APPLICATION OF THE TAGUCHI METHOD IN CEREAL TISSUE CULTURES PROCESS: MAXIMIZATION OF GREEN REGENERANTS

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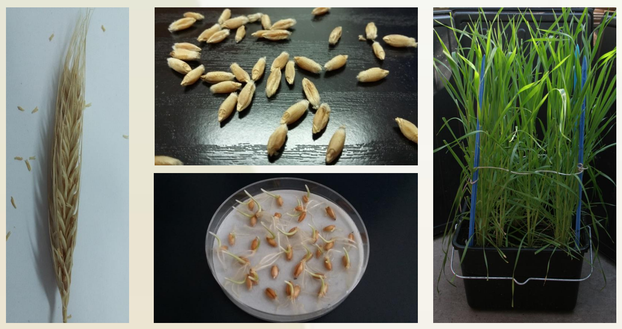
INTRODUCTION The discovery of plant doubled haploids (DHs) provided researchers with multifunctional material valuable in studies in many areas. In cereals, where the androgenesis is the general method to obtain DHs, the albinism is the bottleneck affecting the efficient production of green DH regenerants. To overcome the problem and raise the productivity of green regenerants via androgenesis the selection of maternal plants, proper developmental stage of microspores, choice of stress factor that triggers androgenesis or manipulation of media composition should be considered. The regeneration medium composition and stress factor need investigations. As some data suggest the supplementation of the induction medium with $CuSO_4$ and $AgNO_3$ may increase green regenerants production. Moreover, the varying longevity of induction medium treatment was also applied in the case of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and triticale (x *Triticosecale* spp. Wittmack ex A. Camus 1927) materials. Multifactorial optimization could be achieved via either via grid or statistical approach. The latter might be based on Taguchi method allowing a significant reduction of experiments and reducing optimization costs.

AIM The study aimed at simultaneous optimization of three factors (concentration of $CuSO_4$, $AgNO_3$, and tissue culture age) suggested affecting the regeneration of DH green plants by using Taguchi method in the case of barley, wheat and triticale.

PLANT MATERIAL AND METHODS

Figure 1. A scheme of preparing plant material



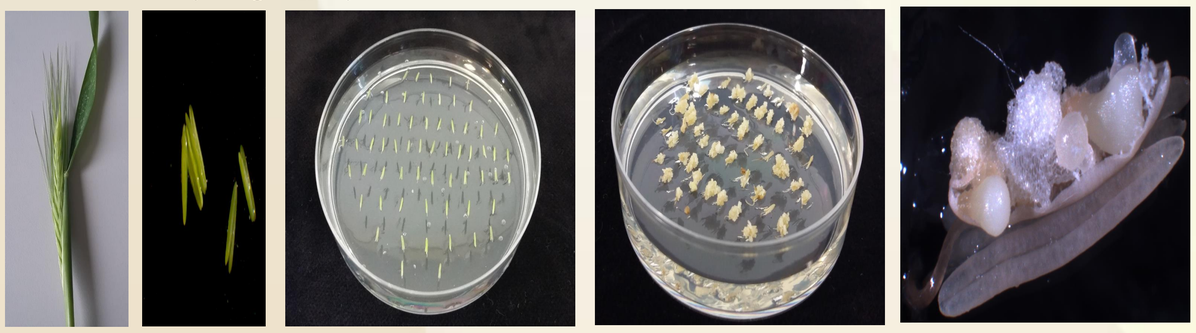
Preparing donor plants DH

The generative progeny of DH spring **B**arley (2dh/8), winter **W**heat (P2/9), and winter **T**riticale (28/2) plants were used as a source of explants. Anthers from stressed spikes were placed on solid induction media (IM):

B – barley**:** N6L with macro- and microelements according to Chu (1978) with 2 mg l^{-1} 2.4D, 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} kinetin.

W – wheat: C17 medium (Wang and Chen 1983) with 2 mg l^{-1} 2.4D and 0.5 mg l^{-1} kinetin.

T – triticale**:** modified 190-2 (Zhuang and Xu 1983) with 2 mg l^{-1} 2.4D, and 0.5 mg l^{-1} kinetin.

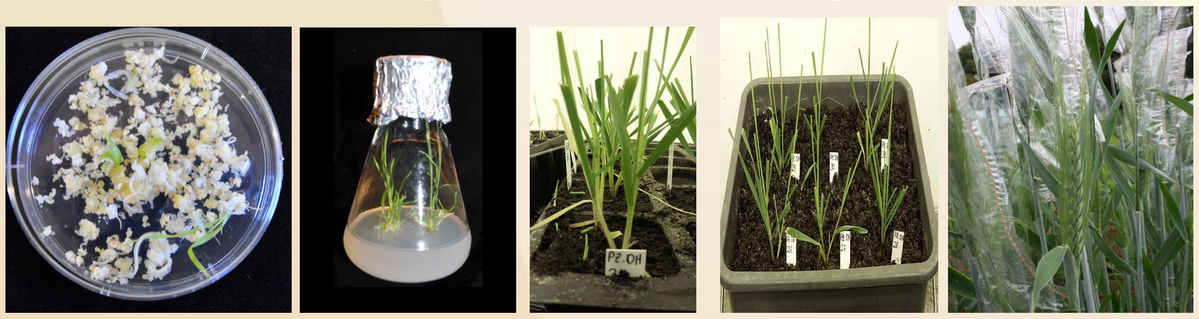


Collection of spikes and cooling; anthers extraction and transferring on induction medium (IM)

The calli were transferred onto regeneration media (RM):

B - (barley): K4NB medium (Kumlehn et al. 2006) with 0.225 mg l^{-1} BAP

W - (wheat) and **T** - (triticale): 190-2 medium (Zhuang and Xu 1983) with 0.5 mg l^{-1} NAA and 1.5 mg l^{-1} kinetin.



Anthers on regeneration medium RM; transferring plantlets to flask, rooting, potting and growing to maturity

Next plantlets were transferred to Erlenmeyer flask with the N6I rooting medium (Chu 1978) with 2 mg l^{-1} IAA.

After acclimatization plants were grown to maturity in the greenhouse under control conditions.

RESULTS

I. Optimisation conditions (OC)

The optimization conditions demonstrated that all tested factors were essential for the regeneration of green plants. Moreover, each of the tested species had different requirements concerning factors used. Among nine investigated trials, the M7 conditions appeared to be the most promising in the RGPs of barley (2.91), M3 for wheat (14.62) and M9 for triticale (6.06) (Fig. 2). All of those results were significant according to ANOVA.

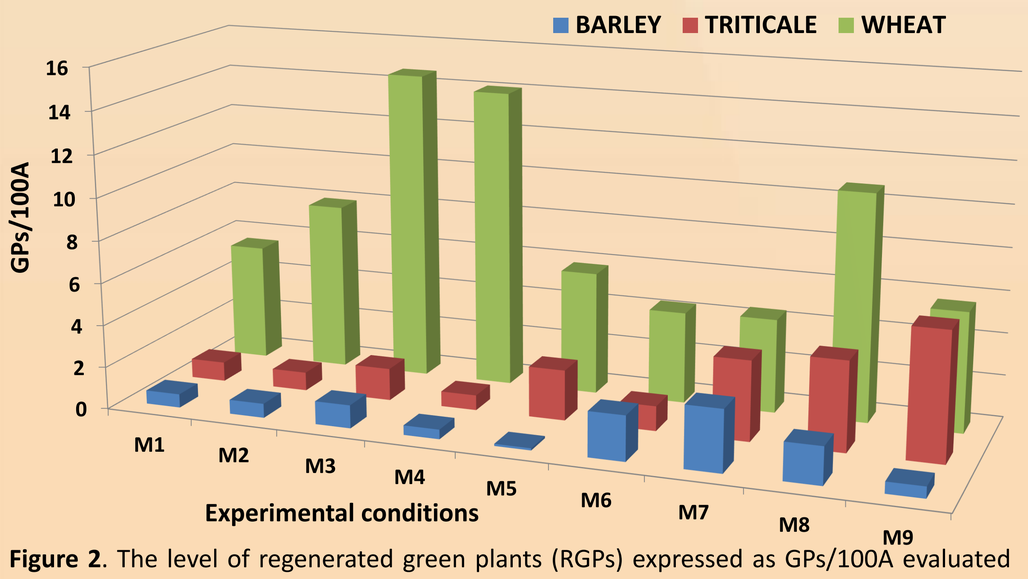


Figure 2. The level of regenerated green plants (RGPs) expressed as GPs/100A evaluated for the M1-M9 experimental conditions in barley, wheat and triticale under OC.

II. Optimised regeneration conditions (ORC)

The Taguchi approach allowed to identify the ORC that increased the RGPs compared to controls (Tab. 3). The ORC in the case of barley and wheat increased the RGPs value as much as twice compared to control but the difference was not significant. In triticale, the gain was almost triple and significant according to ANOVA.

Table 3. The arrangement of the RGP (expressed as GPs/100A) results evaluated under optimized regeneration conditions (ORC) (Tab 2). B states for barley, W for wheat, T for triticale, M1 reflects control, M10 for experimental condition used in the ORC. Values marked with the same letter do not differ according to Tukey's HSD test at α=0.05.

Experimental conditions	Cereals		
	B	W	T
M1	0.95[a]	4.95[a]	0.55[b]
M10	1.97[a]	7.99[a]	1.61[a]

There were nine experimental designs in optimization conditions (OC) M1-M9. Varying concentrations of $CuSO_4$, $AgNO_3$, and longevity of androgenesis performed in the induction medium (IM) were tested. The OCs were chosen based on Taguchi approach (Tab. 1).

Table 1. The array with three factors and three levels of each of them (the OCs) used for the induction of green regenerants according to Taguchi method. The basic induction medium was as indicated in Table 1. As mentioned earlier B states for barley, W for wheat, T for triticale, M1- reflects control conditions (control design) whereas M2-M9 optimization designs.

Optimization designs	Optimization factors (Taguchi design)		
	Ingredients		Time (days) (B/W/T)
	$CuSO_4$ (μM)	$AgNO_3$ (μM)	
M1	0,1	0	21/35/35
M2	0,1	10	28/42/42
M3	0,1	60	35/49/49
M4	5	60	28/42/42
M5	5	0	35/49/49
M6	5	10	21/35/35
M7	10	10	35/49/49
M8	10	60	21/35/35
M9	10	0	28/42/42

After transferring plantlets onto regeneration medium (RM) the level of the regenerated green plants (RGPs) expressed as the number of green plants (GPs) regenerated per 100 plated anthers (A) was calculated: RGPs-GPs/100A. The appropriate data were implemented in QI Macros230T software to perform the optimization towards maximum regeneration of green plants. The optimized regeneration conditions (ORC) ($CuSO_4$ and $AgNO_3$ concentration as well as the longevity on IM (Table 2)) were applied for each of the tested species to calculate the RGPs.

Table 2. Optimized regeneration conditions (ORC) used for the induction of green plants regeneration, where B states for barley, W for wheat, T for triticale and M1 reflects control.

Optimized conditions	ORC		
	Ingredients		Time (days) (B/W/T)
	$CuSO_4$ (μM)	$AgNO_3$ (μM)	
M1	0.1	0	21/35/35
B	10	30	21
W	0.1	60	37
T	10	0	49

CONCLUSION

1. The application of Taguchi approach allowed the reduction of the number of experiments needed for the optimization of regeneration conditions where several factors are considered simultaneously. This is especially important if available plant materials are limited and experimental time is rather long.
2. The Taguchi approach proved to be useful for the evaluation of the experimental conditions leading to the increase in the number of green regenerants compared to the once derived under control conditions in all tested species despite numerous factors affecting green plant regeneration that are tricky to control.
3. The Taguchi approach should be considered as an alternative way of improving green plant regeneration leading to the reduction of experimental costs.
4. The optimized regeneration conditions may not necessarily be optimal but suboptimal.
5. Due to the number of factors controlling plant regeneration that are not known or are tricky to control the increase in the number of green regenerants derived under either control or optimized conditions may vary from experiment to experiment and from species to species.

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