In vitro gynogenesis in some varieties of durum wheat (Triticum durum. L.)

Alaoui M.M., Gaboun F., Cherkaoui S.

in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 223-228

In vitro gynogenesis in some varieties of durum wheat (*Triticum durum*. L.)

Meriem Mdarhri Alaoui[1], Fatima Gaboun[1], Souad Cherkaoui[2]

[1] Unité de Recherche en Biotechnologie, INRA, CRRA-Rabat, Maroc
[2] Faculté des Sciences de Rabat, Université Mohamed V, Rabat, Maroc

Abstract. To succeed in the production of doubled-haploid plants of durum wheat *(Triticum durum*. L), gynogenesis was studied in six durum wheat varieties Anwar (AN), Jawhar (JW), Yasmine (YS), Be bachir (BB), Sebou (SB) and Kyperonda (KP). The MS medium (Murashige and Skoog 1966) supplemented with 9% sucrose and 0.8% agar was used as basal medium. 2mg /l of three auxins [2,4-D (2,4-dichloro- phenoxy acetic acid), NAA (naphthalene acetic acid), and 2.4.5-T (trichlorophenoxy acetic acid)] were added to the induction medium. The embryogenic calluses were transferred onto regeneration medium R9 without growth regulators for two weeks then the same medium supplemented with 2 mg /l BAP (benzyl adenine purine) and 0.1mg /l NAA. The results showed that 2,4-D was the most reactive auxin for all varieties studied. The effect of genotype was significantly very marked in the presence of the three auxins tested. The best rate of embryogenic callus induction was 63% obtained from the Anwar variety. The presence of light (16h per day) favorably affected the kinetics of appearance of gynogenetic embryogenic callus. The first results appeared in the second week of culture whereas, in darkness, this period lasted up to nine weeks. The study also showed a genotypic effect on the regeneration phase with the best rate (27%) obtained with the variety Anwar.

Keywords. *Triticum durum* – Gynogenesis – Haploid methods – Genotypes – Green plants.

Gynogenèse in vitro dans certaines variétés de blé dur (Triticum durum. L.)

Résumé. *Pour réussir la production d' haploïdes doublés de blé dur (Triticum durum. L), on a étudié la gynogenèse dans six variétés de blé dur Anwar (AN), Jawhar (JW), Yasmine (YS), Belbachir (BB), Sebou (SB) et Kyperonda (KP). Le milieu MS (Murashige et Skoog, 1966), additionné de 9% de saccharose et 0,8% de gélose, a été utilisé comme milieu de base. 2 mg/l de trois auxines [2,4-D (2,4-dichlorophénoxyacétique), NAA (acide naphtalène acétique) et 2.4.5-T (acide trichlorophénoxyacétique)] ont été ajoutés au milieu d'induction. Les cals embryogènes ont été transférés sur un milieu de régénération R9 sans régulateurs de croissance pendant deux semaines, puis le même milieu a été additionné de 2 mg/l de BAP (benzyle-adénine-purine) et de 0,1 mg/l de NAA. Les résultats ont montré que le 2,4-D était l'auxine la plus réactive pour toutes les variétés étudiées. L'effet du génotype était significativement très marqué en présence des trois auxines testées. Le meilleur taux d'induction du cal embryogène était de 63% pour la variété Anwar. La présence de la lumière (16h par jour) avait une incidence favorable sur la cinétique d'apparition de cals embryogènes gynogénétiques. Les premiers résultats ont été obtenus dans la deuxième semaine de culture alors que, dans des conditions d'obscurité, cette période s'est étendue jusqu'à neuf semaines. L'étude a également montré un effet du génotype sur la phase de régénération avec le taux le plus élevé (27%) observé chez la variété Anwar.*

Mots-clés. *Triticum durum – Gynogenèse – Méthodes haploïdes – Génotypes – Plantes vertes.*

I – Introduction

The application of haplodiploidisation to cereals is a technique, at present, integrated into breeding programs and selection (Gallais, 2011). The cultures of anthers, microspores or pollen grains have led to spectacular results in many cereal crops: corn, wheat, rice and barley (Devaux, 1998, Cherkaoui *et al*., 2000, Bordes *et al*., 2006,).

However, few successes are noted in durum wheat. The species is considered recalcitrant to this technique because of the low induction rates of androgenic embryos and the abundance of albinism. The use of gynogenesis (culture of unfertilized ovaries) as a second way of production of pure green lines has been successful since the 1960s for barley (San Noeum 1967), and subsequently in other barley species (Wang and Kuang 1981, Zhou and Yang 1980), corn (Gbaguidi, 2010) rice (Zhou and Yang, 1980) bread wheat (Zhu and Wu, 1979, Devaux, 1998). In durum wheat, few research results are published and the successes achieved remain limited. Determining factors and culture conditions of *in vitro* culture of the female gametophyte would allow the improvement of the potential of durum wheat for the production of doubled haploid green plants. Several studies have shown the significant effect of culture conditions of unfertilized ovaries in some cereals (Mdarhri *et al*., 1998, Mdarhri, 2000, Chlyah *et al*., 2001, Mdarhri *et al*., 2005, Bordes, 2006, Gbaguidi, 2010). In this study, we try to determine the effect of the season, of three auxins and of the photoperiod on gynogenesis of six durum wheat varieties We have already shown the haploid nature of the green plants produced (Mdarhri *et al.*, 1998).

II – Material and methods

In late binucleate stage before anthesis, ears of six varieties of durum wheat (*Triticum durum* L.) Anwar (AN), Jawhar (JW), Yasmine (YS), Belbachir (BB), Sebou (SB) and Kyperonda (KP) were harvested and submitted to a pretreatment in the cold (4°C) during 10 days, then they are treated 3 minutes in 70% ethanol prior to disinfection. This involves, in sterile conditions, immersion in a solution of 2% Tween 20 for 2 min followed by a 20 min treatment in commercial bleach. Finally ears are rinsed three times with sterile distilled water at a rate of 3 min for every rinse. Disinfected ears are stripped of their husks and chaff.

Embryogenic calluses were initiated by culturing unfertilized ovaries on MS basal medium containing 2mg /l 2,4-D, NAA or 2.4.5-T. Cultures were incubated in a growth chamber at (25 ± 1) °C in the absence of light or under a photoperiod of 16h/8h (light /dark).

The embryogenic calluses formed were subcultured onto R9 regeneration medium without growth regulators for two weeks. Then they were transferred onto the same medium supplemented with 2 mg /l BAP and 0,1 mg /l NAA. The shoots obtained were transferred to a rooting medium: MS containing 1mg/l IAA and 1mg/l kinetin.

Results focused on the rate of induction of embryogenic calluses, the kinetics of their appearances and the rate of chlorophyll regeneration. The results of the various experiments were treated by analysis of variance and the significantly different averages were separated by Student's t-test and Newman Keuls at the probability level of 5%.

III – Results

1. Harvest season of spikes and ovaries

A preliminary study was made to determine the best time for harvesting spikes and excision of unfertilized ovaries. The results showed (Fig. 1) that the best rates were noted for the induction period which runs from early March to mid-April with an average rate of 3%. For this study all genotypes were grouped.

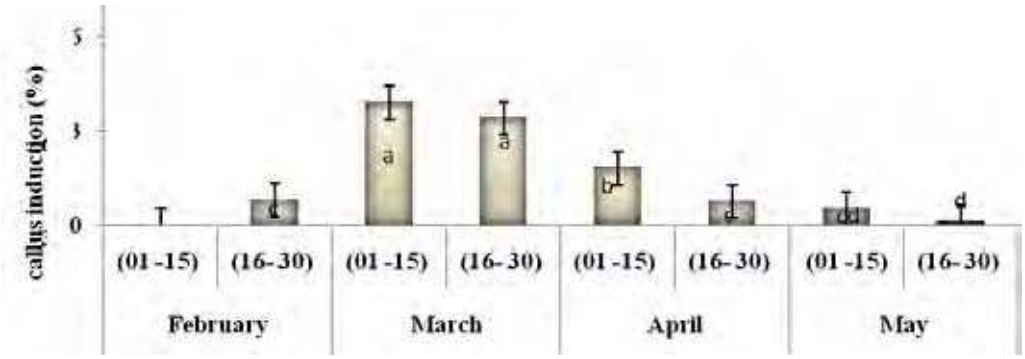
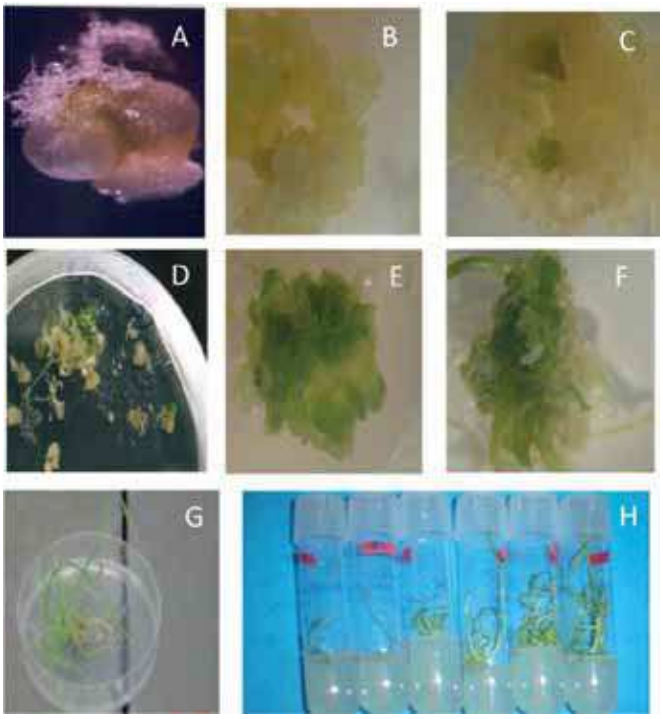


Figure 1. Callus formation as function of the date of culture.

2. Effect of growth regulators and genotype on the induction phase

For the six varieties studied in the presence of the three auxins tested, whitish friable calluses were induced. Transplanting to the regeneration medium resulted in the formation of tufts of green shoots. When these were transplanted to MS rooting medium, they formed roots (Fig. 2). However, genotypic differences significantly affected the rates of callus induction. The variety Anwar (An) has always given the best response allowing the production of 63.52% , 47.21 % and 29.34% in the presence of 2,4- D, T 2.4.5 or NAA respectively (Table 1).



Figue 2. Stages of haploid plant production in durum wheat from unfertilized ovary culture. A) Gynogenetic callus coming out at the base of the ovary. B) Gynogenetic callus transferred to the regeneration medium. C) Green shoots appear in the callus. D) Shoots develop into small plants. E) and F) Tufts of green plants. G) Rooted green plants. H) Multiplication and rooting of plants.

Table 1. Comparison of the effect of three auxins on the induction rate (%) of embryogenic callus from unfertilized ovary culture in six durum wheat varieties.

	Growth Regulator (2mg/l)		
Variety	**2,4-D**	**2,4,5 T**	**NAA**
AN	63.52a	47.21a	29.34a
JW	59.06b	38.64c	17.02c
YS	27.64f	27.87d	19.32bc
BB	45.87c	25.21d	21.41b
KY	38.74e	13.26e	7.61d
SB	40.39d	41.06b	28.07a
Mean	45.87	32.20	20.46
LSD	1.53	0.76	0.97

The mean values of the same column with the same letter are not significantly different at the 5% level (test of Student-Newman Keuls).

The study of the effect of three auxins on the induction rate showed that 2,4-D gave the best results. Figure 3 shows that the rate of callus induction decreased significantly in the presence of NAA. This variability was marked and highly significant for all varieties. Genotypes were classified according to their mean rates of callus formation after variance analysis (student –Neuman and Keuls test).

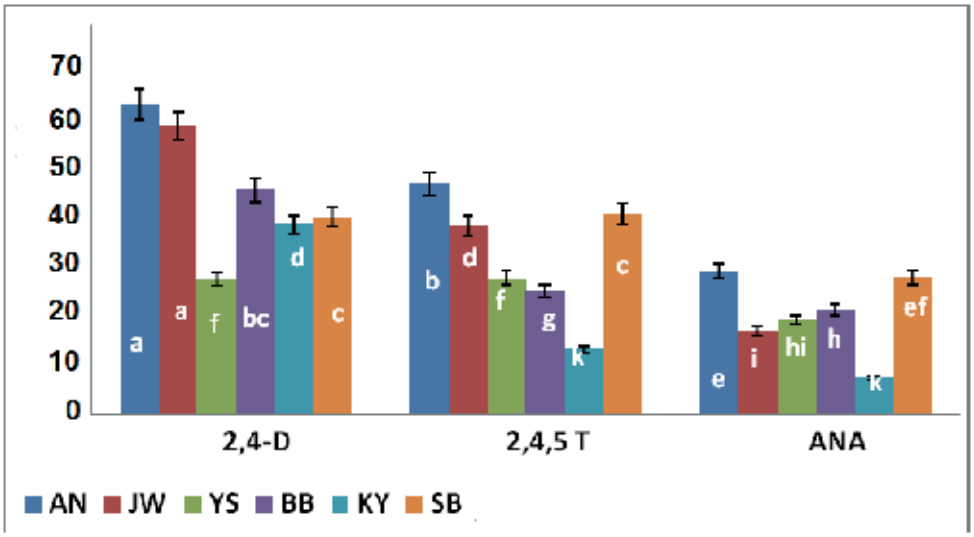


Figure 3. Effect of plant growth regulators on the rate of induction of gynogenetic callus for six varieties of durum wheat. Blocks of the same color with the same letter are not significantly different at the 5% level (test of Student-Newman Keuls).

3. Induction kinetics as a function of photoperiod

After monitoring the kinetics of appearance of embryogenic callus from cultures incubated in the dark and cultures submitted to a photoperiod of 16h per day we concluded the beneficial effect of alternating light and dark (Figure 4). In this last case, the initial responses were observed from the third week of culture with an optimum at the sixth week with an induction rate of 49.78%.

The percentage of induction fell gradually after the seventh week and ended in the 13th week. Cultures incubated in darkness started to produce callus only in the 11th week of culture, this rate increased up to the 17th week with the optimum at the 16th week at 27.89%.

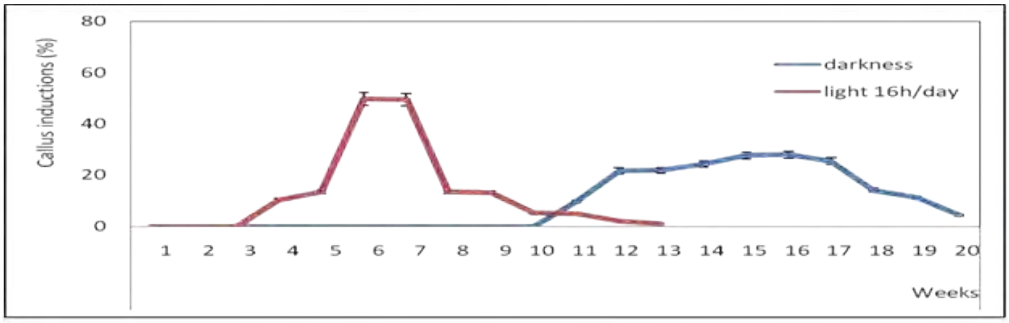


Figure 4. Kinetics of callus induction according to the light conditions.

4. Regeneration of haploid plants

Embryogenic callus obtained from the induction phase were transferred onto the R9 medium without growth regulators at first and then in the presence of BAP and NAA (2,01 mg /l) . The observations showed that all the regenerations were green in the form of tufts. The genotypic effect was striking (figure 5). Average rates of regeneration were between 15 % and about 4.4%, respectively for Anwar and Belbachir. No plants were regenerated from callus derived from the Yasmine and Kyperounda varieties.

The ANOVA showed significant differences among genotypes at a probability level of 5% by the Student- Newman and Keuls test.

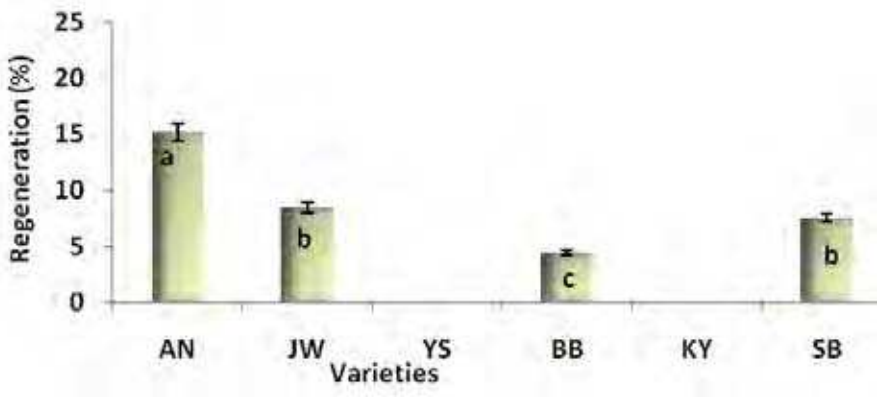


Figure 5. Regeneration of gynogenetic plants.

IV – Conclusion

Improvement of the technique of gynogenesis in durum wheat could bring about its integration into breeding and selection programs. This study showed the importance of culture conditions

and the variable performances of different genotypes. The Anouar variety gave best results both for callus induction and for green plant regeneration.

Acknowledgments

Special thanks to Professor Averil B. Chlyah for translating and reviewing this article.

References

Bordes J., 2006. Création de lignées haploïdes doublées de maïs par gynogenèse induite *in situ*: amélioration de la méthode et intégration dans les schémas de sélection. *Thèse Présentée à l'Université Blaise Pascal,* France.

Bordes J., Charmet G., Dumas R., Pollacsek M., Beckert M., Gallais A., 2006. Doubled-haploid versus S1-family recurrent selection for testcross performance in a maize population. *Theor. Appl. Genet.,* 112, pp. 1063-1072.

Cherkaoui S., Lamsaouri 0., Chlyah A., Chlyah H., 2000, Durum wheat x maize crosses for haploid wheat production: Influence of parental genotypes and various experimental factors. *Plant Breed*., 119, pp. 31-36.

Chlyah H., Cherkaoui S., Saidi N., Lamsaouri O., Mdarhri-Alaoui M., Chlyah O., Benkirane H., Amail O., Chlyah A.B. 2001. Production d'haploïdes chez le blé dur. Sélection en milieu salin. In: *Des modèles biologiques à l'amélioration des plantes.* Hamon S. (ed). IRD Editions, Collection Colloques et Séminaires, pp. 235-254.

Devaux P., 1998. Les plantes haploïdes chez l'orge avec extension sur le blé tendre, méthodes d'obtention et relation avec l'organisation de leur génome. *Sciences et Technologies,* Université Lille1.

Gallais A., 2011. Méthodes de création de variétés en amélioration des plantes. *Edition QUAE, INRA,* pp. 278.

Dobo M., Yao N.K., Monty J.P., 2010. Influence de l'écosystème et du stade de développement des panicules sur l'androgenese chez le riz. *Agronomie Africaine,* Vol. 22(2), pp. 109-119.

Mdarhri Alaoui M., Moussa-Labé P., Chlyah A., 2005. Combined effect of 2,4-D and sucrose concentrations on the gynogenetic response in durum wheat. *Al Awamiam,* 113, vol. 2(1), pp. 15-27.

Mdarhri-Alaoui M., 2000. Nouvelles voies d'haploïdie chez le blé dur: androgenèse après croisements interspécifiques avec le blé tendre et gynogenèse *in vitro* par culture d'ovaires non fécondés. *Thèse Université Mohamed V, Faculté des Sciences,* Rabat. Morocco.

Mdarhri-Alaoui M., Saidi N., Chlyah A., Chlyah H., 1998. Obtention par gynogenèse *in vitro* de plantes haploïdes chlorophylliennes chez le blé dur. *C. R. Acad. Sc. Paris*, 321, pp. 25-30.

San Noeum L., 1976. Haploïdes d'*Hordeum vulgare* L. par culture *in vitro* d'ovaires non fécondés. *Ann. Amelior. Plantes*, 290, 26, pp.751-754.

Wang C., Kuang B., 1981. Induction of haploid plants from the female gametophyte of *Hordeum vulgare* L. *Acta Bot. Sin.,* 23, pp. 329-330.

Zhou C., Yang H., 1980. *In vitro* induction of haploid plantlets from unpollinated young ovaries of *Oryza sativa* L. *Acta Genet. Sin*., 7, pp. 287-288.

Zhu Z., Wu H., 1979. *In vitro* production of haploid plantlets from the unpollinated ovaries of *Triticum aestivum* and *Nicotiana tabacum*. *Acta Genet. Sin*., 5, pp. 181-183.