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Callus formation and plant regeneration from young wheat spikes: Effect of genotypes

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SUMMARY – Calli formation and plant regeneration from dicotyledonous species were successfully and widely reported. However, for monocotyledonous species (cereal), this success did not have the same level. This study highlights a rapid technique of wheat plant regeneration based on in vitro culture of young spike explants. This technique could be used to exert selection pressure to abiotic stress or to transfer genes of agronomic interest and produce transgenic plants. It shows that callus induction and development is under genotype and medium control. This technique is faster and less exposed to contamination than immature embryo methods.

Key words: Tissue culture, cereal, young spikes, immature embryos.

RESUME – *"Formation de cals et régénération de plantes à partir de jeunes épis de blé : Effet des génotypes". La formation de cals et la régénération de plantes dicotylédones ont été largement rapportées avec succès. Cependant, cette réussite n'a pas eu la même ampleur chez les monocotylédones (céréale). Cette étude met en lumière une technique rapide de régénération de plantes de blé, basée sur la culture* in vitro *d'explants de jeunes épis. Cette technique pourrait être utilisée pour exercer des pressions de sélection aux stress abiotiques et pour le transfert de gènes d'intérêt agronomique, en cas de création de plantes transgéniques. Elle montre que l'induction et la formation de cals dépend du génotype et de la composition du milieu. Elle est plus rapide et moins exposée aux contaminations que celle basée sur la culture d'embryons immatures*

Mots-clés : *Culture de tissus, céréales, jeunes épis, embryons immatures.*

Introduction

Plant tissue and cell cultures are usually initiated from pieces or parts of whole plants. For dicotyledonous species, callus formation and plant regeneration were widely carried out (Fellner and Lebeda, 1998; Rady, 1998). For cereal crops, regeneration has been set up in cell culture programmes with various degrees of success. Nevertheless, callus formation and plant regeneration have been reported in a number of cereals (Hanzel *et al.,* 1985; Bregitzer, 1992).

Anthers and immature embryos have been used frequently as an explant source in cereal tissue culture (Cheng *et al.,* 1997). But there are no reports to date dealing with repetitive somatic embryogenesis in young wheat spikes.

Thus, the aim of our study is to develop a protocol for wheat callus formation and plantlet regeneration from young spikes, to identify the genotypes which have favourable response in tissue culture and to use this system, later, to transfer some genes of interest into cereals.

Materials and methods

Triticum aestivum L. cv: Florence-aurore, Salombo, Tubica, Nesma, Achtar, Ariana, Vaga and *Triticum turgidum* L. var. *durum* Desf. cv: Maghrebi, Med Ben Bachir, Razzak, Khiar, Oum Rabia, INRAT69, were used in this study.

Plants were cultivated at National Agronomic Research Institute field (Tunisia). Three months after sowing, elongation stage was reached and young spikes were collected from the plants (elongation stage is located between tillering and earing stage). They were sterilized in 70% ethanol, thoroughly washed with sterilized distilled water and placed into medium.

The medium used was Murashige and Skoog medium (MS-salt) supplemented with 3% sucrose, 2.3 g/l phytagel, 2 mg/l 2,4-D and 0.5 mg/l KT. pH was adjusted to 5.7 then autoclaved at 121ºC for 20 min. Explants were cultured in the dark at 25ºC and medium was refreshed at 4 week-interval. After 2 subcultures, percentage of explants proliferated calli, formation and frequency of embryogenic calli, morphology and friability of the proliferated calli were recorded.

To regenerate plants, calli were transferred to regeneration medium which contained MS basal salts without hormone (Bregitzer, 1992; Daaloul *et al.,* 1992) or MS basal salts supplemented with 0.5; or 1 mg/l of 2,4-D or with KT (1 mg/l) and NAA (0.5 mg/l). The remainder of medium is the same as previously described. Individual cultures were then scored for the regeneration of green and/or albino plants.

Results and discussion

To evaluate callus initiation, the MS-(2,4-D + KT) was used because it had been used successfully to initiate callus cultures in many cereals (Weeks *et al.,* 1993).

There was a wide range of variation among genotypes for callus initiation (Table 1).

Table 1. Percent of young wheat spikes forming callus 4 weeks after culture initiation in MS medium supplemented with 2 mg/l (2,4-D) and 0.5 mg/l (KT)

Genotype	% of explant forming callus	Friability†	Morphology††
Triticum aestivum			
Florence-Aurore (short beard)	81	3	3
Nesma (short beard)	81	3	3
Ariana (short beard)	80	3	3
Salombo (short beard)	75	3	2
Tubica (short beard)	62.5	3	3
Achtar (short beard)	56	2	2
Vaga (short beard)	50	1	1
Triticum turgidum			
Khiar (long beard)	60	3	2
Med Ben Bachir (long beard)	60	2	2
Razzak (long beard)	25	2	2
INRAT69 (long beard)	25	2	1
Maghrebi (long beard)	20	2	1
Oum Rabia (long beard)	16	2	1

†1 = Nonfriable; 3 = Friable; 2 = Intermediate.

††3 = Yellow and compact; 1 = White callus; 2 = Intermediate.

The percentage of explants that developed calli ranged from 16 to 81%, for all genotypes. But there was significant differences between *T. turgidum* and *T. aestivum* genotypes for callus initiation. The average was 69.36% for *T. aestivum* against 34.33% for *T. turgidum.* This result agrees with that reported by many authors in wheat and in other cereals (Bregitzer, 1992; Daaloul *et al.,* 1992). Not all genotypes responded similarly to this medium. Variability was also observed among genotypes of the same type of wheat. Thus Florence-Aurore showed more proliferate callus than Vaga variety (*T. aestivum*) and Khiar than Oum Rabia one (*T. turgidum*). Whatever genotypes were used it seems that short beard wheat had higher initiation frequency than long beard ones, as was demonstrated by Daaloul *et al.* (1992) (Fig. 1).

In other studies, the rate of calli obtained from anther culture did not exceed 19% (Piri, 1991), while frequency of callus initiation obtained from immature embryo did not exceed 44% for barley (Hanzel *et al.,* 1985) and 44% for wheat (Sears and Deckard, 1982). Many more contaminations related to various handlings were observed in these two cases than in young spikes protocol.

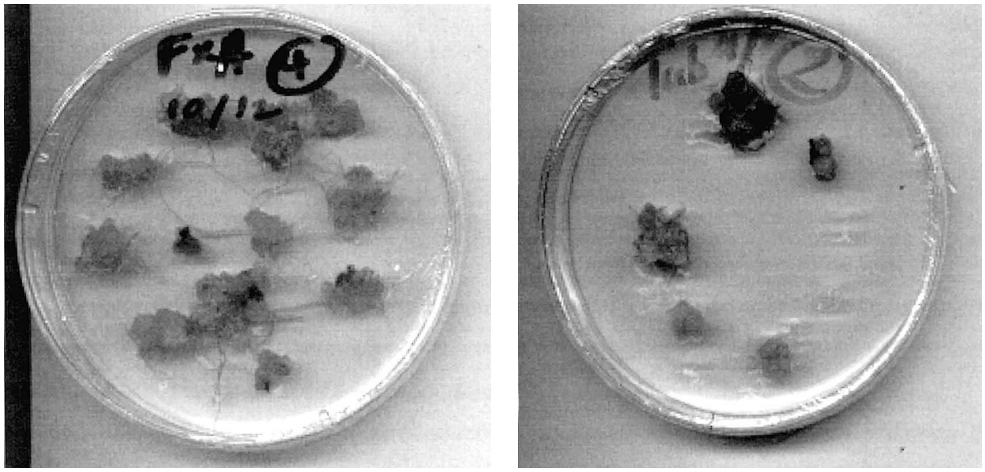

Fig. 1. Variability of proliferate callus according to genotype (Florence-Aurore and Tebuca genotypes).

The time required, from sowing to having explants and from subculturing the first callus to regenerate plants was longer in immature embryos than in young spikes ones, as is shown in Table 2.

Table 2. Time needed for each development stage from sowing to regenerate whole plant

	Time required (days)				
	From sowing to explants	From first explant to calli	From calli to third subculture	From last subculture to regenerate plant	Whole period
Young spikes	90	15	63	39	207
Immature embryos	120	21	63	60	264

In our study, the relative ability of a genotype to form a callus was not correlated with ability to regenerate green plants. For example, “Med Ben Bachir” genotype, which has an intermediate friability and morphology of callus and which formed callus at frequency of 60%, against 81% for “Florence-Aurore”, showed the first green plant developed in medium 2 (Fig. 2). The frequency of plant regenerated was 33% in “Med Ben Bachir” genotype against 25% for Tebuca one. This indicates that regeneration is under genotypic control as has been reported in other plant species (Rines and McCoy, 1981; Sears and Deckard, 1982).

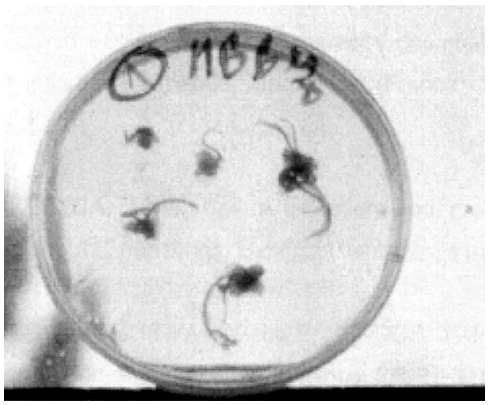

Fig. 2. Wheat plant regenerated from young spikes in medium 2 (Med Ben Bachir variety).

To identify the best combination which would initiate organ formation in tissues of wheat, a variety of growth regulators were tested. The medium 1 (MS + 1 mg/l of 2,4-D) cannot initiate plant regeneration and it is considered as a maintenance medium.

KT and NAA (medium 2) were effective for organ formation, in some varieties (Med Ben Bechir and Florence-Aurore). However further work is required to increase the efficiency of the method and to determine the growing conditions to regenerate whole plant.

The medium 4 (MS + IAA + KT) produces a synergistic increase in embryogenic callus, in some other varieties like Tubeca, Florence-Aurore and Khiar.

The other media (MS basal salt supplemented with 0 and 0.5 mg/l of 2,4-D) (medium 3) need longer time to regenerate plants. Besides, these media occasionally give rise to shoots or roots by organogenesis.

Conclusions

The present results indicate that young spikes can be used as anthers and immature embryos to regenerate wheat plant. This method showed that callus induction and development is under genotype and medium control. Thus *T. aestivum* is more easy to handle than *T. turgidum* genotypes. However further work is required to optimize the method and to determine the growing conditions. This technique is faster and less exposed to contamination than the immature method.

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