Iran Agricultural Research 17:91-102 (1998)

ANTHER CULTURE OF IRANIAN WHEAT GERMPLASM USING DIFFERENT MEDIA

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(Received: April 21, 1998)

ABSTRACT

An experiment was conducted to study the androgenic response of five wheat (*Triticum aestivum* L.) cultivars and to identify an appropriate medium for doubled-haploid production for the Iranian wheat breeding programs. Four induction media including modified C17 (MC17), B5, N6 and MS media were used in this study. The overall response involved two independent phenomena of embryo induction and plant regeneration. An Iranian cultivar, 'Falat', carrying the 1BL/1RS translocation was particularly responsive in MC17 medium. Results of analysis of variance for percentage of anthers responding (percentage of embryoid induction) showed that there were highly significant differences both among wheat cultivars and among the tested media. The cultivar×medium interaction was only significant for plant regeneration. However, a positive and significant correlation was obtained for percentage embryoid induction and percentage green plant regeneration. Chromosome counting of meristemic cells in root tips of regenerated green plants indicated that 17 percent of the regenerants were spontaneous doubled haploids.

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تحقیقات کشاورزی ایران

۱۷:۹۱-۱۰۲ (۱۳۷۷)

کشت بساک ژرم پلاسم گندم ایرانی با استفاده از محیط کشت های مختلف

احمد ارزانی و کیانوش چغا میرزا

به ترتیب استاد یار و دانشجوی سابق کارشناسی ارشد (اکنون مربی دانشکده کشاورزی دانشگاه رازی، کرمانشاه، ایران) گروه زراعت و اصلاح نباتات، دانشکده کشاورزی دانشگاه صنعتی اصفهان، اصفهان، ایران.

چکیده

آزمایشی به منظور مطالعه واکنش به نرزایی در پنج رقم گندم (*Triticum aestivum* L.) و همچنین برای شناسایی محیط کشت مناسب برای تولید هاپلوئید مضاعف شده در برنامه به نژادی گندم ایرانی اجرا گردید. چهار محیط کشت انگیزش شامل C۱۷ تغییر یافته (MC ۱۷)، B۵، N۶ و MS در این مطالعه مورد استفاده قرار گرفت. پاسخ کلی به کشت بساک ناشی از دو پدیده مستقل انگیزش رویان و باززایی گیاه بود. رقم ایرانی فلات حاوی جابجائی ژنی 1BL/1RS به ویژه در محیط کشت MC۱۷ واکنش مطلوبی داشت. نتایج تجزیه واریانس برای درصد بساک های پاسخ داده (درصد انگیزش رویان) نشان داد که تفاوت های بسیار معنی داری بین ارقام و بین محیط های کشت وجود داشت. برهمکنش رقم × محیط کشت تنها برای باززایی گیاه معنی دار گردید. با این وجود، همبستگی مثبت و معنی داری بین درصد انگیزش رویان و درصد باززایی

گیاه سبز بدست آمد. شمارش کروموزومی سلول ها در نوک ریشه گیاهان سبز باززایی شده نشـان داد که ۱۷ درصد از گیاهان باززایی شده به صورت هاپلوئید مضاعف شده خود بخودی بودند.

INTRODUCTION

Anther culture provides an efficient tool for achieving homozygosity of plant materials and reducing the time for the development of new cultivars in self-fertilizing crops. It has been shown that a number of factors influence the production of haploid regenerants of which donor plant genotype is the most important. Thus, significant genotype differences in anther culture response for both induction and regeneration have been widely reported in bread wheat (3, 9, 12, 14, 20, 21), durum wheat (7, 18) and triticale (2, 8).

Efficiency of haploid production is also affected by the donor plant growth environment and by the applied media (10, 23). The genotypic dependency as well as genotype × medium interactions in anther culture techniques have restrained plant breeders from a wider production of doubled haploids in their programs. Recently, a significant improvement in anther culture efficiency was demonstrated in wheat (11) and triticale (1). The improvement was due to the modification of the previously used C17 medium (24) to MC17, and due to the utilization of hydroponics as an optimal donor plant environment.

The aim of this study was to assess anther culturability of Iranian wheat genotypes, which are predominantly utilized in the breeding of local cultivars, using four induction media MC17, B5, N6 and MS.

MATERIALS AND METHODS

Plant Materials

Five wheat (*Triticum aestivum* L.) cultivars ('Omid', 'Navid', 'Roshan' and 'Falat' originated from Iran and 'Hartog' from Australia) were used in this study. 'Hartog' was included in this experiment because of its

good response in anther culture (S. Venkatangappa, personal communication). Plants were grown both in a recirculating hydroponic system (1) and in 25-cm diameter pots within a greenhouse with an average minimum and maximum temperature of 18° and 34°C, respectively, and 150-400 μEs^{-1} m^{-1} light intensity.

Anther Culture

Spikes were harvested in the late booting stage (corresponding with mid-uninucleate stage of microspore development) in the hydroponic system. Spikes were harvested from pots when the midpoint of the inflorescence was level with the leaf immediately below the flag leaf. This stage was highly correlated with the mid-uninucleate stage of microspore development. Spikes from each of the environments (hydroponics and pots) were used equally for all anther culture treatments. Spikes were cold pre-treated at 4°C for 4-7 days before excision of anthers. Sixty anthers from a single spike were plated in a 6 cm petri dish containing the induction medium. These were then incubated in the dark at 27°C. After 4-8 weeks, embryoids were placed on the regeneration medium and incubated at 25°C with 16 hr light per day.

Culture Media

Four induction media were used including modified C17 (MC17) (11), B5(6), N6(4), and MS (15). The B5, N6, and MS media contained 100 mg l^{-1} myo-inositol, 30 g l^{-1} sucrose, 150 mg l^{-1} glutamine, 2 mg l^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid), 0.5 mg l^{-1} kinetin and 2.5 g l^{-1} agarose (Sigma Type 1A). These components were the same in MC17 medium, except that 98 g l^{-1} maltose (monohydrate) was used instead of sucrose. For plant regeneration, MS medium was supplemented with 1mg l^{-1} indoleacetic acid (IAA), 1 mg l^{-1} benzylaminopurine (BAP) and 2.8 g l^{-1} agarose (Sigma Type 1A).

Statistical Analysis

A 5×4 factorial experiment in a completely randomized design with unequal numbers of replications (i.e., 12 to 18) was used in this study. Five

variables were obtained: the number of embryoids per 100 anthers plated (EA); the number of green plants per 100 anthers plated (GPA); the number of green plants per 100 embryoids (GPE); the number of albino plants per 100 embryoids (APE); and the number of total plants (green+albino) per 100 embryoids (TPE).

Statistical analysis was conducted using GLM procedure of SAS computer package (19). The variable EA was transformed by arcsin $\sqrt{x}$ and the regeneration variables were transformed by log (x+1) as they had a range of $0 \leq x \leq 10$ (22). The comparison of means was made by the LSD test.

Cytological Examination

Chromosome counts were made on root tip cells from 15 regenerated green plants by the Feulgen stain technique. Root tips were stored overnight in 2% monobromonaphthalene solution at 4°C. Root tips were then hydrolyzed for 10 min in 1M hydrochloric acid at 45-60°C, stained in Feulgen stain, and squashed in 1% acetocarmine.

RESULTS

Analyses of variance for the induction and regeneration responses of wheat cultivars in four induction media are presented in Table 1. The results of the analysis of variance showed that there were highly significant differences ($P<0.001$) among genotypes and media for anther culture responce [the number of embryoids per 100 anthers plated (EA)]. However, genotypic effects were not significant but media effects were significant ($P< 0.05$ or $P<0.01$) for most of plant regeneration criteria (Table 1). The genotype × medium interaction was not significant for embryoid induction (EA), but it was significant ($P<0.05$) for all of the regeneration variables (GPA, GPE, APE, and TPE).

The means for both the induction and regeneration responses of wheat cultivars averaged over media are presented in Table 2. The genotype means for pollen embryoid induction (EA) showed the highest mean for cv. 'Hartog' and lowest mean for cv. 'Roshan'. Cultivar 'Falat' also performed

well with respect to embryoid induction, and was not significanly different from 'Hartog' cultivar in this aspect. The genotypes 'Omid' and 'Navid' were ranked second after the first group ('Hartog' and 'Falat'), and showed a medium induction response.

Table 1. Analysis of variance for induction and regeneration responses of wheat cultivars using four induction media.

Source of variation	Degree of freedom	Mean square				
		EA§	GPA†	GPE†	APE†	TPE†
Genotype (G)	4	289.13***	0.34	0.28	0.32	0.45
Medium (M)	3	449.44***	2.34**	0.93*	0.24	1.74**
G × M	12	57.76	1.30*	0.63*	0.30*	0.76*
Residual	235	37.18	0.40	0.28	0.15	0.29

† Based on log-transformed data.

*, **, *** Significant at P<0.05, P<0.01 and P<0.001, respectively.

§ Embryoids per 100 anthers plated (EA); the number of green plants per 100 anthers plated (GPA); the number of green plants per 100 embryoids (GPE); the number of albino plants per 100 embryoids (APE); and the number of total plants (green+albino) per 100 embryoids (TPE).

Although, the genotype × medium interaction was not significant for embryoids per 100 anthers plated (EA), it was significant for the regeneration variables comprising GPA, APE, GPE and TPE (Table 1). Fig 1. shows the means of green plants per 100 anthers plated (GPA) for the genotype × medium interactions. The genotypes 'Hartog' and 'Falat' produced the highest GPA in MC17 medium. However, genotype 'Roshan' also performed well in MC17 medium, and there were no significant differences among these genotypes.

The means of total plants regenerated per 100 embryoids (TPE) for genotype × medium interactions are presented in Fig 2. 'Falat' in MS medium, and 'Navid' in MC17 medium had the highest means for TPE. However, no significant difference was observed for genotype when cultured in MC17 or MS medium. Fig. 3 shows plant regeneration of 'Falat' in MC17 and N6 media.

Table 2. Genotypic effect (A) and the effect of induction medium (B) on induction and regeneration responses (means, %) of wheat cultivars.

Main effects	EA†	GPA	GPE	APE	TPE
(A) Genotype					
'Hartog'	9.2	8.76	95.3	13.5	108.8
'Falat'	6.9	8.47	122.8	57.4	180.2
'Omid'	5.5	3.26	59.4	16.1	75.5
'Navid'	5.3	3.38	63.9	55.3	119.2
'Roshan'	4.4	3.40	77.4	28.8	106.2
(B) Medium					
MC17	9.1	10.78	118.5	65.3	183.8
N6	6.7	5.57	83.2	34.3	117.5
MS	4.8	4.72	98.5	25.7	124.2
B5	4.3	1.50	34.9	11.6	46.5
$LSD_{0.05}$	3.6	1.43	35.5	26.0	36.2
$LSD_{0.01}$	5.2	2.03	50.2	36.7	51.2

† Embryoids per 100 anthers plated (EA); the number of green plants per 100 anthers plated (GPA); the number of green plants per 100 embryoids (GPE); the number of albino plants per 100 embryoids (APE); and the number of total plants (green + albino) per 100 embryoids (TPE).

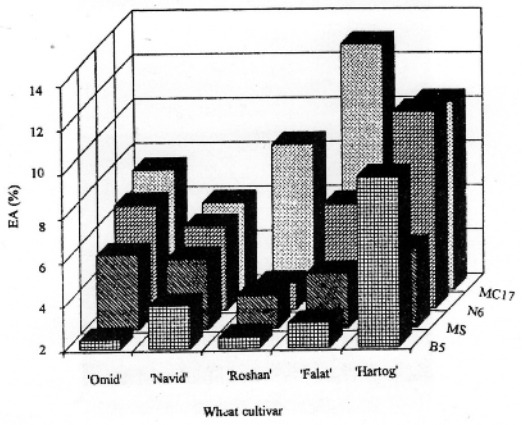

Fig. 1. Genotype × medium interaction for green plant regeneration (GPA).

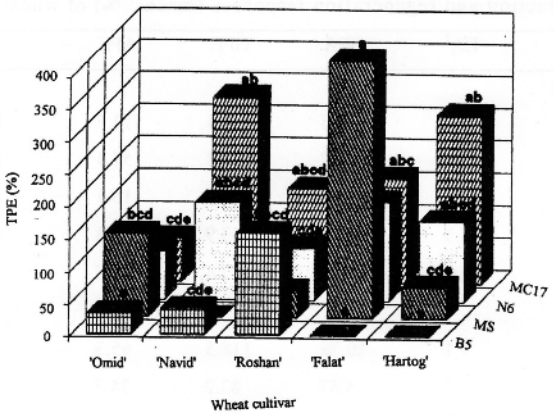

Fig. 2. Genotype × medium interaction for total plant regeneration (TPE).

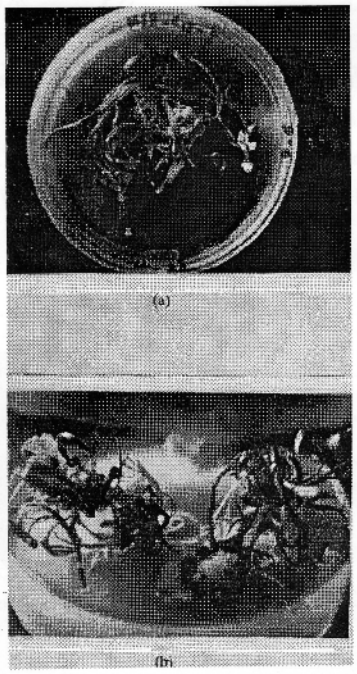

Fig.3. Plant regeneration of 'Falat' cultivar in MC17 medium(a) and N6 medium(b).

The medium means for induction and regeneration responses averaged over genotypes (Table 2) indicated that MC17 medium generally ranked best, followed by N6 medium.

Correlation coefficients of anther culturability of wheat genotypes showed that GPA correlated significantly ($P<0.01$) with EA ($r=0.71$), GPE ($r=0.65$), and TPE ($r=0.54$). However, EA was not correlated significantly with GPE ($r=0.28$), TPE ($r=0.22$), and APE ($r=0.02$).

Chromosome numbers were determined in 15 randomly selected green regenerants representing five cultivars. The results indicated that 83% of green regenerants were haploid ($n=21$), and the remainders (17%) were spontaneously doubled haploids ($2n=42$).

DISCUSSION

Genotype is a critical factor affecting the variability of response in plant tissue culture. The different responses to anther culturability in the cultivars studied, confirmed earlier observations of a strong genetic effects in wheat (3, 12, 14, 20, 21). The relatively high degree of responsiveness of Iranian cultivar 'Falat' (Seri-82/Veery#2) is attributed to the presence of the 1BL/1RS wheat-rye translocation in this cultivar. This finding is consistent with those of Masojc *et al.* (12) and Luckett *et al.* (11), who observed the positive effect of the short-arm chromosome 1R of rye in wheat androgenic response. In addition to specific nuclear genes for androgenic response, the positive effect of cytoplasmic DNA and in particular *Triticum timopheevii* cytoplasm has been reported in wheat (5, 17) and in triticale (2).

The use of androgenesis to obtain double-haploid plants of potential value in basic studies or plant breeding programs is dependent on the proportion of green haploid or spontaneous double-haploid plants recovered. The final androgenic response of each plant type can be estimated as the product of EA×GPE [GPA=(EA×GPE)/100] and from a practical point of view, this product is the most useful variable in the classification of genotypes. Our study for GPA suggest that GPA is greatly dependent on genotype (see Table 1).

The superiority of MC17 medium over the others in embryo formation and in regeneration, might result from its rich composition of micronutrients, its optimal nitrogen content or the presence of biotin which has been shown (24) to favor androgenesis. In addition some studies (11, 16) have shown the superiority of maltose in wheat anther culture media.

The cytological data were generally in agreement with those of previous studies (12, 13). The mechanisms of genome duplication of microspores (spontaneously doubled-haploid) were ascribed to two phenomena, nuclear fusion and endomitosis (9). The possibility that some of spontaneous diploids may be derived from the anther walls is unlikely . It has been shown that all anther-derived diploid plants were homozygous and therefore were microspore origin (13).

Although no direct comparison can be made between our results and those of others, due to genotypic and environmental differences, the level of embryoid induction in the present study was lower than that previously reported in wheat (11, 12, 20). However, the level of green plant regeneration was comparable, and this reinforces an earlier conclusion that embryogenesis and regeneration potential are controlled by different genes (9). Hence, the overall response involved two independent phenomena of embryoid induction and plant regeneration, as previously described (9). In the present study, the different patterns of regeneration and induction variables observed for the genotype × medium interaction validated this explanation.

In conclusion, the employment of cv. 'Falat' in Iranian wheat hybridization breeding programs using doubled haploids, coupled with MC17 induction medium for anther culture, is recommended.

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