INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 2, p. 87-98, 2013

RESEARCH PAPER OPEN ACCESS

Callus growth and plant regeneration in durum wheat (*Triticum durum* Desf.) immature embryos under abscisic acid (ABA) treatment

El Houssine Bouiamrine[1*], Mohamed Diouri[1], Rachid EL Halimi[2], Lahcen Chillasse[3]

[1]*Plant Biotechnology and Molecular Biology Laboratory, Moulay Ismail University, B.P 11201 Zitoune Meknès, Morocco*

[2]*Computational statistics research team, Moulay Ismail University, B.P 11201 Zitoune Meknès, Morocco*

[3]*EBZH team, Moulay Ismail University, B.P 11201 Zitoune Meknès, Morocco*

Key words: Durum wheat, somatic embryogenesis, Abscisic acid, plant regeneration, immature embryos.

doi: http://dx.doi.org/10.12692/ijb/3.2.87-98 Article published on February 25, 2013

Abstract

The effect of abscisic acid (ABA) on callus growth and plant regeneration was studied in Four cultivars of durum wheat considered to have a good ability for *in vitro* culture. calluses induced from immature embryos on MS medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) were transferred onto the same medium supplemented with different concentrations of ABA (0, 1, 2, 4 and 10 µM). The regeneration medium used was MS medium supplemented with 0.2 mg L-1 of 2,4-D, 10µM of benzylaminopurine (BAP) and 5µM of naphthaleneacetic acid (NAA). callus growth decreased with increasing concentration of ABA in the medium. Best regeneration rates were obtained from calli that were grown on media containing a low concentration of ABA (1 µM).The best number of plantlets per regenerating callus was also obtained from cultures on media containing 1µM ABA. Among the regenerated plants, some rare albino plants were obtained from calluses of Karim and Anouar varieties.

* **Corresponding Author:** El Houssine Bouiamrine ✉ bouiamrine@yahoo.fr

Introduction

In view of the economic importance of wheat, great interest has been focused on applying cell culture methods to the breeding program of this crop. One of the prerequisites for the utilization of cell culture techniques in plant breeding is the high frequency of plant regeneration. In durum wheat, callus formation and plant regeneration following somatic embryogenesis have been widely reported (Bennici *et al.,* 1988; Bouiamrine *et al.,* 1999, Benkirane *et al.,* 2000; Satyavathi *et al.,* 2004). Previous studies had showed that the response of wheat to callus induction and plant regeneration in tissue culture were commonly influenced by genotype (Mzouri *et al.,* 2001; Vendruscolo *et al.,* 2008), explant source (Ozias-Akins and Vasil, 1982; Redway *et al.,* 1990), physiological status of the source plant (Redway et al., 1990; Hess and Carman, 1998) and medium composition (Mzouri and Amssa, 2002; Tamas *et al.,* 2004, Ren et *al.,* 2010). However, the influence of nutrient composition and plant growth regulators of the culture is considered a major factor influencing embryogenic response and regeneration. Auxins, cytokinins, gib-berellins, abscisic acid and ethylene are commonly known as naturally occurring plant hormones. Among these, auxins and cytokinins have been shown to play an important role in the induction and control of morphogenesis for a large number of cereals (Carman *et al.*, 1987; Gaspar *et al.,* 1996; Mzouri and Amssa, 2002). However, other plant hormones and a number of new natural growth substances, such as polyamines, oligosaccharins, jasmonates, brassinosteroids, sterols, salicylic acid and phosphoinositosides, also have specific regulatory roles which must not be ignored in culture systems (Gaspar *et al.*,1996; Jimenez, 2005). The use of those plant hormones in tissue culture also has possible effects on morphogenesis. but most effects are attributed to auxins and cytokinins because of a lack of information regarding other hormone types and their interactions.

ABA is one of the five classical growth regulating substances that have not been extensively studied for its application in callus induction and regeneration (Fazelienasab *et al.,* 2012). However, it was reported that this hormone plays an important role in inducing the expression of maturation genes in wheat embryos (Morris *et al.,* 1990), in maturating somatic embryos (Carman *et al.,* 1987, Brown *et al.,* 1989, Rai *et al.*, 2011) and in inhibiting early germination of cultured embryos (Morris at al. 1986). In addition, application of exogenous ABA improves *in vitro* conservation and the adaptive response of plant cell and tissues to various environmental stresses (Rai *et al.*, 2011). The exogenous ABA can also serve as antitranspirant during the acclimatization of vitroplants and reduces relative water loss of leaves during the ex vitro transfer of plantlets (Pospíšilová *et al.,* 2007).

The phytohormone abscisic acid is mainly defined as a stress hormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that help plant survival over the stresses. ABA regulates many important aspects of plant growth and development and its main function is to regulate plant adaptive responses to various adverse environmental conditions (Kumari *et al.,* 2010)

ABA was also used as a selective agent for the selection to detect useful variants for freezing (Sapina *et al.,* 1994) and drought tolerance (Lu, 1988).

The aim of the present work is to study the effect of different concentrations of ABA in the culture medium on growth and regeneration in four genotypes of durum wheat with a high potential of in vitro morphogenesis. This work is undertaken as part of a breeding program initiated in vitro in our laboratory to improve the tolerance to water stress in durum wheat using ABA.

Material and methods

Plant materials and explant preparation

Four durum wheat (*Triticum durum* Desf.) cultivars with high ability for somatic embryogenesis and plant regeneration were used (Bouiamrine *et al.,* 2012a). These varieties were: Anouar, Karim, Sebou and Ourgh. The seeds were provided by INRA (National Institute for Agricultural Research (Morocco)).The

explant source consisted of immature embryos (about 0.5-1.5 mm long), collected from seeds in the milky phase, approximately 14-16 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 20% commercial bleach for 20 min followed by three rinses with sterile water.

Preparation of media, and cultivation

The basal medium used was that of Murashige and Skoog (1962) modified for callogenesis (MC), for regeneration (MR1) and for rooting (MR2) (Table 1). Prepared media were sterilized by autoclaving at 120 °C for 20 minutes. Abscisic acid was added aseptically into autoclaved media by filtration through a sterile 0.5 µm porous membrane.

Immature embryos collected under a binocular microscope were placed skullcap face up in Petri dishes (10-cm diameter) containing callogenesis medium MCT (Table 1). The cultures were then incubated in the dark inside an air conditioned room maintained at 25±2 °C. 3-week old Calluses were then subcultured on media containing different concentrations of ABA. After 4 weeks of culture, calli were then subcultured on regeneration medium MR1 and placed in a growth chamber under a photoperiod of 16 hours of light/24 hours.

After 5 weeks of culture on regeneration medium MR1, calli with shoots were transferred to the rooting medium MR2.

Parameters Evaluation

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

• Relative growth of callus fresh material: 2 grams of 3-week old calluses initiated on an MCT medium are subcultured on media containing different concentrations of ABA. After 4 weeks of culture, the relative growth of the fresh material is determined using the formula: FCW = [(PFF (final fresh matter weight)-PFI (initial fresh matter weight)] / PFI.

• Callus relative growth (CRG): 2 grams of 3 week old calli initiated on MCT medium were transferred to media containing various concentrations of ABA. After 4 weeks of culture, the CRG is calculated by the following formula CRG = [(FFW (final fresh weight)-IFF (initial fresh weight)] / IFF.

• Percentage of regeneration = (number of regenerated 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

• The percentage of calli regenerating roots = [(number of calli regenerated with roots / total number of calli)] x 100.

Acclimation of regenerated plants

Plantlets with at least five well-developed roots were subjected to acclimation, transplanted to potting soil under high humidity by covering the plant with plastic envelopes. Pots were placed in a growth chamber at 25±1°C under a 16-h photoperiod. After acclimation, plantlets were transplanted to field conditions.

Statistical analysis

Statistical analysis of data was carried out using the R statistical environment (R Development Core Team, 2012). Data were analyzed using the analysis of variance technique and comparison of means was done by LSD test (Steel et *al.,* 1997). Means were also separated using Duncan's Multiple Range Tests at 0.05 level.

Results

Observations made during callus growth phase enabled us to distinguish different evolutions of the callus texture depending on the medium ABA concentration. Calluses from MC1 media were more compact and more nodular. Under a binocular microscope we were able to clearly observe embryoids undergoing maturation and well developed somatic embryos (Figure 1 a, b). Conversely, calluses from high ABA concentrations media are more brittle and show a development of whitish mucilaginous-texture secondary calluses.

Effect of ABA concentration

Table 2 presents the results concerning the evolution of callus fresh weight (FCW), callus relative growth (CRG), regeneration rate, the average number of plants per regenerating callus and the percentage of calli regenerating roots. FCW decreased significantly with increasing medium ABA concentration. The highest weight (10.87 g) was obtained on control medium (MCT) without ABA and the lowest (3.37 g) in the middle MC4 containing the highest concentration. Likewise, the highest relative growth (4.43) was observed in calluses grown on control medium without ABA and gradually decreased to reach 0.65 in medium MC4 containing 10 µM ABA.

Table1. Composition of callogenesis (MC) and regeneration (MR) Media.

Medium	**Callogenesis media MC**					**Regeneration media MR**	
Components	**MCT**	**MC1**	**MC2**	**MC3**	**MC4**	**Caulogenesis medium MR1**	**Rhizogenesis medium MR2**
Macroelements	MS	MS	MS	MS	MS	MS/2	MS/2
2,4-D (mg L^{-1})	2	2	2	2	2	0.2	-
ABA (µM)	-	1	2	4	10	-	-
BAP (µM)	-	-	-	-		10	-
ANA (µM)	-	-	-	-		5	-

2,4-D = 2,4-Dichlorophenoxyacetic Acid. ABA: Abscisic Acid. BAP = benzylaminopurine. NAA = naphthalene-acetic acid.

Table 2. Effect of different concentrations of ABA on the studied characters.

Culture medium	**Characters**				
	FCW (g)	CRG	% regeneration	NPRC	%CRR
MCT	10.87± 0.47 a	4.43 ± 0.23 a	88.73±1.42a	19.08 ± 0.63 ab	1.16±056d
MC1	8.75 ± 0.28 b	3.37± 0.14 b	88.90±1.31a	20.91 ± 0.66 a	8.83±1.16c
MC2	6.72 ± 0.26 c	2.35 ± 0.13 c	83.21±1.82b	17.66 ±1.01 b	16.75±1.69b
MC3	4.15 ± 0.15 d	1.07 ± 0.07 d	65.25±2.79c	11.91 ± 0.69 c	20.50±2.42b
MC4	3.375 ± 0.12e	0.65 ± 0.06 e	52.16±2.76d	5.41 ± 0.60 d	29.58±4.27a

Means, within the same column, followed by the same letter are not statistically different according to the LSD test (P <0.05)

FCW: Fresh callus weight. CRG: callus relative growth. NPRC = Number of plantlets per regenerating callus. CRR: callus regenerating roots

CFW : LSD = 0.4897605	NPRC: LSD = 1.750303
CRG : LSD = 0.2236413	CRR: LSD = 4.667474
% de regeneration : 4.22404	

The germination of somatic embryos began after 4-6 days of culture on regeneration medium MR1 (Figure 1 c). This germination was sometimes accompanied by rooting. Different types of calli obtained on different media responded differently to culture conditions of the regeneration phase. Calli that lost their embryogenic capacity because of high ABA concentrations sometimes regenerated only roots that are sometimes with chlorophyll (Figure 1 d). The obtained regeneration percentages significantly decreased with increasing concentrations of ABA in the growth medium (Table 2). The highest percentages were obtained in calluses grown in media containing 1 µM ABA (88.91%) or control medium (88.73%). Calluses from medium MC4 containing the highest concentration of ABA showed a lower regeneration rate (52.17%).

Table 3. Effect of genotype on the studied characters.

Variety	Character				
	FCW (g)	CRG	% regeneration	NPRC	%CRR
Anouar	7.34±0.75 a	2.64±0.38 a	76.83±4.41 b	16.2±1.55 a	19.00±4.50a
Karim	6.38±0.71 b	2.19±0.35 b	71.79±5.23 c	15.80±2.01ab	16.60±2.40a
Sebou	7.08±1.00 a	2.53±0.50 a	72.32±3.44 c	14.46±1.42bc	17.33±3.56a
Ourgh	6.3±0.58 b	2.15±0.29 b	81.66±3.44 a	13.53±1.42c	8.53±1.29 b

Means, within the same column, followed by the same letter are not statistically different according to the LSD test (P <0.05)

FCW: Fresh callus weight. CRG: callus relative growth. NPRC = Number of plantlets per regenerating callus. CRR: calluses regenerating roots

CFW : LSD = 0.4380551	NRPC: LSD = 1.565518
CRG : LSD = 0.2236413	CRR: LSD = 4.174715
regeneration% : LSD = 3.778096	

Table 4. Effect of variety × ABA interaction for all studied characters.

Effect		Character				
variety	culture media	FCW	CRG	% regeneration	NPRC	%CRR
Anouar	MCT	10.9a	4.45 a	9.66 a	19.66 a	0 d
	MC1	9.50b	3.75 b	90.30 a	22.00 a	5.00 cd
	MC2	8.06 c	3.03 c	88.20 a	19 a	18.00 bc
	MC3	4.53 d	1.26 d	59.66 b	14 b	30.33 ab
	MC4	3.70 d	0.71 e	55.33 b	6.33 c	41.66 a
Karim	MCT	9.9 a	3.75 a	90.30 a	21.33 a	1.66 c
	MC1	8.53 b	3.26 b	91.33 a	23.00 a	13.00 b
	MC2	6.46 c	2.23 c	78.00 b	20.00 a	22.00 a
	MC3	4.00 d	1.00 d	56.00 c	11.00 b	20.66 a
	MC4	3.00 d	0.50 d	43.33 d	3.66 c	25.66 a
Sebou	MCT	13.16 a	5.58 a	82.30 a	17.33 a	1.33 c
	MC1	9.33 b	3.66 b	82.66 a	19.00 a	10.00 bc
	MC2	6.16 c	2.05 c	77.00 ab	18.33 a	17.00 b
	MC3	3.66 d	0.83 d	70.00 b	12.33 b	19.66 b
	MC4	3.06 d	053 d	49.66 c	5.33 c	38.66 a
Ourgh	MCT	9.5 a	3.75 a	91.66 a	18.00 ab	1.66 b
	MC1	7.66 b	2.83 b	91.33 a	19.66 a	7.33 ab
	MC2	6.20 b	2.10 b	89.66 a	13.33 bc	10.00 a
	MC3	4.40 c	1.20 c	75.33 b	10.33 cd	11.33 a
	MC4	3.73 c	0.86 c	60.33 c	6.33 d	12.33 a

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.

FCW: Fresh callus weight. CRG: callus relative growth. NPRC = Number of plantlets per regenerating callus. CRR: calluses regenerating roots.

The transfer of regenerating calluses to the hormone-free medium MR2 has enabled the development of roots and a healthy growth of the regenerated green plants (Figure 1 e, f). Few albino seedlings were also observed among varieties Anwar and Karim (Figure 1 g). After five weeks of culture on rooting medium MR2, and just before the transfer of plantlets to soil, the average number of plantlets per regenerating callus (NPRC) was also affected by the concentration of ABA in the medium (Table 2). The best yields of regenerated plantlets were obtained in calluses derived from MC1 medium containing 1 µM ABA (20.91) followed by those from the control medium (19.08).

Table 5. Analysis of variance (Mean square) for all studied characters from four durum wheat varieties**.**

		Character				
Source of variance	DF	FCW	CRG	% regeneration	NRPC	%CRR
Cultivar (A)	3	3.97 ***	0.909 ***	318***	22.6 **	326.4 ***
medium (B)	4	117.39***	29.690***	3192***	480.4***	1424.1 ***
AB	12	2.41 ***	0.620 ***	81**	9.2*	127.2 ***
Residuals	40	0.35	0.092	26	4.5	32.0

(***) Significatif à 0,1%, (**) à 1%, (*) à 5%

FCW: Fresh callus weight. CRG: callus relative growth. NPRC = Number of plantlets per regenerating callus. CRR: calluses regenerating roots.

High concentrations of ABA in the growth medium significantly reduce the NPRC down to 5.41 in calluses from medium MC4. Transfer of plantlets to the field was made after an acclimatization period of about 15 days. The transferred plantlets generally showed normal growth and maturity phenotype (Figure 1 h).

On medium MR1, the percentage of callus regenerating roots significantly increased with increasing concentrations of ABA in the growth medium (Table 2). The highest percentage of calluses with roots (29.58) was observed in calluses from medium MC4 containing 10 µM ABA. Calluses from the control medium without ABA MCT showed a very low percentage (1.16%).

Genotype effect

Significant differences were observed among genotypes for all parameters considered (Table 3). Calli of Anouar and Sebou varieties, for example, showed superior growth compared to other varieties (callus weight of respectively 7.34g and 6.38g), and superior relative growth (2.64 and 2.53 respectively).

About the morphogenetic capabilities, Ourgh variety showed the highest (81.66%) percentage of regeneration. The lowest percentages were recorded in Karim (71.79%) and Sebou (72.32) varieties. NPRC also differed among the four genotypes in the different media. It was highest in Anwar (16.2) and Karim (15.80), and lowest in Ourgh (13.58).

Rooting induced by high concentrations of ABA was also affected by genotype. Calli of varieties Anouar, Sebou and Karim showed percentages of calluses with roots of respectively 19, 17.33 and 16.60%. The lowest percentage (8.53%) was recorded in Ourgh variety.

Effect of variety × ABA interaction

The response of the four varieties to ABA concentration in the growth medium was substantially similar (Table 4).

The increase in concentration of ABA in the culture medium affected the response of the varieties in the same manner by a decrease in the growth of the callus, the percentage of regeneration and NPRC and by an increase in root regeneration. On growth, the highest results were observed in calluses from media without ABA (MCT) and lowest in the medium MC4 containing 10 µM in the four varieties studied. Also, the percentage of regeneration and NPRC decreased progressively in four varieties, reaching its lowest level in the medium MC4.

Regarding calli regenerating roots, the increase in the concentration of ABA enhanced the induction of roots on regeneration medium in the four varieties. In fact, the highest percentages of root regeneration were observed for the four genotypes at 10 µM ABA, and lower on the medium without ABA.

Par ailleurs des différences génotypiques ont été enregistrées par rapport à la réponse aux concentrations de l'ABA dans le milieu de culture. L'analyse de la variance (Table 5) montre en effet une interaction significative génotype/ milieu de culture pour l'ensemble des caractères étudiés.

Moreover, genotypic differences were recorded in relation to the response to concentrations of ABA in the culture medium. Analysis of variance (Table5) shows a significant interaction effect genotype / culture medium for all the characters studied.

Discussion

Growth regulators and their interactions are often considered important factors in in vitro morphogenesis (Gaspar *et al.,* 1996). However, the optimal concentrations which allow proper morphogenesis depend on several factors including genotype and nature of explant (Liu *et al.,* 1990). A proper hormonal balance in the culture medium is necessary to promote the induction of somatic embryogenesis and subsequently stimulate the regeneration of plants. The addition of ABA, a key component of plant adaptation to abiotic stress, in the culture medium appears to interact with other regulators such as cytokines and auxins (Gaspar *et al.,* 1996). Its use in the induction medium inhibits zygotic germination and at the same time promotes the maturation of embryoids (Querschi *et al.,* 1989).

The study we undertook clearly showed the effect of ABA on callus growth and in vitro morphogenesis in four varieties studied. The results showed that high concentrations are detrimental to callus growth and regeneration. Our results are in agreement with those reported by Fazelienasab *et al.* (2012). These authors showed that the increase of ABA in the callogenesis medium significantly reduced callus growth and rate of regeneration from mature soft wheat embryos.

Adding low concentrations of ABA in the medium improved the yield in plants regenerated per callus. This improvement may be explained by the role played by this hormone in stimulating embryoids development and maturation. Indeed, the beneficial role of this hormone in the maturation of somatic embryos has been reported both in Dicots (Li and Wolyn, 1996) and in Monocots (Carman *et al.,* 1987, Brown *et al.,* 1989; Rai *et al.,* 2011).

The ABA also enhances the ability of embryogenesis by reducing the rate of abnormal embryoids while promoting normal embryonic development (Carman *et al.,* 1987; Querschi *et al.,* 1989). The increase in the number of plants regenerated per callus by adding low concentrations of ABA can be interpreted by the increase in the number of somatic embryos produced. In fact, Brown *et al.* (1989) reported that the addition of low concentrations of ABA (0.4 to 4 µM) increased the callus embryogenic surface in soft wheat. ABA promotes embryoids maturation probably through LEA proteins accumulation. The correlation between the accumulation of these proteins and the development of somatic embryos was indeed reported (Wurtel *et al.,* 1993; Kiyosue *et al.,* 1993).

The results revealed that the addition of ABA at high concentrations in the medium increases the percentage of callus root regeneration. The works of Abou-Mandour and Hartung (1986) have indeed indicated that treating calluses with ABA increases the percentage of root induction from calluses in maize. Also, in soybean young shoots, the application of ABA at low concentrations inhibited shoot growth, whereas root elongation was slightly stimulated (Creelman *et al.,* 1990).

The effect of ABA on callus growth and regeneration is substantially similar in all four varieties. This observation has also been reported in wheat from mature embryos (Fazelienasab *et al.,* 2012). The observed Genotypic differences with respect to callus growth and morphogenesis may be explained by the

variation in endogenous tissue levels of this hormone in the different varieties studied. These differences may also be explained by genotypic differences in tissue sensitivity to exogenous ABA.

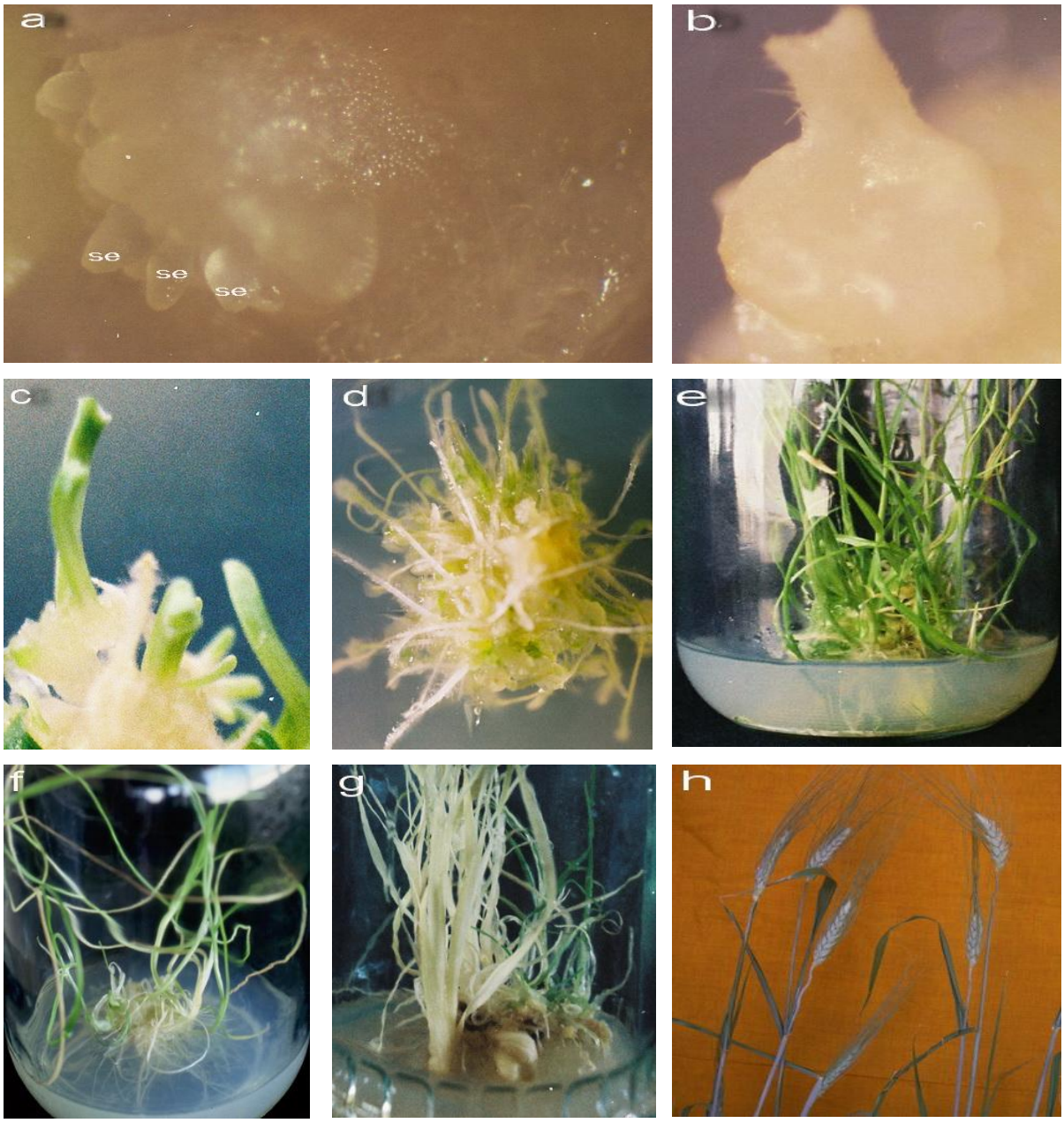

Fig. 1. Somatic embryogenesis and regeneration from calluses on different media.
(a) and (b) maturation of embryoids on MC1 medium. se: somatic embryo (c) Germination of somatic embryos on MR1 medium. (d) Calli regenerating only roots on MR1 medium. (e) Regeneration on MR2 from calluses of MC1 medium (f) Regeneration of green plantlets from calluses of MC3 medium. (g) Regeneration of albino and green plantlets on MR2 medium. (h) Mature plants of normal phenotype after *ex vitro* transfer.

It is also known that the content of endogenous ABA in explants differs with their age. This content decreases and they develop (Morris *et al.,* 1988). A correlation between the age of the embryo and the endogenous levels of growth regulators, particularly ABA, was reported by Qureshi *et al.* (1989). In fact, their work showed that embryos at early stages synthesize enough ABA, which consequently stimulates somatic embryogenesis and decreases the ability of early embryo germination. Mature embryos, on the contrary, contain less ABA, which promotes germination in culture medium.

In addition, it was reported that the level of endogenous ABA in zygotic embryos decreases during embryonic development whereas the sensitivity to this hormone increases (Finkelstein *et al.,* 1985). Studies have shown that the levels of ABA in somatic embryos are much smaller than in zygotic ones (Gawronska *et al.,* 2000, George *et al.,* 2008). The explanation of the effects of ABA on morphogenesis in vitro, however, remains limited due to the complexity of this hormone. Indeed, ABA is involved in a number of physiological processes during the plant life, including the adaptation of plants to various stresses, such as dehydration, salinity, cold or injury. Its use as a selective agent is poorly documented.

Furthermore, chlorophyll deficiency or albinism is a standard marker of variation in the cytoplasmic genome. This variation is common in androgenesis in durum wheat and is a major problem for the application of haplodiploidisation in breeding programs of this species (Ghaemi and Sarrafi, 1994). In somatic embryogenesis, conversely, few reports have mentioned regeneration of albinos (Maddock *et al.,* 1983; Bouiamrine *et al.,* 1999; Bouiamrine *et al.,* 2012b). In our culture conditions, the number of albino plantlets regenerated remained very low.

Conclusion

MCT media (without ABA) and MC1 (containing 1 µM ABA) are favorable for callus growth and regeneration in the varieties studied. The medium MC1 proved beneficial for plant regeneration by allowing the increase of both the percentage of regeneration and the number of plantlets per regenerating callus. The gradual increase of ABA in the growth medium has a negative effect on all the studied parameters. The low concentration of ABA (1 µM) will be chosen to be combined with PEG (polyethylene glycol) in our future work.

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

Abou-mandour AA, Hartung W. 1986. The effect of abscisic acid and increased osmotic potential of the media on growth and root regeneration of Zea mays callus. Journal of Plant Physiology **122(2),** 139-145, http://dx.doi.org/10.1016/S0176-1617(86)80054-2

Bennici A, Caffaro L, Dameri RM, Gastaldo P, Profumo P. 1988. Callus formation and plantlet regeneration from immature *Triticum durum*. Euphytica **39(3),** 255-263, http://dx.doi.org/10.1007/BF00037104

Benkirane H, Sabounji K, Chlyah A, Chlyah H. 2000. Somatic embryogenesis and plant regeneration from fragments of immature inflorescences and coleoptiles of durum wheat. Plant Cell, Tissue and Organ Culture **61,** 2107-113.

Bouiamrine EH, Mzouri K, Amssa M. 1999. Effet du génotype et du milieu de culture dans la culture d'embryons immatures de blé dur (*Triticum durum* Desf.) et de blé tendre (*Triticum aestivum* L.). In: Aupelf-Uref ed.. Actualités Scientifiques, Biotechnologies, amélioration des plantes et Sécurité alimentaire. Estem, Paris, 533-534. (In French).

Bouiamrine EH, Diouri M, El-Halimi R. 2012a. Somatic embryogenesis and plant regeneration capacity from mature and immature durum wheat embryos. International Journal of Biosciences **9(2),** 29-39.

Bouiamrine EH, Diouri M, El-Halimi R. 2012b. Assessment of somaclonal variation in regenerated plants from immature embryos culture of durum wheat. International Journal of Agriculture and Biology **14,** 941-946.

Brown FC, Brooks FJ, Pearson D, Mathias RJ. 1989. Control of embryogenesis and organogenesis in immature wheat embryo callus using increased

medium osmolarity and abscisic acid. Journal of Plant Physiology **133(3),** 727-733, http://dx.doi.org/10.1016/S0176-1617(89)80080-X

Carman JG, Jefferson NE, Campbell WF. 1987. Induction of embryogenic *Triticum aestivum* L. calli. II. Quantification of organic addenda and other culture variable effects. Plant Cell Tissue and Organ Culture **10(2),** 115-128, http://dx.doi.org/10.1007/BF00035909

Creelman RA, Mason HS, Bensen RJ, Boyer JS, Mullet JE. 1990. Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings. Plant Physiology **92,** 205-214.

Fazelienasab B, Omidi M, Amiritokaldani M. 2012. Callus induction and plant regeneration of wheat mature embryos under Abscisic Acid treatment. International Journal of Agriculture and Crops Sciences **4 (1),** 17-23.

Finkelstein RR, Tenbarge KM, Shumway JE, Crouch ML. 1985. Role of ABA in maturation of rapeseed embryos. Plant Physiology **78,** 630-636.

Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. 1996. Plant hormones and plant growth regulators in plant tissue culture. *In Vitro* Cellular and Developmental Biology-Plant **32,** 272-289, http://dx.doi.org/10.1007/BF02822700

Gawronska H, Burza W, Bolesta E, Malepszy S. 2000. Zygotic and somatic embryos of cucumber (*Cucumis sativus* L.) substantially differ in their levels of abscisic acid. Plant Science **157,** 129-137, http://dx.doi.org/10.1016/S0168-9452(00)00277-6

George EF, Hall MA, De Klerk GJ. 2008. Plant Propagation by Tissue Culture, Vol. 1, Third Edition, Springer, Dordrecht, Netherlands, 501.

Hess JR, Carman JG. 1998. Embryogenic competence of immature wheat embryos: genotype, donor plant environment, and endogenous hormone levels. Crop Science **38,** 249-253.

Ghaemi, M, Sarrafi A. 1994. The effect of the 'D' genome from synthetic wheat lines in anther culture response. Plant Breeding **112(1),** 76-79, http://dx.doi.org/10.1111/j.1439-0523.1994.tb01279.x

Jiménez VM. 2005. Involvement of plant hormones and plant growth regulators on in vitro somatic embryogenesis. Plant Growth Regulation. **47,** 91-110.

Kiyosue T, Yamaguchi-Shin K, Shinozaki K, Kamada H, Harada H. 1993. cDNA cloning of ECP40, embryogenic-cell protein in carrot, and its expression during somatic and zygotic embryogenesis. Plant Molecular Biology **21,** 1053-1068.

Kumari M, Patade VY, Arif M, Ahmed Z. 2010. Effect of iba on seed germination, sprouting and rooting in cuttings for mass propagation of Jatropha Curcus L strain DARL-2. INSInet Publication. Research Journal of Agriculture and Biological Sciences. **6(6),** 691-696.

Li B, Wolyn DJ. 1996. Abscisic acid and ancymidol promote conversion of somatic embryos to plantlets and secondary embryogenesis in Asparagus officinalis L. *In Vitro* Cellular & Developmental Biology - Plant. **32 (4),** 223-226, http://dx.doi.org/10.1007/BF02822691

Liu HJ, Misso SH, Kamijima O, Sawano M. 1990. Effects of plant growth regulators on the response of immature wheat embryo culture. Plant Tissue Culture Letters **7,**170-176.

Lu DB. 1988. Charactenzation of abscisic acid-induced heat and drought tolerant wheat plants selected from tissue culture. Ph.D dissertation, Kansas State, USA, 80.

Maddock SE, Lancaster VA, Risiott R, Franklin J. 1983. Plant regeneration from cultured

immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum* L.). Journal of Experimental Botany **34,** 915-926.

Morris PC, Kumar A, Bowles DJ. 1990. Osmotic stress and abscisic acid induce expression of the of the wheat Em genes. European Journal of Biochemistry **190,** 625-630.

Morris PC, Maddock SE, Jones MGK, Bowles DJ. 1986. Lectin levels in tissues of cultured immature wheat embryos, Plant Cell Report **5,** 460-463.

Morris PC, Weiler EW, Maddock SE, Jones MGK, Lenton JR, Bowles DJ. 1988. Determination of endogenous abscisic acid levels in immature cereal embryos during *in vitro* culture. Planta **173(1),** 110-116, http://dx.doi.org/10.1007/BF00394495

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15,** 473-497.

Mzouri K, Amssa M, Bouiamrine EH. 2001. Somatic embryogenesis from immature embryos of wheat cultivars (*Triticum aestivum* L.): genotype effect. Acta Botanica Gallica **148(3),** 215-225.

Mzouri K, Amssa M. 2002. Amélioration de l'embryogenèse somatique à partir d'embryons immatures chez le Blé tendre (*Triticum aestivum* L.). II : Effet des régulateurs de croissance sur la callogenèse. Acta Botanica Gallica **149(4),** 357-368. (In French).

Ozias-Akins P, Vasil IK 1982. Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L. (wheat): evidence for somatic embryogenesis. Protoplasma **110,** 95-105.

Pospisilova J, Synkova H, Haisel D, Semoradova S. 2007. Acclimation of plantlets to *ex vitro* condition: Effects of air humidity, irradiance, CO2 concentration and abscisic acid (a review). Acta Horticulturae **748,** 29-38.

Qureshi JA, Kartha KK, Abrams SR, Steinhauer L. 1989. Modulation of somatic embryogenesis in early and late-stage embryos of wheat (*Triticum aestivum* L.) under the influence of (plus orminus) abscisic acid and its analogs. Plant Cell Tissue and Organ Culture **18,** 55 - 69.

Rai MK, Shekhawat NS, Harish, Gupta AK, Phulwaria M, Ram K, Jaiswal U. 2011. The role of abscisic acid in plant tissue culture: a review of recent progress. Plant Cell, Tissue and Organ Culture **106,** 179-190.

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.

Redway FA, Vasil V, Lu D, Vasil IK. 1990. Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **79(5),** 609-617, http://dx.doi.org/10.1007/BF00226873

Ren J, Wang X, Yın J. 2010. Dicamba and Sugar Effects on Callus Induction and Plant Regeneration from Mature Embryo Culture of Wheat. Agricultural Sciences in China **9 (1),** 31-37, http://dx.doi.org/10.1016/S1671-2927(09)60064-X

Sapina NF, Karasev GS, Trunova TI. 1994. Abscisic acid as an inducer of freezing tolerance in wheat cell suspension cultures. Russian Journal of Plant Physiology **41,** 546-551.

Satyavathi VV, Jauhar PP, Elias EM, Rao MB. 2004. Effects of Growth Regulators on *In Vitro* Plant Regeneration in Durum Wheat. Crop Science **44,** 1839–1846.

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and procedures of statistics - a biometrical approach (3rd edition). McGraw Hill Book Co. Inc., New York, USA, 400-428.

Tamás C, Szucs P, Rakszegi M, Tamás L, Bedo Z. 2004. Effect of combined changes in culture medium and incubation conditions of the regeneration from immature embryos of elite varieties of winter wheat. Plant Cell Tissue and Organ Culture **79,** 39-44.

Vendruscolo ECG, Schuster I, Negra ES, Scapim CA. 2008. Callus induction and plant regeneration by Brazilian new elite wheat genotypes. Crop Breeding and Applied Biotechnology **8,** 195-201.

Wurtele ES, Wang H, Durgerian S, Nikolau BJ, Ulrich TH. 1993. Characterization of a gene that is expressed early in somatic embryogenesis of *Daucus carota*. Plant Physiology **102,** 303–12.