesur *et al*., 2013; Boersma & Heaton, 2014a; Iqbal *et al*., 2015). The low-input character of miscanthus can be mainly attributed to its perennial nature, with a lifetime of more than 20 years and efficient nutrient recycling (Cadoux *et al*., 2012). Given its high yield potential and benign environmental profile, miscanthus is seen as a promising crop to provide sustainably produced biomass for a growing bioeconomy (Lewandowski, 2015).

Despite these advantages, miscanthus is currently only grown on about 19 000 ha in Europe (Lewandowski *et al*., 2016). Reasons for this are a lack of higher value utilization options and high initial investment costs for establishment of the plantation. Novel higher value utilization options have only recently been identified and need to be implemented in practice to create a market for miscanthus biomass (Kiesel & Lewandowski, 2017; Lewandowski *et al*., 2016; van der Weijde *et al*., 2017). However, expensive propagation is still one of the main reasons for the low cultivation rate. Therefore, various studies have tested alternative propagation

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methods (Lewandowski *et al*., 2003; Atkinson, 2009; Zub & Brancourt-Hulmel, 2010; Xue *et al*., 2015). The conventional propagation method for miscanthus is via rhizomes. This is currently the cheapest and easiest of all available propagation methods. However, a rhizome harvest is not possible every year because harvesting all rhizomes leads to the destruction of the complete mother field and leaving some rhizomes in the field to regrow the following year requires time for the plants to recover. The low dividing efficiency of 1:10–50 is a further disadvantage of rhizome propagation (Xue *et al*., 2015; Clifton-Brown *et al*., 2017). Heaton *et al*. (2010) showed that rhizomes harvested from 0.4 ha can result in about 3.6 ha of miscanthus, and a such vegetative reproduction is still economically viable. To improve the multiplication rate and reduce costs, other vegetative propagation methods have been sought. One alternative propagation method is micropropagation, which is very effective due to its high multiplication rate (1:960) and has the additional benefit of being able to prevent the transmission of diseases (Lewandowski, 1998; Xue *et al*., 2015). This method is the most expensive way of propagating miscanthus, because it is very labour-intensive (Xue *et al*., 2015), and is therefore mainly used for scientific trials. Propagation via seeds is another promising method and with the development of novel hybrids that produce fertile seeds, this method is becoming increasingly interesting and relevant. It has a much higher multiplication rate than rhizome propagation (Clifton-Brown *et al*., 2017) and enables long use of the mother plants, as seeds can be harvested without destroying the propagation fields. For this reason, seed propagation seems a viable method for fertile hybrids. However, propagation via seeds increases the danger of invasiveness, an important factor when selecting plants to be used as new biomass crops (Raghu *et al*., 2006; Boersma & Heaton, 2014b). Thus, future genotypes of miscanthus may be sterile, rendering seed production impossible. For such genotypes, an improved vegetative, nondestructive and low-cost propagation method is required.

Collar propagation is a vegetative propagation method, which could be used for sterile genotypes. Collars are built at the junction between the rhizome and the stem (Fig. 1) and usually have buds. Theoretically, every bud has the potential to generate a new shoot or even a new plant (Klimešová & Klimeš, 2007). The collars can be harvested less destructively than rhizomes by pulling out the stems of senesced plants, leaving the rhizomes in the ground. As the collars are strongly attached to the bottom of the stem, the chance is quite high that stem and collar can be harvested in this way. As enough of the rhizome is left in the ground, the propagation field is not destroyed. This ensures long-term use of the field, avoiding the establishment of new propagation fields.

So far, there have been no reports on the potential of using this propagation method for miscanthus. Hence, the objective of this study was to test whether it is possible to raise new miscanthus plants from collar fragments. For this purpose, three different fragment types (rhizomes, collars with additional rhizome pieces and collars only, see Fig. 1) from one genotype of each of the species *M. sacchariflorus*, *M.* × *giganteus* and *M. sinensis* were tested in three trials. Establishment success, yield and plant performance were analysed. Additionally, the best harvest date for collars in terms of establishment success was investigated.

Materials and methods

The plants used as material source were taken from plots of the field trials established in the European Miscanthus Improvement (EMI) project (Clifton-Brown *et al*., 2001) in 1997 at the experimental station Ihinger Hof (IHO, 48°45′N, 8°56′E, 480 m a.s.l.). Of the 15 genotypes tested in this trial, three were chosen (EMI numbers 4, 5 and 11) for this study to give a genotypic diversity: one genotype from each of the species *M.* × *giganteus (M*×*G), M. sacchariflorus (MSac)* and *M. sinensis (MSin)*, as shown in Table 1 (in accordance with Clifton-Brown *et al*., 2001). In late April 2014, the stubble and rhizomes were harvested in a nondestructive way by pulling the whole stems with attached collar and rhizome parts out. They were washed,

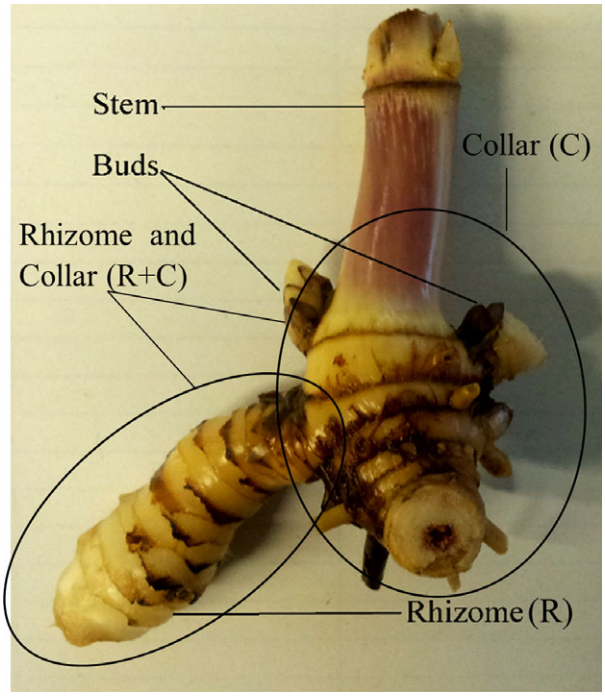

Fig. 1 Photograph of a *Miscanthus sacchariflorus* Rhizome + Collar fragment used in the experiments.

cut and separated into three treatment categories according to fragment size: 5-cm-long collar fragments (C), 5-cm-long collar fragments with additional 3-cm-long rhizome pieces (R+C) and 5-cm-long rhizome cuttings (R) as a control. The 5-cm collar fragments were cut in such a way that they were made up of 2 cm aboveground stem and 3 cm belowground collar part. All these prepared materials were then placed in plastic bags and stored in the fridge at 4 °C until they could be used in the experiments. The maximum storage period was 3 weeks. Four different experiments were conducted to analyse the viability of the collar fragments, with genotype and fragment size as treatment factors. Table 1 shows all material types for all genotypes.

An overview of the different trials is provided in Table 2, including the genotypes and fragment types used and the parameters tested. The different treatments are combinations of genotype (*M×G*, *MSac* or *MSin*) and fragment type (C, R+C or R) and are thus abbreviated, for example, to '*MSac* R+C' for the fragment collar + rhizome of the genotype *Miscanthus sacchariflorus*.

Shoot emergence from fragments in a chamber study

The first experiment (Trial 2.1) started in mid-May 2014 when the propagules had been stored for 20 days. The fragments were planted in 54 pots measuring 18 cm (length) × 12 cm (width) × 6 cm (height) each, which were filled with 550 g soaked potting media (100% water-holding capacity) with a high clay content of 10–12%, a pH value of 5.5–6, and containing all necessary micronutrients (Ensinger Kulturerden, Pikiersubstrat Premium). For each treatment combination, six randomly selected fragments were planted in each pot and covered with an additional 150 g soaked potting media (1 cm minimum soil coverage). In addition, each pot was covered with a thin transparent film to avoid water loss. On the fifth day after planting, the film was removed and, from this point onwards, the pots were watered with 50 ml water a day. The pots were placed in a randomized complete block design in a climate chamber with a 16 h/8 h light/dark period and a 23 °C/18 °C day/night temperature for a period of 18 days, creating optimal conditions for establishment. During this time, the sprouting of new shoots was recorded on a daily basis, counting shoots that had emerged from soil by at least 1 cm.

Field performance of transplanted chamber study plantlets

At the end of the chamber study in June 2014, the strongest plantlets of each genotype from Trial 2.1 were taken and manually transplanted into a clayey loam research field at the University of Hohenheim campus (48°42′N, 9°13′E) (Trial 2.2). To ensure good soil conditions, the field was harrowed before planting. The three different fragment types of two genotypes (*M×G* and *MSac*) were transplanted in a randomized complete block design with three replicates, that is a total of 18 plots. Due to the low emergence of *MSin* in the chamber study, this genotype was neglected in this trial. In each of the 18 plots (1.0 m × 0.3 m), four plantlets of either *M×G* and *MSac* were

Table 1 Description of the different miscanthus genotypes with their EMI numbers and their fragment weights (n = 3)

		Fresh weight (g)/fragment		
Genotype	EMI No.	5-cm collar (C)	5-cm collar with 3-cm rhizome (R+C)	5-cm rhizome (R)
Miscanthus sacchariflorus (*MSac*)	5	4.4 ± 1.4^{b}	8.3 ± 1.3^{a}	4.5 ± 0.7^{b}
Miscanthus × *giganteus* (*M×G*)	4	5.6 ± 1.8^{B}	7.7 ± 1.3^{A}	4.3 ± 0.7^{C}
Miscanthus sinensis (*MSin*)	11	3.0 ± 0.9$^{\boldsymbol{b}}$	4.7 ± 1.1$^{\boldsymbol{a}}$	2.9 ± 0.7$^{\boldsymbol{b}}$

Further information on these genotypes is available in the references of Clifton-Brown *et al.* (2001) and Iqbal & Lewandowski (2014). Significant differences of weight within a genotype are indicated by different lower-case letters (a, b) for *MSac*, different upper-case letters for *MxG* (A, B, C) and different bold, italic letters (***a***, ***b***) for *MSin* (α = 0.05).

Table 2 Overview of the four trials

Trial	Abbreviation	Trial type	Genotypes	Fragments	Measured traits	Date range
2.1 Shoot emergence in a climate chamber	Chamber study	Chamber study	*MSin* *M×G* *MSac*	C; R+C; R	Shoot emergence	19/05/2014 to 06/06/2014
2.2 Field performance of transplanted greenhouse plantlets	Transplanted	Field trial	*M×G* *MSac*	C; R+C; R	Plant traits Establishment success	06/06/2014 to mid-April 2015
2.3 Field performance of fragments directly planted into the field	Directly planted	Field trial	*MSin* *M×G* *MSac*	C; R+C; R	Plant traits Establishment success	06/06/2014 to mid-April 2015
2.4 Influence of collar harvest date on emergence	Harvest trial	Chamber study	*M×G* *MSac*	C	Shoot emergence	Early November 2014 to early February 2015

planted with 0.2 m spacing between plants within the rows. The plots were irrigated twice in the first 2 weeks after planting, and then, no additional water or fertilizer was given during the whole experimental period. Weeding was conducted several times in all plots to ensure better establishment conditions. The establishment success was determined in July 2014.

At the end of the 2014 growing season (3rd November 2014), plant survival was assessed prior to harvesting. After that, the three strongest plants per plot were selected for morphological measurements including plant height, stem number and stem diameter. Plant height was measured from the soil surface to the node of the uppermost fully expanded leaf on the highest stem of each selected plant. Stem diameter was measured on the same stem between the collar and the first internode. For stem number per plant, all stems with a height of at least 10 cm were counted and the number divided by the planting density. The harvested plants were then oven-dried (60 °C for 7 days) and weighed for dry matter biomass yield assessment. To calculate overwintering survival rate, the plants still alive after the winter (mid-April 2015) were counted.

Field performance of fragments directly planted into the field

A second field experiment started at the same time as Trial 2.2, but this time planting the stored fragments directly into the field (Trial 2.3). In each 1.2 m × 0.6 m plot, one fragment type of one genotype was planted. The trial had a randomized block design with four replications, giving 36 plots in total. Per plot, 10 fragments were planted at a soil depth of 5 cm. Prior to planting, the field was harrowed to ensure good establishment conditions. The plots were irrigated twice in the first 2 weeks after planting, and then, no additional water or fertilizer was given during the whole experimental period. To minimize weed pressure, manual weeding was conducted several times in all plots. In July 2014, 1 month after planting, the establishment success was calculated as a percentage of the planted fragments.

Morphological measurements were taken of the three strongest plants at the end of the 2014 growing season. Stem number, stem diameter, plant height, biomass yield and number of plants at harvest were determined according to the methods described for Trial 2.2. In April 2015, overwintering survival was assessed.

Influence of harvest date on shoot emergence from collars

During the period November 2014 to February 2015, collar pieces of *M*×*G* and *MSac* were collected each month from the EMI project fields at Ihinger Hof (Trial 2.4). A separate shoot emergence experiment was conducted for each harvest date. Thirty collar fragments were randomly selected from each genotype and planted into five pots with six collars each, in the same way as in the chamber study (Trial 2.1). The emergence ratio of collar pieces from each harvest date was calculated 21 days after planting.

Statistical analysis

Data analysis was performed using the Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). Metric plant traits were analysed by mixed models using the PROC MIXED procedure. A test for normal distribution and variance homogeneity was conducted for each plant trait. As emergence rate and establishment success in each trial are binomially distributed, a generalized linear mixed model was performed using the PROC GLIMMIX procedure and a logarithm was used for the link function. The model allows overdispersion. In Trials 2.1–2.3, establishment success was measured by shoot emergence, and where appropriate, survival rate after transplanting, at harvest and after winter, was analysed using the generalized linear mixed model shown in Eqn (1). In the Trials 2.2 and 2.3, plant traits were analysed by the linear mixed model shown in Eqn (2). In both models, genotype, fragment and their interactions were taken as fixed effects. In Trials 2.1–2.4, the effects of replicates were assumed to be random effects. In Trial 2.4, Eqn (3) was used to analyse shoot emergence. This model is the same as Eqn (1) but with fragment instead of harvest date. In this trial, shoot emergence of collars from different harvest dates was measured in different experimental runs, and thus, the estimated error only accounts for errors within the chamber experiment and ignores errors in the different experimental runs. As such, it underestimates the true error variance. Multiple *t*-tests with a significance level of $\alpha = 0.05$ were conducted only where significance was found in a type 3 test for fixed effects. The genotype *MSin* was partially omitted from the statistical analysis and presentation of results due to its poor emergence.

$$y_{ijk} = \mu + \log\,(g_i + f_j + (gf)_{ij} + r_k) + e_{ijk} \quad (1)$$

$$y_{ijk} = \mu + g_i + f_j + (gf)_{ij} + r_k + e_{ijk} \quad (2)$$

$$y_{ihk} = \mu + \log\,(g_i + d_h + (gd)_{ih} + r_k) + e_{ihk} \quad (3)$$

y_{ijk} = measurement of the *g*-th genotype with the *f*-th fragment in *r*-th replication; μ = general effect; g_i = main effect of the *g*-th genotype (*MSac*; *M*×*G*; *MSin*); f_j = main effect of *f*-th fragment (C, C+R, R); d_h = main effect of *h*-th harvest date (November, December, January, February); bc_{gf} = interaction of *g*-th genotype with *f*-th fragment; r_k = random effect of *r*-th replication; e_{ijk} = residual error term for y_{ijk}; e_{ihk} = residual error term for y_{ihk}.

Results

Establishment success

Overall, the trials showed that the establishment success of the collars was either better than or at least as good as that of the rhizome fragments. To gain an overview of the development of fixed effects over the 18 days of the chamber study (Trial 2.1), days 6, 12 and 18 were taken for analysis. The fixed effects showed that genotype and fragment were significant, whereas the

interaction between genotype and fragment was not significant on these days (Table 3). It was found that the rhizome fragments of all three genotypes had the lowest emergence rate on all 3 days (except *MSac* on day 6; Fig. 2). The fragment 'R+C' had the highest emergence rate at each of the days 6, 12 and 18 (except for *MSin* R+C at day 6). The fragment 'C' was in between. At least 54% of the collar fragments of all three genotypes had emerged after 18 days under controlled conditions. Information on shoot emergence of each day can be found in Table S1.

When the pregrown plantlets were transplanted into the field (Trial 2.2), there was no significant difference in survival rate between the fragment types (Fig. 3a). When planted directly into the field (Trial 2.3), at least 40% of the collar fragments of genotypes *M*×*G* and *MSac* emerged (Fig. 3b). Fragment R+C had the highest emergence rates in all three genotypes. The rhizome fragments had lowest emergence rate, except for *MSin*, where the collars had the lowest emergence, but without a significant difference. For *MSac*, the fragment R+C had significantly higher emergence rates than the rhizomes. For *M*×*G*, the R+C fragment had significantly higher emergence rates than both other fragments (Fig. 3b).

Field establishment success was determined twice, at harvest in November 2014 (Fig. 4a) and after the winter in April 2015 (Fig. 4b). On both dates, survival of the transplanted plants (Trial 2.2) was higher than that of the directly planted fragments (Trial 2.3). The directly planted collar fragments showed a similar or significantly higher survival rate than the rhizome fragments, whereas none of the *MSin* collar fragments had survived (Fig. 4a,b) on either assessment date. For the transplanted plants, no significant effect was found between the different fragment types within the two genotypes tested. Although there were some losses (about 5%) from counted plants between November 2014 and April 2015 for *MSac* C, the general losses of Trial 2.2 over winter were low. In Trial 2.3, however, the overwintering losses for *MSac* *R*+C, *M*×*G* C and *M*×*G* R were between 10 and 15%, and thus higher than for Trial 2.2 for these combinations. It should be pointed out that in the case of *MSac* C (directly planted), more plants were counted in November 2014 and after winter in April 2015 than at the first counting in July 2014. There could be two reasons for this: First, the planting distance was narrow at only 20 cm. Therefore, it was difficult to differentiate between plants and their tillers

Table 3 Type 3 test for the significance of main effects and their interactions (genotype, fragment, genotype*fragment) on shoot emergence of the three genotypes (*MSac*, *MxG* and *MSin*) in the chamber study (Trial 2.1). Level of significance was α = 0.05

	Day 6		Day 12		Day 18	
	F-value	Pr>*F*	*F*-value	Pr>*F*	*F*-value	Pr>*F*
Genotype	6.67	0.0032	5.54	0.0076	8.93	0.0006
Fragment	5.82	0.0061	14.86	<0.0001	13.80	<0.0001
Genotype × Fragment	2.5	0.0582	2.34	0.0724	1.68	0.1740

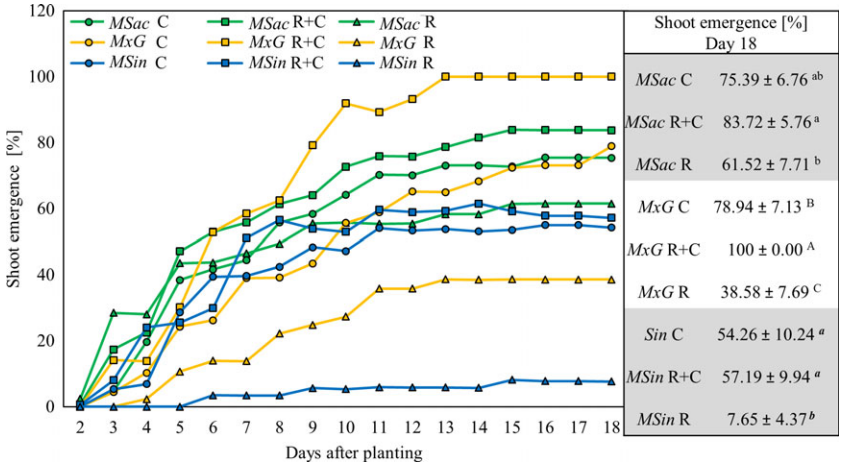

Fig. 2 Shoot emergence (*n* = 6) over 18 days after planting in a chamber study (Trial 2.1) with the three genotypes (*MSac*, *M*×*G* and *MSin*) and the three fragment types (collar = C, rhizome and collar = R+C, rhizome = R). Significant differences within each genotype are indicated by different lower-case letters for *MSac*, upper-case letters for *M*×*G* and bold italic letters for *MSin* (α = 0.05).

and double counting may have occurred. In addition, it is possible that some plants emerged later than July 2014 and therefore were not included in the first counting.

Influence of fragment type on plant performance

This section shows the results of Trials 2.2 and 2.3, referred to as 'Transplanted' and 'Directly planted', respectively.

The type 3 test (Table 4) showed that overall only very few significant impacts were observed for the main effects genotype and fragment. The interaction of genotype and fragment only showed a significance for stem number. Fragment only showed significant effects in Trial 2.3 for the traits dry matter content and height. The trait stem diameter was not significantly influenced by the main effects or their interaction, and for this reason, these results are not shown in detail below. Where the pregrown plantlets were transplanted into the field, R+C fragments resulted in the shortest and R fragments in the tallest plants, for both genotypes (Fig. 5a). However, the differences were not significant. Where the fragments were directly planted into the field, the results were the reverse: R+C fragments had in the tallest, and R fragments the shortest plants, for both genotypes (Fig. 5b). However, this difference was only significant for *M*×*G*.

In Trial 2.2, no significant differences were detected between the different fragments for *MSac* (Fig. 5a). For *M*×*G*, however, there was a significant difference between the fragments R+C and R. There was a difference in stem number between genotypes *MSac* and *M*×*G* for the fragment R. It was highest in *M*×*G* and lowest in *MSac*. In Trial 2.3, for *MSac*, fragment C had a significantly higher stem number than R, with R+C in between the two (Fig 5b). For *M*×*G*, the same trend was observed, but the differences were too small to be significant.

The stem diameter of plants from the three fragments was not significantly different within each genotype in either trial. On average, *MSac* and *M*×*G* showed a stem diameter of 0.68 cm and 0.79 cm in Trial 2.2 and

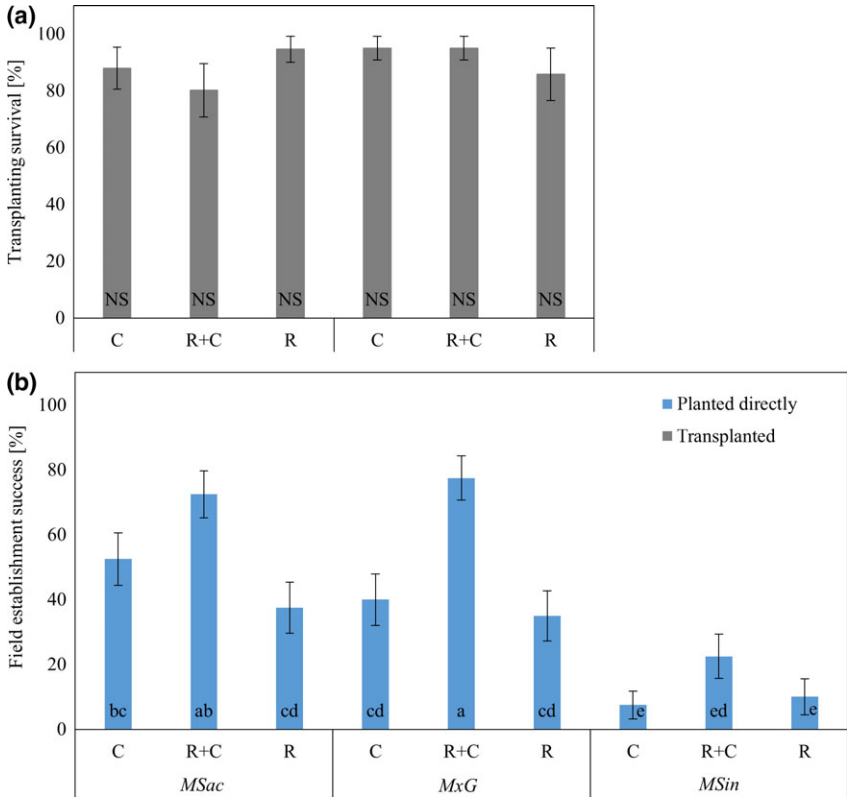

Fig. 3 Transplanting survival of pregrown plantlets (a) and field establishment success of fragments planted directly into the field (b) one month after transplanting/planting. Transplanting survival was assessed for two genotypes (*MSac* and *M*×*G*) and three fragment types (collar = C, rhizome and collar = R+C, rhizome = R). Field establishment was conducted for all three genotypes (*MSac*, *M*×*G* and *MSin*) in combination with the three fragment types. Significant differences between the genotypes in combination with the fragments are indicated by different upper-case letters for transplanted pregrown plantlets and different lower-case letters for fragments planted directly into the field ($\alpha = 0.05$). Bars represent standard deviation.

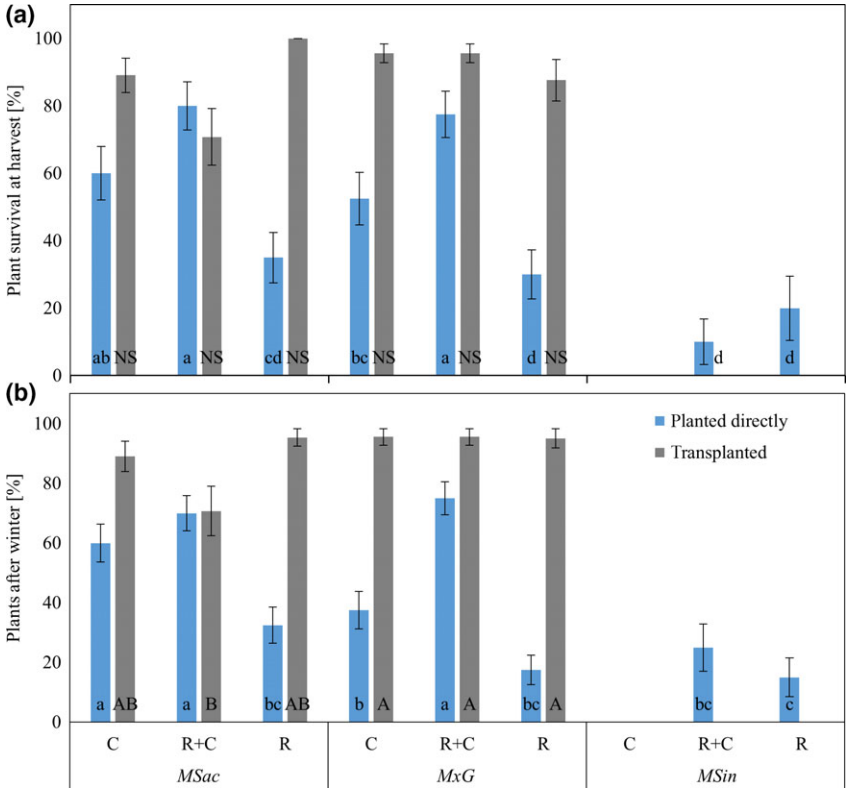

Fig. 4 Plant survival [%] at harvest in November 2014 (a); and after winter in mid-April 2015 (b); for the three genotypes (*MSac*, *M*×*G* and *MSin*) and the three fragment types (collar = C, rhizome and collar = R+C, rhizome = R) for transplanted pregrown plantlets (Trial 2.2) and fragments planted directly into the field (Trial 2.3). In Trial 2.2, only two genotypes (*MSac* and *M*×*G*) were transplanted. Significant differences between the genotypes in combination with the fragments are indicated by different upper-case letters for transplanted pregrown plantlets and different lower-case letters for fragments planted directly into the field ($\alpha = 0.05$). Bars represent standard deviation.

Table 4 Type 3 test for the significance of the main effects and their interactions (genotype, fragment, genotype*fragment) on yield and plant traits for two genotypes (*MSac* and *MxG*) in two field trials (transplanted, directly planted) ($\alpha = 0.05$).

		Transplanted		Directly planted	
Trait	Effect	*F*-value	Pr>*F*	*F*-value	Pr>*F*
Dry matter yield	Genotype	7.87	0.0205	3.43	0.0838
	Fragment	2.64	0.1252	0.09	0.9158
	Genotype × Fragment	1.05	0.3877	0.22	0.8077
Dry matter content	Genotype	0.23	0.6396	2.03	0.1750
	Fragment	1.66	0.2432	5.48	0.0163
	Genotype × Fragment	0.06	0.9434	0.40	0.6772
Stem number	Genotype	0.29	0.6026	4.84	0.0439
	Fragment	0.55	0.5937	3.37	0.0617
	Genotype × Fragment	5.01	0.0345	1.61	0.2333
Stem diameter	Genotype	2.61	0.1409	2.04	0.1738
	Fragment	0.29	0.7584	0.61	0.5552
	Genotype × Fragment	0.16	0.8553	0.01	0.9911
Height	Genotype	7.42	0.0234	37.39	<0.0001
	Fragment	4.03	0.0562	5.04	0.0212
	Genotype × Fragment	0.11	0.8987	1.01	0.3869

0.65 cm and 0.73 cm in Trial 2.3, respectively. In Trial 2.2, the largest stem diameter was found in plants of *M*×*G* C (0.83 cm) and the smallest in *MSac* R+C (0.64 cm). In Trial 2.3, *M*×*G* C (0.76 cm) also had the largest stem diameter and *MSac* R (0.61 cm) had the smallest.

Dry matter yield (DMY) of the plants from the directly planted fragments (Trial 2.3) was not significantly different within each genotype (Fig. 6b). In Trial 2.2, significant differences were observed between the different fragments of genotype *MSac*. *MSac* C had the significantly highest DMY (36.17 g plant^{-1}) and *MSac* R+C the lowest (26.41 g plant^{-1}). For *M*×*G*, no significant differences were visible; fragment R had the highest yield (25.27 g plant^{-1}; Fig. 6a). For the transplanted plants, the DMC ranged from 41.7% to 47.6% (Fig. 6a). Fragment R+C had highest DMC for both genotypes. Genotype *MSac* showed no significant differences in DMC between the fragments. *M*×*G* R+C, however, had a significantly higher DMC than *M*×*G* R. When planted directly, the DMC varied from 34.9% to 52.7%. Fragment R showed the highest DMC in both genotypes, but without significant differences (Fig. 6b).

Influence of harvest date of collars on emergence ratio

The effect of harvest date of the collar fragments on the emergence rate was not significant at a significance level of $\alpha = 0.05$, whereas the genotype effect was (Table 5). However, the November harvest resulted in the lowest emergence rate in both genotypes: 66.9% for *MSac* and 70% for *M*×*G* (Table 6). On the other harvest dates, the *M*×*G* collars in particular showed very high emergence rates of on average 91.2%. By contrast, for *MSac*, the highest emergence rate of 83.4% was observed for collars harvested in December.

Discussion

This study showed that the tested miscanthus genotypes of the two species *MSac* and *M*×*G* can be successfully propagated and established via collar fragments. These collars can be harvested by pulling out the stems of senesced plants with low impact compared to the current commercial practice of rhizome harvesting. However, the tested genotype of the species *MSin* showed that collar propagation is not suitable for all miscanthus genotypes. The following sections discuss (i) the suitability of the three lower stem parts for

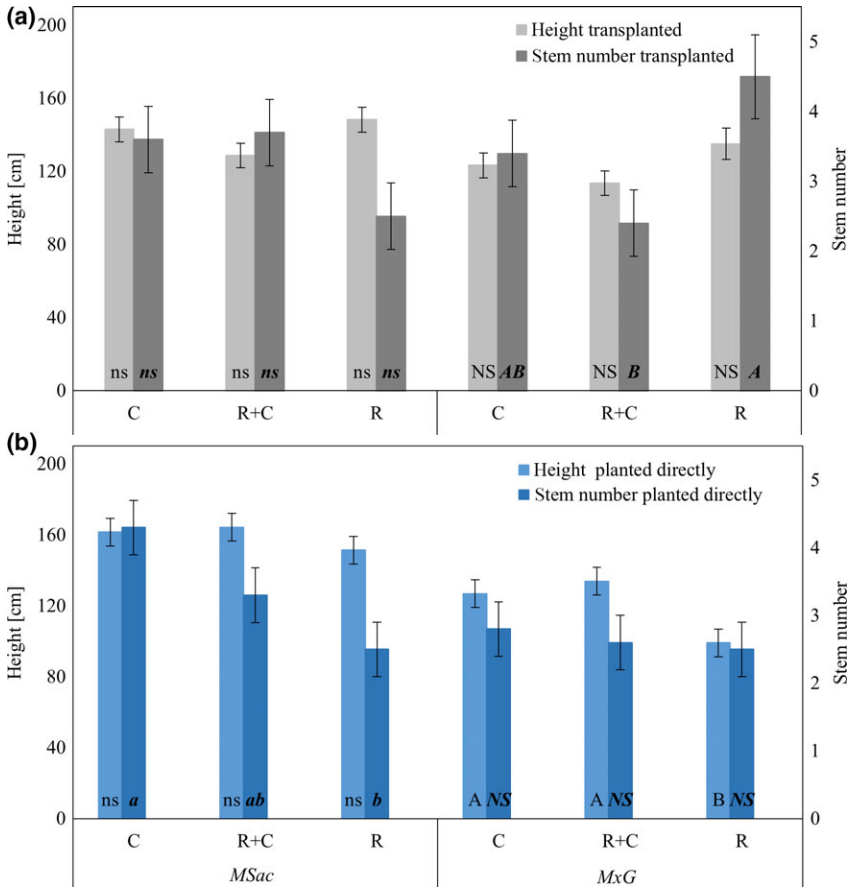

Fig. 5 Height (cm) and stem number of the two genotypes (*MSac* and *M*×*G*) for transplanted pregrown plantlets (a) and fragments planted directly into the field (b). Significant differences in height are indicated by different lower-case letters for *MSac* and different upper-case letters for *M*×*G*. Significant differences in stem number are indicated by bold italic letters, lower-case for *MSac* and upper-case for *M*×*G*. Level of significance was $\alpha = 0.05$. Error bars represent standard deviation in height and stem number.

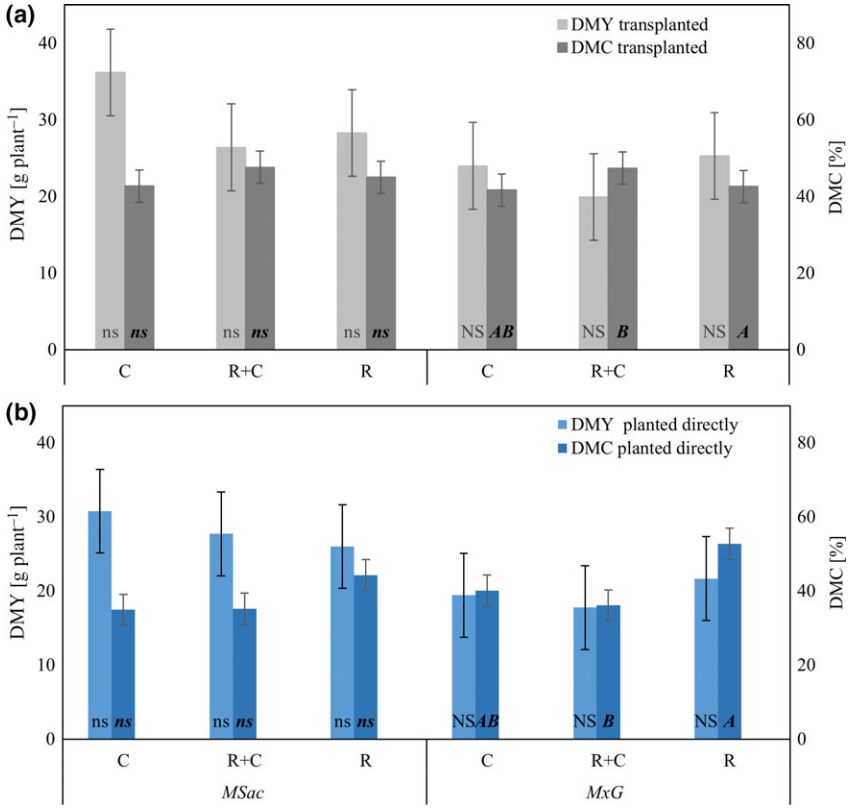

Fig. 6 Average dry matter yield (DMY) [g plant^{-1}] and dry matter content (DMC) [%] of the two genotypes (*MSac* and *M*×*G*) for transplanted pregrown plantlets (a) and fragments planted directly into the field (b). Significant differences in DMY are indicated by different lower-case letters for *MSac* and different upper-case letters for *M*×*G*. Significant differences in DMC are indicated by bold italic letters, lower-case letters for *MSac* and upper-case letters for *M*×*G*. Level of significance was $\alpha = 0.05$. Error bars represent standard deviation for DMY and DMC.

Table 5 Type 3 test for the significance of main effects and their interactions (genotype, harvest date, genotype*harvest date) on emergence in two genotypes (*MSac* and *MxG*) ($\alpha = 0.05$)

Effect	*F*-value	Pr>*F*
Genotype	5.63	0.0291
Harvest Date	2.22	0.1169
Genotype × Harvest date	0.85	0.4860

Table 6 Emergence rate [%] of collars in the two genotypes (*MSac* and *MxG*) when collars were harvested at four different harvest dates

	Emergence rate (%)	
	Msac	*M*×*G*
08/11/14	66.91 ± 9.171^{ns}	70.26 ± 8.859^{ns}
10/12/14	83.38 ± 6.970^{ns}	90.04 ± 5.491^{ns}
12/01/15	73.39 ± 8.488^{ns}	93.37 ± 4.502^{ns}
12/02/15	76.76 ± 8.057^{ns}	90.07 ± 5.480^{ns}

ns, not significant ($\alpha = 0.05$).

propagation, (ii) the performance of the novel collar propagation method compared to conventional miscanthus propagation methods and (iii) the further development of the collar propagation method for practice.

Suitability of the three lower stem parts for propagation

The results showed that all three lower stem parts, that is collar, rhizome and the combination of collar and rhizome, are suitable for miscanthus propagation.

Where pregrown plantlets were transplanted, no significant differences in establishment and overwintering success were found between the fragments. In contrast, where the fragments were planted directly into the field, the establishment success was significantly higher (depending on genotype) for R+C than for C and R alone (Figs 3 and 4). The R+C fragment is larger and also significantly heavier (Table 1) than the single fragments C and R. It can be assumed that field survival was positively influenced by the weight of the planting material. Previous investigations have observed that shoot emergence rate increases with rhizome weight and size (Christian *et al.*, 2009; Xue *et al.*, 2015). More reserve

substances can be stored in a larger fragment, probably leading to a better overwinter survival of R+C than for single R or C fragments. Therefore, when planting directly into the field, the use of R+C fragments is recommended in order to ensure higher establishment success.

The establishment success of collars was either better than or not significantly different from that of rhizomes when planted directly. Both these fragment types are parts of the stem and had a similar size and weight, except *M*×*G*, where fragment C was significantly heavier than fragment R (Table 1). The rhizomes used were only 1 year old and so can be expected to have a better establishment success than older rhizomes. The age of collars is more homogeneous than that of rhizomes, because collars grow annually together with the stems. By contrast, the age of harvested rhizome parts is heterogeneous and depends strongly on the plantation age of the mother field. The vitality and capacity to regenerate a full plant decreases with age of the mother field and thus rhizome age (Christian *et al.*, 2009). Therefore, collars are more advantageous, as they are more homogenous in age.

Comparison with other propagation methods

The optimal miscanthus propagation method should be characterized by simplicity, low costs, high reproduction rate of the mother material, low labour and energy inputs, and should ideally be nondestructive for the mother field (Xue *et al.*, 2015). Applying these criteria to the results of the four trials of this study, it can be seen that miscanthus propagation via collar fragments is feasible and preferable to conventional rhizome propagation. Harvesting of rhizomes is very labour-intensive, requires heavy machinery and causes soil disturbance, which can lead to soil carbon losses (Boersma & Heaton, 2012) and soil compaction. After harvesting, the rhizomes have to be separated from the roots, cleaned of soil (Xue *et al.*, 2015), graded by size and dried out fragments discarded. For this propagation method, viability of the rhizomes is the most important criterion for success and therefore the most important quality criterion for customers purchasing the rhizomes. In addition, it can take up to a maximum of 5 years before sufficient planting material is available in the mother field to be harvested (Christian *et al.*, 2005). This means that the harvested rhizomes vary in age, which can influence field emergence. Christian *et al.* (2009) showed that rhizomes from a 9-year-old plantation had a lower viability than rhizomes from a 5-year-old plantation. Therefore, quality screening of the harvested rhizomes is often performed manually to guarantee high-quality rhizomes. This, however, increases the labour intensity of rhizome harvesting.

By comparison, the harvesting of collars is relatively simple. Collars are harvested in a nondestructive way from the mother field by pulling out the stems. As they can be harvested annually, the propagation material is more homogenous than for rhizomes, and the collar fragments can be easily cut to the required size. In addition, harvesting of collars does not disturb the soil, and the remaining mulch layer avoids potential erosion problems. Moreover, collar harvesting delivers fragments of the same age with similar emergence rates, rendering quality screening of viable collars easier than for rhizomes. Future research is required to determine whether, or to what extent, the mother plants are impacted by the harvesting of its collars. If the crop is impacted, a 2-year cycle of collar harvesting may solve the problem. Further research should also clarify whether the age and planting density of the mother field, from which the collars are harvested, influence the viability of collars in different ways.

Establishment success and overwintering losses are also important indicators for the comparison of different propagation methods. In a field trial of Boersma & Heaton (2014a), the establishment loss 2 months after planting reached up to 25% for nodal-stem-propagated plants (transplanted) and up to 34% for (directly planted) rhizome-derived plants. The losses after the first winter were 1–2%. Clifton-Brown *et al.* (2007) also rated establishment survival of micro- and rhizome-propagated *M*×*G* plants in Ireland. At the end of the first growing season, 95% of the plants of both propagation types had survived, whereas after 5 years, 86% of rhizome- and 53% of micro-propagated plants had survived. Although establishment success in our field trial was lower than in those two studies, it was shown that the emergence of collar fragments was as high as for rhizome fragments. As there were virtually no plant losses between the counting at harvest and after winter in Trial 2.2, the overall overwintering survival of the transplanted plants is comparable to the results of Boersma & Heaton (2014a). Apart from *MSac* R+C (70.8%), the plant survival was at a similar level to the results of Clifton-Brown *et al.* (2007), as 87% (*M*×*G* R) to 100% (*MSac* R) of the transplanted plantlets survived until harvest. The higher overwintering losses in Trial 2.3 (15%) were probably caused by the short growing season (June to November) available to the fragments planted directly into the field. This short vegetation period led to lower yields in the directly planted than in the transplanted fragments (Fig. 6). There was obviously not enough time for the plants to establish sufficiently and build up enough reserve substances in the rhizome for regrowth the following year. This can be seen by the plant performance analysed in Trials 2.2 and 2.3. Here, the transplanted plantlets not only had

higher DMY and DMC but, with a few exceptions, also higher stem number and stem diameter (Fig. 5). The transplanted plantlets had more time to develop and, for example produce more stems, and build up enough reserve substances for a regrowth.

As mentioned above, a high multiplication rate is an important factor for an economically successful propagation method. The multiplication rate of collars is lower than that of seed production (>1.500 m^{-2}), and lies more in the region of rhizome propagation (10–50 m^{-2}) (Clifton-Brown *et al.*, 2017). This is due to the fact that every tiller can only deliver one collar. Kalinina *et al.* (2017) found average shoot densities of 29–74 shoots per plant (29 for *M*×*G*) with a planting density of 2 plants m^{-2} in various miscanthus genotypes across Europe. Thus, for those genotypes, a harvest of 58–148 collars m^{-2} would be possible if every stem was harvested. However, as this was not analysed in our study, further research is needed to clarify how many stems per plant could be harvested without negatively affecting the mother plant. The multiplication rate of collars is also lower than that of stem cuttings (max. 200 possible; Boersma & Heaton, 2012). However, as described by Xue *et al.* (2015), stem cuttings cannot easily be planted into the field, as they deteriorate rapidly. Therefore, it is better to pregrow them under controlled conditions and then transplant them into the field (Xue *et al.*, 2015). The field experiments described above showed that collars could be planted directly into the field, with some improvements discussed below. When planted directly into the field, collar propagation would be cheaper than stem propagation.

Seeds are easier to store and transport (Clifton-Brown *et al.*, 2017) and need less space than rhizomes and collars. However, propagation via seeds also has some disadvantages compared to collar propagation. First, dedicated seed nurseries need to be established. These are often in warmer regions than the productive miscanthus plantations to allow the seeds to ripen. This means the propagules cannot be produced locally by farmers, leading to further transport costs. In addition, miscanthus seeds are very small, and thus, direct sowing into the fields is not yet a reliable option. Plug plants are currently used instead of sowing seeds directly, but this involves many additional logistical steps, again increasing the costs. Methods for coating seeds to allow direct sowing are still at the experimental stage. The genetic variability of seeds is another problem yet to be solved. Crossing out can lead to genetically inhomogeneous seeds and consequently an inhomogeneous miscanthus stock (Lewandowski *et al.*, 2016). This complicates field management and can result in additional work for farmers as well as lower yields and inhomogeneity of biomass quality. As described above, invasiveness of fertile miscanthus genotypes is also a huge problem. It can result in additional environmental costs, as the native habitat is changed by the invasive grass, and economic costs to curb the invasiveness of the crop (Raghu *et al.*, 2006). Jorgensen (2011) pointed out that sterility is an important goal in future miscanthus breeding to rule out invasiveness before planting. Quinn *et al.* (2010) refer to regulatory restrictions in the United States for certain miscanthus genotypes and therefore recommend sterile or at least functionally sterile genotypes. There are also some other disadvantages of miscanthus propagation via seeds. For example, in areas with low spring temperatures, the earliest possible sowing date may be too late for the crop to develop sufficient rhizome biomass to survive the first winter. Secondly, the risk of overwinter losses increases in plants without fully developed rhizomes (Clifton-Brown *et al.*, 2017). Finally, seed propagation is only feasible for fertile genotypes, mostly *MSin*, and for new hybrids yet to be developed, but a directly sowing is not yet reliable.

To summarize, collar harvest is nondestructive for the mother field, less labour-intensive and therefore cheaper than rhizome propagation. It could become the best propagation method for those miscanthus genotypes that either cannot reproduce via seeds or where genetically homogenous plantations are to be established in temperate regions, using the propagation material from nearby fields.

Development of the collar propagation method in practice

Presently, harvesting of collars has to be performed manually, as no specific machinery is available. Suitable collar harvest machinery needs to be developed to upscale this propagation method. The machine should remove stems and collars from the ground, for example using rubber rollers or a robot arm and, ideally, separate them at the same time.

The harvest trial (Trial 2.4) showed that it is in principle possible to harvest collars throughout the whole winter, as no significant differences were found between the four harvest dates. However, the very early harvest in November had the lowest emergence rate for both genotypes and therefore may be less suitable for commercial application. According to Atkinson (2009), rhizomes are measurably affected by their harvest date, as contents of protein, nitrogen and soluble carbohydrates decrease and contents of lipids increase with a later harvest date. Lipids in particular provide an important energy store for overwintering and regrowth in spring (Atkinson, 2009). Future research therefore needs to determine whether this is also valid for collars, in order to identify the most suitable harvest time.

There were no significant differences in emergence rate of collar fragments from *MSac* and *M*×*G* between the harvest dates December to February. Thus, February harvest is recommended because it reduces storage costs and the drying out of collar fragments, which can reduce establishment success.

Both *MSac* and *M*×*G* showed good establishment success and plant performance when propagated via collar fragments. However, this method does not seem suitable for the genotype of the species *MSin* tested, which can be attributed to the rhizome and collar morphology of *MSin*. This genotype has short, thin rhizomes, whereas *MSac*, and *M*×*G* have thick rhizomes (Lee *et al*., 2012; Xue *et al*., 2015). As described above, thicker rhizomes lead to better establishment of *MSac* and *M*×*G*, as they are able to store more reserve substances. Thus, for these two species tested, propagation via collar fragments is recommended. By contrast, for the tested genotype of the *MSin* species with its thinner rhizomes and fertile seed production, seed propagation is recommended.

The genotypes *MSac* and *M*×*G* showed similar emergence and survival rates for C and R under controlled and field conditions. Therefore, both options, planting collars directly and transplanting pregrown plantlets into the field, may represent opportunities for commercial application of collar propagation. Direct planting of the collar fragments would be preferable, because no additional propagation in the greenhouse is required, saving costs, energy and labour. However, direct planting would necessitate a late collar harvest, for example in April, so that the collars can be planted into the field directly after harvest without any storage. This, however, has not yet been tested and requires further research. To increase establishment success of collars directly planted into the field, one option could be to cover them with a plastic mulch film, which increases soil temperature. O'Loughlin *et al*. (2017) showed that miscanthus rhizomes had a better establishment success, higher stem numbers and higher biomass yields, when they were covered with a plastic mulch film. Another option to improve establishment success and storage suitability of collars could be to encapsulate the collars in a beneficial coating, possibly also including nutrients and growth-promoting substances, to improve emergence rate, as it has been shown for seeds and other propagules (NEF, 2015; Greenfield Mantelsaat®, 2017). Collars could also be coated using such technologies to improve their establishment success when directly planted into the field. The coating would reduce the drying out of the collar fragments, which is one of the main reasons for low emergence rates. In addition, by covering the collars, it may be possible to standardize their size, enabling mechanical planting and thus further reducing establishment costs. Lower establishment costs would also facilitate miscanthus cultivation on marginal land, where establishment is the most challenging phase in the lifetime of a miscanthus crop. Adapting the coating materials to marginal conditions could further enhance establishment success and rooting of the crop in such areas, improving the efficiency of crop production.

An alternative to coating is pregrowing the collars in the greenhouse. In our study, the transplanting of pregrown plantlets resulted in a higher establishment success than the direct planting of collar fragments into the field. However, it should be mentioned that only the stronger plants were transplanted into the fields; we cannot say how the weaker plants would have developed. In practice, it could be possible to transplant the plantlets with a conventional planting machine as used in vegetable production. However, transplanting requires additional production steps, which lead to additional costs, energy and labour and also logistic efforts, as green plantlets have to be shipped. For marginal sites or regions with low temperatures in spring, field establishment via plantlets may be advantageous and result in a higher establishment success.

In conclusion, miscanthus propagation via collars was shown to be viable and a promising alternative to rhizome propagation. Collar propagation enables the generation of homogenous planting material and thus a uniform miscanthus stock. As the harvesting of collars is likely to be less labour-intensive and is less destructive for the mother field than rhizome propagation, this method is more favourable than rhizome propagation for both economic and ecological reasons. However, whereas collar propagation is the most suitable method for the two *MSac* and *M*×*G* genotypes tested, this is not true of the *MSin* genotype. These can already best be propagated by seeds. If collars are directly planted into the field, the fragment R+C should be used. Separated C and R fragments could be used for coated propagation material, which can be easily stored and transported and used for the establishment of homogenous miscanthus plantations, possibly also under marginal site conditions.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Mean shoot emergence over 18 days in the chamber study.