

EFFICIENCY OF POTATO MEDIA FOR ANDROGENIC RESPONSE OF IRANIAN WHEAT CULTIVARS

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ABSTRACT

The efficiency of potato media (P1, P2 and P4) prepared from tuber extract of twelve commercial cultivars of potato, grown in Iran, on anther culturability of five wheat cultivars was investigated. Analysis of variance for embryoid induction (EI) showed significant differences ($P < 0.001$) among wheat cultivars, between media, and among potato cultivars. The interactions of wheat genotype \times medium, wheat genotype \times potato genotype, medium \times potato genotype, and wheat genotype \times medium \times potato genotype were also highly significant ($P < 0.001$) for EI. Among the five genotypes tested, the means of EI, averaged on the potato media, ranged from 6.8 for 'Falat' to 2.01 for 'Navid'. Potato tuber extract prepared from 'Primor' was ranked as the best for EI. P4 medium prepared from the extract of 'Aola' and 'Primor' potato cultivars was best for plant regeneration. In conclusion, the use of 'Falat' in Iranian wheat hybridization programs using doubled haploidy, coupled with the P4 induction medium for anther culture can be recommended.

KEY WORDS: Androgenic response, Embryoid induction, Potato media, Wheat cultivars.

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کار آبی محیط های کشت سبب زمینی برای پاسخ به نرژائی ارقام

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چکیده

کار آبی محیط های کشت سیب زمینی (P1, P2, P4) تهیه شده از عصاره غده های دوازده رقم مورد کشت در ایران بر قابلیت کشت بساک پنج رقم گندم مورد بررسی قرار گرفت. تجزیه واریانس برای انگیزش رویانواره تفاوت معنی داری ($P < 0.001$) را بین ارقام گندم، بین محیط های کشت و بین ارقام سیب زمینی نشان داد. برهمکنش ژنوتیپ گندم × محیط کشت، ژنوتیپ گندم × ژنوتیپ سیب زمینی و ژنوتیپ گندم × محیط کشت × ژنوتیپ سیب زمینی نیز برای انگیزش رویانواره بسیار معنی دار ($P < 0.001$) بود. در بین پنج ژنوتیپ گندمی که مورد آزمون قرار گرفتند، میانگین های انگیزش رویان در محیط های کشت P1, P2, P4 دامنه ای از ۶/۸ برای رقم 'فلات' تا ۲/۰۱ برای رقم 'نوید' داشت. عصاره غده های سیب زمینی 'پریمور' به عنوان بهترین نوع عصاره برای تولید جنین رتبه بندی گردید. محیط کشت P4 تهیه شده از عصاره ارقام سیب زمینی 'آشولا' و 'پریمور' برای باززایی گیاه برتری نشان دادند. در نتیجه، بکار گیری رقم 'فلات' در طرح های دورگه گیری گندم ایران از طریق دابل هاپلوئیدی به همراه محیط کشت انگیزشی P4 برای کشت بساک توصیه می شود.

INTRODUCTION

The use of anther culture as a routine tool in wheat breeding for rapidly attaining homozygosity may save research costs and provide an increased return to growers (10). In order to apply this technique effectively for plant breeding and genetic research, the steps in the wheat anther culture system should be simplified. In recent years, some laboratories have tried to substitute aqueous potato (*Solanum tuberosum* L.) extract for the majority of organic and inorganic components of synthetic media in order to simplify the procedure of medium preparation. Potato extract has been demonstrated to be very efficient in wheat anther culture (1). This potato medium, containing 20% potato extract was designated as potato-1(P₁) medium. The components of potato extract, like those of many other natural plant extracts, are subjected to various genotypic and environmental factors,

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thus leading to inconsistencies in the effects of the potato medium. Hence, a potato medium containing 10% potato extract and half strength synthetic medium was used for wheat anther culture, and designated as potato-2 (P2) medium (5). Another medium, potato-4 (P4) medium was reported by Ouyang *et al.* (18), which is essentially the same as P₂ medium except for an increase in concentration of KNO₃ from 1000 mg l⁻¹ to 1150 mg l⁻¹. In wheat, many researchers used the potato medium as induction medium (6, 8, 12, 17, 21, 22, 23). The potato medium was compared with MS, N6, and C17 media for wheat anther culture, and was superior to all of the synthetic media (8, 22). However, C17 medium containing 100 g l⁻¹ maltose (as the sugar source) performed better than P2 medium for two of the wheat cultivars, while P2 medium gave a better result for two of the other wheat cultivars (17). The potato genotype was also considered as a major factor that influenced the quality of potato extract (8).

In the present study, the efficiency of potato media (P1, P2 and P4), prepared from tuber extracts of 12 commercial cultivars of potatoes, grown in Iran, on anther culturability of five wheat cultivars was investigated.

MATERIALS AND METHODS

Plant materials and the anther culture procedure used in this study was described earlier (3).

Culture Media

The potato media [P1 (1), P2 (5) and P4 (18)] prepared from extracts of 12 potato cultivars ('Primor', 'Aola', 'Sandra', 'Marfona', 'Picaso', 'Draga', 'Scort', 'Drizah', 'Kosima', 'Moron', 'Diamond' and 'Alpha') were used as induction media. The potato cultivars grown in commercial potato production areas in Iran, were supplied by the Department of Vegetables, Seed and Plant Improvement Institute, Karaj, I.R. Iran.

Potato-1 medium containing a potato extract described by a Chinese research group (1), potato-2 medium (5), and potato-4 medium (18) were used. The potato extract was prepared by boiling diced debudded potatoes

for 25-30 min. The extract was then squeezed through several layers of cheesecloth to remove the insoluble residues. For one liter of medium containing 10% or 20% potato extract, 100 or 200 g of potato tubers were used.

For plant regeneration, MS medium (16) supplemented with 1 mg l⁻¹ IAA (indoleacetic acid), 1 mg l⁻¹ BAP (benzylamino purine) and 2.8 g l⁻¹ agarose (Sigma Type 1A) was used. Sixty anthers from a single spike were plated in a 6-cm petri dish containing the induction medium.

Statistical Analysis

A 5×3×12 factorial experiment in a layout of completely randomized design with four replications was used in this study. Five variables were obtained: the number of embryoids per 100 anthers plated (EI); 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).

The general linear model procedure (GLM) of SAS/PC (19) was used to analyze the data by analysis of variance (13). The LSD test was used for comparing means.

RESULTS

Several major factors were analyzed for their influences on the formation of haploid embryoids and on the regeneration of plants.

Embryoid Induction

Analysis of variance for EI showed significant differences ($P < 0.001$) between wheat cultivars, between media, and between potato cultivars (Table 1). Wheat genotype × medium, wheat genotype × potato genotype, medium × potato genotype, and wheat genotype × medium × potato genotype interactions were also highly significant ($P < 0.001$) for EA (Table 1). Among the five wheat genotypes tested, the means of EI, averaged on

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the media (P1, P2 and P4), ranged from 6.8 for 'Falat' to 2.01 for 'Navid' (Fig. 1). Cultivar 'Hartog' also ranked in the first group as its mean was not significantly different from 'Falat' in EI.

Table 1. Analysis of variance for embryoid induction of wheat cultivars using potato media prepared from twelve potato cultivars.

Source of variation	Degree of freedom	Mean square
Wheat genotypes (WG)	4	1514.0***
Media (M)	2	3359.2***
Potato genotypes (PG)	11	851.5***
WG × M	8	205.0***
WG × PG	44	174.2***
M × PG	22	114.3***
WG × M × PG	88	205.0***
Residual	540	17.6

*** = Significant at P<0.001.

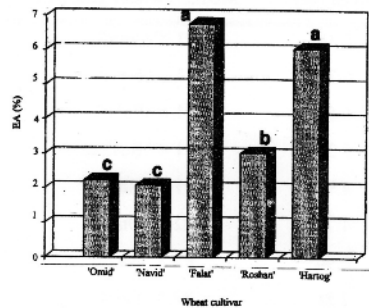


Fig. 1. Effect of wheat genotypes on embryoid induction using potato media.

Although genotype × medium interaction was significant (Table 1), the highest EA was produced on the P4 medium. The medium used was a highly significant factor in EI (Fig. 2). Potato tuber extract prepared from 'Primor' was ranked as the best for EI, and this was followed by a group comprising 'Sandra', 'Draga', 'Picaso', 'Aola' and 'Marfona' (Table 2). The high ranked group for potato genotype × media interaction comprised 'Aola' × P4 and

for wheat genotype \times potato genotype interaction comprised 'Aola' \times 'Falat' and 'Primor' \times 'Falat'.

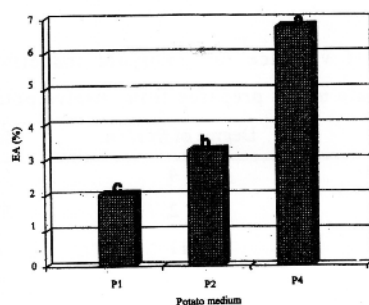


Fig. 2. Effect of potato media on wheat embryoid induction.

Table 2. Effects of potato genotypes and media \times wheat genotype interaction on embryoid induction.

Potato cultivar	Mean (%)	Media \times wheat genotype	Mean (%)
'Primor'	7.38	P1 \times 'Omid'	1.36
'Aola'	6.72	P1 \times 'Navid'	1.79
'Sandra'	5.54	P1 \times 'Falat'	2.04
'Draga'	5.21	P1 \times 'Roshan'	1.25
'Marforma'	4.96	P1 \times 'Hartog'	3.40
'Picaso'	4.79	P2 \times 'Omid'	1.76
'Kos'	2.94	P2 \times 'Navid'	1.25
'Scort'	2.88	P2 \times 'Falat'	6.14
'Drizah'	2.58	P2 \times 'Roshan'	2.19
'Moron'	2.37	P2 \times 'Hartog'	5.12
'Alpha'	1.17	P4 \times 'Omid'	3.59
'Diamond'	0.94	P4 \times 'Navid'	3.23
		P4 \times 'Falat'	11.77
		P4 \times 'Roshan'	5.68
		P4 \times 'Hartog'	9.53
LSD 0.01	0.56		0.56

Plant Regeneration

The best quality and quantity of embryoids were produced by 'Falat'. Hence, its embryoids, 1 mm or more in diameter, were transferred to the regeneration media (MS). Means of plant regeneration (GPA, GPE, APE and TPE) for 'Falat' as affected by the induction media and the potato genotypes are presented in Table 3. P4 medium prepared from the extract of 'Aola' and 'Primor' potato cultivars appeared superior for plant regeneration. However, the means on P4 and P2 media for plant regeneration did not differ significantly, as seen in Table 3, thus all of the eight media were grouped together.

Table 3. Means of plant regeneration[†] for anther culture response of 'Falat' on potato media.

Potato medium	GPA (%)	GPE (%)	APE (%)	TPE (%)
'Aola' × P4	6.3	73.5	23.5	107.0
'Primor' × P4	3.5	68.6	27.1	95.7
'Scort' × P4	3.5	85.0	10.0	95.0
'Picaso' × P4	3.3	66.7	8.3	75.0
'Sandra' × P4	3.8	65.7	17.2	82.9
'Draga' × P4	3.3	52.2	0.0	52.2
'Sandra' × P2	2.5	55.0	8.3	63.3
'Draga' × P2	1.7	50.0	12.5	62.5

[†] Plant regeneration variables include number of green plants per 100 anthers (GPA); number of green plants per 100 embryoids (GPE); number of albino plants per 100 embryoids (APE) and number of total regenerants (albino + green) per 100 embryoids (TPE).

DISCUSSION

The gene effects on androgenic response revealed in the present study is in agreement with our earlier report, using synthetic media (3) and other studies conducted with wheat (3, 4, 6, 8, 11, 13, 21, 23) and triticale (2, 7), using either synthetic or potato media. The high degree of

responsiveness of 'Falat', an Iranian wheat cultivar (Seri-82/Veery #2), is attributed to the presence of the 1BL/1RS wheat-rye translocation in this cultivar, and corroborated our earlier observation of a superior androgenic response of this cultivar in the synthetic media (3).

In this study, a strong influence of potato genotypes on anther culturability of wheat cultivars was observed. Furthermore, different demands of wheat genotypes to the composition of the induction medium could be deduced from the highly significant wheat genotype \times potato genotype interaction. There is little information available in the literature on the impact of potato genotypes in androgenesis, but potato tuber of 'Bintje' is commonly used as the source of potato media in France (8) and China (Ding Xiao-Ling, personal communication). In Iran, cultivar 'Bintje' is not grown, due to its poor adaptation to the environment. It was eliminated from local yield trials a few years ago (A. Mortazavi-Baek, personal communication). Therefore, assessment of available Iranian potato cultivars for anther culture was unnecessary. Nevertheless, Marburger *et al.* (12) and Henry and de Buyser (8) claimed that the quality of potato tuber extract and in turn of the resulting embryoids depended on the potato genotype, environmental conditions of the donor potato plants, and storage conditions of potato tubers.

Superiority of P4 medium compared with P2 medium could be ascribed to the difference in potassium nitrate content. According to Lindsey (9), optimum growth of cells for most purposes of *in vitro* culture requires a medium containing at least 30 mM of inorganic nitrogen and potassium, as the major salt. The poor performance of the P1 medium compared with P2 and P4 could be attributed to the unsteadiness of the effects of the potato medium when used as the major source of nutrient components for plant cells. Lockett and Darvey (10) noted that some of the best media are undefined since they contain chemically unspecified components, principally coconut milk or potato tuber extract.

In conclusion, the employment of 'Falat' in Iranian wheat hybridization breeding programs using anther culture for the production of doubled haploids (3), coupled with the P4 induction medium, is recommended

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