



Minimizing albinos among DH plants derived via *in vitro* anther cultures

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Introduction

The level of albinos could be affected by the age of the culture, the presence of callus phase during embryo formation as well as by ingredients present in the culture (i.e. CuSO_4 , AgNO_3 , etc.) or many others. It is documented that copper sulfate may positively regulate plant regeneration and green plantlets formation whereas silver nitrate may intensify the embryogenic callus production and stimulate callus growth during *in vitro* procedures. On the other hand, the age of tissue cultures, as well as callus stage, may result in increased number of mutants. Thus, it is hardly difficult to evaluate the approach that could mimic all of the numerous factors at the same time. Nevertheless, one may try to use a limited number of them or their combinations that are known to guarantee decreased level of albinos and increased number of green regenerants. Among many CuSO_4 and AgNO_3 as well as the culture age could be more or less controlled.

Aim

The objective of the study was to verify whether varying concentration of CuSO_4 and AgNO_3 included into tissue culture medium and the age of tissue cultures could be optimized reducing the number of albinos and increasing the amount of green regenerants that would be in type with parental plants. For such purposes, anther cultures of triticale (x *Triticosecale* spp. Wittmack ex A. Camus 1927) were used.

Plant material and methods

DH triticale plants (Mungis x Presto) were used as a source of explants (Fig. 1). Anthers from stressed spikes were placed on solid induction medium 190-2 supplemented with 2 mg/l 2,4D and 0.5 mg/l kinetin. The induction media were prepared in nine different variants (M1 (K) - M9) (Tab. 1) concerning the concentration of CuSO_4 , AgNO_3 and time of androgenesis induction (4, 5, 6 weeks). The regeneration medium (190-2 with 0.5 mg/l NAA and 0.5 mg/l kinetin) was the same for all variants. Next plantlets were transferred to Erlenmeyer flask with rooting medium (190-2 with 2 mg/l IAA). After acclimatization plants were grown to maturity in the greenhouse.

Figure 1. A scheme of preparing plant material



Results

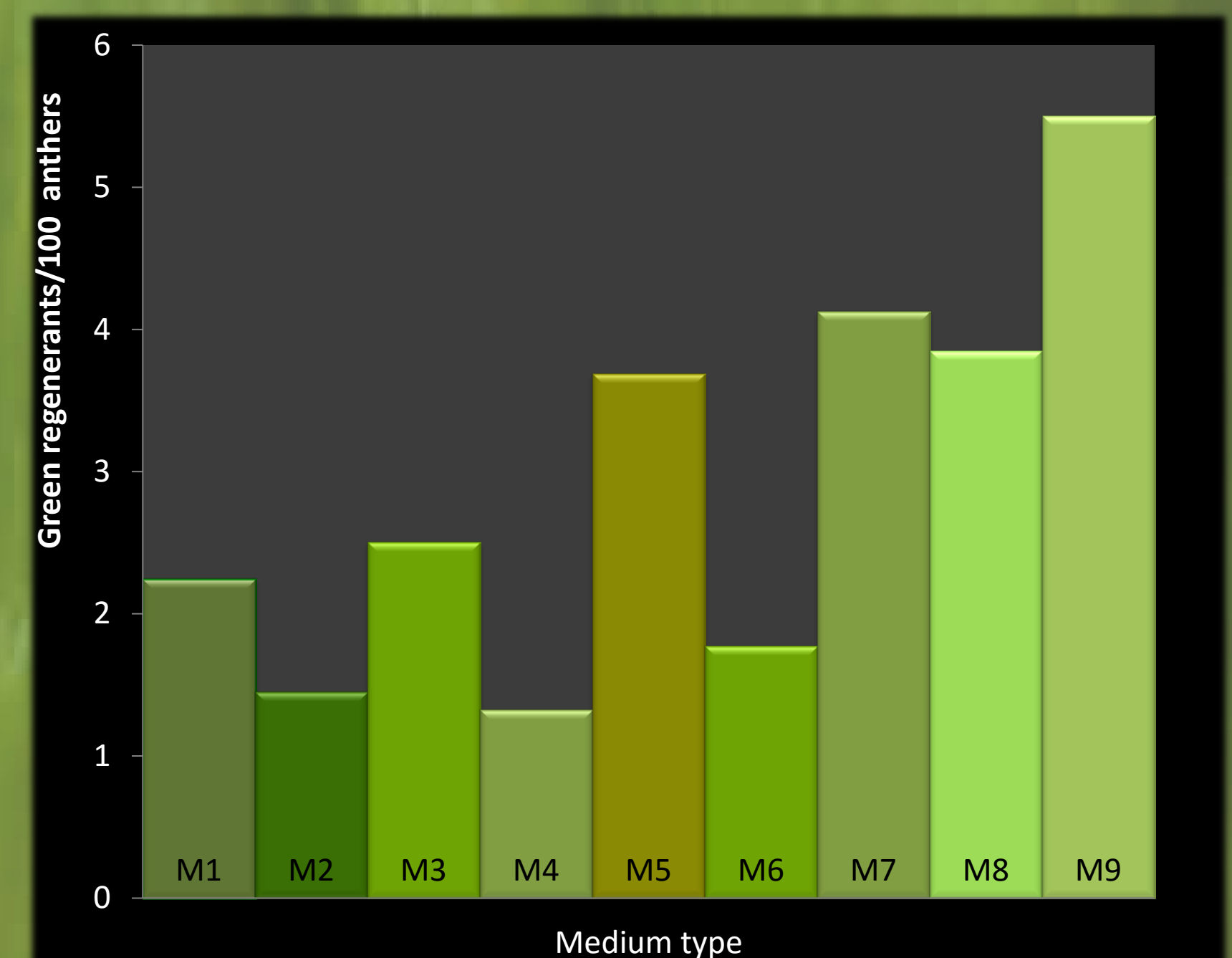
Circa 7200 anthers were cultured on induction media. The androgenesis in anther cultures resulted in the formation of 15 to 43 green regenerants depending on media variant. The highest number of regenerants was found in the case of media: M5 (3.69 regenerants per 100 anthers), M7 (4.13), M8 (3.85) and M9 (5.50) supplemented with 10 μM of CuSO_4 and 12-60 μM AgNO_3 . The number of green regenerants was almost two-fold higher than the amount of regenerants achieved under conditions without ingredients (2.24). The most proper time of induction process was five weeks; there was observed the highest amount of green regenerants per 100 anthers (5.50).

Conclusions

The presence of optimized concentrations of CuSO_4 and AgNO_3 , as well as the five weeks induction process used in triticale tissue cultures, may enhance the efficiency of green plant regeneration and reduction of the number of albinos.

Medium symbol	AgNO_3 (μM)	CuSO_4 (μM)	Time (weeks)
M1 (K)	0,1	0	4
M2	0,1	12	5
M3	0,1	60	6
M4	5	60	5
M5	5	0	6
M6	5	12	4
M7	10	12	6
M8	10	60	4
M9	10	0	5

Table 1. Composition of media used for induction process in triticale anther cultures. K-control



The number of green triticale regenerants per 100 anthers. M1 control condition