EFFECT OF STRESS FACTORS AND MEDIA COMPOSITION ON THE APPEARANCE OF ALBINISM WITHIN ANDROGENIC RYE REGENERANTS

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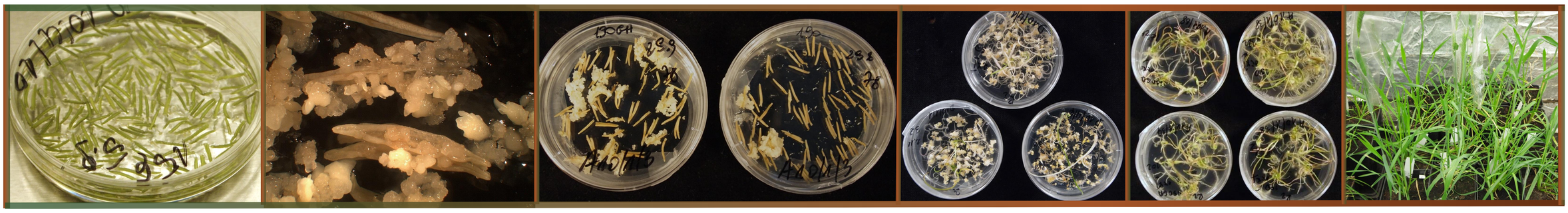
Rye androgenesis – anther culture



Introduction

Regeneration of rye plants has been described for different tissues and organs. The process of androgenesis allows to obtain doubled haploids (DH) – completely homozygous lines in a short time. They are widely used in plant breeding , basic and molecular research. Since the 1970s the aim of researchers was to develop an efficient method of rye doubled haploids production. It seems that our understanding of the rye androgenesis process is still questionable. Despite good results in some laboratories and for some genotypes, anther culture response of rye remains unpredictable. Production of DH plants depends on efficiency in anther culture, survival rate of green plants, fertility and frequency of albino plants among regenerants. The aim of the presented study was to analyze how a certain stress and induction medium composition can reduce the number of anther culture regenerants lacking chlorophyll.

Materials and methods

Four breeding lines: NS 1396/1 (1), NS 1282/4 (2), S01715/14 (3), S554N/15 (4), provided by Poznan Plant Breeding Ltd and Plant Breeding Danko Ltd. were tested for anthers culture (AC) experiments. Three different stresses and two modified induction media 190-2 (Zhuang and Xu, 1983) and 190-2 with 10 mg/l of gum arabic (GA) were applied. The various treatment methods were tested: colling of tillers at 4°C for 3 weeks (A); colling of tillers at 4°C for 2 weeks and then incubation of isolated anthers in 0.3 M mannitol at 4°C for 7days (B); colling of tillers at 4°C for 2 weeks followed by preculture of anthers for 24h in mannitol at 32°C (C). Each combination was tested on 20 spikes. AC was performed followed by triticale methodology (Warzecha et al. 2005). Plant regeneration was according to the protocol of Pauk et al. (1991). The following parameters were monitored: % of reacting anthers among whole plated once; the green plant regeneration rate (the number of green plants per 100 anthers), the level of albinism % of albino plants among total number regenerants.

Results and conclusion

All applied stresses induced haploid embryogenesis. The type of used microspore reprogramming shock had a significant effect on particular androgenesis stages. At the first stage of experiment the impact of stress factors was determined based on percentage of responsive anthers after 4 weeks of culture. The highest value this parameter was achieved after stress B treatment (Fig. 1).

Obtained results show the role of donor plants genotype on qualitative and quantitative plants regeneration. For individual genotypes we observed greater effect of the stress combination on the number of regenerating albino plants than the induction medium variant. Although GA in media had no significant effect on albino regeneration, its increased the total number of regenerants (Tab.).

Among all tested combination, the lowest level of albinism was observed for cooling of tillers for a period of 21 days (A) (Fig.2). After this treatment, for the best genotype, the average number of regenerated green plants was 39 per 100 plated anthers. For NS 1282/4 and NS 1396/1 all regenerated plants (619) were green. In case of other lines the level of albinism ranged from 37% to 70% for S554N/15 and S01715/14, respectively, independent from media used.

Colling stress combined with anthers starvation in mannitol solution (B) revealed low albino plants regeneration level (in well responsive genotypes), ranged from 9% to 19%. For more recalcitrant genotypes, in case of which regeneration was low, the albino level ranged from 57% to 76%.

The use of high temperatures (C) during the reprogramming of microspores was reflected in the increased number of regenerated chlorophyll-deficient plants. The lowest percentage of albino plants was observed in NS 1396/1 (56%), however other genotypes were characterized by albino level higher than 80%.

parameter	medium		190-2				190-2+GA			
	genotypes		NS 1396/1	NS 1282/4	S0 1715/14	S5 54N/15	NS 1396/1	NS 1282/4	S0 1715/14	S5 54N/15
% of albino plants	treatment methods	A	0	0	70	37	0	0	64	64
		B	0	19	66	57	19	9	63	76
		C	81	56	0	0	91	56	100	0

Fig. 1. **Effect of stress treatment on anthers response**

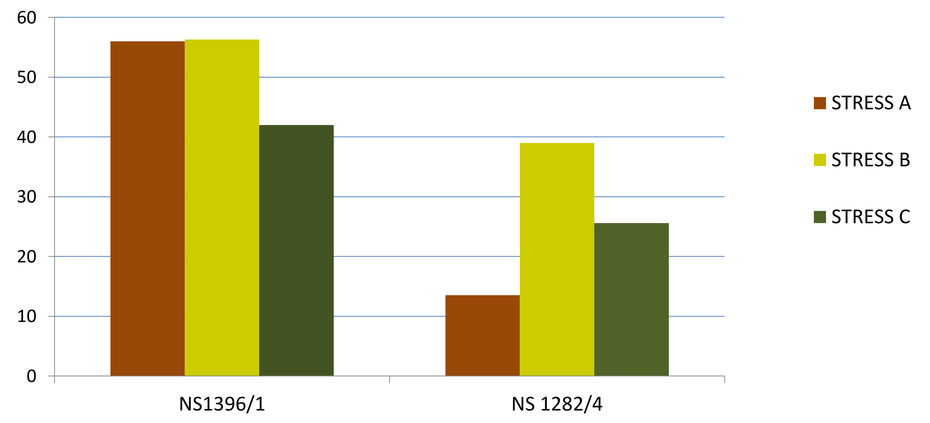
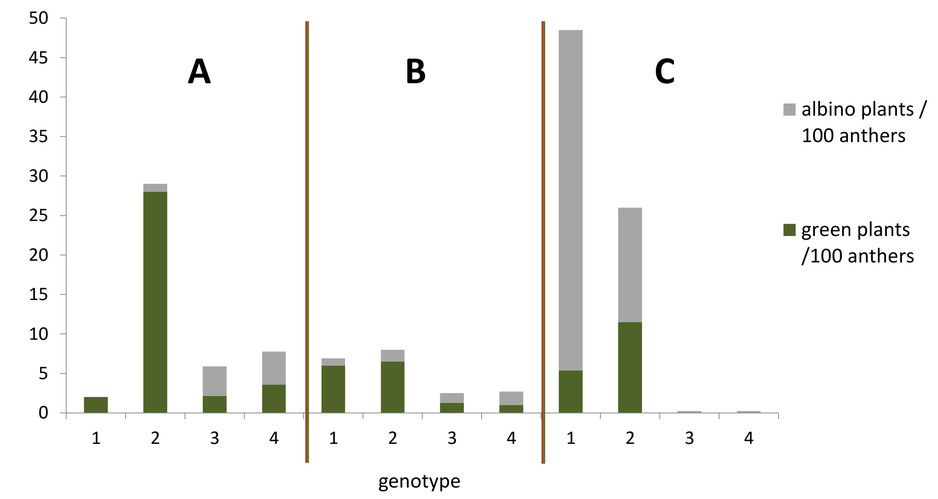


Fig. 2. **Effect of stress treatment on plant regeneration**



Acknowledgment

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References

Zhuang and Xu 1983, 431. Science Press, Beijing; Warzecha et al. 2005, APP 27 (2) 245-250; Pauk et al. 1991, Plant Breed. 107 18-27;