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In vitro androgenesis response of French durum wheat

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تحليل درة اكون اذوري دال ازاج

إن تحليل در ة اكو ن اذور ي دال از اج ميع 100 نمط ور اي ن اقمح اصلب كون الأاس هذا اث. اطلاا ن 100 نمط ور اي ار، 50 ات ضور ي لو ادر ن لى ادد و ار و ن 17 نمط ور اي نهم 4 لالات لة (Mondur, Primadur, Eurodur, Megadur) و 1,16 لات ضور ة ل 100 ر عد زر اهم از اية و انة المائو ة المهمة انت ند لالة Mondur إن أير انمط اور اي و و د دى ميع او ات المحللة دى انة المائو ة لدد ايضور ي لان ذا الأير قى ضعيفا و للي مميز. إن اعدد المر فع ن ادد الجذور ب المحصل ليه ات ن ين المواد المحللة.

اكلمات المفاية : اكو ن اذور ي ـ دال از اج ـ اقمح اصلب ـ لات ضوري

Aptitude à l'androgenèse *in vitro* du blé dur de France

L'analyse de l'aptitude à l'androgenèse *in vitro* d'une collection de 100 génotypes de blé dur de France a montré que 50 plantes chlorophylliennes ont pu être régénérées à partir de 17 génotypes dont 4 lignées fixées (Mondur, Primadur, Eurodur, Megadur). 1,16 plantes chlorophylliennes pour 100 anthères mises en culture est le pourcentage le plus intéressant obtenu chez la lignée Mondur. L'effet génotypique est très significatif pour tous les paramètres analysés sauf pour le pourcentage de régénérations chlorophylliennes, car ce dernier reste assez faible et, par conséquent, peu discriminatoire. Le nombre de régénérations racinaires obtenu est un parametre qui a été inclus dans cette analyse, étant donné son niveau élevé.

Mots clés: Androgenèse - *In vitro* - Blé dur - Plantes chlorophylliennes

***In vitro* androgenesis response of French durum wheat**

The aim of this investigation was to analyse the *in vitro* androgenesis response of 100 French genotypes of durum wheat. From the 100 tested genotypes, we obtained about 50 green seedlings of 17 different genotypes comprising 4 fixed lines (Mondur, Primadur, Eurodur, Megadur). The percentage of green seedlings obtained from Mondur was 1.16 per 100 cultured anthers. The response to androgenesis was affected by genotype for all studied parameters except for the percentage of green regenerations because it was still very low. The high number of embryos developed into root regenerations, was the reason for using the percentage of root regeneration as an androgenesis parameter.

Key words: Androgenesis - *In vitro*- Durum wheat - Green seedlings - Root regeneration

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INTRODUCTION

During the 15 last years, *in vitro* androgenesis has shown an important improvement. This method has been largely improved for most cereal crops, although, some species are still recalcitrant. Since the first success in obtaining haploid embryos of *Datura inoxia*. Mil, via androgenesis (Guha and Maheshwari, 1964), the investigations on understanding this phenomenon resulted in regeneration of plants via androgenesis of many species. This method could be used on crops breeding when the rate of androgenetic doubled haploid plants is sufficiently high (Griffing, 1975). On cereals, success to produce new varieties was reported in china (Hu *et al.*, 1983-1986) and in France (De Buyser *et al.*, 1987). Despite the interesting progress achieved in this way, important constraints remain and limit a large application of *in vitro* anther culture in practical crops breeding.

The production of embryos and plants via *in vitro* androgenesis is controlled by many factors. The most important are the microspore stage, the donor plants growth conditions and the genotype.

The genetic factors affecting results in anther culture involve a complex traits with at least two different components: the ability of microspores to divide and produce embryos and the ability of embryos to regenerate into seedlings (Fourouhi Wehr *et al.*, 1982).

For *in vitro* cereal androgenesis, the major problems are the high rate of albinos regenerated plants and the very low rate of regenerated green plants, especially in the case of durum wheat. Therfore, the comparison between the albinos regenerated plants rate and the green regenerated plants rate has been introduced as a third component of anther culture response in cereals (Wenzel *et al.*, 1977; Fouroughi Werh *et al.*, 1982). Several authors demontrated that this rate is strongly influenced by genotype (Anderson *et al.*, 1987; Tuvson *et al.*, 1989).

In the last few years, a succefull production of haploid green plants was realized via the intergeneric crosses between bread wheat and maize (Laurie *et al.*, 1986, 1987; Moieni *et al.*, 1994; Lefebre *et al.*, 1996) and durum wheat and maize (O'Donoughue *et al.*, 1994; Saidi *et al.*, 1998). This approach could be another way for the production of haploids in durum wheat.

The aim of the present investigation is to screen a durum wheat collection of GAE (Private French Durum Wheat Breeder), for the ability to regenerate androgenetic green seedlings. Very few research studies has been reported on durum wheat androgenesis. Many problems are still not resolved and investigation on some of them will contribute to understand the refractoriness of durum wheat to give a good rate of androgenetic green plantlets.

MATERIALS AND METHODS

The material tested in this experiment consisted of 100 durum wheat genotypes including 17 fixed lines (Agridur, Alphadur, Alpidur, Biodur, Brindur, Capdur, Chandur, Exodur, Eurodur, Flodur, Lhyod, Megadur, Minodur, Mondur, Neodur, Primadur, Supradur). The other genotypes are listed in table 1. Two different conditions have been used for the cultivation of donor plants. For the genotypes used in the hydroponics culture condition, the germination of seeds has carried out in 4 liters pots. For the green house soil condition, the seeds has been germinated on earthenware. The compost "Orgaflore" was the substrate used for both conditions. The germination was conducted in the green house until the seedlings developed into two leaves. This phase took two weeks. After the seedlings were transferred into the vernalization room for four weeks at 5°C with a 8 hours photoperiod. Then, the plants for the cultivation on hydroponics conditions were placed in a large box fed with the hydroponic solution. The irrigation was assured by half immersion of the pots into this solution for ten minutes every day. The solution was composed of 1% of Hydrocani H, 83.33 g/l of Sequesren Fe and 0.06 ml of microelements. The pH was adjusted to 5.8 every two days.

For the second condition of cultivation, the plants were planted into the soil in a green house. All plants were placed into the same green house at 20°C^{+}.2°C. When the microspores reached the uninucleate stage the spikes were collected and stored at 3°C for 10 to 40 days for the cold shock.

For anther culture, the spikes were sterilized with 2% calcium hypochlorite solution for 3 min. The anthers of each spike were plated on solid P2 medium (Chuang *et al.*, 1978) additioned with 100 mg/l of glutamin, then stored in the dark at 25/ 29°C as day temperature and 22/24 h as night temperature. A minimal number of 5 spike anthers were plated for each genotype .

About twenty days after the inoculation of anthers, the first embryogenic structure were obtained and transferred to regeneration medium (R9S). The embryos were placed in the same conditions as anthers but with a photoperiod of 16h for two days, after the storage in the dark for 10 days. Then the embryos were transferred to the conditions and exposed to light (16 h). During the storage in the dark, most of embryos began to differentiate into green, albinos are root regenerations. The statistical analysis concerned the percentage of responding spikes, the number of embryos, green regenerations, albinos regenerations, total (green and albinos) regenerations and root regenerations, for 100 plated anthers. The analysis of variance was carried out on the data to determine the genotypic effect for previous parameters. Comparison of the means was performed via SNK grouping test. All statistical analysis were carried out with the SAS program (1988).

RESULTS

The results are presented on tables 1, 2. and 3. The number of responding genotypes depends on the studied parameter.

For the embryogenic spikes 92 genotypes responded (91%). Only 17 genotypes differentiated into green seedlings (17%). The embryos of 58 genotypes developped into albinos regenerations (57%). For the total regenerations 65 genotypes responded (64%). But the most imprtant percentage of response was observed for the for root regenerations because 83 genotypes responded (82%). A total of 50 seedlings was regenerated from 17 different genotypes.

For the all parameters, the genotypic effect was significant except for the percentage of green plants. For this parameter, the percentage of green regenerations was very low and insufficient to discriminate between genotypes of high and low ability to regenerate into green plants. GA6 was the best genotype according other parameters.

The total regeneration was the most discriminatory parameter. According to this parameter, the best genotypes were, GA6, Mondur, Primadur, GA13 and GA48.

Even if GA6 has gave the best percentage of embryos, it was not sufficient for the production of a good rate of green regeneration. For all genotypes, the root regeneration percentage was higher than the other regeneration percentage parameters.

Mondur was the unique genotype who kept a good percentage for the six studied parameters, showing good stability.

Table 1. Androgenetic ability of 100 tested genotypes. Comparison of the percen tage for each parameter, via SNK test

Genotype	.....Percentage				
	Embrg. spikes	Embryos	Green pl.	Albinos pl.	Root reg.
GA6	100.0a	38.2a	0.0a	7.3a	14.1a
MONDUR	95.5ab	23.1b	1.1a	3.0b	7.2bc
GA27	88.9ab	9.7c	0.0a	1.0c	1.6cd
GA53	85.7ab	4.4c	0.0a	0.3c	0.6cd
GA120	83.3ab	1.7c	0.0a	0.0c	0.3cd
GA44	81.1ab	5.4c	0.0a	0.9c	0.7cd
GA28	80.0ab	3.7c	0.7a	0.6c	1.2cd
GA45	77.8ab	1.5c	0.0a	0.3c	7.1 b
GA58	77.8ab	9.3c	0.0a	0.9c	5.7cd
GA21	76.9ab	5.6c	0.0a	1.9c	2.6cd
GA26	72.2ab	4.0c	0.0a	0.9c	1.0cd
GA35	71.4ab	4.1c	0.1a	0.2c	1.7cd
GA47	71.4ab	3.7c	0.0a	0.4c	0.2cd
GA25	66.7ab	4.0c	0.0a	1.2c	1.3cd
GA49	66.7ab	12.3c	0.0a	0.0c	2.0cd
PRIMADUR	63.6ab	10.6c	0.7a	0.7c	4.1cd
GA103	63.6ab	6.6c	0.0a	0.9c	1.4cd
GA102	62.5ab	2.7c	1.2a	0.3c	0.9cd
GA114	60.0ab	3.1c	0.0a	0.0c	1.6cd
GA98	60.0ab	3.7c	0.2a	0.3c	1.2cd
GA145	60.0ab	5.2c	0.0a	0.4c	1.3cd
GA67	58.8ab	3.1c	0.0a	1.8c	0.9cd
GA94	57.1ab	3.6c	0.1a	0.8c	2.1cd
GA50	56.2ab	4.0c	0.0a	0.8c	1.0cd
GA153	54.4ab	3.8c	0.4a	0.4c	2.1cd
GA29	54.4ab	2.5c	0.1a	0.3c	0.9cd
GA96	53.8ab	2.4c	0.0a	0.1c	0.7cd
MEGADUR	53.3ab	5.4c	0.1a	0.9c	0.9cd
GA24	52.6ab	3.3c	0.0a	0.4c	1.0cd
GA36	50.0ab	3.7c	0.2a	0.6c	0.9cd
GA39	50.0ab	2.6c	0.0a	0.5c	0.3cd
EURODUR	50.0ab	3.2c	0.2a	0.0c	0.4cd
CAPDUR	50.0ab	1.9c	0.0a	0.2c	0.9cd
GA154	50.0ab	6.6c	0.5a	0.3c	3.4cd
GA97	50.0ba	1.8c	0.0a	0.4c	0.8cd
GA54	50.0ab	6.8c	0.0a	1.2c	2.0cd
GA1	50.0ab	2.4c	0.0a	0.0c	0.0 d
GA48	50.0ab	8.2c	0.0a	3.1c	1.8cd
GA13	50.0ab	9.8c	0.5a	3.4c	1.5cd
GA104	50.0ab	2.8c	0.0a	0.6c	0.3cd
GA69	50.0ab	3.4c	0.0a	0.7c	0.7cd
GA117	50.0ab	1.4c	0.0a	1.0c	0.0 d
EXODUR	50.0ab	5.8c	0.0a	1.6c	2.2cd
BIODUR	45.5ab	5.8c	0.0a	0.9c	0.1cd
GA95	44.4ab	2.7c	0.0a	0.1c	0.7cd
GA14	44.4ab	1.1c	0.0a	0.3c	0.3cd
GA31	43.7ab	1.2c	0.0a	0.0c	0.7cd

Embrg. : Embryogenic ; pl. : plantlets ; reg. : regenerations
Percentages not followed by the same letter are significantely different at 0.05 level.

Table 1 *(Continued)*. Androgenetic ability of 100 tested genotypes

Genotype	Percentage				
	Embrg. spikes	Embryos	Green pl.	Albinos pl.	Root reg.
GA71	42.8ab	6.9c	0.3a	1.8c	2.4cd
FLODUR	42.8ab	5.0c	0.0a	0.0c	1.3cd
GA100	41.7ab	0.9c	0.0a	0.0c	0.3cd
GA46	40.0ab	1.3c	0.0a	0.0c	1.3cd
GA115	40.0ab	0.9c	0.0a	0.0c	0.0 d
GA64	40.0ab	2.0c	0.0a	0.0c	1.0cd
GA34	40.0ab	0.9c	0.0a	0.1c	0.5cd
GA81	40.0ab	1.8c	0.0a	0.0c	1.1cd
GA90	37.8ab	3.1c	0.0a	0.0c	0.8cd
GA38	37.5ab	1.7c	0.0a	0.2c	0.6cd
GA122	33.3ab	1.1c	0.0a	0.2c	0.2cd
GA113	33.3ab	0.8c	0.0a	0.3c	0.3cd
ALPIDUR	33.3ab	1.8c	0.0a	0.4c	1.1cd
GA33	33.3ab	1.2c	0.0a	0.0c	0.3cd
GA59	33.3ab	4.8c	0.0a	0.5c	1.7cd
GA125	33.3ab	2.7c	0.0a	0.5c	0.5cd
GA89	33.3ab	0.6c	0.0a	0.0c	0.6cd
GA152	33.3ab	0.6c	0.0a	0.0c	0.3cd
GA30	33.3ab	0.7c	0.0a	0.0c	0.4cd
GA101	31.2ab	2.2c	0.5a	0.3c	0.6cd
CHANDUR	30.8ab	1.1c	0.0a	0.2c	0.4cd
GA37	28.6ab	3.2c	0.0a	0.2c	0.5cd
GA70	28.6ab	0.7c	0.0a	0.0c	0.0 d
GA105	28.6ab	0.7c	0.2a	0.2c	0.0 d
GA51	28.6ab	1.2c	0.0a	0.0c	0.5cd
NEODUR	28.6ab	1.4c	0.0a	0.0c	0.3cd
GA22	27.3ab	0.4c	0.0a	0.0c	0.1cd
GA82	25.0ab	1.7c	0.6a	0.0c	0.3cd
GA52	25.0ab	0.8c	0.0a	0.2c	0.2cd
GA116	22.2ab	0.4c	0.0a	0.0c	0.2cd
GA118	22.2ab	0.3c	0.0a	0.0c	0.0 d
GA106	20.0ab	0.6c	0.0a	0.0c	0.0 d
GA8	20.0ab	0.5c	0.0a	0.0c	0.0 d
GA127	20.0ab	0.9c	0.0a	0.0c	0.5cd
GA85	20.0ab	1.2c	0.0a	0.0c	0.5cd
LHYOD	20.0ab	0.4c	0.0a	0.0c	0.0 d
GA132	18.2ab	0.6c	0.0a	0.0c	0.2cd
GA150	16.7ab	0.4c	0.0a	0.0c	0.0 d
GA99	15.0ab	0.4c	0.0a	0.0c	0.4cd
GA5	14.3ab	0.2c	0.0a	0.0c	0.0 d
ALPHADUR	14.3ab	0.9c	0.0a	0.0c	0.5cd
GA63	12.5ab	0.5c	0.0a	0.0c	0.2cd
GA32	12.5ab	0.5c	0.0a	0.0c	0.3cd
GA112	12.5ab	0.3c	0.0a	0.0c	0.0 d
GA23	8.3b	0.1c	0.0a	0.0c	0.1cd
BRINDUR	0.0b	0.0c	0.0a	0.0c	0.0 d
GA40	0.0b	0.0c	0.0a	0.0c	0.0 d
GA119	0.0b	0.0c	0.0a	0.0c	0.0 d
GA148	0.0b	0.0c	0.0a	0.0c	0.0 d
GA156	0.0b	0.0c	0.0a	0.0c	0.0 d
MINODUR	0.0b	0.0c	0.0a	0.0c	0.0 d
AGRIDUR	0.0b	0.0c	0.0a	0.0c	0.0 d
SUPRADUR	0.0b	0.0c	0.0a	0.0c	0.0 d

Table 2. Variance analysis for different studied parameters

	ddl	Percentage				
		Embr. spikes	Embryos	Green pl.	Albinos pl.	Root reg.
R^2		0.5***	0.4***	0.2	0.5***	0.6***
Genotype	99	2.9***	2.2***	1.0	2.6***	5.9***
Residual	719					

Embrg. : Embryogenic ; pl. : plantlets ; reg. : regenerations
*** Significance at level 0.1%

Table 3. Correlation coefficient between different analysed parameters

	Percentage				
	Embr. spikes	Embryos	Green pl.	Albinos pl.	Root reg.
% Embr. spikes	1.00	0.45***	0.33***	0.76***	0.76***
% Embryos		1.00	0.16***	0.36***	0.39***
% Green plantlets			1.00	0.25***	0.22***
% Albinos plantlets				1.00	0;54***
% Root regenerations					1.00

Embrg. : Embryogenic ; pl. : plantlets ; reg. : regenerations
*** Correlation significance at 0.1%

DISCUSSION AND CONCLUSION

The first important phenomenon observed in this experiment was the genotype effect. This effect has been already discussed by Fouroughi-Wehr & Zeller (1990), Hadwiger & Haberles-Bors (1986). The two first authors have tested the genotype Mondur, it was the unique durum wheat genotype that gave green regenerations (0,3%). But in this experiment this percentage reached 1.16% for the same genotype.

The analysis of the correlation between root regeneration percentage and other androgenetic parameters was highly significant (Table 3), especially with the percentage of albinos regenerations.

The most important problem of durum wheat is the low rate of green regenerations and the high rate of albinos and root regenerations. This recalcitrance to regenerate green seedlings could be due to the disturbance of the normal evolution of the proplasts into chloroplasts during *in vitro* androgenesis. For many androgenesis recalcitrant species, the pollen mother cells contain amyloplasts during the pollen maturation phasis (Sangwan and Sangwan Norreel, 1987). In the case of *in vitro* culture of bread wheat immature embryos, Purnhausser *et al.* (1987) explained the

problem of regeneration by the negative effect of auxine via the production of ethylen that could inhibit the primodium formation. Several authors demonstrated that albinos regenerations alter or delet genome (Day & Ellis, 1985; Harada *et al.*, 1991). According to Tuvson *et al.* (1989), there are two different classes of genes affecting green plant regeneration in bread wheat anther culture. The first one with additive effects modifying green plants rate and the second one with few major genes affecting the original frequency of potentially green structures.

For the answer to the problem of green regenerations via androgenesis, further investigation is required, especially via the DNA analysis of the green albinos and the parents, to compare their profiles. Many molecular analysis methods are now easy to use for this aspect. The quantitative and qualitative analysis of the presence or absence of proteins of some recalcitrant and non recalcitrant genotypes could elucidate this problem. Also physiologic and cytological studies could contribute to comprehension of the albinism problem. This needs a good collaboration between different experts on physiology, cytology and molecular biology to insure a successful way for answering the questions about the albinism phenomenon.

In the few last years, the production of doubled haploid lines of durum wheat via intergeneric crosses was used and some encouraging results have been obtained as discussed in the introduction.

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