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THE INFLUENCE OF SUGAR SOURCE IN INDUCTION CULTURAL MEDIA ON THE EFFECTIVENESS OF CALLUS FORMATION AND PLANT REGENERATION IN DURUM CULTURE OF WHEAT ANTHR *IN VITRO*

H. O. Dobrova, I. S. Zambriborsh, O. L. Shestopal

*The Plant Breeding and Genetics Institute – National Center of Seed & Cultivar Investigation
3, Ovidiopol'ska Road, Odessa 65036, Ukraine
e-mail: dobrovaann@gmail.com*

Double haploids are important for durum wheat breeding. Anther culture method for obtaining double haploid is one of the most popular. Effectiveness of this method depends on different factors such as cultural condition and plant genotype. It is important to investigate the impact of cultural condition on the level of callus induction and plant regeneration. Sugar source in cultural media affects a process of androgenesis *in vitro*. Ability of four winter and four spring durum wheat hybrids F2 to androgenesis *in vitro* was studied. Two induction media with different sugar source were compared. C17M media contained maltose in 90 g/l concentration. C17n media contained sucrose in 60 g/l concentration and glucose in 17.5 g/l concentration. It was estimated that the level of callus induction and albino plant regeneration were higher on the media with maltose for all investigated genotypes. The level of green plants regeneration was low. Green plants were obtained only for three genotypes. Three green double haploids of T12 genotype obtained from new formations on C17M media, were successfully cultivated in soil and produced seeds. Analysis of variances (ANOVA) was used for statistical analysis. The percentage of total variance accounted for each factor was calculated. It was established that plant genotype had the most significant influence on callus induction. Interaction between factor of genotype and induction cultural media had the biggest percentage of total variance accounted for albino and green plants regeneration.

Keywords: wheat durum, double haploids, anther culture, maltose.

INTRODUCTION

Double haploid is an efficient biotechnological method in wheat breeding which can significantly accelerate the process of production of new varieties. *In vitro* anther culture is one of the most important methods of haploid plants obtaining. Presently we have only limited knowledge about androgenesis in wheat durum. It was reported that this cereal is recalcitrant regarding anther culture because of its low ability to regenerate green plants [1, 9].

Several factors which influenced anther culture have been studied, including genotype of the explants [1] and culture conditions [17].

Most of the induction cultural media contain high sugar concentration (6–10%). Nutritional requirements for androgenesis and formed embryos growth are different [19]. For many plant species maltose has been known as a better carbohydrate source in comparison to sucrose for androgenesis. The use of maltose instead of sucrose as a carbohydrate source in culture medium led to a substantial stimulation of microspore embryogenesis, plant regeneration, and green plantlet formation [6, 10, 11].

The effects of maltose have been documented in several tissue culture systems, but the reason for their superiority to sucrose is not known. It was demonstrated that maltose improves the osmotic stability of culture medium compared to sucrose [4]. It has also been reported that maltose stimulates embryogenesis at low concentrations [14]. It was estimated that utilization of maltose as sugar source lasted for a longer period due to a slower rate of hydrolysis compared to sucrose [8]. Besides, it is assumed that fructose, which is a side product of sucrose hydrolysis, inhibits androgenesis *in vitro* [5]. Maltose may be more effective source of sugar due to the fact that the rate of hydrolysis is close to the rate of glucose uptake during embryos germination [12].

Effect of sugar agents on durum culture of wheat anther were tested in several investigations [9, 16]. The anthers were cultured on media with different carbon sources: sucrose and maltose. Supplements in culture medium significantly affected calli induction and plant regeneration. The number of calli and plants on the medium with maltose were significantly higher than the medium with sucrose. The green plant regeneration was effected by the initiation medium as well as genotypes. Maximum response was found on the medium with maltose.

The aim of this investigation was to determine the impact of sugar source in the induction cultural media on the effectiveness of calli production and plant regeneration in durum culture of wheat anther *in vitro*.

Two induction cultural media with different sugar source were compared. The influence of plant genotype and cultural media on callus induction and plant regeneration *in vitro* was estimated.

MATERIALS AND METHODS

Four spring and four winter wheat durum hybrids F2 were used as anther donors (Table 1). For spike pretreatment low positive temperatures (+3–+5 °C) in water for 7 days were used [15]. Spikes were sterilized with calcium hypochlorite solution using the method [3]. The experiment was set up with two induction media: C17n and C17M. Induction media were identical except the source of sugar. C17n media contained 6 % of sucrose and 1.75 % of glucose and C17M media contained 9 % of maltose. Both media contained 2 mg/l 2,4-D and 0.5 mg/l kinetin [18]. Anthers were incubated in dark at +24 °C [2].

After 15–25 days of cultivation new formations were transferred to regeneration media MS cultural media with BAP (1 mg/l) and IAA (1 mg/l), proline (200 mg/l) and glutamine (200 mg/l). New formations were incubated in dark. Callus with regenerative zones or embryos were transferred to S MS media without growth regulators [7] and in a growth room at 25 °C with 16 h light / 8 h dark photoperiod [2].

Table 1. The studied hybrids of winter and spring wheat durum**Таблиця 1. Осимі та ярі гібриди пшениці твердої, залучені до дослідження**

Type	No	Genotype
Spring hybrids F2	T1	(Saratovsky Zolotiy × Gidara 2) × Gardemaryn
	T8	Topdy 18/FOCHA1/Altar84 × /(Yav79 × Aliy Parus) × {Koral Odessky × [(LR-1 × 504/67) × Kharkivska 1] × [(Tigris × Aisberg Odessky) × (Aisberg Odessky × Novynka 4)]}/
	T12	Haurani × /(Yav79 × Aliy Parus) × { Koral Odessky × [(LR-1 × 504/67) × Xap.1] × [(Tigris × Aisberg Odessky) × (Aisberg Odessky × Novynka 4)]}/
	T15	Topdy 18/FOCHA1/Altar84 × Bosfor
Winter hybrids F2	T42	DF-900-83/WPB-881 × Novynka4
	T43	DF-900-83/WBK-881 × Linkor
	T44	DF-900-83/WBK-881 × Zolote Runo
	T45	DF-900-83/WBK-881 × Yantar Odessky

The level of induction and regeneration was calculated as a callus and albino and green regenerated plants percentage upon total number of callus and confidence interval for each experimental variant. Analysis of variance (ANOVA) was used for statistical analysis [13].

RESULTS AND DISCUSSION

Level of induction of all tested genotypes was higher on C17M media (Fig.1). Albino plant regeneration of spring hybrids was observed only for callus on C17M media. Level of winter hybrids albino plant regeneration was higher for callus on C17M media (Fig. 2).

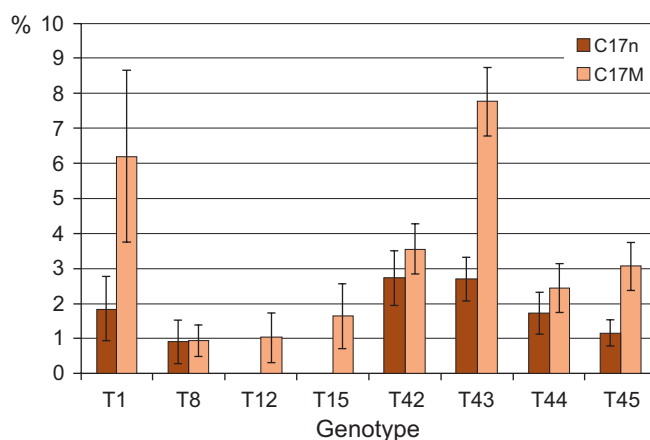
**Fig. 1.** Frequency of callus induction of different wheat durum genotypes on C17n and C17M induction media

Рис. 1. Частота індукції новоутворень різних генотипів пшениці твердої на поживних середовищах C17n і C17M

Level of green plant regeneration was low. On C17M media it was 0.51 ± 0.51 % for T12 and 0.26 ± 0.19 % for T43. On C17n media it was 0.77 ± 0.54 % for T32. Three green plants of T12 were successfully adopted in soil and cultivated in growth chamber. Plants were double haploids and produced seeds. Green plants of T32 and T43 were defective and could not be adopted in soil. In general, the media with maltose was more effective for callus induction and plant regeneration for most of studied plant genotypes.

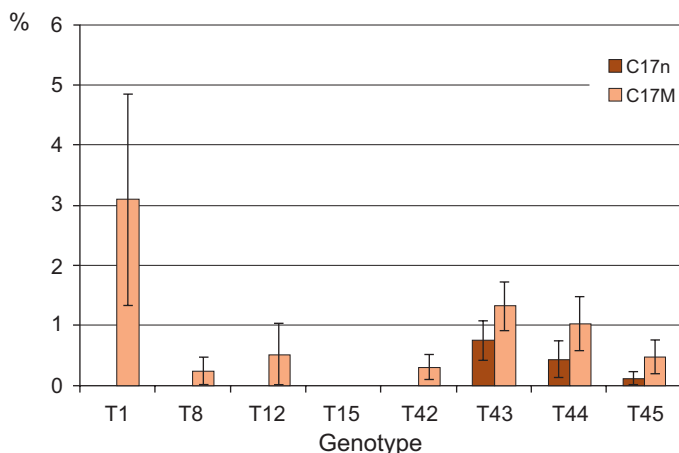


Fig. 2. Frequency of albino plant regeneration of different wheat durum genotypes from new formations obtained on C17n and C17M induction media

Рис. 2. Частота регенерації альбіно рослин різних генотипів пшениці твердої з новоутворень, отриманих на індукційних середовищах C17n і C17M

Table 2. Two factor analysis of variance of callus induction and albino and green plants regeneration level

Таблиця 2. Двофакторний дисперсійний аналіз даних рівня індукції новоутворень і регенерації альбіно та зелених рослин

Process	Variances	F	h, %
Induction	Main effect of factor A: genotype	23.50**	55.89
	Main effect of factor B: induction cultural media	49.80**	16.92
	Interaction A and B	6.86**	16.32
Albino plant regeneration	Main effect of factor A: genotype	7.27**	34.54
	Main effect of factor B: induction cultural media	11.98**	8.13
	Interaction A and B	7.50**	35.62
Green plant regeneration	Main effect of factor A: genotype	6.99**	31.54
	Main effect of factor B: induction cultural media	13.29**	8.56
	Interaction A and B	8.71**	39.28

Comments: ** – significant at $p \leq 0.01$; h – the percent of total variance accounted for each factor

Примітки: ** – достовірно при $p \leq 0,01$; h – відсоток від загальної дисперсії, розрахований для кожного фактора

Two-factor analysis of variance with factors of plant genotype and induction cultural media was used to check the impact of sugar sources and plant genotype on the level of callus induction and albino and green plant regeneration (Table 2). Two factors and its interaction affected significantly both the level of callus induction and plant regeneration. The highest percent of total variance for callus induction was 55.89 % accounted for genotype factor. The highest percent of total variance for albino and green plant regeneration was 35.62 % and 39.28 %, correspondingly. It was accounted for interaction between two factors. Percent of total variance for factor of induction cultural media was 16.92 % for callus induction, 8.13 % for albino plant regeneration and 8.56 % for green plant regeneration. Since induction media were different only with sugar source, it can be concluded that maltose had a significant influence on *in vitro* callus induction and plant regeneration.

CONCLUSIONS

1. The influence of sugar source and genotype on the level of callus induction and plant regeneration in wheat anther durum culture *in vitro* was estimated. It was found that sugar source had a significant influence on wheat anther durum culture effectiveness.
2. Maltose is shown to be more effective sugar source comparing with sucrose. It was established that the level of callus induction and plant regeneration was higher on the media with maltose comparing with media with sucrose and glucose.
3. Three fertile double haploid plants of T12 genotype were obtained.

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ВПЛИВ ВМІСТУ ДЖЕРЕЛА ВУГЛЕЦЮ В ІНДУКЦІЙНОМУ ПОЖИВНОМУ СЕРЕДОВИЩІ НА ЕФЕКТИВНІСТЬ КАЛЮСОУТВОРЕННЯ І РЕГЕНЕРАЦІЇ РОСЛИН У КУЛЬТУРІ ПИЛЯКІВ ПШЕНИЦІ ТВЕРДОЇ *IN VITRO*

Г. О. Доброва, І. С. Замбріборщ, О. Л. Шестопап

Селекційно-генетичний інститут –
Національний центр насіннєзнавства та сортовицвання
Овідіопольська дорога, 3, Одеса 65036, Україна
e-mail: dobrovaann@gmail.com

Подвоєні гаплоїди є важливими для селекції твердої пшениці. Одним із найбільш поширених методів їх отримання є метод культури пиляків. Ефективність цього методу залежить від різних факторів, таких як умови культивування і генотип рослин. Важливим є дослідження впливу умов культивування на індукцію новоутворень і регенерацію рослин. Вміст джерела вуглецю у поживному середовищі впливає на процес андрогенезу *in vitro*. Вивчили спроможність до андрогенезу *in vitro* чотирьох озимих і чотирьох ярих гібридів F2 пшениці твердої. Порівнювали два індукційних поживних середовища з різними джерелами вуглецю. Середовище С17М містило мальтозу в концентрації 90 г/л. Середовище С17н містило цукрозу в концентрації 60 г/л і глюкозу в концентрації 17,5 г/л. Було встановлено, що рівень індукції новоутворень і регенерації альбіно рослин був вищим на середовищі з мальтозою для всіх протестованих генотипів. Рівень регенерації зелених рослин був низьким. Отримали зелені регенеранти лише трьох генотипів. Три подвоєні гаплоїди генотипу Т12, що регенерували з новоутворень, отриманих на середовищі С17М, були успішно адаптовані до умов ґрунту і вирощувались у ростовій камері до отримання насіння. Для статистичного аналізу використовували двофакторний дисперсійний аналіз. Було розраховано ступінь впливу кожного фактора. Встановили, що генотип рослин мав найбільший вплив на індукцію новоутворень. Взаємодія факторів генотипу й індукційного поживного середовища найсильніше впливала на регенерацію альбіно і зелених рослин.

Ключові слова: тверда пшениця, подвоєні гаплоїди, культура пиляків, мальтоза.

ВЛИЯНИЕ ИСТОЧНИКА УГЛЕРОДА В СОСТАВЕ ИНДУКЦИОННОЙ ПИТАТЕЛЬНОЙ СРЕДЫ НА ЭФФЕКТИВНОСТЬ КАЛЛУСООБРАЗОВАНИЯ И РЕГЕНЕРАЦИИ РАСТЕНИЙ В КУЛЬТУРЕ ПЫЛЬНИКОВ ПШЕНИЦЫ ТВЕРДОЙ *IN VITRO*

А. А. Доброва, И. С. Замбриборщ, О. Л. Шестопап

Селекционно-генетический институт –
Национальный центр семеноводства и сортоизучения
Овидиопольская дорога, 3, Одесса 65036, Украина
e-mail: dobrovaann@gmail.com

Получение удвоенных гаплоидов важно для селекции твердой пшеницы. Одним из самых распространенных методов получения удвоенных гаплоидов

считается метод культуры пыльников. Эффективность этого метода зависит от разных факторов, таких как условия культивирования и генотип растений. Важно исследовать влияние условий культивирования на индукцию новообразований и регенерацию растений. Источник углевода в питательной среде влияет на процесс андрогенеза *in vitro*. Изучили способность к андрогенезу *in vitro* четырех озимых и четырех яровых гибридов F2 пшеницы твердой. Сравнивали две индукционные питательные среды с разными источниками углевода. Среда С17М содержала мальтозу в концентрации 90 г/л. Среда С17н содержала сахарозу в концентрации 60 г/л и глюкозу в концентрации 17,5 г/л. Установили, что уровень индукции новообразований и регенерации альбино растений был выше на среде с мальтозой для всех протестированных генотипов. Уровень регенерации зеленых растений был низким. Получили зеленые регенеранты только трех генотипов. Три удвоенных гаплоида генотипа Т12, которые регенерировали из новообразований, полученных на среде С17М, были успешно адаптированы в почве и выращивались в ростовой камере до получения семян. Для статистического анализа использовали двухфакторный дисперсионный анализ. Рассчитали степень влияния каждого фактора. Установили, что генотип растений влиял на индукцию новообразований в наибольшей степени. Взаимодействие факторов генотипа и индукционной питательной среды имело наибольшее влияние на регенерацию альбино и зеленых растений.

Ключевые слова: твердая пшеница, удвоенные гаплоиды, культура пыльников, мальтоза.

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