Journal of Genetic Engineering and Biotechnology (2017) **15**, 239–247

HOSTED BY

ELSEVIER

Academy of Scientific Research & Technology and National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ORIGINAL ARTICLE

In vitro screening of durum wheat against water-stress mediated through polyethylene glycol

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Received 10 November 2016; revised 21 February 2017; accepted 8 April 2017
Available online 22 April 2017

KEYWORDS

Callus;
Durum wheat;
Mature embryo;
PEG;
Selection;
Water stress

Abstract Three durum wheat (*Triticum durum* Desf.) genotypes with three levels of drought tolerance were screened in order to evaluate their response to water stress at callus induction and plant regeneration levels. Significant differences were observed among the genotypes, and polyethylene glycol (PEG) levels used, and their interactions were however, significant for all the studied characters. Increase in PEG concentration increased the time required for callus initiation and reduced the number of calli frequency of embryogenic structures and number of plants regenerated, showing the adverse effect of PEG on the somatic embryogenesis developmental., under *in vitro* conditions tested, and Djenah Khetifa was the most tolerant genotype, followed by Oued Zenati and Waha. This pattern was per their drought tolerance behavior under field conditions. Principal component analysis (PCA) showed that 95.56% of the total variation was explained by the first two principal components. Biplot analysis allowed the stress-tolerant genotype to be distinguished from the two less tolerant genotypes. Time required for callus initiation was strongly negatively correlated with all other studied traits. These traits can be recommended as suitable selection criteria for screening drought-tolerant genotypes. The selected cells and plants will provide a tool for determining the mechanisms involved in tolerance to water stress.

1. Introduction

Environmental conditions in agricultural settings are highly variable, leading to suboptimal crop yields and survival rates. The frequency and intensity of environmental extremes are expected to increase with climate change [24]. How plants cope with drought stress is a topic of an intense debate. In addressing this problem, geneticists and breeders have focused mainly

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Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2017.04.004

on exploiting high yield potential and genotype selection for morphological, physiological and agronomic traits indicative of drought tolerance under field conditions [12]. Developing an understanding of plant responses to drought is a fundamental part of developing stress-tolerant varieties [31,36]. Screening for drought tolerance under field conditions involves considerable resources (land, people and power) and requires suitable environmental conditions for the effective and repeatable phenotypic expression of drought tolerance attributable to the genotype. It is therefore necessary to use simple but effective early screening methods that relate to the field phenotypes [14]. However, *in vitro* selection for tolerance to abiotic stress depends on the development of efficient and reliable callus induction and plant regeneration systems. In wheat species, various explants sources have been used for embryogenic callus formation and plant regeneration [10,11,32,33]. These tissues differ in their ability to regenerate whole plants [11]. If the mature embryos can be used as the explants for tissue culture, this offers many important advantages over immature tissues as explants. For example, the dry seeds would be available for isolating mature embryos in large quantities with no seasonal influence throughout the year. The physiological states of mature embryos are similar and dry seeds are easy to manipulate in tissue culture [7]. Water stress could be induced in plant cell cultures by adding osmotica, such as mannitol, polyethylene glycol, sucrose or sorbitol. For drought stress induction, however, one of the most popular approaches is to use high molecular weight osmotic substances, such as polyethylene glycol (PEG) [27,34,41]. These agents have no detrimental or toxic effects on the plant; they inhibit the plant's growth, however, by reducing the water potential of the culture medium in a way similar to soil drying, so that cultured explants are unable to take up water [9]. The *in vitro* culture system is based on inducing genetic variation among cells, tissues and/or organs in cultured and regenerated plants. However, there are genetic, biochemical and physiological constraints to obtaining stress-tolerant plants through *in vitro* culture [7,22]. Several authors have used this technique successfully to screen various genotypes for water tolerance. The present study sought to identify the superior genotypes in terms of water stress tolerance with the objective to develop *in vitro* screening method for drought tolerance.

2. Material and methods

2.1. Plant material

The experiments were carried out on three durum wheat (*Triticum durum* Desf.) genotypes. Based on field trials, one genotype was classified as drought sensitive (Waha) and two as drought tolerant (Oued Zenati and Djenah Khetifa) [23]. The wheat germplasm was obtained from the Technical Institute of Field Crops (ITGC) Institut Technique des Grandes Cultures (Station El-khroub Constantine, Algeria).

2.2. Callus induction and in vitro selection procedures

Callus cultures were initiated using mature embryos. The seeds were surface sterilized with 70% ethanol for 15 min, followed by 12% sodium hypochlorite (NaClO) for 20 min, and then rinsed five times with sterile dH_2O. Mature embryos about 2–4 mm long were aseptically excised and then incubated with the scutellum side down on MS induction medium (Table 1) [30], supplemented with 2 mg l^{-1} 2.4-dichlorophenoxyacetic acid (2.4-D), 3% sucrose and 2.5 g l^{-1} phytagel (Sigma-Aldrich). The pH value of the medium was adjusted to 5.7 prior to autoclaving at 110 °C for 30 min. The cultures were maintained at 25 ± 1 °C under 30 µm^{-2} S^{-1} cool fluorescent light intensity, with a 16 h/8 h (light/dark) photoperiod. Each treatment was performed in five replicates (20 mature embryos per Petri dish). The calli were maintained by subculturing every 20 days on the same MS medium with different PEG 6000 concentrations: 0% (control), 10% (SI: −0.49 MPa) and 20% (SII: −1.2 MPa). The osmoticum was added to the media before autoclaving.

2.3. Plant regeneration and acclimatization

The surviving calli on media containing 10% and 20% PEG 6000 were transferred into test tubes containing MS basal salt medium (Table 1) [30] supplemented with 1.0 mg l^{-1} benzylaminopurine (BAP), 0.5 mg l^{-1} naphthalene acetic acid (AIA), 30 g l^{-1} sucrose and 2 g l^{-1} Phytagel. The cultures were maintained at 25 °C under cool-white fluorescent light (30 µm m^{-2} S^{-1}) 16 h/8 h (light/dark) photoperiod. Rooted plantlets were transferred to Jiffy peat pellets containing a mixture of garden soil and sand (2:1) for acclimatization in a greenhouse.

Table 1 MS basal media composition [30].

Ingredients	Conc. of stock solution (mg/l)	Concentration in medium (mg/l)
Macroelements		
NH_4NO_3	33,000	1650
KNO_3	38,000	1900
$CaCl_2{\cdot}2H_2O$	8800	440
$MgSO_4{\cdot}7H_2O$	7400	370
KH_2PO_4	3400	170
Microelements		
KI	166	0.83
H_3BO_3	1240	6.2
$MnSO_4{\cdot}4H_2O$	4460	22.3
$ZnSO_4{\cdot}7H_2O$	1720	8.6
$Na_2MoO_4{\cdot}2H_2O$	50	0.25
$CuSO_4{\cdot}5H_2O$	5	0.025
$CoCl_2$	5	0.025
Iron source		
$FeSO_4{\cdot}7H_2O$	5560	27.8
$NA_2{\cdot}EDTA{\cdot}2H_2O$	7460	37.3
Vitamins		
Myo-inositol	20,000	100
Nicotinic acid	100	0.5
Pyridoxine HCl	100	0.5
Thiamine HCl	100	0.5
Glycine	400	2
Carbon source		
Sucrose	Added as solid	30,000