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RESEARCH ARTICLE

Selection for salanity tolerance and molecular genetic markers in Durum Wheat (*Triticum durum Desf.)*

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Abstract

Response of six genotypes of durum wheat (*Triticum durum* Desf.) Waha, Beliouni, Gumgoum Rkhem, Adna-2, Beni mestina and Adna-1; to mature embryo culture, embryogenic callus production and *in vitro* salt tolerance, were the main objective of this study. The effect of salt stress induced by NaCl on MS medium (Murashige and Skoog., 1962), the callus embryogenic medium was added to 3.5mg/l of 2.4-D (2.4-dichlorophenoxyacetic acid) and were subcultured on regeneration media containing 1mg/l of kinetin and 2 mg/l of AIA (β-indoleacetic acid), for roots proliferation, plantlets regenerated was transferred to MS/2 medium added to 1 mg/l. The embryogenic callus induction and regeneration rate were determined. The results showed that salt influence the regeneration rate and embryogenic callus induction. The influence of these two parameters is very marked when NaCl concentration reached $16g.l^{-1}$ in medium culture. For RAPD (Random Amplified Polymorphic DNA) analysis, the six genotype of durum wheat was cultivated *ex vitro* in absence and under salt stress. DNA (RAPD) markers were used to evaluate the genetic variability of the regenerated plants. Nine arbitrary primers were used to amplify genomic DNA of the original durum wheat cultivars and regenerated plants. Twenty two bands of 557 were polymorphic. The presence of polymorphic bands showed the presence of somaclonal variation, which can be used for selection of durum wheat toward desirable traits.

INTRODUCTION

Algeria is the second importing country of wheat in *Africa* after *Egypt*, with a global import market average of 5.5 million tons (68% of needs) in 2014 against a demand of 8 million tons. The observed deficit of the cereal sector in Algeria is caused by different stress conditions including salt stress. As wheat is considered as salt sensitive species (Ayolié *et al*.,2011), there is a need to develop stress tolerant cultivars.

Plant tissue culture plays an important role in plant amelioration and in the manipulation of plants for improved agronomic performance (Zale *et al*., 2004). *In vitro* culture of plant cell tissues has shown a growing interest over recent years. This approach is considered as a powerful mean to study plant physiological, genetic process and to increase genetic variability which can be used in breeding programs (Karp *et al*., 1987). Somatic embryogenesis is a process in which any somatic tissue is potent to regenerate into a whole plant through formation of embryo-like structures (Rao., 1996, Jiménez., 2005), and represents a unique developmental pathway that involves a number of characteristic events: cells dedifferentiation, cell division activation, reprogramming of their physiology, metabolism and gene expression patterns (Pathak *et al.*, 2008, Yang *et al.*, 2010). The phytohormones, auxins and cytokinins are important regulators and play a role in the developmental fate of pluripotent plant cells to regenerate plants which is well known, nevertheless, the mechanism is quite complicated (Ray *et al*.,1996, Pernisová *et al*.,2006.). Auxins are

known to be associated with plants genetic instability, a phenomenon called somaclonal variation (Karp., 1989, Phillips *et al*., 1994, Cullis., 1999). Although somaclonal variation can be used as a source for variation to obtain superior clones (Karp., 1993; Cassells *et al*., 1999). This somaclonal variation can be caused by exogenous factors, as osmotic pressure or mutagenic treatment .The salt stress application during the regeneration process constitutes a convenient way to study the effects of salinity on the morphogenic step developments. Several studies showed that the selective pressure can be applied during the callus formation phase and/ or regeneration (Bouiamrin *et al.,* 2012, Soliman *et al.,* 2013,Balkishna *et al.*,2013), In wheat species, different explant sources have been used for embryogenic callus formation and plant regeneration including mature and immature embryos (Ozgen *et al*.,1996) immature inflorescences and coleoptiles (Benkirane,H *et al*.,2000), shoot apical meristems (Ahmed *et al*., 2002) and others. Indeed, these tissues vary in their ability to regenerate into a whole drought stressed plants in durum wheat (*Triticum durum* Desf.) (Delporte *et al*., 2001). Embryo is the most frequently used explants for the initiation of wheat tissue culture either for callus production or direct DNA delivery techniques. Moreover, mature embryo are readily available all over the year and are used for transformation studies in the recent years (Patnaik et *al*.,2006).

The development of RAPD (Randomly Amplified Polymorphic DNA) has allowed simple, easy and less time-consuming genom analysis at the DNA level compared with RFLP (Restriction Fragment Length Polymorphism). Numerous investigators have successfully employed RAPD to find a molecular marker that could be used for genetic analysis of micropropagated and regenerated plants, taxonomic studies and classification, cultivar identification, genotypic screening and breeding programmes (Mahmoud et al., 1998).

The aim objective of our experiment is to select salinity tolerant genotype and to identify molecular markers and somaclonal variations associated with salt tolerance in durum wheat, although it is difficult to identify the genetic variation in regenerated somaclones, but, as RAPD using the arbitrary primers, it can be used to characterize somaclonale variation.

Materials and Methods

Plant Materials and Explant Preparation: This study was carried out by Genetics, Biochemistery and Plant Biotechnology Laboratory, Faculty of Nature and Life Science, Frères Mentouri University, Constantine, Algeria, during the period from 2012 to 2015.

Six durum wheat (*Triticum durum* Desf.) genotypes: Waha, Beliouni, Gumgoum Rkhem, Adna-2, Beni mestina and Adna-1 were used as the material for this study. The seeds were provided by ITGC, Institut Technique des Grandes Cultures (Station El-khroub Constantine, Algeria). The explant source was mature embryos collected from mature seed.

Callus induction and selection for salt tolerance callus: The mature seeds have been disinfected with 75% ethanol for 5 min, then washed with sterile distilled water and have been sterilized in 0.1% HgCl2 supplemented with 1-2 drops of tween 20 for 30 min with shaking and then have been washed five times with sterile distilled water in order to remove excess of the chemical, and have been imbibed in sterile water 24-36h, at room temperature (22-25°C) in complete darkness. The mature embryos of each cultivars have been aseptically isolated from the freshly imbibed seeds with a sharp knife, and ten mature embryos have been cultured with scutellum side by scalpel and forceps in petri dish containing MS medium salt (Murashinge and Skoog,1962). Callus induction salt media have been supplemented with 3.5 mg/l 2,4-D, 30g/l sucrose, then, solidified with 10g/l agar and various concentration of NaCl (0, 4,16 g/l) . The pH of the medium was adjusted to 5.8 prior to autoclaved at 120°C for 20 min. petri dishes have been sealed with polyethylene film and have been placed in a growth culture room under a photoperiod of 16h light/8h dark, at a temperature between 22-25°C.

After four weeks of incubation on callus multiplication and salt media, the obtained calli have been sub-cultured on fresh callus and salt medium, the experiments have been repeated three times.

Plant regeneration under salt treatment: the obtained calli have been shifted to regeneration medium supplemented with 30g/l sucrose, 10g/l agar and plant growth regulators (1mg/l Kinetin and 2mg/l AIA). The calli have been incubated at 25±2°C temperature with 16h light and 8h dark photoperiod for four weeks, then the regenerated shoots cultures have been transferred to MS/2 medium containing 1mg/l kinetin for rooting regeneration.

The callogenesis, Somatic embryogenesis and regeneration rate have been determinated.

The percentages of callus induction, embryogenic calli induction and regeneration percentage have been calculated by the following formula:

- $Percentage\ of\ callus\ induction = \frac{\text{number of calli}}{total\ number\ of\ explants\ cultured} \times 100;$
- $Percentage\ of\ embryogenic\ callus = \frac{\text{number of embryogenic calli}}{total\ number\ of\ calli\ induced} \times 100;$
- $Regeneration\ percentage = \frac{number\ of\ calli\ with\ regenerated\ seedlings}{\text{total number of calli}} \times 100.$

Acclimatization of plant regenerated: Three-to four-weeks after regenerated rooting, *in vitro* raised healthy plantlets have been taken out from the culture bottles and washed gently with sterile water to remove the adhering medium completely. Thereafter, they have been transferred to pots containing autoclaved compost mixture and vermiculite (3:1 v/v). The plantlets have been covered with transparent polyethylene bags to prevent desiccation and have been maintained in room-culture at temperature 28±2°C with a photoperiod 16h light and 8h dark for four weeks.

Plants germination experiments:

Seeds have been sterilized and pre-germinated in Petri dishes for 48h at room temperature and then transferred into pots containing compost and grown under greenhouse conditions (16h light/8h dark photoperiod, 27 ± 10°C, 50 ± 10% relative humidity). Salt stresses have been imposed watering by salt solution (16g/l NaCl).

Molecular analysis:

Regenerated plantlets produced from plant regeneration medium and plant germination experiment were subjected to RAPD analysis and comparison between the responses to slat stress of two culture condition.

DNA isolation: DNA has been extracted from leaf tissue of the six regenerated and germinated wheat genotype using a cetyltrimethyl ammonium bromide (CTAB) method according to (Maniatis *et al*., 1982), after freezing by liquid nitrogen, leaves has been grounded in a mortar with a pestle. 150 mg from the powder has been used for DNA extraction. DNA quality has been tested using 0.8 % agarose gel electrophoresis, the measuring DNA concentrations using Nano Drop instrument and has been diluted to 20 ng with addition sterile distilled water.

PCR amplification: A 25µl mixture contained 1µl of genomic DNA, 12.5 µl of ampli Taq DNA polymerase, 1 µm of GC Enhancer, 1µl of RAPD Primer and 9.5 µl of distilled sterile water. Nine RAPD primers have been used in the study for detect a marker related to salt tolerance. The list of primers is shown in Table1. Amplification has been carried out using a thermal cycler (Applied Bio systems 9700) programmed for 40 cycles as follows: initial denaturing at 94°C for 5 min, further denaturation at 94°C for 1 min, annealing at 37°C for 1 min, extension at 72°C for 1 min and final extension for 7 min at 72°C. The PCR products have been analyzed by electrophoresis on 1.5% agarose gels containing ethidium bromide in 1 X TBE buffer. The RAPD bands have been visualized under UV light and photographed using E-BOX VX2 system. The O'GeneRuler Express DNA leader 1 Kb (Thermos scientific) has been used as a standard molecular weight marker.

Statistical Analysis: Analysis of variance (ANOVA) has been executed and treatment means have been compared by using Duncan multiple range test. Means are the result of three replicated.

Results:

Callus induction with NaCl: Mature embryos cultured on MS medium with 3.5 mg.l^{-1} 2,4-D supplemented with 0,4 and 16g.l^{-1} of NaCl for six durum wheat genotype and incubated under a photoperiod of 16h light/8h at temperature varied between 22-25°C for eight weeks. Observation made during the incubation of cultures showed that the callus is induced after 5 to 8 days of seeding, NaCl have a great effect on regeneration capacity of callus medium. The regeneration rate of callus change with the different concentration of NaCl in the culture medium, callus initiated on MS medium added to 4g.l^{-1} NaCl produced a percentage of regeneration identical significantly to callus initiated on MS medium without NaCl (control), whereas, the percentage of callus initiated on MS medium added to 16g.l^{-1} was significantly different. Also, wheat has different behaviors in their ability to produce plants via callus cultivated in salt medium. The varieties Beliouni, Gumgum Rkhem and beni mestina have better responded better with high concentration of NaCl (16g.l^{-1}) (Table 2). Significant effects were observed between the wheat varieties studied. So, according to their sensibility to NaCl, wheat varieties can be divided into two groups. The regeneration media supplemented with 0 and 4 g.l^{-1} NaCl are the first group produces more regenerated callus and the regeneration media containing 16g.l^{-1} is the second group. Calli of Waha et Adnan-1 are more sensitive to salinity than others varieties. With 16g.l^{-1} NaCl the calli of Beni mestina Gumgum Rkhem and Beliouni gives a good callus regeneration rate.

Somatic embryogenesis induction with NaCl: Table 3 illustrates the results obtained from embryogenic competence for plantlets for the six genotypes tested in different concentrations of salt medium. These results show that the production means of somatic embryogenesis calli for all genotype in the medium added to 16g.l^{-1} (53.67%)

are more important than the means obtained in medium added to 4 $g.l^{-1}$ (42.94%) beside means obtained in control medium with a rate of (69.59%). the genotype effect and concentration of NaCl is also remarked, in control medium; the highest percentage of somatic embryogenesis calli have been registered in Waha, Gumgum Rkhem and Beliouni these percentages were respectively 74.38%, 91.02% and 83.24%. nevertheless, with 16$g.l^{-1}$ NaCl the results showed that it's not the same genotypes witch registered the highest percentage, in these case Adna-1, Adna-2 and Beliouni are the varieties with the highest results, (73.07%,75.43% and 79.47%) respectively.

Regeneration via Somatic Embryo and transplanting: the regeneration started with the appearance of green dots on callus after 4 weeks incubation on regeneration medium and generally produced normal stem and leaves. The results showed that best regeneration percentage in control medium was observed on Beliouni (30.36%) and Adna-2 (24.74%) genotypes; the minimum number of regenerated plantlets was recoded in Gumgum Rkhem genotype (1.3%). While at 16$g.l^{-1}$ concentration the highest rate was reported in Waha and Gumgum Rkhem genotypes (14.15% and 15.6%) respectively, and minimum rate was reported in Adnan-2 (3.38%) and Beni Mestina (3.27%) genotype.(Table 4, Figure 1) resume the results obtained.

Molecular Genetic Marker:

Somaclonal variation among 18 regenerated plant, their original cultivars control and cultured under 16$g.l^{-1}$ NaCl were tested by RAPD analysis. Nine arbitrary oligonucleotide primers were used, number of fragment amplified using these different primers showed that: the numbers of amplified fragment with some primers different from on genotype to another and these primers have amplified 557 PCR bands in all tested genotypes. A maximum of 128 fragments were amplified with primer OPC-05 and minimum of 32 fragments were amplified with primer OPA-17. Eight primers (OPC-05, OPG-09, OPO-13, OPF-20, OPA-17, OPO-05, OPO-06, OPO-03) produced polymorphic RAPD profiles only one primer (B-19) gave monomorphic bands and with a low concentration of DNA. The OPO-13 primer recorded the percentage of polymorphism (11.11%) as it revealed 8 bands in 72 amplified fragments (Table 5). The size of amplified ranged from 1500bp to 100bp approximately (figure 2). The comparison of amplification with OPO-13 primer highlights a difference between the six genotypes of wheat and their response to salt pressure that what is observed in (figure 2). When we want to associate these markers with the salt tolerance we observed positive markers: the amplified band in the regenerated *in vitro* plant in 16 $g.l^{-1}$ NaCl (lane 1: waha, lane 8: Gumgum Khem lane 15: Adnan-1) and negative markers: the bands amplified in controls plants (lane 7: Beliouni control, lane 9: Gemgum Rhem control and lane 12 Adnan-2 Control). The same results were observed in the OPG-09 primer, which recorded the least percentage (1.12%) by revealing one polymorphic band in 89 amplified fragment (table 5). The OPG-09 primer amplified 89 band in all genotypes the size of amplified was ranged from 100bp to 1000bp, number of band Is different from a genotype in another one: Waha,Gemgum Khem, Adnane 2, Beni Mestina and Adnan-1 genotypes cultured *in vitro* and their plants control amplified tree bands in every tested plant (170bp, 220bp and 888bp) Whereas Beliouni genotype amplified only two bands (170bp and 220bp) in control tested plants and only one band *in vitro* cultured plants with 16$g.l^{-1}$ NaCl. The results of RAPD analysis using primer OPG-09 are illustrated in (figure 2).

Table 1: Nucleotide sequences of the 09 primers used in the study.

No	Primer	Sequence (5'to3')
1	OPC-05	GATGACCGCC
2	OPG-09	CTGACGTCAC
3	OPE-13	CCCGATTCGG
4	OPF-20	GGTCTAGAGG
5	OPA-17	GACCGCTTGT
6	OPO-05	CCCAGTCACT
7	OPO-06	CCACGGGAAG
8	OPO-03	CTGATACGCC
9	B-19	ACCCCCGAAG

Table 2: Regeneration of callus induced in medium with different concentration of NaCl after 8 weeks.
Means with the same letter(s) in the same column are not significantly different at 5% using Duncan multiple range test

	Percentage of callus regeneration (%)		
	NaCl Concentration ($g.l^{-1}$)		
Genotypes of wheat	0	4	16
Waha	71.63^{bcd}	68.83^{cd}	27.4^{gh}
Gumgum Rkhem	85.01^{ab}	86.34^{a}	38.72^{fg}
Beliouni	76.91^{abc}	60.14^{de}	49.64^{ef}
Adnan-2	76.66^{abc}	73.34^{abc}	32.98^{gh}
Beni mestina	69.16^{cd}	62.11^{de}	35.89^{fg}
Adna-1	34.7^{gh}	29.65^{gh}	21.82^{h}
Means	$69,03^{a}$	$61,36^{b}$	$34,48^{c}$

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan multiple range test

Table 3: Regeneration of embryogenic callus induced in medium with different concentration of NaCl after 8 weeks.

	Percentage of embryogenic callus (%)		
	NaCl Concentration ($g.l^{-1}$)		
Genotypes of wheat	0	4	16
Waha	74.38^{bc}	61.45^{de}	2.6^{j}
Gumgum Rkhem	91.02^{a}	54.49^{efg}	44.6^{gh}
Beliouni	83.24^{ab}	31.21^{i}	79.47^{b}
Adnan-2	57.55^{def}	35.99^{hi}	75.43^{bc}
Beni mestina	43.45^{gh}	43.58^{gh}	46.85^{fgh}
Adna-1	67.92^{cd}	30.95^{i}	73.07^{bc}
Means	69.59^{a}	42.94^{b}	53.67^{c}

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan multiple range test

Table 4: Regeneration via Somatic Embryo induced in regeneration medium with different concentration of NaCl after 4 weeks of incubation.

	Percentage of Regeneration (%)		
	NaCl Concentration ($g.l^{-1}$)		
Genotypes of wheat	0	4	16
Waha	13.33	22.28	14.15
Gumgum Rkhem	1.3	4.67	15.6
Beliouni	30.36	5.26	4.07
Adnan-2	24.74	16.11	3.38
Beni mestina	5.43	7.8	3.27
Adna-1	6.2	5.39	5.71
Means	13.56	10.25	7.68

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan multiple range test.

Table 5: polymorphism rate for the six durum wheat using OPC-05, OPG-09, OPO-13, OPF-20, OPA-17, OPO-05, OPO-06, OPO-03 and B-19 random primers

Primer code	Sequence (5'-3')	Total amplified band	Polymorphic band	Polymorphism (%)
OPC-05	GATGACCGCC	128	2	4.54
OPG-09	CTGACGTCAC	89	1	1.12
OPE-13	CCCGATTCGG	72	8	11.11
OPF-20	GGTCTAGAGG	80	4	5
OPA-17	GACCGCTTGT	32	2	6.25
OPO-05	CCCAGTCACT	44	2	4.54
OPO-06	CCACGGGAAG	69	1	1.44
OPO-03	CTGATACGCC	43	2	4.64
B-19	ACCCCCGAAG	/	/	/
Total		557	22	/

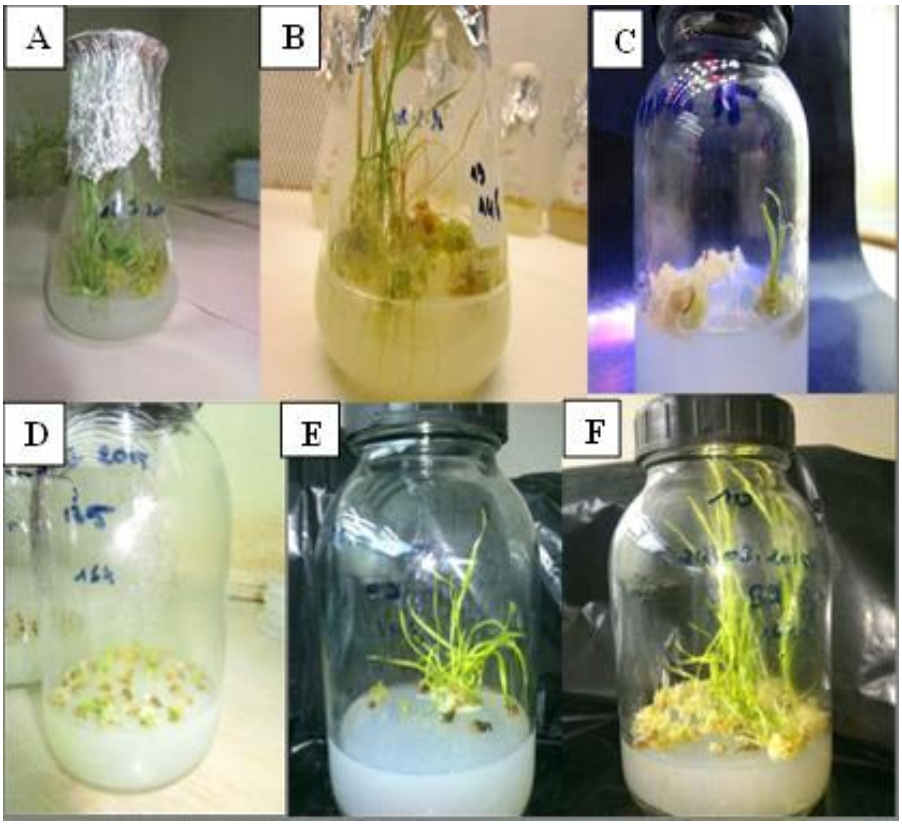

Figure.1: Regeneration of wheat varieties: (A) regeneration of Beliouni genotype on control medium. (B) regeneration of Adnan-2 genotype in control medium. (C) regeneration of Waha genotype on MS medium supplemented with 4g.l^{-1} NaCl. (D) regeneration started of Adnan-2 genotype on MS medium supplemented with 16g.l^{-1} NaCl. (E) (F) regeneration of Adnan-2 genotype on MS medium supplemented with 16g.l^{-1} NaCl.

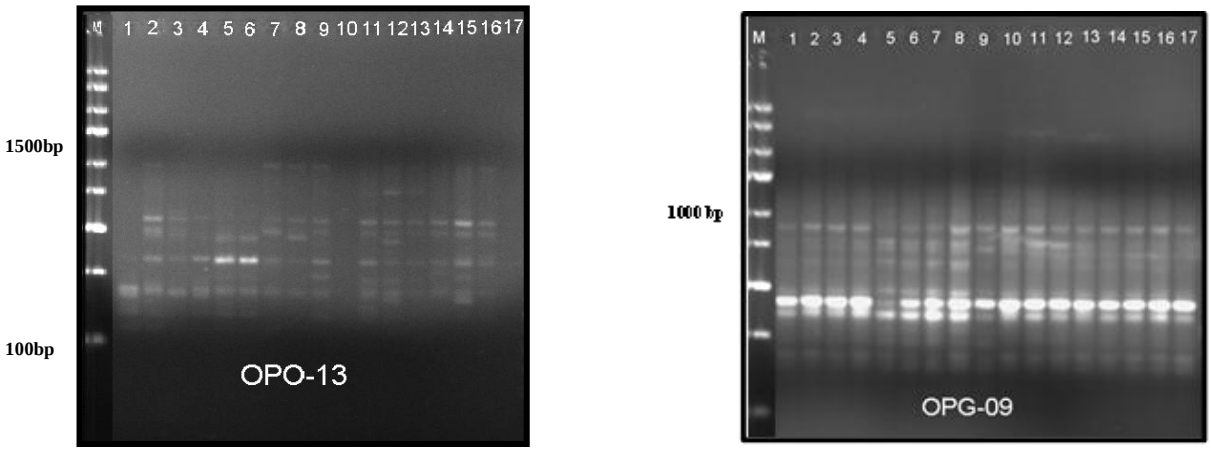

Figure.2: RAPD products obtained by PCR amplification and different primers OPO-13 and OPG-09 of DNA isolated from six durum wheat genotypes in different condition of culture.1-Waha regenerated *(in vitro)* with 16% NaCl after acclimatization. 2-Waha regenerated *(in vitro)* with 16% NaCl before acclimatization. 3-Waha control regenerated *(ex vitro)* in absence of NaCl. 4-Waha control regenerated *(ex vitro)* in 16% NaCl. 5-Beliouni regenerated *(in vitro)* with 16% NaCl. 6-Beliouni control regenerated *(ex vitro*) in 16% NaCl. 7-Beliouni control regenerated (*ex vitro*) in absence of NaCl. 8-Gemgum Khem regenerated *(in vitro)* with 16% NaCl. 9-Gemgum Khem control regenerated *(ex vitro)* in absence of NaCl.10-Adna-2 regenerated *(in vitro)* with 16% NaCl. 11-Adna-2 control regenerated *(ex vitro*) in 16% NaCl. 12-Adna-2 control regenerated *(ex vitro)* in absence of NaCl. 13-Beni mestina control regenerated *(ex vitro*) in 16% NaCl. 14-Beni Mestina control regenerated *(ex vitro*) in absence of NaCl. 15-Adna-1 regenerated *(in vitro)* with 16% NaCl. 16-Adnan-1 control regenerated *(ex vitro)* in absence of NaCl.17- Adna-1 control regenerated (*ex vitro*) in 16% NaCl. M: DNA Marker shown to the left of the figure.

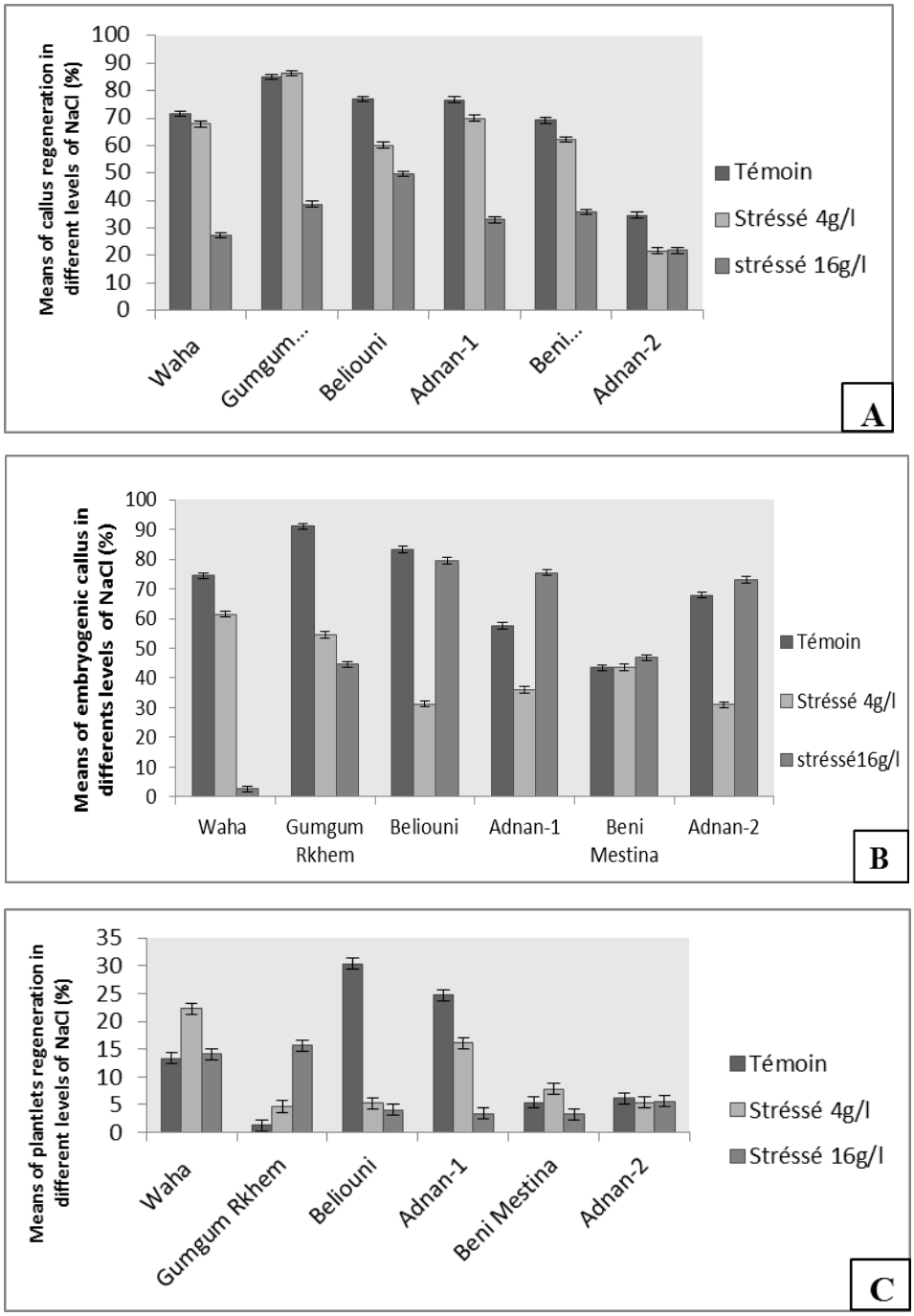

Figure 3: *in vitro* culture response of mature embryo of six different durum wheat genotypes. A) Means of callus regeneration in the six genotypes of wheat in different levels of NaCl. Values are means + SD (n=3). B) Means of embryogenic callus in the six genotypes of wheat in different levels of NaCl. Values are means + SD (n=3). C) Means plantlets regeneration in the six genotypes of wheat in different levels of NaCl. Values are means + SD (n=3).

Discussion:

The callogenesis was initiated from mature embryos, which are the most frequently used explants for the initiation of wheat tissue culture and DNA delivery techniques, moreover, mature embryos are readily available throughout the year, these results are confirmed by different works (Delporte *et al*.,2001, Salama *et al.*, 2013, Almobasher *et al.*,2014), the production of callus in wheat is limited by addition of 2.4-D at the medium induction. 2.4-D was reported as the most widely used growth regulator for induction and callus maintenance in wheat (Quiroz-Figeroa *et al*.,2006, Al. Abdellah *et al.*, 2012), the regulator was added in low concentration, several researches indicated that for callus and embryogenesis induction in wheat the optimal concentration is between 2 mg/l and 4mg/l it depend on the genotypes (Aydin *et al*., 2011, Bouiamrine *et al.*, 2012, Rjurkar *et al*.,2013) in our experimentation the concentration of 3.5mg/l gives a good response to culture these results agree with those reported in (Umer *et al.*, 2009, Ayolié *et al.*, 2011).

For purpose of studied the effect of the NaCl on the callogenesis, the development of somatic embryo and selection tolerant genotypes of wheat; different concentrations of NaCl were added on the culture medium, the results showed that the regeneration rate of callus was reduced with increasing NaCl level in the culture medium, but the effects were different among various NaCl levels. This level is very important with $16g.l^{-1}$ NaCl. Callus initiated on MS medium added to $4g.l^{-1}$ NaCl produced a percentage of regeneration identically significant to callus initiated on MS medium without NaCl (control), the same results was observed in (Ayolié et al., 2011) works. In our case the rate of regeneration of callus represent 88.88 and, 49.94% of the one of control respectively with 4, $16g.l^{-1}$ NaCl, also wheat has different behaviors in their ability to produce plantlets via callus cultivated in salt medium. The analyses of (Figure. 3) highlights that the six varieties of wheat behaves differently in the different stage of cultures, in the callus induction; Gumgum Rkhem, Beliouni and Waha have the highest percentage of response in control medium whereas, in $16g.l^{-1}$ NaCl medium, the varieties react differently in the culture. Beni mestina genotype product a highest percentage of callus in $16g.l^{-1}$ than in control medium it means that Beni mestina varieties Resist better to the salt stress than anthers varieties (Figure.3 A). Our results are in agreement with those obtained by (Benderradji et al., 2007). That shows that the efficiency of callus proliferation differed significantly between two bread wheat genotype at each of the tested salt stress levels. However when we analyse the responses of the six durum wheat genotype in the somatic embryogenesis induction (Figure. 3 B), we note that: in the control medium the varieties which have the highest rate of induction of callus are not the ones which produced the highest rate of somatic embryos, however in $16g.l^{-1}$ NaCl Adnan-1, Adnan-2 and Beliouni are the varieties witch has de highest rate of somatic callus, it means that these varieties are more tolerant to salt than the first one. When we compared the rate of somatic callus induction in the two levels of NaCl (4 and $16g.l^{-1}$) the results showed that the rate in $16g.l^{-1}$ is more important than in $4g.l^{-1}$, we deduct that salt stress at $16g.l^{-1}$ stimulates embryogenic callus development, this information is in agreement with the results reported by (Solis-Marroquin *et al*.,2011) . Salts stress was applied during callus induction and regeneration phase, the results showed that salinity causes a significant reduction in plantlets regenerative capacity the results in (figure. 3 C) prove that the rate of regeneration is decreasing whit increasing the concentration of salt stress NaCl, our results agree with those reported by (Nawaz *et al.*, 2013) ,and we observed that the percentage of regeneration is very low 13.5, 10.25 and 7.68% respectively in 0, 4, $16g.l^{-1}$ NaCl, this weakness is due at recalcitrant nature of durum wheat (Yones *et al.*,2013) , thus Somatic embryo formation and plant regeneration under saline conditions was shown to be strongly genotype dependent. The genotype effect on salt stress response has been recently shown by (Zair *et al.*, 2003).

The results of the *in vitro* culture are confirmed by the Molecular Genetic Marker; the aim of the present study was to provide polymorphic RAPD markers suitable for detected somaclonal variation produced under salt stress. RAPD amplification polymorphism is a powerful technique for detection of somaclonal variation (Khateb *et al*.,2011).

Eight positive markers (OPC-05, OPG-09, OPO-13, OPF-20, OPA-17, OPO-05, OPO-06, OPO-03) were found to be related with salt stress which are in conformity with the results obtained by (Rajurkar et al.,2013) with found six positive markers related with salt stress in maize. (Younis *et al.*,2007) developed four RAPD markers for salt tolerance in sorghum. In our study the result showed that the primer used and there amplification in the plant regenerated *in vitro* under salt stress, can be the proof that the six genotypes of durum wheat has developed a somatic variation under salt stress, We notice that There is a difference between the numbers of fragment amplified, its means that all durum wheat genotypes are not always identical in their DNA capacity to be amplified. The same results was fond in the work of (Hamaid et al., 2013).

Conclusion:

In vitro tissue culture could be an important means of improving crop tolerance and yield through genetic transformation as well as by induced somaclonal variation. Therefore, it is important to control well all the parameters during the *in vitro* culture: The choice of the explant, the best concentration of regulator and the level of

tissue culture to start the stress selection. The results of this study indicated that the effect of callus production, embryogenic callus induction and plantlets regeneration was influenced by addition NaCl in the culture medium, in a low concentration of NaCl ($4g.l^{-1}$), there is no a significates effects on the tissue culture, but depressive effect accentuated by high concentration of salt ($16g.l^{-1}$). Differential genotypic response was also noted in callus ability to proliferate and regenerate plantlets under concentration of NaCl. Also, the results showed that all durum wheat genotypes were not always identical in their DNA ability to be amplified. RAPD analysis is useful molecular tools to indicate genetic polymorphism between the durum wheat genotypes under salt stress.

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