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Regeneration of haploid green plants through anther culture in *durum* wheat (*Triticum turgidum* ssp. *durum*) using liquid media

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إنتاج نباتات خضراء أحادية الصيغة الصبغية بواسطة زرع مآبر القمح الصلب(Triticum turgidum var. durum) في وسط سائل

تمت محاولة جديدة للحصول على نباتات خضراء أحادية الصيغة الصبغية وذلك بواسطة زرع مآبر القمح الصلب مستعملين وسطا سائلا وعددا من الأنماط الوراثية المغربية.تمت دراسة تسعة أصناف "سبو، كوكوريت، مرزاق، كريم، سريف، أم الربيع، ماسة، أسلي، تانسيفت" إستعملت الأوساط السائلة التالية :BPTG، C17و BAC1 هذا الأخير يحتوي على الكلوكوز زيادة على السكروز وتركيز من 2,4D أكثر من الآخرين. خمسة أصناف أعطت نباتات بنسب تتراوح بين 29,7% (سبو) و 5,3% (كريم) غير أن الصنفين سبو وماسة وحدهما أنتجا نباتات خضراء. ولوحظ أن BPTG أعطى أكبر نسبة من تكوين الأجنة. رغم أن نسبة إنتاج النباتات كانت 6,9% في وسط BPTGو 7,9% في وسط C17 لم نحصل إلا على نبات أخضر واحد والباقي كان غير أخضر. أمافي الوسط BAC1 وصلت نسبة إنتاج النباتات إلى 30,3% منها 18,2% من النباتات الخضراء (ستة نباتات خضراء) مع العلم أن خمسة نباتات خضراء أحادية الصيغة الصبغية المكررة (Double haploide) بلغت حد الإكتمال وأنتجت بذور.

الكلمات المفتاحية : *Triticum turgidum* var. *durum*– القمح الصلب – زرع المآبر – تكوين الأجنة – إنتاج النباتات – نباتات خضراء أحادية الصيغة الصبغية المكررة

Régénération de plantes vertes haploïdes par culture d'anthères du blé dur (*Triticum turgidum* var. *durum*) en milieu liquide

Une nouvelle tentative d'obtention de plantes haploïdes vertes par culture d'anthères du blé dur a été entreprise en utilisant un milieu liquide et plusieurs génotypes marocains dont certains encore non testés. Neuf cultivars de *Triticum turgidum* var. *durum* sont étudiés : 'Sebou', 'Cocorit', 'Marzak', 'Karim', 'Sarif', 'Oum Rabia', 'Massa', 'Isly' et 'Tensift'. Les milieux liquides testés sont BPTG, C17 et BAC 1; ce dernier contenait du glucose en plus du saccharose et une concentration plus élevée en 2,4-D que les autres milieux. La régénération de plantes s'est produite pour cinq cultivars à des taux variant de 29,7 % ('Sebou') à 5,3 % ('Karim') mais des régénérations vertes n'ont été observées que pour des génotypes 'Sebou' et 'Massa'. Le milieu BPTG a donné un pourcentage de formation d'embryons supérieur à ceux du C17 et du BAC 1. Les taux de régénération étaient faibles pour les milieux BPTG (6,9 %) et C17 (7,9 %) et seule une régénération verte a été observée, le reste étant albinos. La régénération a atteint 30,3 % dans le cas du milieu BAC 1 et six plantes (18,2 %) étaient vertes. Cinq plantes haploïdes doublées vertes ont atteint la maturité et produit des graines.

Mots clés : *Triticum turgidum* var. *durum* - Blé dur - Culture d'anthères- Embryons androgénétiques- Régénération - Plantes vertes haploïdes doublées

Regeneration of haploid green plants through anther culture in *durum* wheat (*Triticum turgidum* ssp. *durum*) using liquid media

A new attempt has been made to obtain haploid green plants through anther culture in durum wheat, using liquid media and some as yet untested genotypes grown in Morocco. Nine cultivars of *Triticum turgidum ssp. durum* were studied: 'Sebou', 'Cocorit', 'Marzak', 'Karim', 'Sarif', 'Oum Rabia', 'Massa', 'Isly' and 'Tensift'. The liquid media tested were BPTG, C17 and BAC 1, this last containing glucose as well as sucrose and a higher concentration of 2,4-D. Plant regeneration occurred in five cultivars at rates ranging from 5.3% ('Karim') to 29.7 % ('Sebou') but green regenerations were only observed in 'Sebou' and 'Massa'. BPTG gave a higher percent of embryo formation than C17 or BAC 1. Although regeneration rates were 6.9 % and 7.9 %, respectively, for BPTG and C17 media, only one green plant was obtained, the rest were albinos. For BAC 1 medium, regeneration attained 30.3 %, of which six plants (18.2 %) were green. Five doubled haploid green plants were grown to maturity and produced seed.

Key words : *Triticum turgidum ssp. durum*- Durum wheat - Anther culture - Androgenic embryos - Regeneration - Doubled haploid green plants

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INTRODUCTION

Androgenesis through *in vitro* anther culture is the method the most often used to obtain haploids and doubled haploids in cereals (Picard *et al.*, 1994). The bread wheat 'Florin', obtained using this method, was registered in the French catalogue many years ago (De Buyser *et al.*, 1987). Progressive improvements in this technique have brought about higher rates of haploid embryo and plant formations (Foroughi-Wehr & Zeller, 1990 ; Picard *et al.*, 1994).

Androgenesis in wheat species has been shown to be dependent on genotype (El Haddoury *et al.*, 1993), physiological state of the donor plant (Lazar *et al.*, 1990 ; Lu *et al.*, 1991), microspore stage of development and culture conditions (Ouyang, 1986; Henry & De Buyser 1990). Among many physical and chemical factors, a cold pretreatment of wheat spikes has been shown to have a positive effect on androgenesis (Ouyang *et al.*, 1987).

The tetraploid *durum* wheat has been shown to produce few embryosfrom anthers and very few or no green plants (Foroughi-Wehr & Zeller 1990). This species is particularly prone to albinism with a rate approaching 100% in most cultivars (Cattaneo *et al.*, 1991) compared with *aestivum* wheats often producing over 30 % green plants.

The first results on androgenesis in *durum* wheat obtained in our laboratory were achieved on agar media (Chlyah & Saidi, 1991). In spite of the culture of over 60000 anthers and the high rates of embryo formation (up to 22.4 %) and regeneration obtained (up to 37.5 %), only one green plant was regenerated, the rest being albinos. The positive effect on androgenesis of liquid medium, with the addition of ficoll as supporting agent, has been shown by Lettre *et al.* (1990) and Trottier *et al.* (1993) for bread wheat. The latter authors also showed the beneficial effect of adding amino acids to the induction medium.

In this study, we have attempted to improve the rate of haploid green plant formation in *durum* wheat using three liquid induction media containing starch as supporting agent, and several as yet untested genotypes grown in Morocco.

MATERIAL & METHODS

Nine genotypes of *durum* wheat varieties grown in Morocco were provided by the Guich Station, I.N.R.A., Rabat: 'Cocorit', 'Isly', 'Karim', 'Marzak', 'Massa', 'Oum Rabia', 'Sarif', 'Sebou' and 'Tensift'. Seeds were sown from November to January. Spikes were excised when the tip of the growing head reached the upper third of the sheath: microspores were then at the mid-uninucleate, vacuolized stage. Spikes were usually kept at 4°C for 2 to 8 days. After the cold pretreatment, spikes were surface sterilized with a 4 % sodium hypochlorite solution for 7 mn, and rinsed 3 times in sterile distilled water. Anthers were excised and placed at the surface of the liquid induction medium.

Three basal media, defined in the following references, were used: C17 (Wang & Chen, 1986), BPTG (Chuang *et al.*, 1978) and BAC 1 (Marsolais & Kasha, 1987; Trottier *et al.*, 1993). C17 contains macro and microelements as well as biotin and 2,4-dichlorophenoxyacetic acid (2,4-D) at 2 $mg.l^{-1}$. The BPTG medium does not contain microelements and is supplemented with potato extract, vitamins, particularly inositol, as well as 2,4-D (2 $mg.l^{-1}$), and kinetin (0.5 $mg.l^{-1}$) except for medium 6 (Table 1) which contains 0.2 $mg.l^{-1}$ 2,4-D, 1$mg.l^{-1}$ naphtalene acetic acid (NAA) and 2 $mg.l^{-1}$ benzyladenine (BA). The BAC 1 medium is composed of macro and micro nutrients enriched with inositol, vitamins, organic acids, and a relatively high concentration of 2,4-D (8 $mg.l^{-1}$). The first two induction media contain 9 % sucrose (except medium 6 which contains 9 % glucose) whereas BAC 1 contains 6 % sucrose and 1.75 % glucose. Casein hydrolysate and a few amino acids were also added in various combinations to some of these three media: 17 variants were tested in all (Table 1). Starch (2.8 %) was used as support agent in the case of C17 and BAC 1 media. When no starch was present, the produced embryos generally sank into the medium and browned. Since the BPTG medium containing potato extract was quite buoyant, variants of this medium were tested without starch.

Approximately 90 anthers obtained from 2 or 3 spikes of the same genotype were placed in petri dishes (5 cm) containing three ml of autoclaved culture medium.

After 3 to 6 weeks incubation at 27°C in the dark, the androgenic embryos which appeared were transferred to the agar regeneration medium R9 (M3 medium in Picard & De Buyser, 1977) in petri dishes and kept at 22°C under 16h of light per day. After about a month, young green plants were transferred to tubes containing R9 medium supplemented with 1 mg. l^{-1} indole acetic acid (IAA). When plants reached approximately 10 cm and had a good root system, they were placed in

individual pots containing organic soil, sand and peat.

Table 1. Composition of media with starch (%) and amino acid (mg.l^{-1}) additives for anther culture

Basal medium	Medium N°	Starch %	Cas. hydr.	Glu. mg.l^{-1}	Cyst. mg.l^{-1}	Gly. mg.l^{-1}	Arg. mg.l^{-1}
BPTG	1	-	-	-	-	-	-
	2	-	-	-	20	-	-
	3	-	-	500	-	-	-
	4	-	-	500	20	-	-
	5	-	-	-	20	-	60
	6	-	-	-	-	-	-
C17—	7	2,8	-	-	-	-	-
	8	2,8	-	1000	-	-	-
	9	2,8	-	-	20	-	-
	10	2,8	-	-	-	-	20
	11	2,8	-	1000	20	-	-
	12	2,8	-	1000	20	20	20
BAC 1	13	2,8	300	-	-	-	-
	14	2,8	300	2000	20	-	20
	15	2,8	300	-	20	-	-
	16	2,8	-	2000	20	20	20
	17	2,8	300	2000	20	20	20

Cashydr: Casein hydrolysate;
Glu : Glutamine;
Cyst: Cysteine;
Glyc: Glycine; Arg: Arginine
[a] Different from medium n ° 1 by its hormone content (see text)

Chromosome doubling took place 2 or 3 weeks later when tillering had started. Roots were immersed for 4h in a 0.2 % colchicine solution and rinsed three times with water before replanting. When plants attained 20-25 cm, they were placed in bigger pots and kept in a glasshouse until seed maturation.

Since all genotypes were cultured on the three basal media, all results related to genotype effect are presented as means of all media. Results relating to media effects are compounded from all genotypes.

The X^2 test ($p = 0.05$) was used to appreciate the statistical significance of the genotypic effect on androgenetic embryo formation. A test of comparison of percentages was employed to compare different media, two by two, as to their relative effects on androgenetic embryo formation. The variable between two percentages (P1 and P2) from independant samples was calculated using the following formula :

$$\frac{d}{\delta d} = \frac{P_1 - P_2}{\sqrt{P_0 q_0 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

When $d/\delta d < 2$, the difference is significant ($p = 0.05$); if $d/\delta d > 2$, the difference is not significant.

RESULTS & DISCUSSION

1. Morphogenic observations

After 3 or 4 weeks of culture on the induction medium, rounded androgenic embryos could be seen under a microscope emerging from the anthers (Figure 1). After transfer to the regeneration medium, varied responses were recorded. Some produced only roots or callus which was occasionally accompanied by green, leafy structures. Others continued their development forming either albino plantlets (Figure 2), or chimera regenerations with green and white striped leaves. Others still turned green during embryo development (Figure 3) and formed green plantlets (Figure 4). Only green plantlets developed into adult plants after chromosome doubling (Figure 5) and produced seeds (Figure 6).

2. Genotypic effects

The important effect of genotype (genetic capacity) was confirmed in our experiments. The percentage of embryos (embryos per 100 anthers) obtained varied from 7.1 % for 'Sebou' to 0.5 % for the cultivar 'Tensift' (Table 2). These differences due to genotype were highly significant ($P = 0.01$ level using the chi-square test). All varieties showed some androgenic capacity. This genotypic effect was even more marked concerning regeneration (Table 2). Apart from four cultivars which showed no regeneration, the rate of regeneration varied from 29.7 % for 'Sebou' to 5.3 % for 'Karim'. Given the small numbers of embryos involved, regeneration values must be taken with precaution. Only two cultivars gave green regenerations: 'Sebou' and 'Massa'. Of the seven green plants formed by the 'Sebou' cultivar, four survived and grew into adult plants, as did the one formed by the cultivar 'Massa'.

The genotypic effect on androgenesis has already been shown in a previous study on twenty Moroccan cultivars of *durum* wheat (four of which are included in the present study) on solid media (Chlyah & Saidi, 1991). In *Triticum aestivum*, fifty

Figure 1. Androgenic embryos formed in anther culture after 5 weeks of culture on BAC 1 medium. Bar : 1 mm

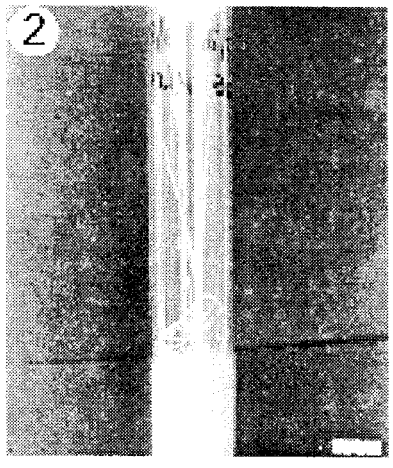

Figure 2. Albino plant (cv. 'Cocorit') on R9 medium, after 9 weeks of culture. Bar : 2 cm

Figure 3. Early embryo development and greening on medium R9 (cv. 'Massa'). Bar : 1 mm

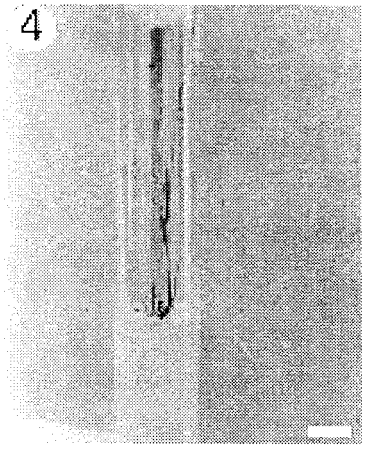

Figure 4. Green plant ('Sebou') on R9 medium after 10 weeks culture. Bar : 2 cm

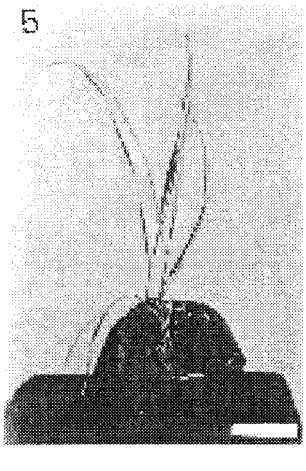

Figure 5. Green plant ('Sebou') after transfer to soil and chromosome doubling. Bar: 5 cm

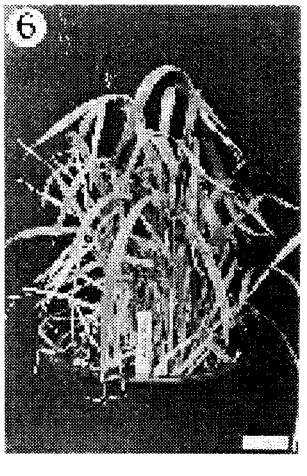

Figure 6. Tillering and seed formation in doubled haploids of cultivar 'Sebou'. Bar : 5 cm

spring cultivars showed large differences in androgenic capacity with a clear superiority of cultivars carrying the 1BL/1RS wheat-rye translocation known for its positive effect on the rate of androgenesis (Foroughi-Wehr & Zeller, 1990). Another study on nine cultivars of *T. turgidum* ssp.*durum* showed that only one of these, into which the 1BL/1RS translocation had been introduced, produced 2 green plants from 124 embryos formed (Cattaneo *et al.*, 1991).

In *durum* wheat, the induction of haploid embryo formation in anther culture is relatively easy to achieve whereas green regeneration remains a major problem. Except for 'Sebou', almost 100 % of the plants obtained were albinos. These results support the idea of separate genetic capacities for regeneration in general and for green regeneration in *durum* wheat as Henry *et al.*, (1994) have already shown.

Table 2. Genotypic effects on embryogenesis and regeneration in anther culture on liquid media (all media grouped)

Genotype	Number of anthers	Number of embryos	% emb.[a]	Regenerations	% regen.[b]	% green regen.[c]
Sebou	519	37	7.1	7 green plants 3 albino plants 1 chimera 1 green callus 2 roots	29.7	18.9
Marzak	1190	41	3.4	4 roots	0	0
Cocorit	2380	52	2.2	8 albino plants 1 chimera 10 roots	17.6	0
Karim	1098	22	2.0	1 albino plant 1 root	5.3	0
Sarif	859	17	2.0	2 albino plants 1 root	11.8	0
Massa	3291	64	1.9	1 green plant 1 albino plant 1 chimera 1 root	4.3	1.4
Isly	1069	8	0.8	0	0	0
Oum Rabia	2097	15	0.7	0	0	0
Tensift	1071	5	0.5	2 roots	0	0

[a] embryos per 100 anthers; [b] total plants (green, albino, chimera) formed per 100 embryos; [c] green plants formed per 100 embryos

3. Effect of culture medium

Embryo induction on three different basal liquid media showed that BPTG was the most favorable with a significantly superior yield (3.1 %) followed by C17 (1.2 %) and BAC 1 (1.1 %) (Table 3). These results are low when compared with those obtained on the former two media when solidified with agar (15.5 and 23.8 % respectively) (Chlyah & Saidi 1991).

After transfer to solid R9 medium, the highest percent regeneration was obtained when the induction medium was BAC 1: 30.3 % of which 18.2 % were green regenerations. With the two other induction media, total regeneration was significantly lower with 6.9 % for BPTG and 7.9 % for C17. Only one green plant (0.5 %) was observed in BPTG and one non viable plant (not surviving the 4 leaf stage) was obtained in C17 (2.6 %). Our results confirm the beneficial effect of BAC 1 medium, which result has also been obtained in the case of *aestivum* wheat (Trottier *et al.*, 1993).

More detailed results showing the effects of various amino acid additives are presented in Table 4. Among the BPTG variants, medium 6 with no amino acids (but with a richer hormonal composition and sucrose replaced by glucose) gave the highest rate of embryo formation (16.6 %). The five other BPTG variants, including medium 1 which was also lacking in amino acids, gave much

lower percentages (0.6 - 3.3 %). Medium 3, containing only glutamine, gave the highest total regeneration in this group but none were green plants. Medium 6 was the only BPTG medium in which a green plant was regenerated.

For C17 variants, medium 7, lacking in amino acids, gave the highest percentage embryos (16.9%) but no green plants. Medium 8, containing only glutamine, gave one green regeneration which did not develop. The other C17 variants containing one to four amino acids, gave few embryos and no regenerations.

Concerning BAC 1 variants, media 15 and 13 (with casein hydrolysate and respectively one or no other amino acid) gave higher rates of embryo formation than variants with three or more amino acids. Highest total regeneration was achieved in media 13 (30.8 %) and 14 (55.5 %) which produced respectively one and five green regenerations. On medium 14, containing casein hydrolysate and three amino acids, four viable green plants were recovered. This result seems to show a positive effect of amino acids in the BAC 1 medium on green regeneration. However, the genotype might be an important interacting factor since all five plants obtained are of the 'Sebou' cultivar.

Despite the fact that yields of embryos were generally lower on the liquid induction media compared with our earlier results for *durum* wheat on agar media, the number of green plants obtained was higher. Eight green plants were recovered from approximately 12400 cultured anthers whereas only one had been obtained after agar culture of about 60000 anthers (Chlyah & Saidi, 1991). Five of these green plants have grown to maturity and produced seed after chromosome doubling.

Table 3. Action of three culture media on regeneration in anther culture (all genotypes grouped)

Medium	Number of anthers	Number of embryos	% embryos	Total regeneration	% regeneration	Number of green regenerations	% green regenerations	Number regeneration viable green plants
BPTG	6058	189	3.1	13	6.9	1	0.5	1
C17	3299	38	1.2	3	7.9	1	2.6	0
BAC 1	3046	33	1.1	10	30.3	6	18.2	4

BPTG - C17: d/ $\delta d = 5.92$ 2. The difference is significant at 0.05. ; BPTG - BAC 1: d/ $\delta d = 5.97$ 2. The difference is significant at 0.05.
C17 - BAC 1: d/ $\delta d = 0.31$ 2. The difference is not significant.

Table 4. Results of anther culture for three basic media containing various combinations of amino acids

Basal medium	Medium N°	Number of anthers	Number of embryos	%	Total nuber of embryos	% regeneration*	% green regeneration	Number of viable green regenerations plants	
BPTG	1	1425	43	3.0	2	4.7	0.0	0	
	2	877	29	3.3	2	6.9	0.0	0	
	3	1167	25	2.1	6	24.0	0.0	0	
	4	1331	37	2.8	2	5.4	0.0	0	
	5	963	6	0.6	0	0.0	0.0	0	
	6	295	49	16.6	1	2.0	2.0	1	
C17	7	89	15	16.9	2	13.3	0.0	0	
	8	412	13	3.2	1	7.7	7.7	0	
	9	225	0	0.0	0	0	0.0	0	
	10	378	1	0.3	0	0	0.0	0	
	11	316	5	1.6	0	0	0.0	0	
	12	1879	0	0.0	0	0	0.0	0	
BAC 1	13	519	13	2.5	4	30.8	7.7	0	
	14	451	9	2.0	5	55.5	55.5	4	
	15	327	10	3.1	1	10.0	0.0	0	
	16	851	0	0.0	0	0.0	0.0	0	
	17	898	5	0.6	0	0.0	0.0	0	

* Green, chimera or albino plants

According to Lettre *et al.* (1990), liquid media increases the number of viable embryos by avoiding the presence of toxic impureties contained in agar. The low survival rate of microspores in anther culture of wheat would be the cause of low rates of embryo formation (Tuvesson *et al.*, 1991) and these tended to improve in liquid media.

The support agent used can also affect the obtained results: Lettre *et al.* (1990) showed that ficoll was consistently better than agar and Trottier *et al.* (1993) found that a liquid ficoll medium was superior to a gelatinous one (ficoll and gelrite) or an agar medium. Our results showed that starch can also be effective as support agent.

In spite of the efforts of many research groups, the numbers of green plants of *durum* wheat regenerated through anther culture remain relatively low, except in very specific genotypes, such as those carrying the 1BL/1RS translocation. Work is presently being carried out in our laboratory on transferring this translocation from *aestivum* to *durum* wheat through intraspecific crosses (Chlyah *et al.*, in press).

Another promising technique for obtaining haploids in *durum* wheat is wide crossing with *Hordeum bulbosum* or with maize to induce haploidy after chromosome elimination. This technique, already successfully applied to *aestivum* wheat (Snape *et al.*, 1979 ; Laurie & Bennett, 1988 ; Laurie & Reymondie, 1991), has shown promise in *durum* wheat (O'Donoughue & Bennett, 1994 ; Chlyah *et al.*, in press). All plants produced through this technique were green .

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REFERENCES

Cattaneo M., Qias Y.M. & Pogna N.E. (1991) Embryoid induction and green plant regeneration from cultured anthers in a *durum* wheat line homozygous for the 1BL/1RS translocation. *J. Genet. and Breed.* 45: 369-372.

Chlyah H. & Saidi N. (1991) Analyse des capacités androgénétiques de génotypes marocains de *Triticum durum*. In: AUPELF-UREF (ed.), Amélioration des plantes pour l'adaptation aux milieux arides, 135-148, John Libbey Eurotext, Paris

Chlyah H., Saidi N., Cherkaoui S. & Chlyah A. (in press) Production de plantes haploïdes doublées chez le blé dur (*Triticum durum*). Fifth Scientific Days of the AUPELF-UREF Biotechnology Network, Dakar, Senegal, John Libbey Eurotext, Paris.

Chuang C.C., Ouyang T.W., Chia H., Chou S.M. & Ching C.K. (1978) A set of potato media for wheat anther culture. In: Proc. Symp. Plant Tissue Culture, 51-56, Science Press, Beijing

De Buyser J., Henry Y., Lonnet P., Hertzog R. & Hespel A. (1987) "Florin": a doubles haploid wheat variety developed by anther culture method. *Plant Breeding* 98: 53-56

El Haddoury J., Chlyah H. & Picard E. (1993) Etude de l'effet de quelques facteurs génotypiques et environnementaux de l'androgenèse *in vitro* chez des variétés de blé tendre adaptées au Maroc. In (AUPELF-UREF ed.), Le progrès génétique passe-t-il par le repérage et l'inventaire des gènes? 221-232, John Libbey Eurotext, Paris

Foroughi-Wehr B. & Zeller F.J. (1990) *In vitro* microspore reaction of different German wheat cultivars. *Theor. Appl. Genet.* 79: 77-80

Henry Y. & De Buyser J. (1990) Wheat anther culture: agronomic performance of doubled haploid lines and the release of a new variety "Florin". *In* Y.P.S. Bajaj (ed.), Biotechnology in agriculture and forestry 13, pp. 285-352, Springer-Verlag, Berlin, Heidelberg

Henry Y., Vain P. & De Buyser J. (1994) Genetic analysis of *in vitro* plant tissue culture responses and regeneration capacities. *Euphytica* 79: 45-58

Laurie D.A. & Bennett M.D. (1988) The production of haploid wheat plants from wheat x maize crosses. *Theor. Appl. Genet.* 76 : 393-397

Laurie D.A. & Reymondie S. (1991) High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. *Plant Breeding* 106: 182-189

Lazar M.D., Schaeffer G.W. & Baenziger P.S. (1990) The effects of culture environment with genotype on wheat (*Triticum aestivum*) anther culture response. *Plant Cell Reports* 8 : 525-529

Lettre J.J., Kelly S.L., Marsolais A.A. & Kasha K.J. (1990) Wheat anther culture using liquid media. In: Y.P.S. Bajaj (ed.), Biotechnology in agriculture and forestry 13, Wheat, pp. 416-424, Springer-Verlag, Berlin, Heidelberg

Lu C.S., Sharma C.H. & Ohm H.M. (1991) Wheat anther culture: effect of genotype and environmental conditions. *Plant Cell, Tissue and Organ Culture* 24 : 233-236

Marsolais A.A. & Kasha K.J. (1985) Callus induction from barley microspores. The role of sucrose and auxin in a barley anther culture medium. *Can. J. Bot.* 63: 2209-2212

O'Donoughue L.S. & M.D. Bennett M.D. (1994) Comparative responses of tetraploid wheats pollinated with *Zea mays* L. and *Hordeum bulbosum* L. *Theor. Appl. Genet.* 87: 673-680

Ouyang J.W. (1986) Induction of pollen plants in *Triticum aestivum* L. *In*: Hu Han and Yang Hong-Yau (eds.), Haploids in higher plants *in vitro*, pp. 26-38, Springer-Verlag, Berlin, Heidelberg.

Ouyang J.W., He D.G., Feng, G.H. & Jia S.E. (1987) The response of anther culture to culture temperature varies with growth conditions of anther donor plants. *Plant Sci.* 49: 145-148

Picard E. & De Buyser J. (1977) High production of embryoids in anther culture of pollen derived homozygous spring wheats. *Ann. Amélior. Plant.* 24: 483-488

Picard E., Crambes E., Liu C.S. & Mihamou-Ziyyat A. (1994) Évolution des méthodes d'haplodiploïdisation et perspectives pour l'amélioration des plantes. *C.R. Soc. Biol.* 188: 109-141

Snape J.W., Chapman V., Moss J., Blanchard C.E. & Miller T.E. (1979) The crossabilities of wheat varieties with *Hordeum bulbosum* . *Heredity* 42: 281-298.

Trottier M.C., Collin J. & Comeau A. (1993) Comparison of media for their aptitude in wheat anther culture. *Plant Cell Tissue and Organ Culture* 35 : 59-67

Tuvesson I.K.D, Pedersen S., Olesen A. & Andersen S.B. (1991) An effect of the 1BL/1RS chromosome on albino frequency in wheat (*Triticum aestivum*) anther culture. *J. Genet. and Breed.* 45 : 345-348

Wang P. & Chen Y.R. (1986) A study on the application of C17 medium for anther culture. *Acta Bot. Sin.* 28: 41-45