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In vitro-propagated *Miscanthus* × *giganteus* plants can be a source of diversity in terms of their chemical composition

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ABSTRACT

Miscanthus × *giganteus* is a triploid hybrid propagated vegetatively from rhizomes or in a tissue culture, so its clones are characterised by a very low genetic diversity. Giant *Miscanthus* is cultivated mainly for biomass used as biofuel; depending on its chemical composition, it can be used in many industries. The goal of this work was to determine whether regenerants obtained in tissue culture can be a source of new forms characterised by different biomass yield and energy values as well as by their concentration of cellulose, hemicellulose and lignin. In the study the number and height of stems as well as the dry weight, ash and water mass fraction of biomass were estimated. The study was performed on *Miscanthus* plants propagated from rhizomes obtained from two different locations in Poland (Zabierzów – Z and Radzików – R), and on regenerants (Iv) obtained from Z plants. The results indicated that somaclonal variation occurs in *M.* × *giganteus* cultures and that regenerants show low polymorphic variation compared to donor plants. Plants propagated in an *in vitro* culture showed the lowest stem height, but the highest number of stems per plant. Dry matter yield was greatest in the R group, while regenerants demonstrated the highest level of hemicellulose fraction and lowest cellulose and lignin concentration.

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; AFLP, amplified fragment length polymorphism; DW, dry weight of plant samples; DM, dry matter of the biomass; Iv, regenerants propagated in an *in vitro* culture; NDF, neutral detergent fibre; PPFD, photosynthetic photon flux density; R, plants obtained from the Plant Breeding and Acclimatisation Institute in Radzików (Poland); TBE buffer, tris-base and boric acid with ethylenediaminetetraacetic acid; Z, plants obtained from a commercial producer in Zabierzów (Poland).

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Energy value was the highest for Z plants, but Iv plants showed energy values on the same level as those of R plants. The data suggest that *in vitro*-propagated plants may be a source of new *Miscanthus* forms.

1. Introduction

Miscanthus × *giganteus* is a typical C_4 grass characterised by high photosynthetic efficiency and biomass production. This plant species is regarded mainly as an 'energetic' plant, cultivated as a material for pellet or lignocellulosic biofuel production, but it can also be used as building, paper fibre and insulation material and to make hard boards and plant potting mixtures as well as roof thatching [1]. The lifespan of *Miscanthus* plants ranges from 20 to 25 years, so long-term plantations can be treated as a soil carbon sequestration. Additionally, they are planted on river banks and coastal areas to protect the soil from erosion [2–4].

Miscanthus species have the potential for high yields of biomass, reaching yields of 25–35 t ha^{-1} in European climate conditions [2,5]. Due to the rise in demand for renewable energy, *M.* × *giganteus* is becoming more widely cultivated across Europe. The composition of cell walls determines the use of *Miscanthus* in various industries. For example, high-lignin feedstocks confer a higher heating value, which is preferable for combustion [6], but inhibits biological conversion processes such as fermentation and anaerobic digestion due to an increased resistance to microbial and fungal degradation [7]. On the other hand, high-cellulose concentration makes *Miscanthus* preferable for paper or ethanol production [8,9]. Saccharification of a lignocellulose biomass has been recognised as a potential source of mixed sugars for fermentation for fuel ethanol or chemicals [8]. The multilateral use of this plant is sparking a growing interest in its cultivation. The plant, however, has one fundamental flaw: it is sterile. Triploid *M.* × *giganteus* ($3n = 57$ chromosomes) is a hybrid of diploid *Miscanthus sinensis* ($2n = 38$) and tetraploid *Miscanthus sacchariflorus* ($4n = 76$). Due to its sterility, *M.* × *giganteus* reproduces only by rhizome division, so the clones of this plant species are characterised by a pool of very low genetic diversity, as proved by Greef et al. [10]. The latter feature strongly limits breeding work, which calls for high genetic variation. Obtaining fertile plants of *M.* × *giganteus* has been the subject of some studies, but the results have been unsatisfactory [11,12]. New genotypes of *Miscanthus* are obtained by crossing *M. sacchariflorus* and *M. sinensis*, using very important features typical for *Miscanthus* genera: incompatibility and high heterozygosity, which is a prerequisite for biomass yield based on the heterosis phenomenon [13]. Again, sterility makes Giant *Miscanthus* safe for the natural environment and enables control of its spread.

Plants of *M.* × *giganteus* are also propagated via regeneration in *in vitro* cultures [14–18]. It is worth mentioning that plants obtained *in vitro* are more frost-resistant than those reproduced from rhizomes, but only during the first winter [19]. The physiological background for this phenomenon has not yet been explained.

In tissue culture a phenomenon called somaclonal variability occurs [20]. The main factors causing somatic variability are the genetic heterogeneity of initial explant cells and genetic and the epigenetic variability which manifests during *in vitro* cultivation [21]. Moreover, conditions maintained during *in vitro* cultivation and growth regulators, usually applied in high concentration, increase the frequency of this phenomenon. Somaclonal variability may be a source of new genetic variations used in the breeding of new cultivars of crop plants [20]. In this way new forms of wheat and sorghum were obtained [22–24]. These somaclones demonstrated higher yields, an increased concentration of protein, earlier maturation and increased resistance to diseases.

The aim of the present study was to investigate if *M.* × *giganteus* regenerants obtained in an *in vitro* culture can be a source of new forms characterised by different chemical composition than plants propagated from rhizomes. The study was performed on plants in three groups: those of two different clones, propagated from rhizomes (obtained from two producers), and of regenerants obtained *in vitro* conditions. The genetic differences between these plant groups were investigated. Plants used for the chemical analyses were grown in the field for three years. In the dried stems, the following parameters were estimated: dry weight (DW), ash and water mass fraction of DW, energy of biomass, ADF (acid detergent fibre) including cellulose and lignin, NDF (neutral detergent fibre) including cellulose, hemicellulose and lignin, and ADL (acid detergent lignin).

2. Material and methods

2.1. Plant material

Rhizomes of *M.* × *giganteus* were obtained from a horticulture farm located in Zabierzów near Krakow (Poland) and from the Plant Breeding and Acclimatization Institute in Radzików (Poland). Rhizomes were planted in an experimental field (50°08′39″ N, 19°85′37″ E) belonging to the University of Agriculture in Krakow (Poland); these were the source of plants used in the experiment. The experimental field is located on soils of class V (weak arable soils). Regenerants were obtained from tissue culture derived from immature inflorescences. After 6 months of acclimatisation in a glass greenhouse, regenerants (Iv) were planted in the field (0.7 m × 0.7 m) in April 2011 and were cultivated next to plots with plants grown from rhizomes originating from Zabierzów (Z) and Radzików (R), planted with the same spacing and at the same time. Each