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# Application of Biotechnology to Rice Breeding by Enhancing the Bulgarian Anther Culture to Facilitate Rice Variety Development

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## 1. Obtaining of DH lines from F1 hybrid combination Japonica x Indica

In this experiment, 2 solid media were used for callus induction and Plant regeneration: N6 and MS (for callus induction: 2 mg/l. 2.4 D; for regeneration: - 0,5 mg/l IAA + 2 mg/l kinetin). As a Control standard DH variety Mariana/Japonica type was used with high induction and regeneration ability.

*Table 1* presents the results from induction and regeneration by the investigated combination and the following indexes: the number of inoculated anthers and calluses, albino and green regenerants and the percentage.

In N6 media for induction 2,975 anthers were inoculated from 4 F1 hybrid Japonica x Indica combinations, 2 Japonica x Japonica combinations, and Mariana DH standard. 548 calluses were obtained (15.3%). In the same media for regeneration we transferred 379 calluses and the results exceeded 164 albino and 86 green regenerants. In the media for induction (MS) we inoculated 2,988 anthers and obtained 335 calluses (11.2%). In the same media for regeneration we transferred 264 calluses and obtained 54 albino and 93 green plants.

In the future, it is perhaps better to test liquid media, and compare them with solid media. But in this test we used for induction N6 and for regeneration, MS.

The regenerations in the F1 combination Belozem x Tetep being absent from this combination we obtain only 1 albino plant.

**2. Screening *in vitro* for resistance to syringomycin with anther culture** from DH cultivar Mariana/J/ were excised and placed on N6 medium. Toxin syringomycin was added at 10, 20 and 30 active units in ml active ingredient/ml in the MS regeneration medium.

For control no toxin was added. Rhizogenesis and regeneration were measured before and after appearance of plantlets. After screening 121 resistance lines were obtained, and 71 were field tested. Their reactions *in vivo*, checked in field condition with inoculum from 2 strains (*P. atrofaciens* and 43080) with 3 concentrations (10 cfu/ml, 20 cfu/ml, 30 cfu/ml). Only 1 line has shown resistance to field condition. The results are shown in *Table 2*. We formed 7 resistance groups after having calculated the degree of infection.

## 3. Field test trial of 17 dh lines, obtained from mixoploid line N45 by the method of anther culture

The mixoploid line N45 was obtained from the F1 hybrid combination Belozem x Plodiv 22/j/ from 17 lines tested. None exceeded the standard cultivar Mariana.

4. For anther culture after mutagenesis and anther culture by the cultivar jubileini, we obtained 1 line with shorter period, but with low yield. After mutagenesis we isolated the second DH line with a compacted-like broom, and obtained a number of seeds (+ 25) compared with the standard. We tested this line, and the same will be inoculated in state variety tester.

**Table 1.**

F1 combination	Media N6					Media MS				
	Induction		Regeneration			Induction		Regeneration		
	Number anthers	Number calluses (%)	Number calluses	Albino Plants (%)	Green calluses (%)	Number anthers	Number calluses (%)	Number calluses	Albino plants (%)	Green plant (%)
St Mariana-DH	300	82-25	31	8-25	21-67.7	300	60-20	50	12-24	39-78
Belozlruj/J/x										
Belozem/J/	411	47-11	42	3-7	28-66.6	320	39-12	31	11-35.4	4-12.9
Belozem/J/x										
Medica/l/	418	101-24	93	8-8	18-19.3	311	34-10.9	32	3-9.3	1-3.1
Belozem/J/x										
Tetep/l/	450	7-1	7	—	423	2-0.4%	2	1-50	—	
Uspeh ran/J/x										
Belozem/J/	300	101-33	96	45-46	8-8.3	320	103-32.1	70	24-34.2	37-52.8
Jubileini/J/x										
Metica/l/	273	53-199	51	30-58	4-7.8	407	62-15.5	57	7-12.2	12-21
Plovdiv 22/J/x										
Tomi-3/l/	823	64-7	59	40-67	7-11.8	907	35-38.1	22	8-36.3	
<b>Total</b>	<b>2975</b>	<b>458</b>	<b>379</b>	<b>164</b>	<b>86</b>	<b>2988</b>	<b>335</b>	<b>264</b>	<b>54</b>	<b>93</b>

**Table 2.**

Concentrations	Inokolum P. atrofaciens							Inoculum 43080 strain						
	I	VR	R	MR	MS	S	VS	I	VR	R	MR	MS	S	VS
10 fcu/ml	—	1	—	—	22	8	40	—	1	—	—	35	11	24
20 fcu/ml	—	1	—	—	18	29	23	—	1	—	—	6	31	33
30 fcu/ml	—	1	—	—	5	11	54	—	1	—	—	—	35	35

I = Immune, VR = very resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, VS = very susceptible.