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

Nadia Sandra Kacem [a,b,*], **Fabienne Delporte** [a], **Yordan Muhovski** [a], **Abdelhamid Djekoun** [b], **Bernard Watillon** [a]

[a] *Department of Life Sciences, Walloon Agricultural Research Centre, Chaussée de Charleroi, 234, 5030 Gembloux, Belgium*
[b] *Laboratory of Genetic Biochemistry and Plant Biotechnology, Faculty of Nature and Life Sciences, University Frères Mentouri Constantine, 25017 Constantine, Algeria*

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

* Corresponding author at: Laboratory of Genetic Biochemistry and Plant Biotechnology, Faculty of Nature and Life Sciences, University Frères Mentouri Constantine, 25017 Constantine, Algeria.
E-mail address: kacem.nadia@umc.edu.dz (N.S. Kacem).

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

2.4. Measured variables

The following characteristics were recorded for the three durum wheat genotypes:

Time of callus initiation (TCI): Time taken for callus initiation (days) using binoculars.

Callus induction frequency (CIF): The number of embryos producing calli divided by the number of cultured mature embryos plated on Petri dishes × 100.

Callus growth surface (CGS): Evaluated based on the callus surface area (mm^2) measured at 7, 15 and 30 days, using Image Pro Plus 6.2 software (Media Cybernetics).

Relative tolerance (RT): Callus growth surface (mm^2) under stress divided by the mean value of callus growth surface (mm^2) under non-stress × 100.

Embryogenic callus production (ECP): Expressed as the number of embryogenic calli divided by the total number of induced calli × 100.

Reduction percentage (R): Mean CGS value under stress level − mean CGS value at 0% stress level.

Plant regeneration (PR): Number of plants regenerated divided by the total number of embryogenic calli × 100.

Regeneration frequency (RF): Number of plantlets obtained divided by the number mature embryos induced in culture × 100.

2.5. Statistical analysis

Analyses of variance (ANOVA), correlation analysis, principal component analysis (PCA) and biplot analysis were carried out using SPSS20, MINITAB17 and XLSTAT2015 software. Cluster analysis was performed using a Ward method and Euclidean distance [29,42].

3. Results and discussion

3.1. Water stress with PEG 6000

Significant differences were observed among the genotypes, PEG concentrations (0%, 10% and 20%), and their interactions for all the studied characters (Table 2), indicating genetic variability in response to PEG-simulated drought stress in terms of TCI, CIF, CGS, ECP, PR and RF.

3.2. Effect of water stress on time of callus initiation

Callus initiation was visible within a few days of mature embryos being cultured under control conditions. For Djenah Khetifa and Oued Zenati, TCI was 2 days and for Waha it was 5 days. The TCI was greatly influenced by genotype, explant, media composition and hormones. For example, in *Triticum aestivum*, the appearance of primary calli from mature embryos was observed 3–4 days after inoculation as translucent, rough and rather watery structures [44]. For Bi and Wang [7], it was 2 days later; calli formed from mature embryos of durum wheat on 2 mg l^{-1} 2,4-D. For Özgen et al. [32] it was 10–11 days; calli formed from mature embryos of winter durum wheat on 8 mg l^{-1} 2.4-D. For Özgen et al. [33], it was 2–3 days later; calli formed from mature embryos of common winter wheat (*T. aestivum* L.). In the presence of the osmotic agent, however, increased PEG concentration significantly increased the TCI in all varieties compared with the controls, the delay being longer for Waha than those for the Djenah Khetifa and Oued Zenati varieties (Table 2).

3.3. Effect of water stress on callus induction frequency

The results of our study showed that there were no significant differences among the varieties under non-stress conditions (without PEG), with all explants producing calli (Table 2). The frequency of callus induction approached 100%. The high CIF observed demonstrating the high capacity of the durum wheat genotypes tested to induce calli from mature embryo explants. This observation is in agreement with previous reports that showed a high rate of callus production from mature and/or immature embryos of durum wheat [3].

Under water stress, however, the callus response from mature embryos was genotype dependant (Table 2). At 10% PEG, Djenah Khetifa had the highest rate of callus production (84.0%), followed by Oued Zenati (82.56%) and then Waha (77.4%). Increasing the concentration to 20% PEG resulted in a significant reduction in CIF in all genotypes. The highest percentage of callus induction (80%) was recorded for Djenah Khetifa and the lowest (57%) for Waha (Table 2). Our results showed that CIF response under PEG treatment was genotype dependent. The genetic constitution appeared to play a major role in callus induction under water stress. A decrease in CIF is a typical response of the explants of species, including wheat, when subjected to PEG-simulated drought stress [6,15,18,26,35]. Mahmood et al. [26], reported that incubation of wheat calli derived from immature embryos on callus selection media supplemented with PEG-6000 induced osmotic stress of −0.9 MPa for four weeks seemed sub-lethal and can be expected to kill non-tolerant calli and allow only tolerant ones to survive (survival 26.62%) with reasonable regeneration potential.

3.4. Effect of water stress on callus growth surface and relative tolerance

The growth dynamic of callus tissues was measured at 30 days on MS medium supplemented with different concentrations of PEG (0%, 10% and 20%), and digital images were analyzed using Image Pro Plus software. This non-invasive evaluation of growth had previously proved to be effective and helpful in that it allowed the evolution of the calli to be monitored until regeneration without removing them from their *in vitro* environment, and thus not interfering with their development [23]. On average across all the genotypes, the highest CGS (37.90 mm^2) was recorded when calli were cultured for 30 days on 0% PEG, compared with 10% (25.87 mm^2) and 20% (18.54 mm^2) PEG (Table 3).

All culture conditions and for the three measurements taken over time, Djenah Khetifa had the highest CGS (36.22 mm^2), followed by Oued Zenati (28.74 mm^2) and Waha (17.35 mm^2). The most severe growth slowdown was observed in Waha, where the reduction rate was 42.82% at 10% PEG and increased significantly ($p < 0.001$) with increasing stress up to 20% PEG, reaching 77.95%. Oued Zenati seemed less affected by stress than Waha, with a reduction rate of 32.13% at 10% PEG and 47.44% at 20% PEG. Djenah

Table 2 *In vitro* screening of three genotypes for increased water stress tolerance in durum wheat. Mature embryos of three genotypes displaying various levels of drought tolerance (DK: Djenah Khetifa; OZ: Oued Zenati; W: Waha) were exposed to PEG throughout (30 days) the process of somatic embryo induction and formation. **TCI**: time of callus initiation; **CIF**: callus induction frequency; **ECP**: embryogenic callus production; **PR**: plant regeneration; **RT**: relative tolerance; **RF**: regeneration frequency. Mean ± standard deviation from at least 5 replicate experiments ($n = 100$); common letters indicate no significant differences at 5% level of probability.

Variety	DK			OZ			W		
PEG concentration	0%	10%	20%	0%	10%	20%	0%	10%	20%
TCI	2.0 ± 0.0f	3.0 ± 0.2e	5 ± 1c	2.0 ± 0.14f	4.0 ± 0.5d	5.0 ± 0.2c	5.0 ± 0.5c	7.0 ± 0.5b	9.0 ± 0.5a
CIF	100 ± 0a	84.0 ± 1b	80 ± 1.0d	100 ± 0a	82.56 ± 1.2c	79.4 ± 0.5d	100 ± 0a	77.4 ± 2e	57.0 ± 1f
ECP	79.2 ± 3.9b	83.0 ± 2.7ab	56.0 ± 4.2d	61.0 ± 4.2c	58.0 ± 2.7cd	29.0 ± 2.2e	81.0 ± 4.2ab	84.0 ± 2.2a	23.0 ± 2.7f
PR	60.7 ± 5a	46.7 ± 6.2c	34.2 ± 12.7d	49.2 ± 5.4bc	32.7 ± 10.7d	17.3 ± 1.5e	58.1 ± 3.6ab	34.5 ± 3.0d	8.0 ± 10.9e
RF	48.0 ± 4.5a	41.0 ± 8.9b	19.0 ± 6.5d	30.0 ± 3.5c	19.0 ± 6.5d	5.0 ± 3.5e	46.0 ± 2.2ab	29.0 ± 2.2c	2.0 ± 2.7e

Table 3 Effects of PEG concentrations on the callus growth surface (mm^2) in three durum wheat varieties. Presented data are mean values calculated for the three measurements taken over time (at 7, 15 and 30 days) for each variety and each culture condition. DK: Djenah Khetifa; OZ: Oued Zenati: W: Waha; **CGS**: callus growth surface; **RT**: relative tolerance; **R**: reduction percentage. Means followed by the same letter are not statistically different according to the Fisher test ($P < 0.05$).

PEG concentration	0%	10%			20%			Means across treatments
	CGS	CGS	RT%	R%	CGS	RT%	R%	CGS means
DK	45.56 ± 3.1a	34.42 ± 2.45c	75.55	24.45	28.68 ± 1.2d	62.95	37.05	36.22 ± 7.57
OZ	39.12 ± 3.2b	26.54 ± 3.2d	67.84	32.16	20.54 ± 2.07e	52.56	47.49	28.74 ± 8.44
W	29.00 ± 1.4d	16.64 ± 0.7f	57.38	42.62	6.40 ± 0.6g	22.07	77.93	17.35 ± 9.61
Means	37.89 ± 7.48	25.87 ± 7.84	68.26	31.74	18.54 ± 9.62	48.94	51.06	–

Khetifa seemed to be the most tolerant, showing reduction rates of 24.52% and 37.06% at 10% and 20% PEG, respectively (Table 3). Although a progressive increase in CGS was maintained when calli were incubated on 10% and 20% of PEG, an increase in water stress induced by PEG caused a significant decrease in CGS (especially at the highest PEG concentration) compared with the controls. The callus induction response in terms of callus surface was variety dependent, with Djenah Khetifa showing the highest rate growth under control conditions and the least affected rate under PEG treatment (Table 3). Data recorded after the PEG treatments confirmed that all the studied genotypes had different, but nevertheless high, capacities for callusing under water stress. CGS data were also used to calculate relative tolerance (RT) in order to provide a value for relative comparisons among genotypes, by eliminating inherent differences associated with the growth rate of each genotype in response to water stress. For each genotype, RT was calculated based on the growth difference measured between calli exposed to two PEG concentrations [1]. Djenah Khetifa exhibited the highest tolerance of PEG from 10% to 20% (Table 3), whereas Waha exhibited the lowest RT values under these water stress conditions. Cells that continue to grow under severe stress are tolerant, but PEG might improve callus growth capacity. Bressan et al. [9] reported that an enhanced ability to grow in the presence of water stress was achieved by the exposure of cultured cells to a medium containing PEG. Reduced growth in the presence of PEG in the medium has been reported for several plants, including wheat [35], rice [8] and sugarcane [5]. As plant growth is a result of cell division and enlargement, water stress would directly restrict growth by slowing down both processes [25], and the most sensitive process to get affected by water deficit is the cell growth [16]. Our results indicated that Djenah Khetifa and Oued Zenati were better at adapting to higher water stress than Waha, which indicated the superiority of these genotypes for *in vitro* water stress tolerance. The calli that actively grew at this stage were considered to be PEG tolerant and were used for further characterization and their ability to regenerate plantlets [23].

3.5. Effect of water stress on embryogenic callus production

Significant differences were observed among the genotypes (Table 2). The embryogenic and nonembryogenic calli were distinguished on the basis of their external aspect, as reported in several studies [11,20]. Embryogenic calli have a glossy look, are compact and have a whitish-cream color and a nodular structure, whereas non-embryogenic calli are soft and translucent and have a hyperhydric appearance. In our observations, non-embryogenic callus showing necrosis or browning (Fig. 1C) was not selected for regeneration. According to Sharma et al. [37], the browning of the callus cells was considered as an indicator of tissue culture intolerance to PEG induced drought.

The three genotypes showed high embryogenic callus production rates, the average percentage of embryogenic calli formation from unstressed calli being (73.66%) (Table 2). Waha and Djenah Khetifa had the highest ECP rates (81% and 79.2%, respectively), whereas Oued Zenati had the lowest (61%).

Homogenous grouping using the Fisher LSD Method and a 5% probability level showed that under non-stress conditions Waha had very similar behavior to Djenah Khetifa (Table 2). The ECP was slightly higher when mature embryos were exposed to the moderate water stress treatment (10% PEG treatment) compared with the control conditions (Table 2). The calli had a nodular appearance (Fig. 1A), which is considered a typical physical feature of embryogenic calli. The presence of 10% PEG in the medium might increase the number and quality of embryogenic calli, as reported by Heringer et al. [21] for papaya (*Carica papaya* L.), who noted that PEG treatment facilitated the maturation of somatic embryos and their conversion into plantlets similar to those that originated from seed.

The beneficial effect of PEG in the maturation medium was first reported in *Picea glauca* [4] and it has been shown that different genotypes react in various ways. Moon and Park [28], reported a significant increase in embryogenesis, with more than twice the average number of somatic embryos obtained by doubling the PEG concentration. Stasolla et al. [39], however, reported that the inclusion of PEG (up to 10%) in the maturation medium could improve the number and quality of embryos produced.

A significant reduction in ECP was observed at 20% PEG; this effect being found at this high PEG concentration probably corresponds to more severe physiological conditions. Waha had the highest reduction rate (R = 58%). The resistant varieties (Djenah Khetifa and Oued Zenati) showed minimum reduction in callus initiation (23% and 32%, respectively) at high (20%) PEG levels. This result confirmed that the level of the applied stress affects somatic embryo development and that ECP capacity is genotype dependent. The genotype effect on ECP has been reported in previous studies [8,33].

3.6. Effect of water stress on regeneration potential

Calli with green spots developed small shoots and roots simultaneously on regeneration medium (Fig. 1). There was variability among the genotypes in the number of plants regenerated ($p < 0.05$) after a minimum period of 60 days after culture initiation from mature embryos (Table 2). The data indicated that embryogenic calli regenerated at a high frequency on the control medium, particularly Djenah Khetifa (60.7%) and Waha (58.1%). Oued Zenati recorded a mean of 49.2%. These results demonstrated the efficiency of the regeneration

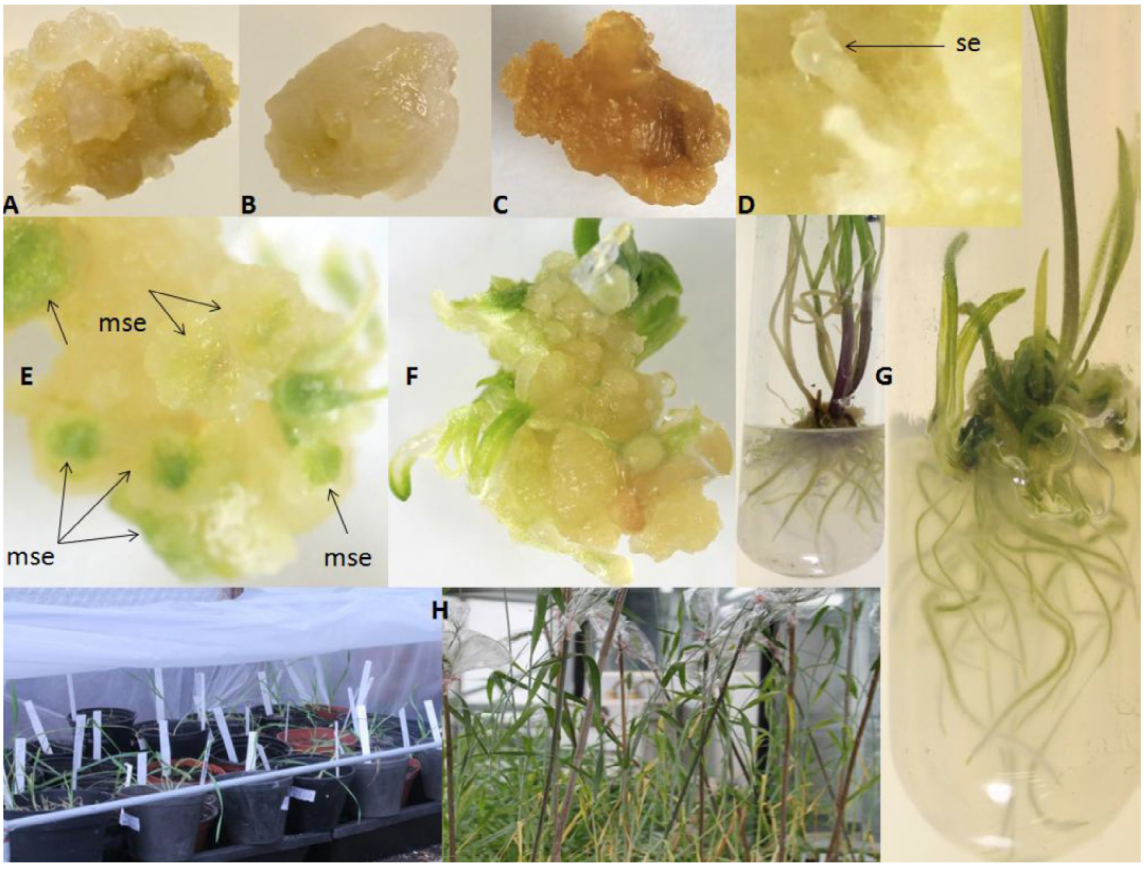

Figure 1 Callus induction, somatic embryo formation and plant regeneration. (A) An 18-day-old embryogenic callus obtained from the inoculated embryo on MS medium in the presence of PEG. (B) An 18-day-old non-embryogenic callus obtained from the inoculated embryo on MS medium in the absence of PEG. (C) A non-embryogenic callus obtained from the inoculated embryo on MS medium in the presence of 20% PEG. (D) A somatic embryo 20 days after incubation. (E) Matured somatic embryos 45 days after incubation. (F) Germination of a somatic embryo. (G) Continuation of the regeneration in a small glass and root formation on regeneration medium 60 days after incubation. (H) Mature plants derived from embryogenic calli acclimated in a greenhouse. **mse**: mature somatique embryo; **se**: somatic embryo.

Table 4 Pearson's correlation coefficients between three mature embryo callus characteristics under stress and non-stress conditions. **CIF**: callus induction frequency, **TCI**: time of callus initiation, **CGS**: callus growth surface, **ECP**: embryogenic callus production, **PR**: plant regeneration, **RT**: relative tolerance, RF: regeneration frequency.

Variables	CIF	TCI	CGS	ECP	PR	RT	RF
CIF	**1**	**−0.833****	**0.878****	0.638	**0.915****	**0.990****	**0.795***
TCI		**1**	**−0.958****	−0.471	**−0.746***	**−0.836****	−0.616
CGS			**1**	0.584	**0.863****	**0.897****	**0.752***
ECP				**1**	**0.844****	**0.697***	**0.917****
PR					**1**	**0.955****	**0.962****
RT						**1**	**0.850****
RF							**1**

* Significant at the 5% probability level.
** Significant at the 1% probability level.

medium used. Effects of genotype on plant regeneration from embryo cultures have been reported previously [7,44]. Genetic variability can be observed at each step of the morphogenic process of somatic embryogenesis, from the early stages of cell proliferation and callus formation to somatic embryo formation and maturation within the embryogenic calli and through to the ability of these embryos to germinate into plantlets. For all the genotypes, plant regeneration (PR) ability decreased significantly with increasing osmotic stress in selective media (Table 2). Djenah Khetifa had the lowest reduction percentage (14%), followed by Oued Zenati (17%), compared with the control (0% PEG), whereas Waha had the lowest PR percentage and the highest reduction percentage compared with the control (23.6%). After exposure to 20% PEG, the calli exhibited significantly lower PR capacity than the control and with 10% PEG. Djenah Khetifa had a higher PR capacity than Oued Zenati and Waha, with the reduction percentages in PR being 26.5%, 32.37% and 50.1%, respectively.

The tested genotypes differed in their ability to regenerate plants after exposure to PEG concentration (10% and 20%). Djenah Khetifa and Oued Zenati were most able to do this and were therefore the most tolerant genotypes, whereas Waha had a high sensitivity response, especially at high PEG concentration levels (20%).

Under non-stress conditions (0% PEG), however, Waha and Djenah Khetifa produced similar results and the regeneration frequency confirmed these observations (Table 2). We fully expect the selected plants to be highly tolerant, although this remains to be seen. Positive correlation between calli behavior in the presence of osmotic stress and between the plants' levels of drought resistance has been demonstrated by several authors [2,38,40]. Adding PEG 6000 to culture media reduces the water potential of the medium that affects cell division, leading to reduced callus growth, which therefore affects the regeneration ability [13]. A parallel decrease in PR with increasing *in vitro* osmotic stress has been reported for rice [8], wheat [1] and sugarcane [5]. PR provides an opportunity to screen genotypes for water stress.

3.7. Correlation analysis

Trait correlation can be a good criterion for screening the best genotypes and indices used. The results in Table 4 showed that the RT% was positively and highly correlated with CIF (r = 0.990), CGS (r = 0.897), PR (r = 0.955) and RF (=0.850).

These results indicate that genotypes with a high RT had high CIF, CGS, PR and RF, suggesting that these traits could be selected simultaneously for their positive effects on water stress tolerance in durum wheat. Our results accord with those reported by Farshadfar et al. [19], who found significant correlations among most of the traits and suggested that RT could be recommended as a suitable selection criterion for screening water stress-tolerant genotypes. Significant negative correlation coefficients were found between TCI and CIF (−0.833), CGS (−0.958), PR (−0.746) and RT (−0.836).

There was no correlation between TCI and ECP, indicating that these variables were not related. It would appear that a variety requiring time to induce calli is not necessarily one with low embryogenic capacity (e.g., Waha), which requires more time to induce calli, but produces the highest rate of embryogenic calli (Table 4).

Also, no significant correlation was observed between CIF and ECP. This indicated that callus induction and embryogenic capacity might be controlled by different mechanisms. Our results were consistent with those reported by Moon and Park [28], who found that more than 90% of explants formed calli, but only 2.5% formed embryogenic calli. There was a highly positive correlation between PR and CIF (r = 0.915), CGS (0.863), ECP (r = 0.844), RT (r = 0.955) and RF (r = 0.962), which might indicate that these traits are controlled by the same mechanisms. Viertel et al. [43] reported that the high correlation observed between the ability of cultivars to produce embryogenic calli and their PR capacity indicates that ECP percentages constitute a good index for callus ability to regenerate later on plantlets.

3.8. Cluster and principal component analysis

Another approach such as a biplot is needed to identify superior genotypes for both stressed and non-stressed conditions. The relationships among different indices are shown in the biplot in Fig. 2, which explained 95.56% of the total variation of the standardized data. PC1 explained 84.02% of the total obtained variation, with the first dimension essentially defined by CIF, CGS, PR, RF, RT and TCI. PC2 explained 11.54% of total obtained variation, with the second dimension essentially defined by the ECP capacity of genotypes. PCI alone should be a good indicator of drought tolerance.

Based on the results given above, the most prominent relationships revealed by the biplot were a strong negative associ-

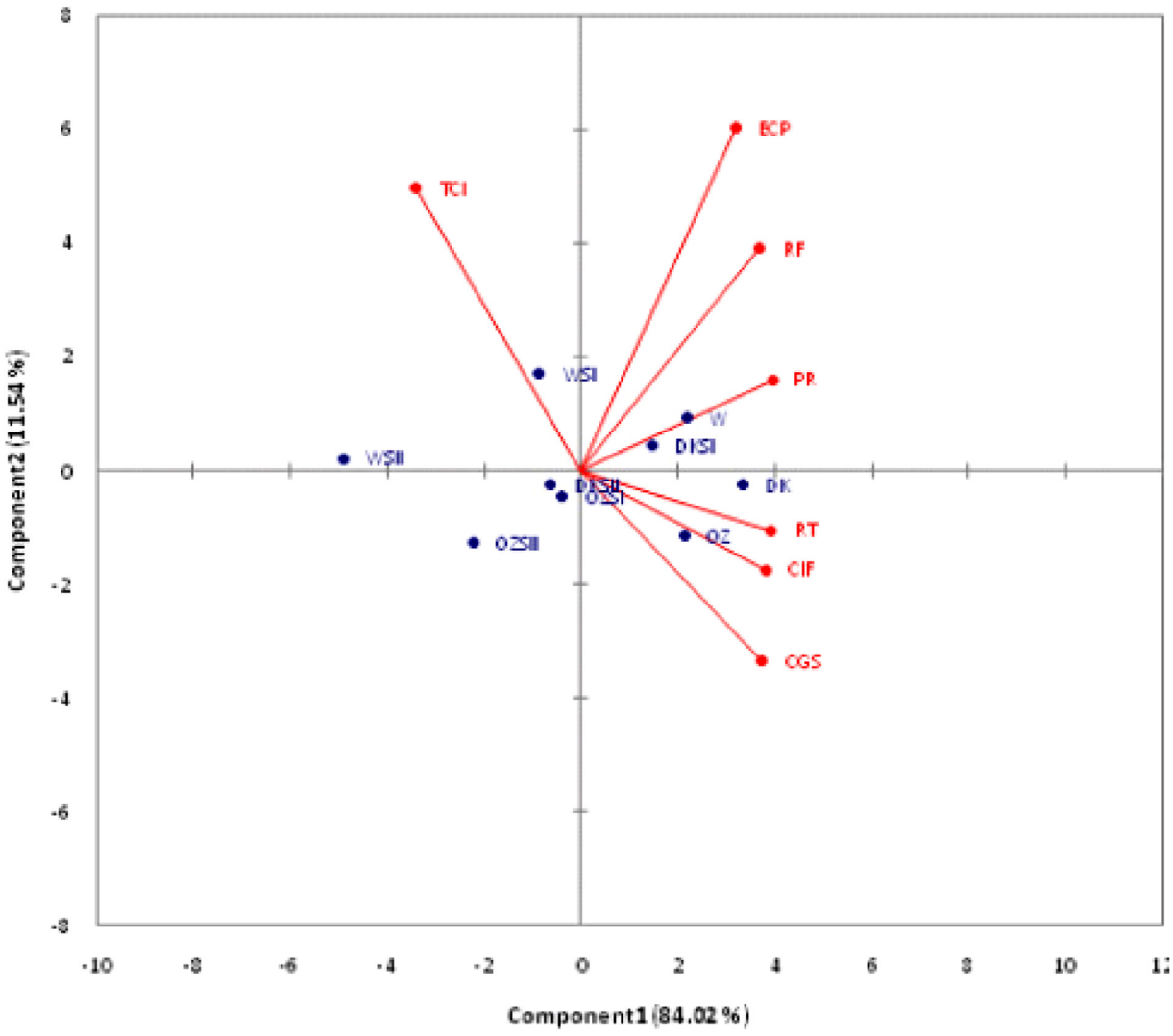

Figure 2 Biplot graph based on the main components PC1, PC2. The symbols combined the durum wheat genotypes (DK, OZ, and W; see "Section 2") with the moderate (SI: 10% PEG) and severe (SII: 20% PEG) water stress.

ation between TCI and all the other parameters (CGS, ECP, RT, CIF, PR, RF), as indicated by the large obtuse angles between their vectors. There was a highly significant positive association among RT, CIF and CGS and among PR, RF and ECP, as indicated by the acute angles (Fig. 2). These traits could be used to select and identify genotypes with high tolerance to water stress and/or good competence under *in vitro* culture. Cluster analysis was performed in order to assess the level of dissimilarity among the genotypes under stress or non-stress conditions (Fig. 3).

Group 1 included Djenah Khetifa, Oued Zenati and Waha varieties under non-stress conditions; all these genotypes had high production rates under non-stress conditions only. Group 2 included Djenah Khetifa SII, Djenah Khetifa SI, Oued Zenati SI and Waha SI; Djenah Khetifa genotype had a stable performance in all conditions, but Oued Zenati and Waha were stable at the 10% PEG level only. Group 3 comprised Oued Zenati SII. Group 4 comprised Waha SII and differed greatly from the other groups; Waha is water-stress susceptible with low capacity at 20% PEG. This classification showed the direct effect of water stress on genotypes and confirmed all previous results and previous classification based on field trials.

This procedure was also employed in bread wheat [15,17,18] and durum wheat [35] for screening selection criteria of drought tolerance. Farshadfar et al. [18] reported that superior genotypes showed drought tolerance at the callus culture level together with their high potential for callus induction and these authors suggested that *in vitro* selection can be used as an effective tool to screen a large number of genotypes to water deficit. However, more investigations such as field and hydroponic conditions studies are needed to corroborate this thought.

4. Conclusion

With regard to all the studied traits, Djenah Khetifa was selected as the most water-stress tolerant genotype under

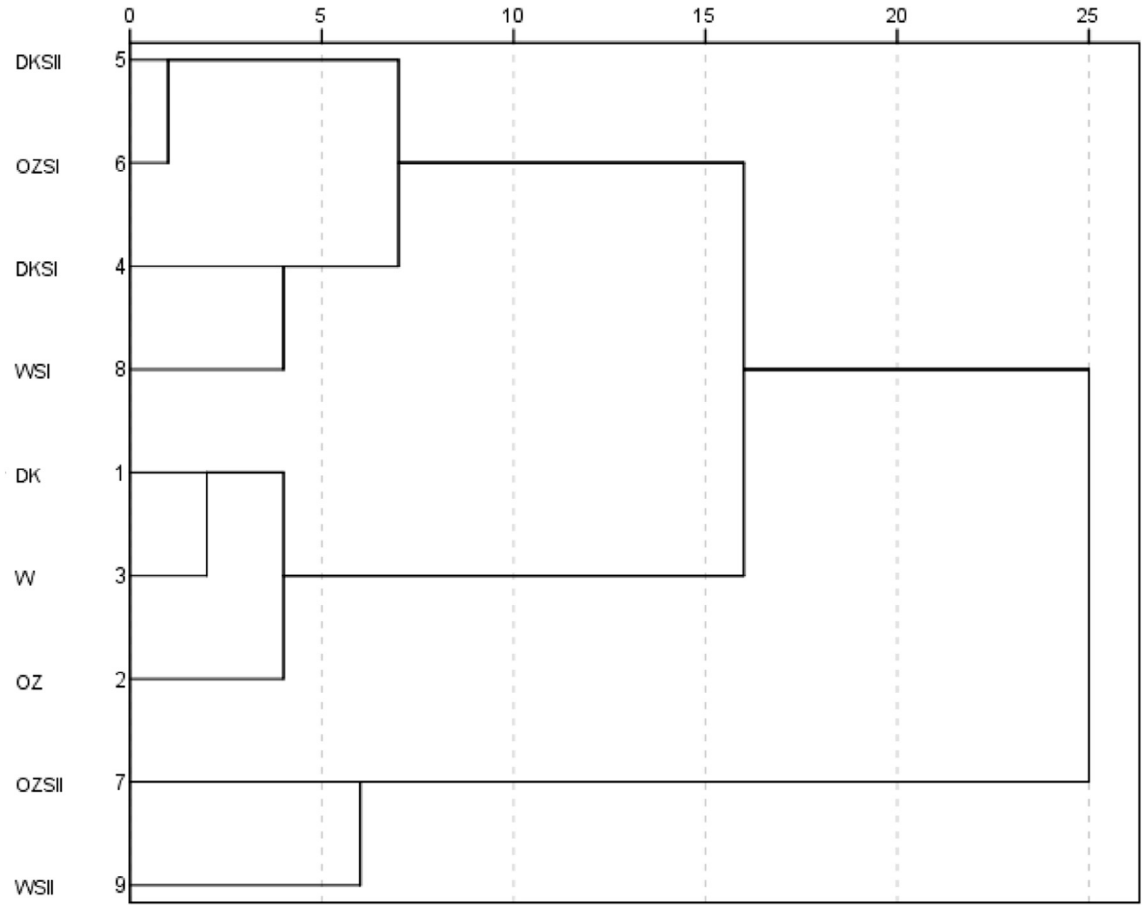

Figure 3 Hierarchical cluster analysis of three wheat genotypes under stress and non-stress conditions (Ward's Method).

in vitro conditions, followed by Oued Zenati. Waha appeared to be the most sensitive, but also the most embryogenic, under non-stress conditions. The results verified a remarkable variation for callus induction ability in genetic materials under water stress condition that can be used in durum wheat breeding program. These genotypes should be tested in a field trial and then looking for the association or correlation between *in vitro* and *in vivo* conditions. The selected cells and plants will provide a tool for determining the mechanisms involved in tolerance to water stress. We suggest that *in vitro* screening method for drought tolerance would be an appropriate route to develop drought-tolerant lines in durum wheat.

Acknowledgments

This work was supported by the Ministry of Higher Education and Scientific Research of Algeria and Walloon Agricultural Research Centre in Belgium. We thank the both institutions for their assistance.

References

[1] A. Abdelsamad, O.E. El-Sayed, F. Ibrahim, Development of drought tolerance haploid wheat using biochemical genetic markers on *in vitro* culture, J. Appl. Sci. Res. 3 (2007) 1589–1599.

[2] T. Ali Dib, P. Monneveux, E. Acevedo, M.M. Nachit, Evaluation of proline analysis and chlorophyll fluorescence quenching measurements as drought tolerance indicators in durum wheat (*Triticum turgidum* L. var. durum), Euphytica 79 (1994) 65–73.

[3] O. Alikina, M. Chernobrovkina, S. Dolgov, D. Miroshnichenko, Tissue culture efficiency of wheat species with different genomic formulas, Crop Breed. Appl. Biotechnol. 16 (2016) 307–314.

[4] S.M. Attree, D. Moore, V.K. Sawhney, L.C. Fowke, Enhanced maturation and desiccation tolerance of white spruce (*Picea glauca* [Moench.] Voss) somatic embryos: effects of a nonplasmolysing water stress and abscisic acid, Ann. Bot. 68 (1991) 519–525.

[5] M.K. Begum, M.O. Islam, M.A.S. Miah, M.A. Hossain, N. Islam, Production of somaclone *in vitro* for drought stress tolerant plantlet selection in sugarcane (*Saccharum officinarum* L.), The Agriculturists 9 (2011) 18–28.

[6] M. Berhan, M. Firew, A. Eyasu, *In vitro* screening of cactus [*Opuntia ficus-indicia* (L.) Mill] genotypes for drought tolerance, Am. J. Plant Sci. 7 (2016) 1741–1758.

[7] R. Bi, H. Wang, Primary studies on tissue culture from mature embryos in diploid and tetraploid wheat, Front. Agric. Chin. 2 (2008) 262–265.

[8] J. Biswas, B. Chowdhury, A. Bhattacharya, A.B. Mandal, *In vitro* screening for increased drought tolerance in rice, Vitro Cell Dev. Biol. 38 (2002) 525–530.

[9] R.A. Bressan, P.M. Hasegawa, A.K. Handa, Resistance of cultured higher plant cells to polyethylene glycol-induced water stress, Plant Sci. Lett. 21 (1981) 23–30.

[10] H. Chauhan, S.A. Desai, P. Khurana, Comparative analysis of the differential regeneration response of various genotypes of *Triticum aestivum, Triticum durum* and *Triticum dicoccum*, Plant Cell Tiss. Organ Cult. 91 (2007) 91–199.
[11] F. Delporte, O. Mostade, J.M. Jacquemin, Plant regeneration through callus initiation from thin mature embryo fragments of wheat, Plant Cell Tiss. Organ Cult. 67 (2001) 73–80.
[12] S.S. Dhanda, G.S. Sethi, R.K. Beh, Indices of drought tolerance in wheat genotypes at early stages of plant growth, J. Agron. Crop Sci. 190 (2004) 6–12.
[13] A.A. Ehsanpour, A. Razavizadeh, Effect of UV-C on drought tolerance of alfalfa (*Medicago sativa*) callus, Am. J. Biochem. Biotech. 1 (2005) 107–110.
[14] M.A. El Siddig et al, Preliminary screening for water stress tolerance and genetic diversity in wheat (*Triticum aestivum* L.) cultivars from Sudan, J. Genet. Eng. Biotechnol. 11 (2013) 87–94.
[15] P. Elyasi, E. Farshadfar, M. Aghaee, Response of bread wheat genotypes to immature embryo culture, callus induction and drought stress, Curr. Res. J. Biol. Sci. 4 (2012) 372–380.
[16] M. Farooq, A. Wahid, N. Kobayashi, D. Fujita, S.M.A. Basra, Plant drought stress: effects, mechanisms and management, Agron. Sustain. Dev. 29 (2009) 185–212.
[17] E. Farshadfar, R. Amiri, In vitro application of integrated selection index for screening drought tolerant genotypes in common wheat, Acta Agric. Slovenica 107 (2016) 335–344.
[18] E. Farshadfar, B. Jamshidi, M. Chehri, Assessment of immature embryo culture to select for drought tolerance in bread wheat, Int. J. Bio-Sci. 4 (4) (2014) 194–203.
[19] E. Farshadfar, S. Kianifar, R. Chaghakabodi, GT biplot analysis of genetic diversity in bread wheat using *in vitro* indicators of drought tolerance, Int. J. Biol. Sci. 7 (2015) 1439–1447.
[20] H. Gandonou, T. Errabii, J. Abrinii, M. Idaomar, F. Chibi, N. Skali Senhaji, Effect of genotype on callus induction and plant regeneration from leaf explants of sugarcane, Afr. J. Biotech. 4 (2005) 1250–1255.
[21] A.S. Heringer, E.M. Vale, T. Barroso, C. Santa-Catarina, V. Silveira, Polyethylene glycol effects on somatic embryogenesis of papaya hybrid UENF/CALIMAN 01 seeds, Theor. Exp. Plant Physiol. 25 (2013) 116–124.
[22] Z. Hossain, A.K.A. Mandal, S.K. Datta, A.K. Biswas, Development of NaCl-tolerant line in *Chrysanthemum morifolium* Ramat through shoot organogenesis of selected callus line, J. Biotechnol. 129 (2007) 658–667.
[23] N.S. Kacem, S. Mauro, Y. Muhovski, F. Delporte, J. Renaut, A. Djekoun, B. Watillon, Diagonal two dimensional electrophoresis (D-2DE): a new approach to study the effect of osmotic stress induced by polyethylene glycol in durum wheat (*Triticum durum* Desf.), Mol. Biol. Rep. (2016), http://dx.doi.org/10.1007/s11033-016-4028-5.
[24] A.G.A. Khaled, M.H. Motawea, A.A. Said, Identification of ISSR and RAPD markers linked to yield traits in bread wheat under normal and drought conditions, J. Genet. Eng. Biotechnol. 13 (2015) 243–252.
[25] J. Levitt, in: Responses of Plants to Environmental Stresses: Water, Radiation, Salt and Other Stresses, second ed., Academic Press, New York, 1980, pp. 365–402.
[26] I. Mahmood, A. Razzaq, I.A. Hafiz, S. Kaleem, A.A. Khan, A. Qayyum, et al, Interaction of callus selection media and stress duration for *in vitro* selection of drought tolerant callus of wheat, Afr. J. Biotech. 11 (2012) 4000–4006.
[27] N.P. Money, Osmotic pressure of aqueous polyethylene glycols: relationship between molecular weight and vapor pressure deficit, Plant Physiol. 91 (1989) 766–769.
[28] H.K. Moon, S.Y. Park, Somatic embryogenesis and plantlet production using rejuvenated tissues from serial grafting of a mature *Kalopanax septemlobus* tree, Vitro Cell Dev.-Pl. 44 (2008) 119–127.
[29] L.C. Morey, R.K. Blashfield, H.A. Skinner, A comparison of cluster analysis techniques within a sequential validation framework, Multivariate Behav. Res. 18 (1983) 309–329.
[30] T. Murashige, F. Skoog, A revised method for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant. 15 (1962) 473–479.
[31] C.D. Oneto, M.E. Otegui, I. Baroli, A. Beznec, P. Faccio, E. Bossio, E. Blumwald, D. Lewi, Water deficit stress tolerance in maize conferred by expression of an isopentenyltransferase (IPT) gene driven by a stress and maturation-induced promoter, J. Biotechnol. 220 (2016) 66–77.
[32] M. Özgen, M. Türet, S. Ozcan, C. Sancak, Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes, Plant Breeding 115 (1996) 455–458.
[33] M. Özgen, M. Türet, S. Altinok, C. Sancak, Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum asetivum* L.) genotypes, Plant Cell Rep. 18 (1998) 331–335.
[34] S. Rao, F.T.Z. Jabeen, *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (*Saccharum officinarum* L.), Physiol. Mol. Biol. Plants 19 (2013) 261–268.
[35] M. Razmjoo, R. Mohammadi, L. Shooshtari, *In vitro* evaluation of durum wheat genotypes for drought tolerance, J. New Biol. Rep. 4 (2015) 33–40.
[36] A.R. Reddy, K.V. Chaitanya, M. Vivekanandan, Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants, J. Plant Physiol. 161 (2004) 1189–1202.
[37] D. Sharma, N. Kumar, V. Pandey, S.C. Shankhdhar, D. Shankhdhar, In vitro evaluation of antioxidant potential under drought stress in endangered drought stress in endangered drought stress in endangered *Withania somnifera*, The Bioscan 11 (2016) 93–99.
[38] B. Simane, P.C. Struik, M.M. Nachit, J.M. Peacock, Ontogenic analysis of yield stability of durum wheat in water-limited environments, Euphytica 71 (1993) 211–219.
[39] C. Stasolla, L. Van Zyl, U. Egertsdotter, D. Craig, W. Liu, R.R. Sederoff, The effects of polyethylene glycol on gene expression of developing white spruce somatic embryos, Plant Physiol. 131 (2003) 49–60.
[40] S. Trivedi, G. Galiba, N. Sankhla, L. Erdei, Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures, Plant Sci. 73 (1991) 227–232.
[41] I. Turkan, M. Bor, F. Ozdemir, H. Koca, Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress, Plant Sci. 168 (2005) 223–231.
[42] M. Vachon, D. Beaulieu-Prévost, A. Ouellette, M. Achille, Analyse de classification hiérarchique et qualité de vie, Tut. Quant. Meth. Psychol. 1 (2005) 25–30.
[43] K. Viertel, A. Schmid, M. Iser, D. Hess, Regeneration of German spring wheat varieties from embryogenic scutellar callus, J. Plant Physiol. 152 (1998) 167–172.
[44] Y. Yu, Z.M. Wei, Influences of cefotaxime and carbenicillin on plant regeneration from wheat mature embryos, Biol. Plant. 52 (2008) 553–556.