Long-Term In Vitro Storage of *Miscanthus* Cultures

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In vitro cultures of *Miscanthus* consisting of single shoots on rooting medium were stored at a temperature of 8, 12, 16, or 20C and a photon fluence of 5, 10, or 20 µmol m-2 s-1 for 0, 4, 8, 16, or 26 weeks. With increasing storage period the survival and root and shoot formation measured after 14 days of acclimatization and 14 days of growth were improved considerably. A storage temperature of 8C resulted in the best survival of plants and a temperature of 16C was optimal for shoot formation. Root and shoot formation were improved with increasing photon fluence during storage.

INTRODUCTION

Miscanthus is a potentially important crop particularly due to its high biomass production (Nielsen, 1987; Schwarz, 1993) and large capability to keep the nutrients in the rhizosphere (Jørgensen, 1994). To avoid the risk of spreading *Miscanthus* seeds to nature where *Miscanthus* will provide a weed problem, only sterile cultivars should be planted. This implies that clones and cultivars have to be propagated vegetatively by division, mechanical separation (Kjeldsen 1994), or in vitro propagation (Nielsen et al., 1993, 1995).

Approximately 10,000 plantlets are needed per hectare. A large demand for plants may provide severe problems for tissue culture laboratories because the planting season only is of 2 to 3 months. In vitro storage of *Miscanthus,* thus, could enable a supply of a large number of plants within a limited period of time without having a large in vitro-laboratory capacity.

MATERIALS AND METHODS

The triploid cultivar Miscanthus ×*ogiformis* Honda 'Giganteus' was used. In vitro cultures of axillary shoots grown on propagation medium (a modified MS medium, Murashige and Skoog 1962) containing 5 mg litre^{-1} benzyladenine were divided into single shoots and transferred to rooting medium (a modified MS medium) containing 1 mg litre^{-1} α-naphthaleneacetic acid. After 3 days on rooting medium the containers with 20 plants each were stored at a temperature of 8, 12, 16, or 20C and a photon fluence of 5, 10, or 20 μmol m^{-2}s^{-1} for 0, 4, 8, 16, or 26 weeks. The experiment was repeated three times at different times of the year.

At the end of the storage period, plants were potted into peat in 5.5-cm diameter pots and acclimatized for 14 days in a controlled environment room at a temperature of 23±0.5C, a photon fluence of 35 μmol m^{-2} s^{-1}, and a photoperiod of 16 h. Thereafter the plants were transferred to a glasshouse and grown for a further 14 days at natural light conditions and a minimum temperature of 23C.

At the end of storage, acclimatization, and 14 days of growth the percentage of dead plants and percentage of plants with roots were calculated and the number of new shoots per plant was measured.

RESULTS AND DISCUSSION

On an average basis, the percentage of dead plants calculated at the end of the storage period increased gradually from 1% after 0 weeks of storage to 8.1% after 26 weeks of storage. At the end of the growth period the results showed a significant decrease in percentage of dead plants with increasing storage period from about 40% after 0 weeks of storage to 16% after 26 weeks.

Table 1. Percentage of dead plants after acclimatization and 14 days of growth. Average of 18 containers per treatment.

Storage temperature (C)	Storage period (weeks)				
	0	4	8	16	26
8	40.5	27.3	12.7	11.8	9.4
12	37.5	31.2	42.3	34.7	20.0
16	39.0	31.2	36.1	-	14.5
20	38.4	30.2	28.7	27.9	24.3

Containers that were stored at 8C generally had the lowest percentage of dead plants at the end of the growth period. Storage at other temperatures resulted in higher percentages of dead plants (Table 1).

With increasing photon fluence during storage a reduction in the percentage of dead plants was observed after acclimatization and 14 days of growth. This reduction was most pronounced when plants were stored for a period of 4 to 8 weeks (Table 2).

Table 2. Percentage of dead plants after acclimatization and 14 days of growth. Average of 24 containers per treatment.

Photon fluence (μmol m^{-2} s^{-1})	Storage period (weeks)				
	0	4	8	16	26
5	38.6	38.5	42.2	28.7	19.3
10	44.0	24.5	23.5	18.2	14.8
20	36.2	21.2	12.9	18.2	12.9

The decrease in dead plants with increasing storage period and photon fluence indicates that a longer period than 3 days on rooting medium before storage is necessary in order to develop plants that can survive short-term storage.

Improved survival probably also would be the result if shoot clusters were stored instead of single shoots. Handling of nonstored shoot clusters with about five shoots grown on rooting medium during the last subculture usually only results in very few dead plants after a period of acclimatization and growth.

Root formation measured at the end of the growth period increased with storage time from 53% after 0 weeks of storage to 83% after 26 weeks of storage. Root formation was only slightly influenced by storage temperature but there was a stimulating effect of increasing photon fluence particularly when stored for 4 or 8 weeks (Table 3).

Table 3. Rooting percentage after acclimatization and 14 days of growth. Average of 24 *containers per treatment.*

Photon fluence (μmol m^{-2} s^{-1})	Storage period (weeks)				
	0	4	8	16	26
5	53.6	56.4	46.8	57.9	81.5
10	49.8	69.0	69.7	69.7	79.4
20	56.5	74.3	83.4	72.7	86.8

Shoot formation measured at the end of the growth period was promoted by increasing temperature during storage with 16C as the optimal temperature (Fig.

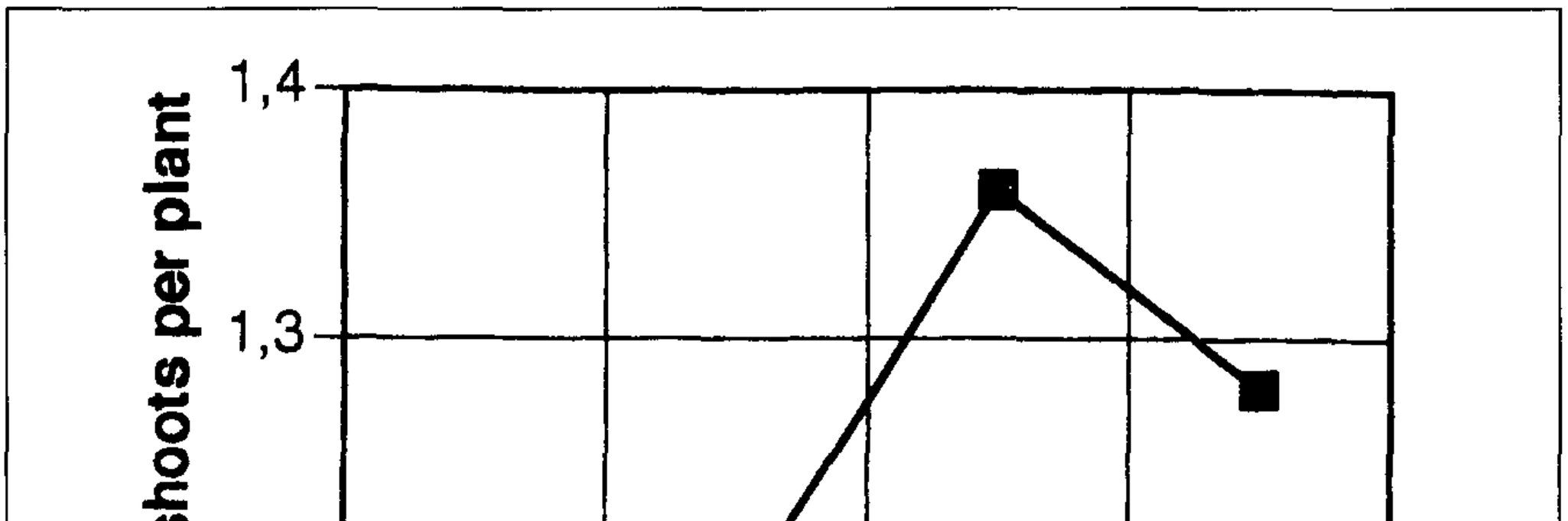

Figure 1. Number of shoots per plant as a function of storage temperature. Average results after 14 days of acclimatization and 14 days of growth.

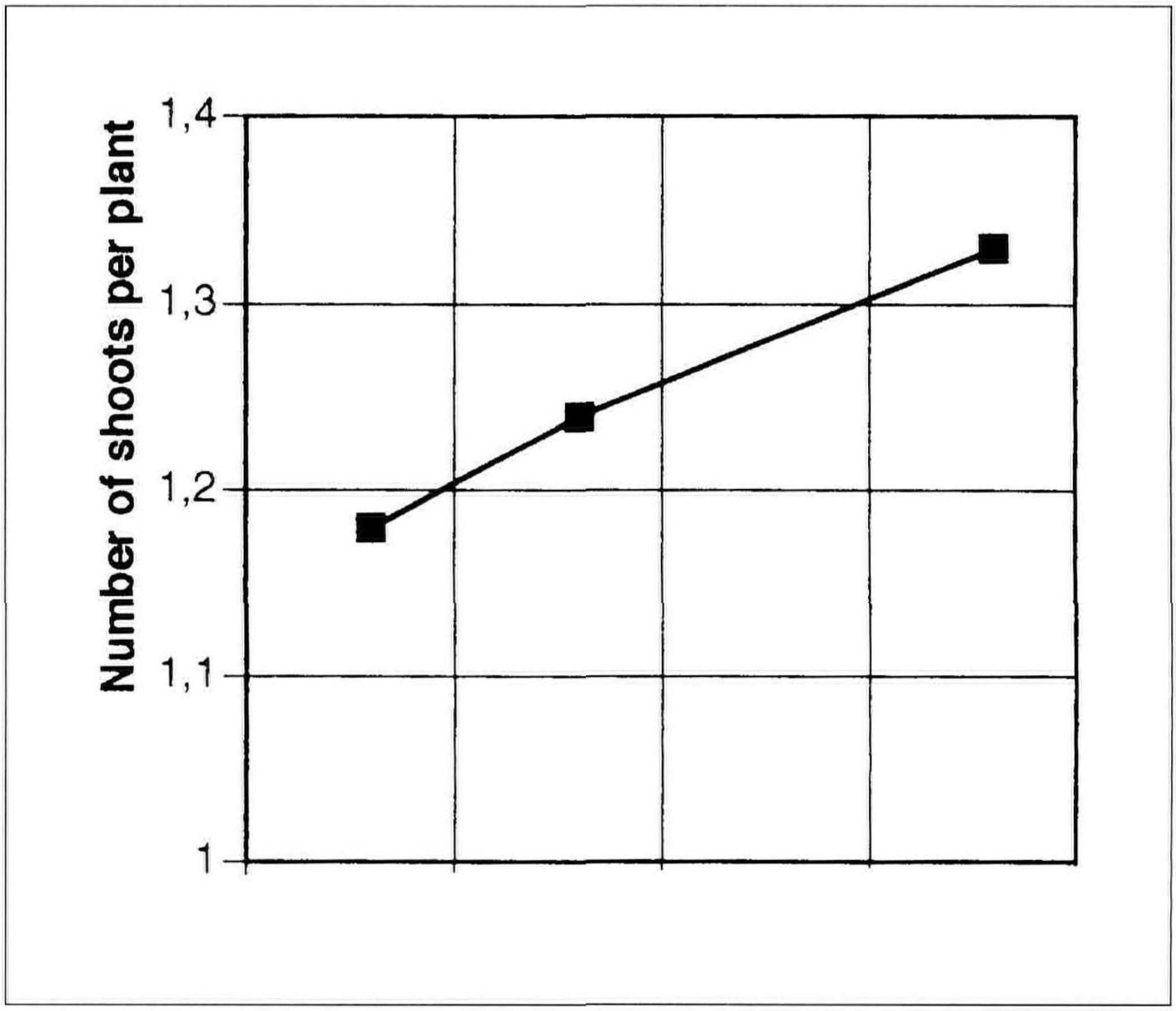

Figure 2. Number of shoots per plant as a function of photon fluence during storage. Average results after 14 days of acclimatization and 14 days of growth.

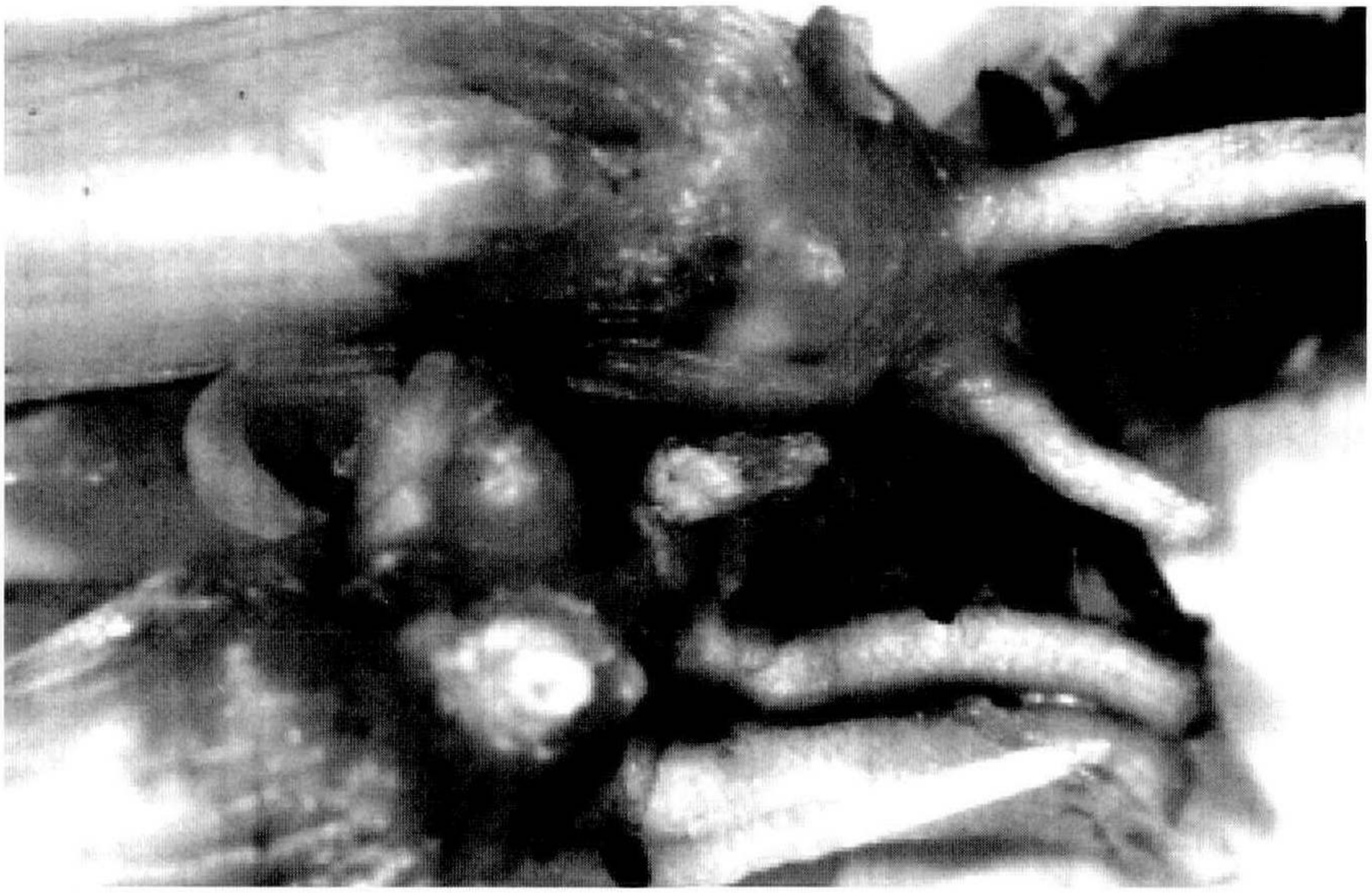

Figure 3. Basal part of a *Miscanthus* plantlet with visible shoot primordia after 26 weeks of in vitro storage.

1). Shoot formation was also improved by increasing photon fluence during storage (Fig. 2).

Leaves of in vitro stored *Miscanthus* plants gradually became more and more necrotic during storage and after 26 weeks of storage almost no green leaves were present. During storage the plants developed a firm rhizome with a few visible shoot primordia (Fig. 3) ready to grow and produce new shoots after transplanting the rhizome to peat.

Long-term in vitro storage of *Miscanthus* cultures at 16C and 20 μmol m^{-2} s^{-1} on rooting medium provides a possibility to efficiently propagate plants more or less continuously thus avoiding bottleneck situations in the laboratory.

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