Effect of exogenously applied sugars on silver birch (*Betula pendula*) *in vitro* shoot rejuvenation

Girgžde E., Samsone I., Gailis A., Ruņģis D.
Latvian State Forest Research Institute "Silava", Rīgas 111, Salaspils LV–2169

SILAVA

INTRODUCTION

Silver birch is one of the most economically important tree species in Latvia covering 30 % of the total forest area. Micropropagation of birches is an effective method for propagation of selected mature genotypes, providing significant practical advantages for tree breeding. Tissue recalcitrance is a major factor limiting micropropagation of mature woody plants. Exogenously applied sugars are essential for successful *in vitro* shoot growth, however, they may affect shoot juvenility state and contribute to recalcitrance during cultivation. Sugars act as signals that mediate vegetative phase change in plants. Recent studies demonstrate their molecular role of regulating miR156 levels – one of the master regulators of phase change. In order to obtain a better understanding of the role of sugars in birch *in vitro* shoot rejuvenation, we investigated the effect of sucrose and glucose on rejuvenated proliferating and mature recalcitrant birch *in vitro* shoots.

MATERIALS AND METHODS

Shoot cultures initiated *in vitro* from 20 year old silver birch were used in this experiment. Shoots were cultured in Woody Plant Medium supplemented with zeatin 0.1 mg L^{-1}. The effects of 2 %, 4 % and 6 % sucrose (Scr), 2 % and 4 % glucose (Glc), as well as 2 % sucrose + 2 % glucose on main shoot length, lateral shoot formation, peroxidase and polyphenol oxidase activity in leaves and stems were assessed as juvenility markers after 30 and 60 days of culturing.

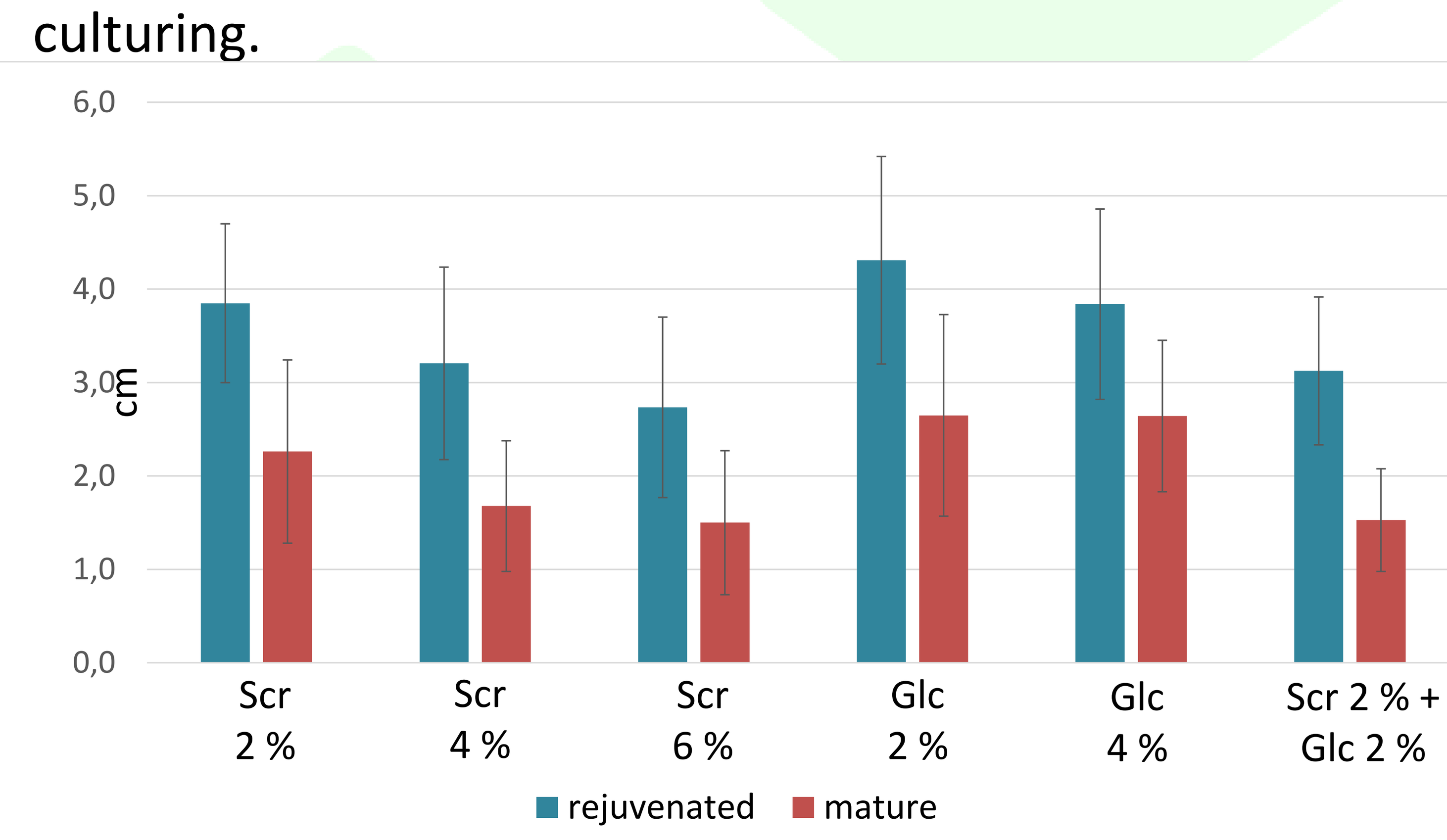

Fig. 1. The main shoot length of rejuvenated and mature *Betula pendula in vitro* shoots after 30 days of culturing at different sucrose and glucose concentrations. Standard deviations are shown.

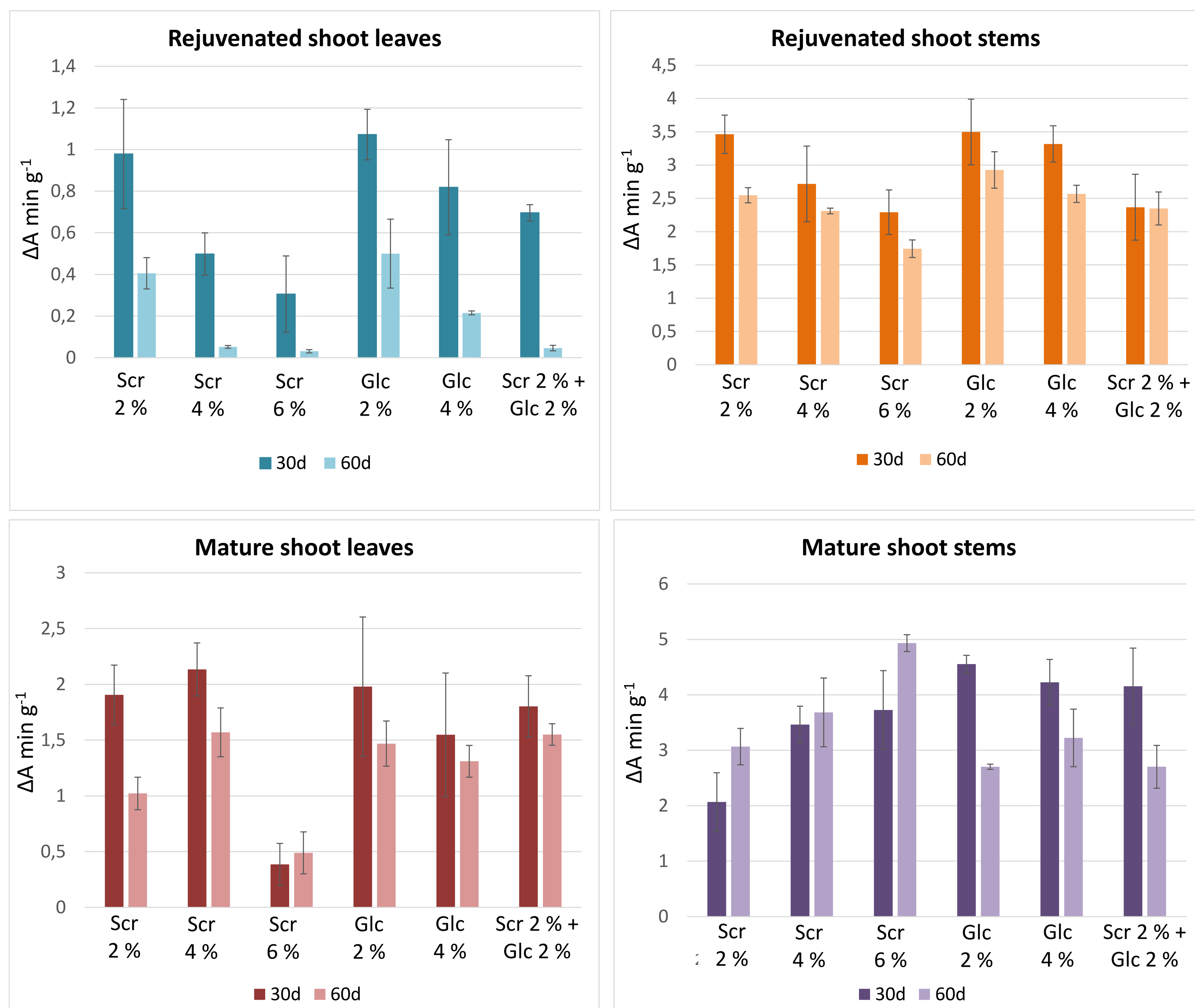

Fig. 2. Peroxidase activity of rejuvenated and mature *Betula pendula in vitro* shoots after 30 and 60 days of culturing at different sucrose and glucose concentrations. Standard deviations are shown.

RESULTS

Our results show that sucrose and glucose have different effects on rejuvenated and mature birch *in vitro* growth. Elevated sucrose concentrations (4 %, 6 %) inhibited shoot growth and proliferation for both juvenile and mature birch *in vitro* shoots, whereas 2 % and 4 % glucose provided better results for rejuvenated shoots and had a rejuvenating effect on mature shoots (Fig. 1., Fig. 3., Fig. 4.).

Active shoot growth correlated with peroxidase activity in leaves. Higher peroxidase activity was observed in both rejuvenated and mature shoots grown on lower sucrose and glucose concentrations (Fig. 2.).

However, peroxidase activity in stems of mature shoots increased with 4 % and 6 % sucrose concentrations, contributing to lignification processes, which was not observed in rejuvenated shoots (Fig. 2.).

Polyphenol oxidase activity was higher in mature shoot leaves and stems compared to rejuvenated shoots, possibly contributing to higher phenol synthesis and oxidative stress in mature shoots, leading to recalcitrance. Polyphenol oxidase activity increased in both types of leaves and stems in the presence of high concentrations of both sugars, enhancing synthesis of phenols, that contributes to recalcitrance. Thus, high sucrose concentration plays an important role in birch *in vitro* shoot maturation processes.

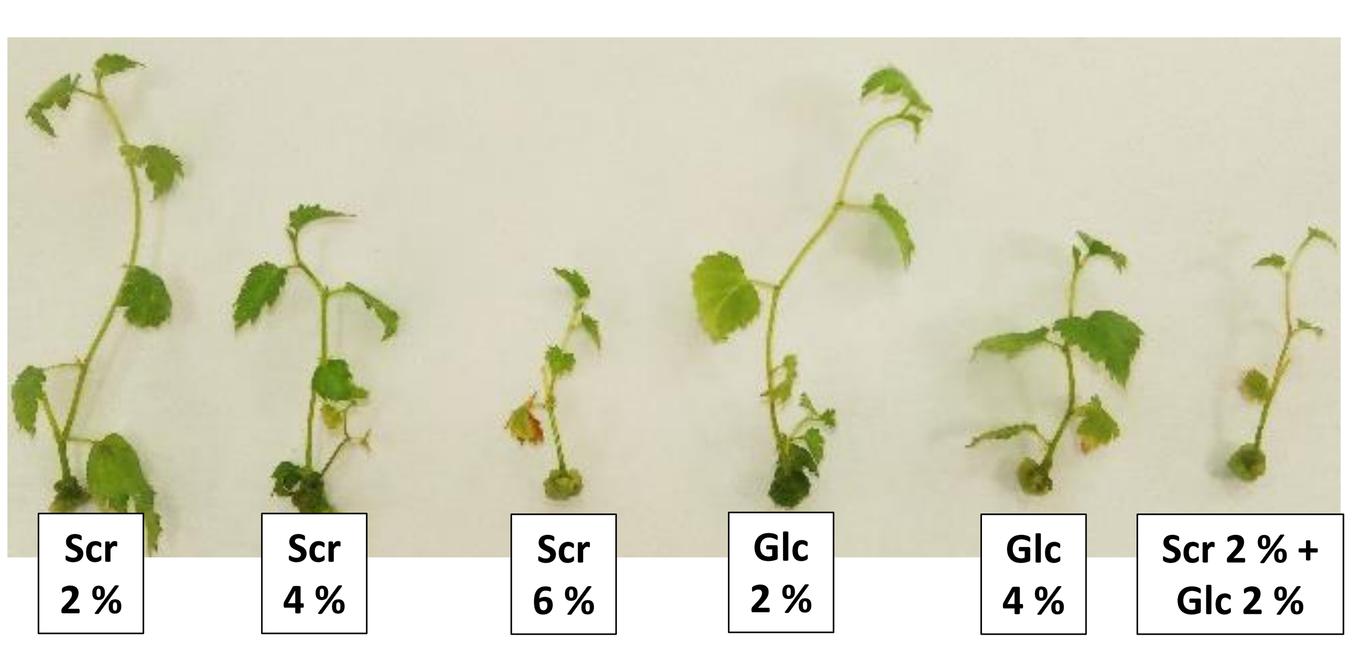

Fig. 3. Rejuvenated *Betula pendula* shoots after 30 days of culturing on different sugar concentrations.

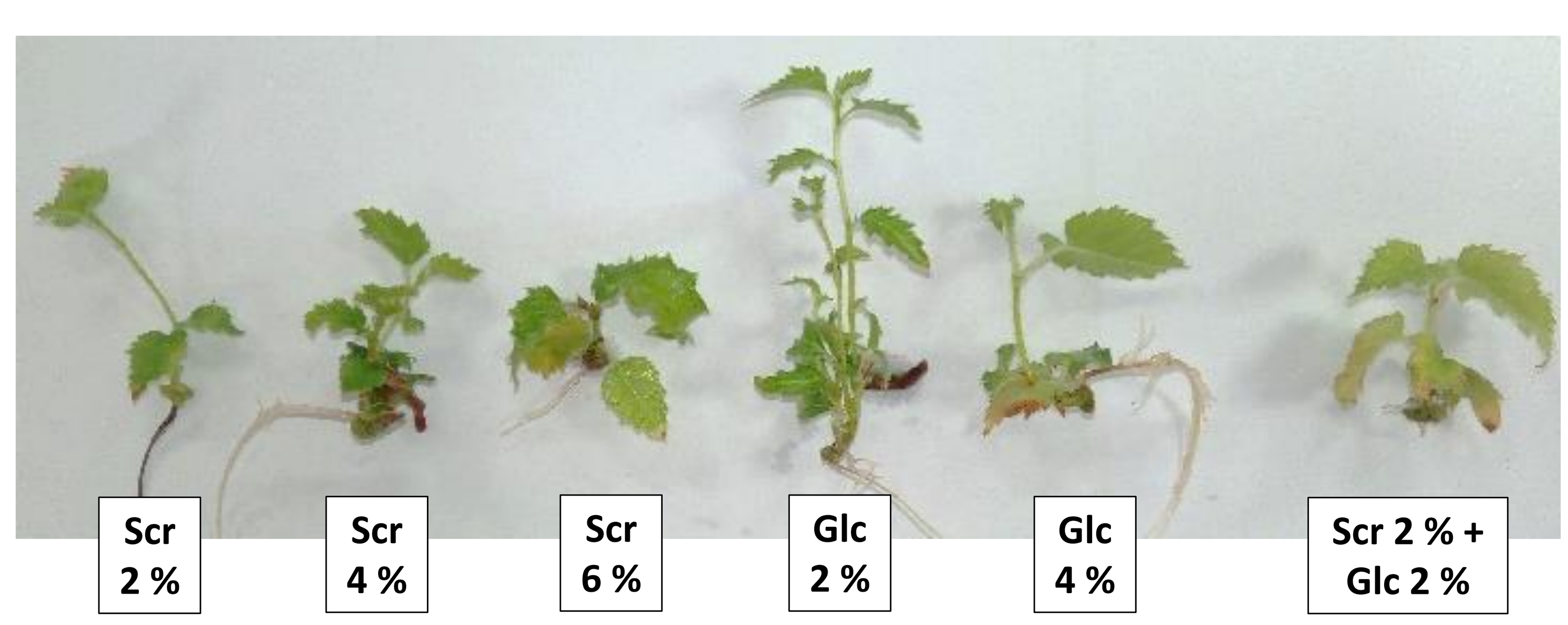

Fig. 4. Mature *Betula pendula* shoots after 30 days of culturing on different sugar concentrations.

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