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CHANGES IN ANDROGENIC RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER *IN VIVO* AND *IN VITRO* WATER STRESS

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Резюме

Белчев, И., Т. Петрова, Е. Пенчев, 2012. Изменения в андрогенната реакция на пшеница (Triticum aestivum L.) при in vivo и in vitro воден стрес. FCS8(1):173-178

Изследван е ефектът на *in vivo* и *in vitro* водния стрес върху реакцията в антерна култура при пшеница (*Triticum aestivum* L.). Осем сорта зимна обикновена пшеница са отгледани при нормално водообезпечаване и условия на засушаване (*in vivo* воден стрес). Подходящите класове са събрани и антерите веднага са заложени върху хранителна среда Potato 2. Калусната индукция съществено е редуцирана от водния дефицит при половината от сортовете. Добивът на зелени растения е намален само при сорт **'Свилена'**, докато регенерацията на албиносни растения е понижена при сортовете **'Безостая 1'**, **'Кристал'** и **'Тодора'**. Андрогенната реакция на моделния сорт **'Свилена'** е изпитана при *in vitro* воден стрес, индуциран от полиетилен гликол 6000 (ПЕГ) в концентрация 0, 3, 6, 9, 12, 15 и 18%. Увеличената ПЕГ концентрация редуцира значително индукцията на калуси и ембриоиди. По-високите концентрации подобряват регенерационната способност, но понижават продукцията на албиносни растения. *In vivo* и *in vitro* водният стрес имат сходен ефект върху реакцията в антерна култура, водещ до намаляване на калусната индукция и регенерацията на албиносни растения.

Ключови думи: Андрогенни параметри – Суша – Полиетилен гликол

Abstract

Belchev, I., T. Petrova, E. Penchev, 2012. Changes in androgenic response of wheat (Triticum aestivum L.) under in vivo and in vitro water stress. FCS8(1):173-178

The effect of *in vivo* and *in vitro* water stress on anther culture response in wheat (*Triticum aestivum* L.) was studied. Eight winter wheat varieties were grown in a greenhouse under normal water supply and drought conditions (*in vivo* water stress). Suitable spikes were collected and anthers were immediately plated on Potato 2 medium. The callus induction was significantly reduced by the water deficit in half of the varieties. The green plant yield was decreased only in variety **'Svilena'** while the albino plant regeneration was lowered in varieties **'Bezostaya1'**, **'Kristal'** and **'Todora'**. The androgenic response of the model variety **'Svilena'** was tested under *in vitro* water stress induced by polyethylene glycol 6000 (PEG) at concentration 0, 3, 6, 9, 12, 15 and 18%. Increased PEG concentrations reduced considerably the induction of calli and embryoids. Higher concentrations improved the regeneration ability but lowered the production of albino plants. *In vivo* and *in vitro* water stress had a similar effect on the anther culture response leading to decrease of callus induction and albino plant regeneration.

Key words: Androgenic parameters – Drought - Polyethylene glycol

INTRODUCTION

Drought is one of the major natural abiotic stresses reducing to a great extent the plant yield and becoming a factor with increasing importance because of the global climate changes. Plants are sensitive to water deficit at any stage of their development but the reproductive period is particularly vulnerable (Saini, 1997). In cereals, Saini and Aspinall (1981) determined microsporogenesis as the most critical stage to water stress leading to male sterility and subsequent grain yield reduction. The male gametophyte is more sensitive to water shortage and also to high temperature in comparison to the female one (Saini & Aspinall, 1982).

Anther culture is a modern biotechnological method for fast production of homozygous lines from microspores. Many factors affect the anther culture response but the genotype is of great importance (Belchev et al., 2000). The environment of growing of plants (Orshinsky& Sadasivaiah, 1997) and culture conditions (Stober & Hess, 1997) can modify the androgenic response. Anther donor plants are usually grown in greenhouses under optimal parameters of the physical factors. However, plants from the field are preferably used because of their better vigour and response (Tuvesson et al., 2000), but soil moisture is sometimes insufficient for supporting the normal physiological status of the plants resulting in changes in their metabolism and characters. On the other hand, adding of an osmoticum to the induction medium simulates drought stress which allows screening of tolerant lines under *in vitro* conditions (Tyankova et al., 2004).

The aim of the present study was to determine the effect of reduced water supply on plants (*in vivo* water stress) and polyethylene glycol added to the induction medium (*in vitro* water stress) on the androgenic response of wheat varieties.

MATERIAL AND METHODS

In vivo water stress

Eight winter wheat (*Triticum aestivum* L.) varieties were grown under two environments. Seeds were sown in rows (each 1 m long, 0.2 m between the rows, 70 seeds/row) in a greenhouse. Soil was primary irrigated with 120 l/m^2, which was the only water the drought-stressed plants received, while the control plants were periodically watered receiving a total of 450 l/m^2 during the vegetation period. Suitable spikes were cut, surface sterilized with 70% ethanol and anthers were immediately plated on Potato 2 medium (Chuang et al., 1978). Fifteen spikes per each variety/treatment were collected and 30 anthers per spike were put in a test tube with 10 ml induction medium. The test tubes with anthers were incubated at 28 ^{0}C in darkness. Embryogenic structures (calli and embryoids) induced from the microspores were transferred to 190-2 regeneration medium (Zhuang & Jia, 1983), cultured at 25 ^{0}C with illumination 3000 lx, 16/8 h photoperiod. Green and albino regenerants were fully developed in approximately 30 days.

In vitro water stress

Donor plants of the highly responsive variety **'Svilena'** were grown under field conditions. Seventy spikes with anthers containing microspores at mid to late uninucleate stage were cut, put in a vessel with water and stored at 4 ^{0}C for 8 days. Liquid Potato 2 medium added with 0, 3, 6, 9, 12, 15 and 18% polyethylene glycol 6000 (PEG) was used for induction of calli and embryoids. Ten spikes were included for each treatment. The next procedures were the same as described above.

The following androgenic parameters were estimated: i) callus induction presented the number of calli and embryoids induced per 100 anthers cultured; ii) plant regeneration was the percentage of green and albino plants regenerated from the calli transferred; iii) green/albino plant yield was the number of green/albino plants produced per 100 anthers

cultured. The data were analyzed using the computer procedure Biostat ver.6.0 (Penchev, 1998).

RESULTS

In vivo water stress

The plants subjected to drought developed faster and stayed smaller in comparison to the normally watered ones. Suitable spikes for anther culture from the plants under drought conditions were collected 1-2 days earlier than the control plants for the varieties **'Bezostaya 1'** and **'Svilena'**, while for the rest varieties this period was 3-4 days (data not shown).

The induction of calli and embryoids was significantly reduced (P = 0.05, 0.01) as a result from the water stress in the varieties **'Kristal'**, **'Svilena'**, **'Todora'** and **'Zora'** (Table 1). Callus induction decreased more than three times in variety **'Charodeyka'**, similar to variety **'Zora'**, but the difference was not significant. The water deficit did not substantially change the production of embryogenic structures in the varieties **'Bezostaya 1'**, **'Elitsa'** and **'Vratsa'**.

Table 1. Androgenic response of winter wheat varieties grown under normal water supply (W) and *in vivo* water stress (D)

Variety	Callus induction,%		Plant regeneration,%		Plants regenerated /100 anthers			
					Green		Albino	
	W	D	W	D	W	D	W	D
Bezostaya 1	4.4	3.3 ns	62.5	14.3 c	0.8	0.2 ns	1.9	0.2 a
Charodeyka	6.4	2.0 ns	60.0	100.0 b	2.3	1.3 ns	1.5	0.7 ns
Elitsa	16.9	14.9 ns	53.0	44.8 a	8.7	5.4 ns	0.3	1.3 ns
Kristal	43.8	29.2 b	45.2	50.0 ns	5.8	8.2 ns	14.0	6.4 a
Svilena	177.9	116.7 a	28.6	32.4 ns	33.3	20.8 a	17.6	16.9 ns
Todora	36.2	18.7 b	63.8	52.1 a	4.1	2.6 ns	19.0	7.2 a
Vratsa	36.4	29.0 ns	38.9	46.7 a	4.7	8,6 ns	9.4	5.0 ns
Zora	17.7	5.8 a	55.1	69.2 b	9.7	4.0 ns	0	0
Mean	42.5	27.4	50.9	51.2	8.7	6.4	8.0	4.7

a, b, c – the differences are significant at P = 0.05, 0.01 and 0.001, respectively; ns – not significant

The plant regeneration decreased drastically from 62.5 to 14.3% in variety **'Bezostaya 1'**. A negative tendency (P = 0.05) was also registered in varieties **'Elitsa'** and **'Todora'**. Contrary, the water stress improved (P = 0.05, 0.01) the regeneration ability of the varieties **'Charodeyka'**, **'Vratsa'** and **'Zora'**. Plants were regenerated from all induced calli of variety **'Charodeyka'**. A positive but not significant effect was observed in varieties **'Kristal'** and **'Svilena'**.

The green plant yield was significantly lowered (P = 0.05) from 33.3 to 20.8 plants/100 anthers cultured in variety **'Svilena'**. The production of green plants was slightly increased by the water deficit in varieties **'Kristal'** and **'Vratsa'**, while in the rest of the varieties it decreased. The albino plant yield was substantially reduced (P = 0.05) as a result from the water stress in varieties **'Bezostaya 1'**, **'Kristal'** and **'Todora'**. The other varieties either regenerated more **('Elitsa'**) or less (**'Charodeyka'**, **'Svilena'** and **'Vratsa'**) albino plants but the differences were not significant. No albino plant was produced from variety **'Zora'** regardless of the treatment.

In vitro water stress

The callus induction of variety **'Svilena'** from the liquid control medium 0 was 197.0%,

the highest value of this trait (Table 2). Adding of 3% polyethylene glycol (3, PEG) did not change considerably the percentage of structure induced. Subsequent increasing of the PEG concentration (6-18) decreased drastically the induction frequency to 1.3 and 0.7% at concentrations 15 and 18%, respectively. There was a significant difference between the control medium and 9% PEG but the value of the latter was rather high (133.7%) within the general tendency of callus induction decrease.

Table 2. Anther culture response of variety **'Svilena'** to *in vitro* water stress (3-18)

PEG concentration,%	Callus induction,%	Plant regeneration,%	Plants regenerated/100 anthers	
			Green	Albino
0	197.0	34.9	16.3	52.3
3	192.6	30.4	17.0	41.5
6	96.3	45.3	15.0	28.7
9	133.7	48.5	29.3	35.6
12	39.7	62.2	15.3	9.3
15	1.3	50.0	0.3	0.3
18	0.7	50.0	0	0.3
LSD 5%	11.4	3.3	1.7	2.1
LSD 1%	14.9	5.1	2.6	3.9
LSD 0.1%	18.1	7.9	4.3	5.3

The regeneration of plants from calli and embryoids induced on medium with 3% PEG was reduced from 34.9 to 30.4% (LSD 5%). Higher concentrations improved regeneration ability, which was as high as 62.2% at 12% PEG. Sporadic calli from the extreme concentrations showed also better plant regeneration (50.0%).

The green plant yield was not so much affected at 3, 6 and 12% PEG in comparison to the control medium. The regeneration of green plants from 9% PEG was increased to 29.3 plants/100 anthers while the value of this parameter at 15% PEG was only 0.3. No green plant was produced at 18% PEG. The albino plant yield was substantially lowered at all PEG concentrations in comparison to the control medium.

DISCUSSION

The callus induction was one of the androgenic parameters affected to the highest degree by the insufficient water supply. All the varieties reduced the induction of calli and embryoids under water stress but the differences were significant only in half of them. Plant regeneration either changed in positive or negative direction, or was not changed significantly. The regeneration of green plants was lowered just in one variety while the production of albino plants was decreased in another three. The androgenic response of variety **'Vratsa'** was relatively stable under water deficit and plant regeneration was even improved. Reduced soil moisture had a partially favourable effect on the anther culture method as the green plant regeneration was kept at the same level while the regeneration of albino plants was reduced in some varieties. Decreasing of the callus induction under drought conditions is probably connected with abnormal anther development (sterility) (Saini & Aspinall, 1981) and mortality of competent but weak microspores. After this selection, the viable competent microspores realized their potential giving rise to green plants mainly. Several studies (Lalonde et al., 1997; Saini & Aspinall, 1981; Saini et al., 1984) have described the morphological and anatomic changes caused by water stress; in our experiment such changes were observed as fast fading of sterile anthers during *in vitro* cultivation. The reasons for male sterility under water stress are indirectly related to changes in the vegetative organs which lead to changes in the carbohydrate metabolic pathways in the reproductive tissue (Barnabas et al., 2008; Dorion et al., 1996; Saini, 1997).

The model variety **'Svilena'** showed a higher callus induction on liquid medium and regeneration predominantly of albino plants which was in accordance with the observations of Konzak and Zhou (1991) for other varieties. In our previous investigation (Belchev et al., 1994) low concentration of PEG (3%) in solid medium stimulated callus induction and regeneration of green plants, which was related to the higher osmotic potential of the induction medium. Kang et al. (2003) observed maximum production of green regenerates at 8% PEG; these data coincided with our results (9% PEG) and confirmed the positive effect of the higher osmotic potential of the nutrient medium. Increased PEG concentration in the induction medium reduced the callus and embryoid formation due probably to mortality of the weak and potentially albinogenic microspores. The extreme concentrations of the osmotic (15, 18%) lead to dying of almost all microspores and the anthers turned white in a few days.

In vivo and *in vitro* water stress had generally equal effect on the androgenic response decreasing the callus induction as well as regeneration of albino plants. The anther culture could be a suitable method for fast production of lines with improved drought tolerance using: i) crosses between parents with different drought tolerance (Khan et al. 2001); ii) varieties and crosses under *in vivo* water stress; iii) *in vitro* screening of varieties and crosses in a medium with an osmoticum.

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