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Effects of NaCl Stress on Callus Proliferation and Plant Regeneration from Mature Embryos of Bread Wheat (*Triticum aestivum* L.) Cultivars Mahon Demias and Hidhab

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Abstract

An *in vitro* experiment with two cultivars, Mahon Demias and Hidhab of bread wheat (*Triticum aestivum* L.) exhibited appreciable callus induction but differed significantly in the capacity of calli proliferation and regeneration under salinity stress; even though Mahon Demias appeared relatively more tolerant than Hidhab. The results indicated the need to optimize a robust protocol for callus induction, maintenance and regeneration before selection process for tolerance to salinity is embarked upon.

Introduction

Plant tissue culture plays an important role in the production of agricultural and ornamental plants and in the manipulation of plant for improved agronomic performance. *In vitro* culture of plant cells and tissue has attracted considerable interest over the recent years because it provides the means to study the physiological and genetic processes of plants in addition of offering the potential to assist in breeding improved cultivars by increasing the genetic variability. Regenerated plants are expected to have the same genotype as the donor plant, however, in some cases somaclonal variants have been found among the regenerated plants (Karp et al. 1987).

Genetic factors are considered to be a major contributor to the *in vitro* response of cultured tissues. Differences in the production of embryogenic calli and regenerated plantlets have been observed, depending on the genotype and source of explant (Ganeshan et al. 2003). Immature as well as mature embryos are currently the most reliable and efficient target tissues for *in vitro* regeneration

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of cereals (Chang et al. 2003). Because of the advantage of easy storage and availability at all times throughout the year mature embryos are a favorable explant source.

The composition of the media mainly the hormonal balance is another important factor influencing *in vitro* culture initiation and plant regeneration from embryos (Jiang et al. 1998). The auxin 2, 4-dichlorophenoxy acetic acid alone or in combination with cytokinins is widely used to enhance callus induction and maintenance (Castillo et al. 1998). Plant response to abiotic stress is a complex phenomenon, and *in vitro* culture could be used to enhance selection process for these stresses. Putative stress-resistant lines derived from conventional breeding or transgenic approaches could be screened using *in vitro* selection. This is particularly attractive for some abiotic stresses, where appropriate screening methods are not available or have a low efficiency.

Salinity is the main abiotic stress that has been addressed by *in vitro* selection, and applications to other stresses such as heat and drought have also been reported (Lutts et al. 1996). Currently, these techniques are considered to be an important complement to classical breeding methods (Zalc et al. 2004). *In vitro* selection for tolerance to abiotic stress is dependent on the development of efficient and reliable callus induction and plant regeneration systems. In Algeria, it is not well-known which bread wheat elite cultivars have good tissue culture response. The goal of this experiment was to study the callus induction capacity and regenerability from mature embryos of two bread wheat (*Triticum aestivum* L.) cultivars Mahon Demias and Hidhab, under salinity stress conditions.

Materials and Methods

The study was conducted in the plant biotechnology laboratory, Faculty of Sciences, Mentouri University, Constantine (Algeria). Mature seeds of the two vars. Mahon Demias and Hidhab of bread wheat obtained from the Agricultural Research Station of the Field Crop Institute (ITGC) of Sétif, were used as explants. Seeds were surface sterilized by immersion into 70% alcohol for one min and into sodium hypochlorite solution (12%) for five min, and washed three times with sterile distilled water.

After disinfection, the embryos were removed with the aid of a pair of forceps and a surgical knife under a laminar flow hood. They were placed, scutellum side up, in Petri dishes containing the callus induction medium, the MS basal salts supplemented with 30 g/l saccharose, 8 g/l agar and 10 mg/l of 2,4-D. The Petri dishes were sealed with household polyethylene film and placed in a growth chamber under conditions of total darkness and 22^{o}C temperature. Eight mature embryos were plated per Petri dish for a total of 96 embryos tested per genotype.

After four weeks of incubation, the induced calli were subcultured, under the same growth conditions, and in the same MS medium to which various NaCl concen-trations (0, 5, 10 and 15 g/l) were added; the incubation period lasted four weeks. Resulting calli were excised and transferred, at a rate of eight calli per flask, onto the MS basal salts medium supplemented with 30 g/l saccharose, 8 g/l agar, 2 mg/l BAP and 0.5 mg/l ANA for shoot initiation, for a three-week period and for another three-week period on to one half strength MS medium supplemented with 30 g/l saccharose, 8 g/l agar, 0.8 mg/l ANA and 0.36 mg/l Kn for root formation.

The flaks were placed in a growth chamber under fluorescent light and ambient temperature of 22°C. The medium is changed every 15 days and after this period, callus with clearly differentiated shoots and roots was scored as regenerating callus. Each piece of regenerating callus was counted as one regardless of the number of shoots and roots. The regenerating calli, showing shoot and root formations, were transferred on to the MS basal medium with no phytohormones and placed in a lighted chamber to sustain the regenerated plantlets growth. The pH of all media was adjusted to 5.8 with 0.2 N KOH prior to autoclaving. The culture medium was autoclaved at 121^{o}C for 25 min. The sterile growth regulator solutions were added to the autoclaved media after they cooled down to 50^{o}C.

The data were obtained on callus induction efficiency, measured as the number of calli/total number of embryos × 100; the embryogenic efficiency as the number of calli-forming shoots/total number of calli × 100; and the regeneration efficiency as the number of plantlets/total number of calli × 100. The number of leaves and roots per plantlet and the maximum root length were also scored.

Results and Discussion

Genotypic callus induction capacity: The overall aim of this experiment was to investigate *in vitro* tissue culture conditions for plant regeneration of two cultivars of bread wheat. The callus induction from mature embryos was assessed as well as the response of the regenerating callus to NaCl-salt stress. The callus induction rates were 88.5 and 58.3%, respectively for Mahon Demias and Hidhab cultivars. This indicated a significant differential genotypic ability for callus induction, with Hidhab being less responsive than Mahon Demias, which appears as best suited for *in vitro* tissue culture (Table 1).

The results of the present study were in agreement with those of Chen et al. (2006), who reported that callus induction frequencies varied from 11.6 to 89.6%. Rashid et al. (2002) mentioned that different genotypes gave different callus induction- and regeneration responses, and observed different callus

induction and regeneration responses in durum wheat. Gonzalez et al. (2001) reported variation in callus induction frequencies from 54 to 100%. He et al. (1988) reported values varying from 44 to 89% in durum wheat. Higher values were recorded by Bommineni and Jauhar (1996).

Table1. Mean values of the measured variables.

	Genotypic callus induction efficiency							
	Mahon Demias				Hidhab			
No. of embryos incubated	96.0				96.0			
No. of embryos showing calli	85.0				56.0			
Callus efficiency (%)	88.5				58.3			
NaCl (g/l)	NaCl salt stress effect							
	Mahon Demias				Hidhab			
	0	5	10	15	0	5	10	15
No. of incubated calli	24	24	24	24	24	24	24	24
No. of proliferating calli	24	24	21	15	22	20	8	6
Callus efficiency (%)	100	100	91.6	62.5	91.6	83.3	33.3	25.0
No. of calli differentiating shoot	2	2	1	0	5	3	1	0
Embryogenic efficiency (%)	8.3	8.3	4.2	0.0	20.8	12.5	4.2	0.0
No. of calli differentiating root	1	1	0	0	1	0	0	0
No. of regenerating calli	1	1	0	0	1	0	0	0
Regeneration efficiency (%)	4.2	4.2	0.0	0.0	4.2	0.0	0.0	0.0
No. of leaves/ plantlet	4	3	0	0	2	0	0	0
No. of roots/plantlet	2	2	0	0	2	0	0	0
Root length (mm)	25	20	0	0	20	0	0	0

Many factors, such as the medium composition, genotype, and the type of explant was found to affect the callus induction, embryogenic differentiation and plant regeneration processes. Ozgen et al. (1996) reported that mature embryos had low callus induction frequency but high plant regeneration capacity, compared to immature embryos. In the present study the variation noted in the callus induction capacity appears to be mainly due to the genotypic effect.

Callus proliferation and plant regeneration response to NaCl stress: The callus proliferation efficiency differed significantly between genotypes at each of the tested salt stress levels (Table 1, Fig. 1). Mahon Demias cultivar was not affected at 5 g/l of NaCl-salt level, while Hidhab was affected. At higher salt stress levels Mahon Demias reacted moderately, while Hidhab showed a sharp decrease in the callus proliferation capacity, reaching 25.0% at 15 g/l NaCl (Table 1). Mahon Demias showed a curvilinear response to salt stress levels, while the response of Hidhab was linear (Fig. 1).

It has been demonstrated in many cases that 2,4-D is usually the best auxin for callus induction and subculture of grasses (Chaudhury and Qu 2000). In the present case 2, 4-D auxin was used at the rate of 10 mg/l of MS medium for callus proliferation. The differential response of the callus proliferation to salt stress was essentially of genotypic origin with Mahon Demias, an old local cultivar, being more salt tolerant than Hidhab, a recently released variety.

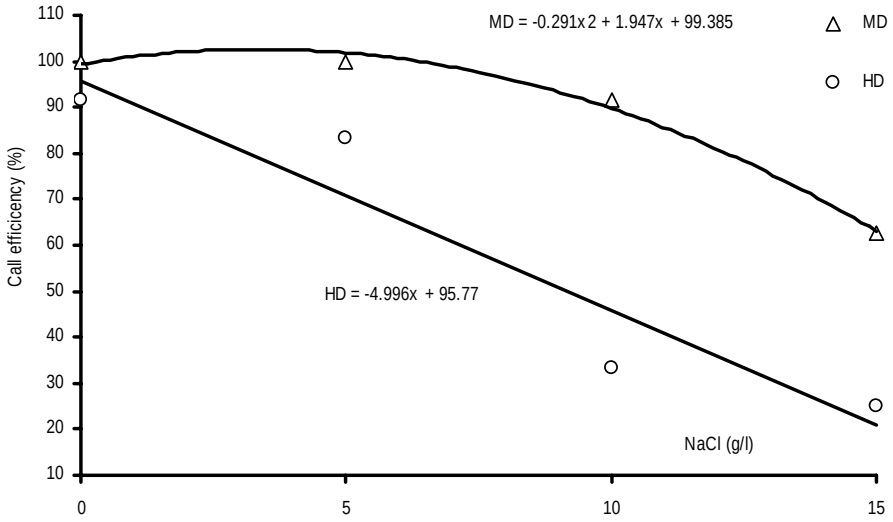

Fig. 1. Variation of the callus proliferation efficiency under four NaCl concentrations of the two tested bread wheat cultivars.

Embryogenic efficiency was dramatically low for Mahon Demias at mild salt stress levels (8.3%) compared to Hidhab (12.5%), but no sizeable differences were observed between the two genotypes at higher salt stress levels (Table 1, Fig. 2). Some experimental results indicated that the addition of a low concentration of cytokinin, particularly BAP, in callus culture medium often enhances embryogenic callus formation (Bradley et al. 2001). In the present experiment MS medium was supplemented with 2 mg/l BAP and 0.5 mg/l ANA for shoot initiation, and with 0.8 mg/l ANA and 0.36 mg/l Kn for root formation.

Plant regeneration rate of the proliferated calli of Hidhab was nil under salt stress and very low (4.2%) at mild salt stress for Mahon Demias. This value was similar to the regeneration efficiency observed for the control treatment of both genotypes (Table 1, Fig. 2). He et al. (1986) mentioned IAA promoted excessive rooting, while Bregitzer et al. (1998) indicated that the number of green plants per incubated embryo was significantly influenced by the genotype and the concentration of the auxin 2,4-D. Ballie et al. (1993) obtained a maximum

regeneration rate with 2.5 mg/l of 2,4-D. Neither IAA nor 2,4-D was used for plant regeneration in the present study.

Generally highly totipotent cell cultures are often derived from yellow pale and friable embryogenic callus tissues. However, in the present study a majority of the explants turned brown in all the salt treatments before shoot formation. Friable embryogenic type- calli were difficult to establish in both cultivars; even though calli with green spots developed shoots rapidly and an average of 2 roots/plantlet on the initiation medium. They grew slowly, producing two to four young curled leaves. However, when placed at the rooting medium, shoot and root growth was much accelerated; the root length varied from 20 to 25 mm (Table 1).

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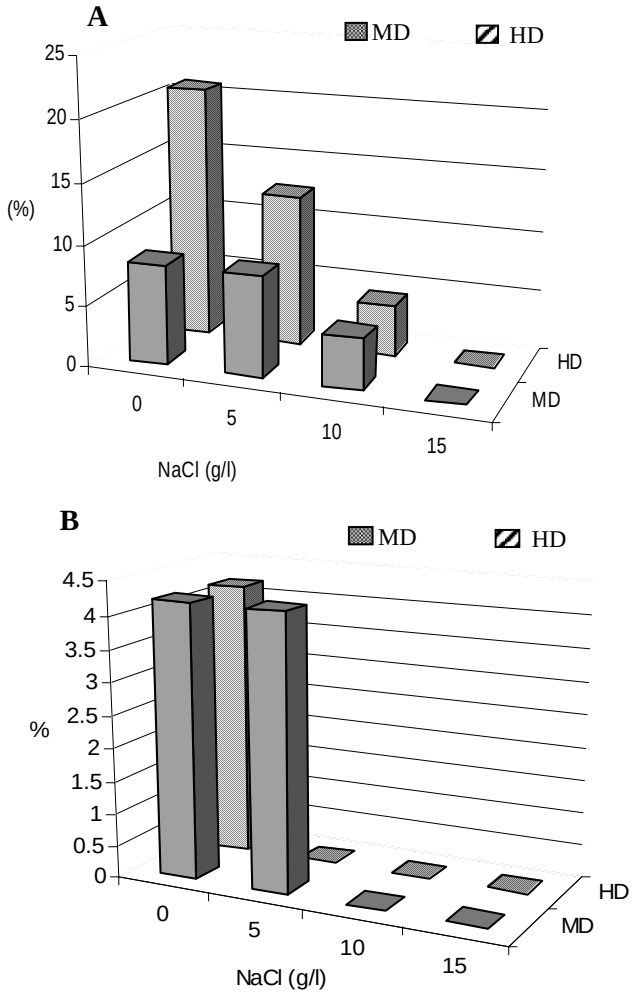

Fig. 2. Embryogenic (A) and regeneration (B) efficiencies observed under salt stress for the two cultivars of bread wheat.

Gonzalez et al. (2001) mentioned that NaCl inhibited plant regeneration. In tomato, a positive correlation between the salt response at the cellular and whole plant levels was found when calli were used for evaluating the salt tolerance at the cell level (Rus et al. 1999). Rus et al. (2000) reported a decrease in the relative growth rate and water content of calli proliferating in saline medium compared to calli incubated in salt-free medium. Chen et al. (1998) mentioned that in *Eucalyptus microcorys* shoot growth was inhibited in saline media and Abebe et al. (2003) observed a 37% reduction in growth of calli in the presence of 100 mM NaCl stress.

Salinity is considered as a major factor limiting plant development and crop productivity and salinization continues to increase particularly in arid and semiarid regions. Salinity tolerance is a polygenic trait difficult to select in the classical breeding procedures under field conditions (Richards 1996). Alternative strategies included regeneration of plants with improved salt tolerance after *in vitro* selection of salt tolerant cells, and/or construction of transgenic plants over-expressing genes expected to increase salt tolerance (Bohnert and Jensen 1996, Wincov 1996, Abebe et al. 2003).

In vitro tissue culture could serve as an important means to improve crop tolerance and yield through genetic transformation as well as induced somaclonal variation. So it is important to device an efficient protocol of callus proliferation in order to start *in vitro* selection for salt and drought stress tolerance, and to broaden the opportunities for genetic manipulation of wheat through tissue culture, including trying various explants and media. .

Conclusion: The results of the present study indicated that Mahon Demias has a good callus induction ability, while that of Hidhab was intermediate to low. A differential genotypic response was also noted in the callus ability to proliferate and regenerate plant under various NaCl stress conditions. Embryogenic efficiency of Mahon Demias was very low at mild salt stress compared to Hidhab. No sizeable differences were observed between the two genotypes at higher salt stress rates. Plant regeneration rate was nil under salt stress for Hidhab and very low at mild salt stress for Mahon Demias. There was a strong genotypic effect on the tissue culture response of wheat embryos. Therefore, to obtain a suitable wheat plant regeneration system for a given genotype of wheat, it is necessary to screen several elite wheat cultivars for their callus induction- and regeneration ability from mature embryos before embarking upon a suitable breeding program for selection for tolerance to salinity.

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