Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (9):4555-4563
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Response of durum wheat (*Triticum durum* Desf.) callus culture to osmosis-induced drought stress caused by polyethylene glycol (PEG)

El Houssine Bouiamrine* **and Mohammed Diouri**

Plant Biotechnology and Molecular Biology Laboratory, Faculty of Sciences, Moulay Ismail University, B.P 11201 Zitoune Meknès, Morocco.

ABSTRACT

The effect of water stress induced by polyethylene glycol (PEG 10000) on callus growth, callus water content, callus necrosis and regeneration was investigated on four cultivars of durum wheat considered to have good in vitro culture ability. 3-week old calluses, initiated on MS medium supplemented with 2 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D), were subcultured on media containing different concentrations of PEG (0, 10, 15, 20 and 25 %). After 4 weeks of culture, calluses growth and water content were determined. The results showed that increasing PEG concentration in the medium causes a gradual decrease in growth and water content of calluses. The results obtained for regeneration, after transferring stressed calluses to regeneration media without PEG, also showed that increasing osmotic pressure in the medium significantly reduces the percentage of regeneration and the number of plantlets per regenerating callus (NPRC). High concentrations of PEG caused callus necrosis as well. However in some cases we observed the development of a whitish, drought tolerant, and embryogenic secondary callus upon the necrotic callus which regenerated after transfer to regeneration medium.

Key words: PEG, drought stress, secondary callus, callus necrosis, plant regeneration

---

INTRODUCTION

Improved yields of wheat depend on many factors, among which one of the most important is tolerance to environmental stress, particularly to water stress. Indeed, in durum wheat (*Triticum durum* Desf.), drought is a major non-biotic stress that causes severe yield loss. In the Mediterranean region, this loss ranges from 10 to 80% depending on the year [35].

Using classic breeding techniques in traditional breeding programs for tolerance to environmental stress was responsible for creating the majority of commercial varieties, but their applications are sometimes limited [37].

The insertion of *in vitro* tissue culture techniques in a breeding program offers considerable opportunities for genetic improvement of plants by saving space and time required by conventional methods [36]. The genetic changes called somaclonal variation [25] during the callogenesis phase of plant cells cultured *in vitro* are now considered a new source of changes intended to enrich the genetic resource for the improvement of plant species [10].

These variations can be exploited by looking in the regenerated plants the one that might have interesting traits [9]. However, methods more directed to *in vitro* selection can exploit these variations by exerting selective targeted pressures [38]. In the case of programs involving water stress tolerance improvement by *in vitro* selection, Polyethylene glycol (PEG), sucrose, mannitol or sorbitol are the best known selective agents that increase the osmotic pressure in culture media [38]. However, the high-molecular-weight PEG is the most selective agent used to induce water stress in the culture media [19]. PEG, which is a water-soluble polymer, nontoxic, non-metabolized

and non-absorbed by the cells, is available in a wide range of molecular weights (e.g., PEG-4000, PEG-4500, PEG-6000, PEG-8000 and PEG-10000) [26]. It simulates the water deficit of *in vitro* tissue cultures in a manner similar to that observed *in vivo* in cells of intact plants subjected to drought conditions [2, 21]. This selective agent was used to select drought tolerant genotypes in sorghum [7], durum [23] and soft [8] wheat.

However, before any *in vitro* breeding program, it is imperative to study and consider the selection criteria such as appearance of calluses, growth rate, regeneration capacity and survival of calluses subjected to osmotic stress [43].
The aim of the present work is to study the effect of different concentrations of PEG in the culture medium on growth and regeneration in four genotypes of durum wheat having a high potential of *in vitro* morphogenesis. The results obtained will allow us to determine the optimum concentrations of PEG to isolate tolerant cell lineages and, in turn, to develop selective media and selection methods to be applied as part of a program of *in vitro* selection we have undertaken.

MATERIALS AND METHODES

Plant material and explant preparation.

Four durum wheat (*Triticum durum* Desf.) cultivars Karim, Sebou, Ourigh and Anouar were used as the material for this study. The seeds were provided by INRA (National Institute for Agricultural Research, Morocco). The explant source consisted of immature embryos collected from seeds in the milky phase, approximately 14-18 days after anthesis. The caryopses were surface sterilized for 1 min in 90% ethanol and rinsed three times in sterile distilled water. Caryopses were disinfected again with 30% commercial bleach for 20 min followed by three rinses with sterile water.

Preparation of media and cultivation

The nutrient medium of Murashige and Skoog [34] was used as the basic medium and was modified for the callogenesis (MC), for regeneration (MR1) and for rooting (MR2) (Table 1). Prepared media were sterilized by autoclaving during 20 min at 120 °C.

Immature embryos were excised aseptically from caryopses, and placed with the embryo axis in contact with a solid agar medium for callogenesis containing induction medium MCi (Table 1). The cultures were then incubated in the dark in a growth chamber maintained at 25±2°C.

After 3 weeks of callogenesis on solid medium MCi, calli were subcultured on liquid media (Table 1) containing different concentrations of PEG (0, 10, 15, 20 and 25 %). In order to remedy the problem of solidification caused by high doses of high molecular weight PEG (PEG 10000), we used the polyester batting as carrier (data not shown). Authors had proposed to use either filter paper bridges [31] or cotton carriers [22].

After 4 weeks of culture on growth medium containing different PEG concentrations, calli were then subcultured on MR1 regeneration medium and placed in a growth chamber under a photoperiod of 16 hours of light/24 hours.

After five weeks of culture on regeneration medium MR1, calli with shoots were then transferred to the rooting medium MR2. Both media MR1 and MR2 were solidified with agar at 7 g L^{-1}.

Table1. Media composition for callogenesis (MC) and regeneration (MR)

Medium	**Callogenesis media MC**						**Regeneration media MR**	
Components	**MCi**	**MC0**	**MC10**	**MC15**	**MC20**	**MC25**	**Caulogenesis medium MR1**	**Rhizogenesis medium MR2**
Macroelements	MS	MS	MS	MS	MS	MS	MS/2	MS/2
2,4-D (mg l^{-1})	2	2	2	2	2	2	0.2	-
PEG (%)	-	0	10	15	20	25	-	-
BAP (µM)	-	-	-	-	-	-	10	-
ANA (µM)	-	-	-	-	-	-	5	-
Agar (g l^{-1})	7	-	-	-	-	-	7	7

2,4-D = 2,4-Dichlorophenoxyacetic Acid. PEG= polyethylene glycol. BAP = benzylaminopurine. NAA = naphthalene- acetic acid.

Parameters evaluation

The studied parameters were calculated for each genotype, using the following formulae:

• Callus relative growth (CRG): 2 grams of 3 week old calli initiated on MCi medium were transferred to media containing various concentrations of PEG. After 4 weeks of culture, the CRG is calculated by the following formula CRG = [(FFW (final fresh weight)-IFF (initial fresh weight)] / IFF [6].
• Calli water content: CWC (%) = 100 x (CFW (Callus Fresh Weight) – CDW (Callus Dry Weight)) / CFW. CDW was determined after a 48-h stay in the oven at 80°C. CFW was determined just before drying.
• Percentage of regeneration = (number of regenerated calli / total number of calli) x 100.
• Percentage of callus necrosis = (number of necrotic calli / total number of calli) x 100.
• The number of plantlets per regenerating callus (NPRC) was estimated by counting regenerated plantlets after five weeks of culture on MR2. Counting was done during the transfer of plantlets to soil for acclimatization.

Statistical Analysis
Statistical analysis of data was carried out using the R statistical environment [39]. Data were analyzed using the analysis of variance technique. The comparison of means was sometimes done by LSD test and sometimes by Duncan's Multiple Range test, at 0.05 level.

RESULTS

After 3 weeks of growth on agar-solidified medium under non-stress conditions, the calli were subcultured on liquid medium with polyester batting as carrier (Figure 1a).

Observation of callus during the stress period allowed us to visually distinguish a first difference in size, color and texture of the calli. Calluses from unstressed media were large, nodular and white to pale yellow. As far as the PEG concentration increased, the calluses lost their compactness and evolved into a mucilaginous texture, their size decreased and they became darker with strong browning and necroses. In some cases there has been a development of a compact whitish embryogenic cell mass that lead after proliferation to the formation of a secondary callus (Figure 1b).

Effect of PEG concentration
Table 2 presents the results concerning the evolution of callus fresh weight (CFW), callus relative growth (CRG) and callus water content (CWC) after undergoing different PEG concentrations. The results concerning CFW showed that increasing PEG in the medium significantly brings down the weight of calluses and therefore the relative growth. The highest weight (10.86 g) was recorded in calluses grown on MC0 without PEG; and the lowest (2.23 g) on MC25. In parallel, the highest CRG (4.43) was noted in calluses grown on control medium MC0 with no PEG and decreased gradually to 0.11 in medium MC25.

Likewise, CWC decreased significantly with increasing PEG concentration in the medium. The highest water content (88.75%) was recorded in the calluses from PEG-free media. The lowest water contents were observed in the calluses from media containing the highest PEG concentrations 20% (MC20) and 25% (MC25). These two contents were respectively 77.76% and 76.57% and were not significantly different.

Table 2. Effects of PEG concentrations on callus fresh weight, Callus relative growth and water content of callus in four durum wheat varieties

Culture medium	CFW (g)	CRG	CWC (%)
MC0	10.86 ± 0.47 a	4.43 ± 0.23 a	88.75 ± 0.53 a
MC10	7.12 ± 0.14 b	2.56 ± 0.07 b	86.46 ± 0.71 b
MC15	4.40 ± 014 c	1.20 ± 0.07 c	81.57 ± 0.80 c
MC20	3.21 ± 0.15 d	0.60 ± 0.07 d	77.76 ± 0.37 d
MC25	2.23 ± 0.03 e	0.11 ± 0.01 e	76.57 ± 0.55 d

Means followed by the same letter, within the same column, are not statistically different according to the LSD test (P<0.05).
CFW: callus fresh weight. CRG: Callus relative growth. CWC: callus water content.

CFW : LSD = 0.4047761
CRG : LSD = 0.202388
WCC : LSD = 1.223868

During the regeneration phase, shoot formation began after 4-6 days of culture on regeneration medium MR1. Types of calli obtained on different media responded differently to culture conditions of the regeneration phase. Calluses from unstressed media regenerated normally and kept their white color (Figure 1c). The calluses that had lost their morphogenesis power because of high PEG concentrations eventually became even more necrotic in the regeneration medium MR1. In many cases there was a regeneration from the secondary non-necrotic callus, which developed more on the regeneration medium (Figure 1d). Non-necrotic secondary calli regenerated, sometimes only roots (Figure 1 e) with chlorophyll, and very rarely albino seedlings (Figure 1 f).

Table 3 shows the percentage of necrosis and regeneration on medium MR1 for calluses from different PEG media. The higher the growth medium PEG concentration, the significantly higher the necrosis rate. The highest necrotic calluses percentage was observed in calluses from medium MC25 containing 25% PEG. Calli from the control medium without PEG MC0 showed no necrosis.

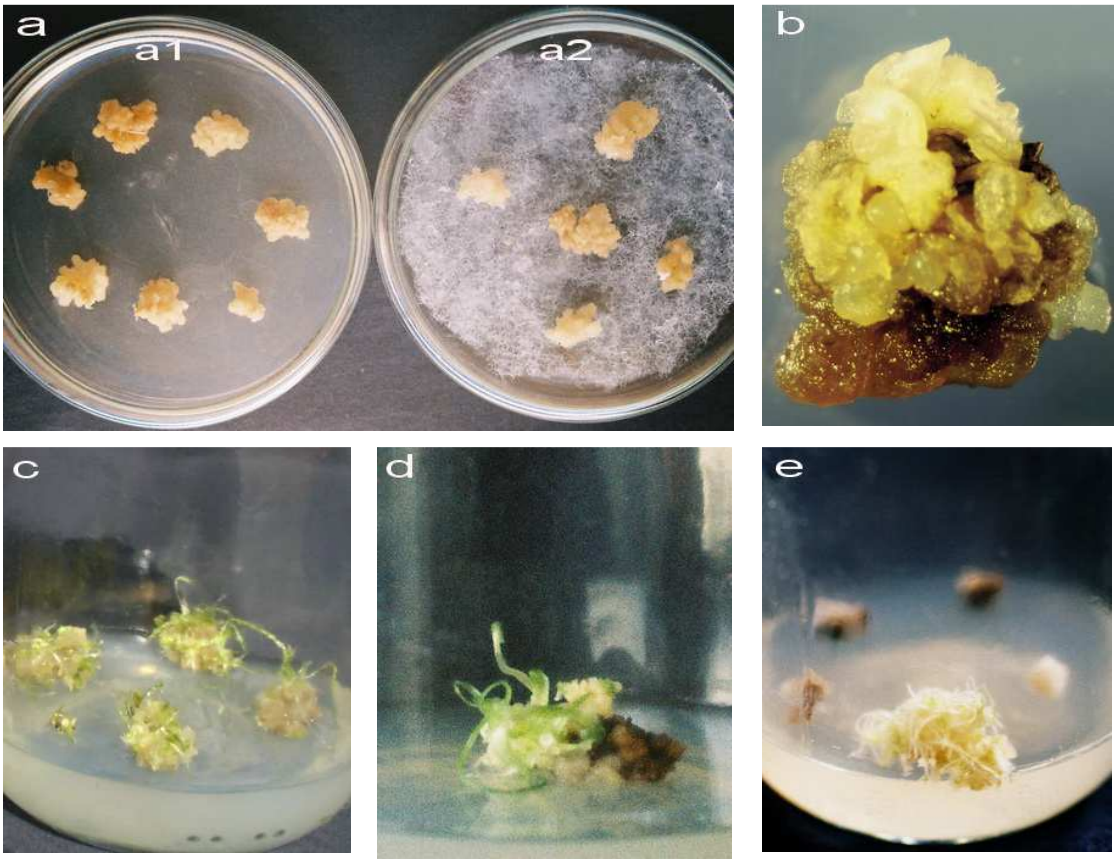
Regeneration results showed that calli morphogenic capacity significantly decreased with increasing osmotic stress in the culture media. Indeed the observed high percentage of necrotic calluses explains this decrease in morphogenesis. Calluses from medium MC0, which showed no necrosis or browning, had a high regeneration capacity (88.73%). The lowest rate (11.41%) was observed in calluses from the medium containing the highest concentration of PEG.

Table 3. Effect of different concentrations of PEG on necrosis, percentage of regeneration and NPRC in four durum wheat varieties

Culture medium	Necrosis (%)	Regeneration (%)	NPRC
MC0	-	88.73 ± 1.42 a	19.08 ± 0.63 a
MC10	8.00 ± 1.34 d	63.66 ± 1.77 b	10.66 ± 0.76 b
MC15	27.08 ± 2.47 c	42.08 ± 5.01 c	5.58 ± 0.51 c
MC20	48.66 ± 1.17 b	26.91 ± 2.39 d	3.58 ± 0.33 d
MC25	64.50 ± 2.30 a	11.41 ± 0.98 e	2.83 ± 0.20 d

Means followed by the same letter, within the same column, are not statistically different according to the LSD test (P<0.05).
NPRC = Number of plantlets per regenerating callus.

% necrosis : LSD = 3.12378
% de regeneration : LSD = 3.469807
NRPC: LSD = 1.112102



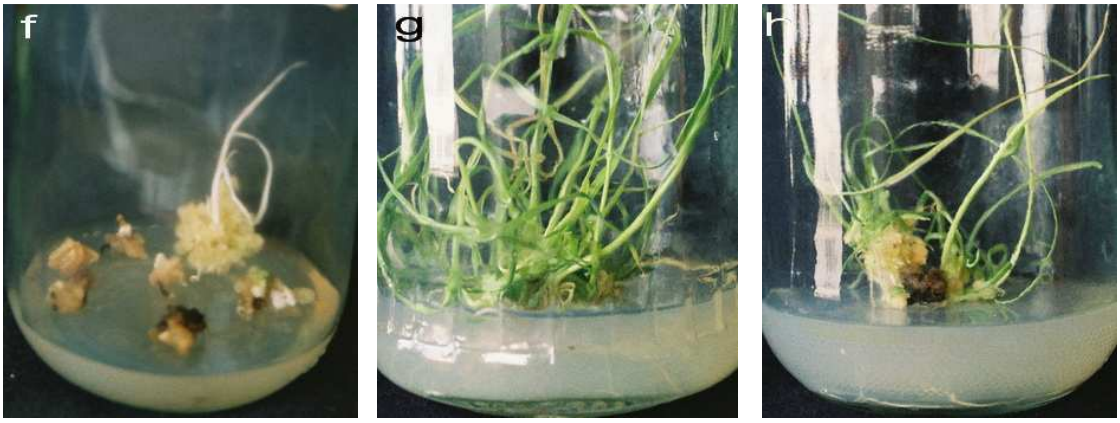


Figure 1. Callus growth and regeneration from immature embryos on media containing different PEG concentrations.

(a) (a1) Callus grown on medium solidified with agar. (a2) Callus grown on liquid medium with polyester batting as carrier. (b) Nodular whitish secondary callus upon necrotic callus. (c) Regeneration of plants from unstressed callus on MR1 medium. (d) Regeneration on MR1 from the necrotic callus whitish area. (e) Root regeneration on MR1 from the necrotic callus whitish area. (f) Regeneration of albino plantlets from well-developed whitish secondary callus on MR1. (g) Regeneration and development and of plantlets from unstressed calli on MR2 medium. (h) Development of seedlings on MR2 medium from necrotic callus whitish area.

Calli that regenerated shoots were transferred to medium MR2 without hormone to allow root development and growth of regenerated plantlets. After 5 weeks of culture on MR2, and just before the transfer of plantlets to soil, the average number of plantlets per regenerating callus (NPRC) was determined by seedlings counting. Results showed that NPRC was also affected by the concentration of PEG in the culture medium (Table 3). The best yield (19.08) of regenerated plantlets was obtained in callus from PEG-free medium MC0 (Figure 1 g).

High concentrations of PEG in the culture medium significantly reduced the NPRC. Indeed only the non-necrotic part of the callus, which was sometimes reduced in size, was able to regenerate plants (Figure 1 h). NPRC decreased significantly, down to 2.83 in the calluses from medium MC25.

Genotype effect

Table 4 summarizes the results obtained in four varieties on the different studied media. Significant differences were observed among genotypes for all parameters considered. Calli of the Sebou variety showed a superior growth compared to other varieties with a callus fresh weight of 6.14 and a callus relative growth of 2.07. Calluses of this same variety also showed water contents (84.91%) higher than those of other varieties.

Necrosis induced by high concentrations of PEG was also affected by genotype. Callus of the variety Karim became necrotic more easily than those of other varieties with increasing PEG concentrations in the medium with a percentage of 34.33%. The Anouar variety showed the highest (53.26%) percentage of regeneration. The lowest percentage (36.79%) was recorded in the Sebou variety. The latter also showed the lowest NPRC equaling 7.53 on average. The highest NPRC was recorded in the Karim variety with an average of 9 regenerated plantlets.

Table 4. Effect of genotype on growth, water content, necrosis and regeneration ability of calluses on the various media

variety	Characters					
	CFW (g)	CRG	CWC (%)	Necrosis (%)	Regeneration (%)	NPRC
Anouar	5.49 ± 0.86 b	1.74 ± 0.43b	81.00 ±1.20 c	26.46±6.38b	53.26±7.58a	8.2±1.75 ab
Karim	5.30 ± 0.77 b	1.65 ± 0.38 b	82.24±1.50 b	34.33±7.18a	49.06±7.10b	9±1.99 a
Sebou	6.14 ± 0.69 a	2.07 ± 0.34a	84.91 ±1.48a	31.6±6.44a	36.79±7.54c	7.53±1.40b
Ourgh	5.33 ± 1.04 b	1.66 ± 0.52b	81.46±1.13bc	26.2±6.26b	47.13±7.74b	8.6±1.45a

Means followed by the same letter, within the same column, are not significantly different according to the LSD test (P<0.05).
CFW: callus Fresh weight. CRG: callus relative growth. CWC: callus Water content NPRC = Number of plantlets per regenerating callus

CFW: LSD = 0.3620427
CRG: LSD = 0.1810214
WCC: LSD = 2.021075
% necrosis: LSD = 2.793994
% de regeneration: LSD = 3.10349
NRPC: LSD = 0.994694

Effect of variety × PEG interaction

The behaviour of the different cultivars to the different PEG concentrations was similar (table 5). Nevertheless, some significant genotype x medium interactions were noted for different studied parameters (table 6 and table 7).

Despite differences, increasing PEG concentration in the culture medium affected the response of varieties in the same way by a decrease of callus growth, callus water content, percentage of regeneration and NPRC, and an increase of callus necrosis. The highest level of necrosis was obtained on medium with 25% PEG in the four varieties. No necrotic callus was observed in four varieties on medium without PEG. Also in the four varieties, callus growth, callus water content and regeneration were highest on control medium without PEG.

Table 5. Effect of variety × medium interaction on callus growth, water content, necrosis and regeneration ability

Effect		Characteristic					
variety	culture media	CFW (g)	CRG	CWC (%)	Necrosis (%)	Regeneration (%)	NPRC
Anouar	MC0	10.9 a	4.45 a	87.6 a	0 e	9.66 a	19.66 a
	MC10	7.26 b	2.63 b	84.36 b	5.66 d	68 66 b	11.33 b
	MC15	4.4 c	1.2 c	79.63 c	17.66 c	64 b	4.66 c
	MC20	2.63 d	0.31 d	76.8 cd	50.b	31.33 c	3.33 cd
	MC25	2.26 d	0.13 d	76.63 d	59 a	11.55 d	2.33 d
Karim	MC0	9.9 a	3.75 a	88.6 a	0 e	90.3 a	21.33 a
	MC10	7.2 b	2.6 b	87 a	13.33 d	61.66 b	13.66 b
	MC15	4.3 c	1.15 c	83.66 b	33.66 c	49.33 c	4.33 c
	MC20	2.96 d	0.48 d	77.6 c	49.33 b	31 d	3 c
	MC25	2.16 d	0.08 d	74.33 d	75.33 a	13 e	2.66 c
Sebou	MC0	13.16 a	5.58 a	91.13 a	0 e	82.3 a	17.33
	MC10	7.46 b	2.73 b	89.5 a	10 d	56.66 b	7.33 b
	MC15	4.13 c	1.06 c	84 b	34.33 c	21.33 c	6.33 b
	MC20	3.66 c	0.83 c	78.66 c	49.33 b	14.66 d	3.33 c
	MC25	2.3 d	0.15 d	77.66 c	64.33 a	9 e	3.33 c
Ourgh	MC0	9.5 a	3.45 a	87.66 a	0d	91.66 a	18 a
	MC10	6.56 b	2.28 b	85 a	3 d	67.66 b	10.33 b
	MC15	4.8 c	1.4 c	79 b	22.66 c	33.66 c	7 c
	MC20	3.6 c	0.8 c	78 b	46 b	30.66 c	4.66 cd
	MC25	2.2 d	0.1 d	77.66 b	59.33 a	12 d	3 d

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

Table 6. Analysis of variance for callus weight, relative growth and water content for four durum wheat varieties

		FCW (g)		CRG		CWC (%)	
Source	DF	Mean Square	F value	Mean Square	F value	Mean Square	F value
Cultivar (A)	3	2.32	9.635***	0.58	9.635***	29.7	13.485***
PEG levels (B)	4	145.48	604.492***	36.37	604.492***	338.4	153.792 ***
AB	12	1.81	7.513***	0.45	7.513***	6.1	2.781**
Residuals	40	0.24		0.06		2.2	

Significant (***) at 0,1%, (**) at 1%, (*) at 5%
FCW: Fresh callus weight. CRG: callus relative growth.. CWC: callus Water content

Table 7. Analysis of variance for necrosis, percentage of regeneration and NPRC

		Necrosis (%)		Regeneration (%)		NPRC	
Source	DF	Mean Square	F value	Mean Square	F value	Mean Square	F value
Cultivar (A)	3	239	16.665***	735	41.546***	5.8	3.190*
PEG levels (B)	4	8792	613.379 ***	11137	629.729***	544.1	299.528***
AB	12	53	3.702***	165	9.313***	7.9	4.359***
Residuals	40	14		18		1.8	

Significant (***) at 0,1%, (**) at 1%, (*) at 5%
NPRC = Number of plantlets per regenerating callus

DISCUSSION

Water stress is one of the limiting factors for plant growth and crop production. Upon exposure to water deficit, plants react by complex mechanisms involving morphological, physiological, biochemical and molecular factors, both at cellular and whole-plant levels [17, 18] At the cellular level, the effect of water stress on the slowdown of cell divisions and elongation by the loss of turgor has been widely reported [27, 32].The addition of PEG in the

medium causes cell dehydration by reducing water availability to cells, Which leads to a loss of cell turgor and hence a loss of growth [20].

The study we undertook showed clearly the effect of PEG on callus growth and morphogenesis *in vitro* in the four varieties studied. The results showed that high concentrations reduce callus relative growth, regeneration ability, water content and morphogenesis capacity. Callus necrosis rate, however, increases with gradual increase in PEG concentrations. Our results on the reduction of callus growth under the effect of PEG are in agreement with previous studies in other species such as rice [1, 43] barley [11] and soft wheat [14]. The 25% Concentration appears to be the dose that inhibits callus growth in the four varieties. These results are consistent with those reported by Hsissou and Bouharmont [23] who also used PEG-10000 in the culture medium. These authors showed that after 6 months of culture, the 25% concentration becomes callus growth inhibiting in durum wheat.

In rice, Al-Bahrany [1] studied the effect of different concentrations of PEG-8000 on growth of callus from mature embryos. These results showed that water stress induced by increasing concentrations of PEG leads to a progressive reduction of callus fresh weight. The significant reduction in fresh weight of callus was observed at 50 g L^{-1}, but starting from 200 g L^{-1}, the concentrations become inhibitory. However, the concentration of PEG inhibiting growth depends on the genotype of the species studied [40, 42].

The progressive increase of PEG in the culture medium caused a gradual decrease in callus water content in the four varieties. Similar results were reported in rice by Al-Bahrany [1]. Indeed, the difference in water potential between callus cells and the culture medium, created by the PEG, causes cellular dehydration [18]. Heyser and Nabors [20] showed that osmotic stress increase, due to the addition of PEG in the medium, was accompanied by a sharp decrease in water content of tissues. This water stress induces also a cell osmotic adjustment by accumulation of solutes such as endogenous proline, which contributes to the protection against cellular damage caused by dehydration, hence triggering an adaptive response [44].

In addition to reduced growth, callus necrosis is considered an indicator of tissue culture intolerance to osmotic stress induced by PEG. Our results showed that increasing the concentration of PEG in the medium caused an increase in the callus necrosis percentage in the four varieties studied. Similar results were also reported in soft wheat [12, 29]. Necrosis observed in calli of wheat subjected to osmotic stress of PEG-6000 (-1.2 MP) is generally accompanied by growth stop [29]. Necrosis of calluses is a phenomenon also observed in cultures subjected to salt stress at high NaCl concentrations in species such as wheat [3, 24] or sugar cane [15]. In the latter, the effect of NaCl on callus necrosis was reduced by pretreatment with PEG [33].

At the *in vivo* plant level, stress also causes growth inhibition and tissue necrosis, followed by a loss of turgor, by leaf drop and eventually by the plant death. Suleman *et al.* [41] reported that the application of a severe water stress on date palm (Phoenix dactylifera) seedlings initially causes necrotic lesions that develop in cankers, then the death of buds, and eventually the death of the plant. Histological studies have shown that tissue necrosis is directly related to water stress that begins with necrotic islands in parenchyma.

As for callus growth, the gradual increase of PEG in the culture medium caused a decrease of callus morphogenic capacity. Similar results were reported in wheat [29] and rice [43]. In corn, Matheka *et al.* [30] reported that the induction of somatic embryogenesis and plant regeneration from callus under water stress are inversely proportional to PEG concentration in the selection media. This decrease in the regeneration capacity, resulting from stress due to PEG, may be explained by the slowdown in all cell physiological processes that affect growth, multiplication and therefore morphogenesis.

The proliferation of whitish embryogenic cell clusters of necrotic calli on medium containing high doses of PEG suggests a major cellular adaptation. These secondary calli were subsequently able to regenerate green plants, roots or rarely albino plants. Mahmood *et al.* [29] showed that 4-week wheat callus culture on medium containing PEG 6000 (-0.9 MPa) caused degeneration of the non-tolerant calluses and proliferation of tolerant calli which were subsequently able to regenerate plants.

The number of albino plantlets regenerated remained very low. Indeed, Chlorophyll deficiency or albinism is common in androgenesis in durum wheat and is a major problem for the application of haplodiploidisation in breeding programs of this species [16]. In somatic embryogenesis, conversely, few reports have mentioned regeneration of albinos [5, 28].

The effect of PEG on growth, water content, necrosis and morphogenesis was substantially similar in all four varieties. However, genotypic differences were observed with respect to the various above mentioned parameters.

These differences could be explained by a variability in tolerance to water stress in the callus, which cannot be correlated with similar behavior in vivo at the scale of the plant. Indeed, Farshadfar *et al.* [13] reported that there was no similarity between the in vivo behavior of seedlings in 20 genotypes of wheat grown in the field under conditions of water stress, and calluses induced from mature embryos derived from these same genotypes.

Bajji *et al.* [4] showed that in durum wheat there is a positive correlation between field performance of cultivars under drought conditions and the responses of callus to osmotic stress, suggesting that resistance to drought in the whole plant depends, at least in part, on the existence of operating mechanisms at the cellular level. Bajji *et al.* [4] added, however, that the behavior of the callus cultures under osmosis-induced drought stress is not always similar to that of the whole plant; and the consideration of these two levels of organization is necessary for a better understanding of the complexity of mechanisms that allow plants to cope with stress.

CONCLUSION

The results we obtained allow us to conclude that water stress induced by high concentrations of PEG 10000 significantly lower callus growth, water content and morphogenesis and, conversely, raise the percentage of necrosis. However, calluses under high osmotic pressure were able to adapt to the stress, created in the medium, by developing non-necrotic secondary calli. These calli continued to grow in the regeneration medium while regenerating seedlings sometimes. The long-term culture of these calli, which seem to tolerate high osmotic pressures, will allow us to regenerate plants with the aim of selecting lines tolerant to water stress, and also to study the mechanism of cell tolerance to high osmotic pressures.

Acknowledgments
The authors are indebted to Dr. Mohammed Amssa, the retired professor and ex-Head of the Plant Biotechnology Laboratory (Moulay Ismail University) for the efforts provided to set up research projects in biotechnology.

REFERENCES

[1] A.M. Al-Bahrany; *Pak. J. of Biol. Sci*., **2002**, 15:1294-1296.
[2] S.M. Attree, D. Moore, V.K. Sawhney, L.C. Fowke. *Annals of Botany*, **1991**, 68, 519–522.
[3] A. Arzani, S.S. Mirodjagh. *Plant Cell, Tissue and Organ Cult.*, **1999**, 58, 67-72.
[4] M. Bajji; S. Lutts; J.M., Kinet, In:C Royo, MM Nachit, N Di Fonzo, JL Araus (ed.) Durum wheat improvement in the Mediterranean region (New challenges, CIHEAM-IAMZ, Zaragoza, **2000**) 227. (In French).
[5] E.H. Bouiamrine, K. Mzouri, M. Amssa, In: Aupelf-Uref (ed.). Actualités Scientifiques, Biotechnologies, amélioration des plantes et Sécurité alimentaire (Estem, Paris, **1999**) 553. (In French).
[6] J.J. Chen, R.Q. Yue, H.X. Xu, X.J. Chen. *Agric Sci China*, 2006, 5(8), 572-578.
[7] R.R. Duncan, R.M. Waskom, M.W. Nabors. *Euphytica*, **1995**, 85, 373-380.
[8] M.K. El-Haris, M.N. Barakat. *Alex. J. Agric. Res*., **1998**, 43, 293-302.
[9] D.A. Evans. *Trends. Genet.*, **1989**, 5, 46-50.
[10] DA. Evans, W.R. Sharp. In: Evans DA, Sharp, WR, Ammirato PV (eds) Handbook of Plant Cell Culture (Macmillan Publishing Company, New York, **1998**) 97.
[11] E. Farshadfar, S. Esmaeili, A. Yaghotipoor. *Ann. Biol. Res.*, **2012**, 3 (3), 1524-1535.
[12] E. Farshadfar, B. Jamshidi, K. Cheghamirza, H. Hashemzadah. *Ann. Biol. Res.*, **2012**, (3)1, 330-338.
[13] E. Farshadfar, B. Jamshidi, K. Cheghamirza, J.A. Teixeira da Silva. *Ann. Biol. Res*., **2012**, 3 (1), 465-476.
[14] V. Galović, Z. Kotaranin, S. Denčić. *Genetika*, **2005**, 37, 165-171.
[15] C. Gandonou, J. Abrini, M. Idaomar, N.S. Senhaji. *Afr. J. Biotechnol*., **2005,** 4, 350-354.
[16] M.Ghaemi, A. Sarrafi, *Plant Breeding*, **1994**, 112: 76-79.
[17] H. Greenway , R. Munns. *Annu. Rev. Plant Physiol.*, **1980,** 31, 149-190.
[18] P.M. Hasegawa, R.A. Bressan, J.K. Zhu, H.J. Bohnert. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **2000**, 51, 463-499.
[19] N.M. Hassan, M.S. Serag, F.M. El-Feky. *Acta Physiol. Plant*., **2004**, 26, 165-175.
[20] J.W. Heyser, M.W. Nabors. *Plant Physiol.*, **1981**, 68, 1454-1459.
[21] M. Hohl, P. Schopfer. *Plant Physiol*., **1991**, 95, 716-722.
[22] D. Hsissou. PhD thesis. Catholic University of Louvain (Louvain, Belgium, **1994**).
[23] D. Hsissou, J. Bouharmont. *Agronomy*, **1994**, 14:65-70.
[24] M. Karadimova, G. Djambova. *In Vitro Cell. Dev. Biol. Plant.*, **1993**, 29: 180-182.
[25] P.J.Larkin, S.C. Scowcroft. *Theor. Appl. Genet.*, **1981**, 60:197–214.
[26] D.W. Lawlor. *New Phytology*, **1970**, 69: 501–514.

[27] J. Levitt. In J. Levitt (ed.). Responses of plant to environmental stress. Water, radiation, salt and other stresses. (Academic Press, New.York, **1980**) 365.
[28] S.E. Maddock, V.A. Lancaster, R. Risiott, J. Franklin. *J. Exp. Bot.*, **1983**, 34: 915-926.
[29] I. Mahmood, A. Razzaq, I.A.Hafiz, S. Kaleem, A.A.Khan, A. Qayyum, M. Ahmad. *Afr. J. Biotechnol.*, **2012**, 11(17): 4000-4006.
[30] J.M. Matheka, E. Magiri, A.O. Rasha, J. Machuka. *Biotechnology*, **2008**, 7(4): 641-650.
[31] A.S. Mohmand, M.W. Nabors. *Plant Cell Tissue Organ Cult.*, **1991**, 26: 187–189.
[32] P. Monneveux. In: Aupelf-Uref (ed.).Quelles stratégies pour l'amélioration génétique de la tolérance au déficit hydrique des céréales d'hiver ? (John Libbey. Eurotext. Paris, **199**1) 165. (In French).
[33] N. Munir, F. Aftab. *Turk. J. Bot.*, **2009**, 33: 407-415.
[34] T. Murashige, F. Skoog. *Physiol. Pl.*, **1962**, 15: 473–97.
[35] M.M. Nachit, E. Picard, P. Monneveux , M. Labhilili, M. Baum, R. Rivoal. *Cahiers Agric*., **1998**, 7:510-515. (In French).
[36] R. Ortiz R (1998). *Electronic Journal of Biotechnology*, **1998**, 1 (3). http://www.ejb.org.
[37] M. Purohit, S. Srivastava, P.S. Srivastava. In: P.S. Srivastava (ed.), Plant Tissue Culture and Molecular Biology: Application and Prospects. (Narosa Publishing House, New Delhi, **1998**) 554.
[38] M.K. Rai R.K. Kalia, R. Singh, M.P. Gangola, A.K. Dhawan. *Environ Exp Bot.*, **2011**, 71(1): 89 -98.
[39] R Development Core Team (**2012**). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
[40] M.S. Santos-Díaz, N. Ochoa-Alejo. *Plant Cell, Tissue and Organ Culture*, **1994**, 37:1-8.
[41] P. Suleman, A. Al-Musallam, C.A. Menezes. *Plant disease*, **2001**, 85 (1): 80-83.
[42] T.G. Tschaplinski, G.M. Gebre, J.E. Dahl, G.T. Roberts, G.A. Tuskan. *Can. J. For. Res*., **1995**, 25:1425-1433.
[43] S.H. Wani, P.A. Sofi, S.S. Gosal, N.B. Singh. *Com. Biometry Crop Sci.*, **2010**, 5 (2), 108-115.
[44] J.K. Zhu. Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol*., **2002**, 53: 247-273.