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In vitro Variability of Morphogenetic Responses of Tomato (*Lycopersicon esculentum* Mill.) Explants under Salt Stress and Thidiazuron as Plant Growth Regulator

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Abstract

For the present investigation, six genotypes of tomato (*Lycopersicon esculentum* Mill.) widely grown in Algeria were selected and used as research material to study the morphogenetic variability under stress conditions using different media treatments and two types of explants (cotyledons and hypocotyls) in order to evaluate their responses to callus induction, embryos production and caullogenic capacity. *In vitro* growth seedlings aged for 21 days old were used as source of explants. These explants were cultivated into a MS-based medium containing a constant concentration of TDZ (3mg/L) used as Cytokinins PGR, combined or not with four increasing levels of NaCl at 0, 25, 50 and 75 mM for eight weeks. This Cytokinins predisposes the explant to stress. Statistical analysis indicated a highly significant variations in responses according the nature of genotype, treatment medium, explant origin and interactions there of possess ample influence on morphogenetic characters under these conditions. Results showed that the morphogenesis responses seems to be dependent NaCl levels added in the media, a moderate concentrations including 25 and 50 mM induces compared to control medium. Whereas, medium MT3 had shown a relative to poor response for the most genotypes tested. It is interesting that the moderate combination NaCl/TDZ was better for the embryos production, which can be used directly or converted into artificial seeds for propagation of high value hybrid. All most every tested explant were differentiated into callus but the favorite explant for carried a research on the variability under stress conditions and embryogenic capacity is the cotyledon. Differences among genotypes were detected. For all effects analyzed, homogeneous groups were established using Newman-Keuls post-hot test. This attempt may be used as efficient protocol for genetic improvement and the use of combination PGRs / Stressor agent could be the option for the somatic embryogenesis investigations.

Key-words: Salt stress, Cytokinins, Organogenesis, Somatic Embryogenesis.

INTRODUCTION

The popularity of the tomato for scientists has increased over the years, until it has become a model organism for research programs, both for applied and theoretical purposes. This is probably due to the possibility of growing tomato in different conditions, allowing an understanding of the adaptability of tomato to different abiotic stresses and its ability to be spread asexually by grafting, or to regenerate whole plants from different parts of the plant [1]. Represents an important pool of resources for breeders as well as for scientists to isolate and understand the function of genes which regulate development and growth of tomato [11].

It is grown in almost every country of the world in the field, green houses and net houses, the tomato crop is very versatile and is grown either for fresh market or processing [24].

The production and productivity of several crops continues to be adversely affected due to the various biotic and abiotic stresses. Damages caused by theses stresses are responsible for enormous economic losses worldwide [34]. Therefore it is needed to deploy the biotechnological tools for addressing the critical problems of crop improvement for sustainable agriculture [4]. *In vitro* regeneration of cultivated tomato (*Lycopersicon esculentum* Mill.) has been a constant subject of research because of the commercial value of the crop [7]. Plant growth regulator are small organic molecules that are produced in specific organs or tissues, and which elicit defined responses either directly at the site where are synthesized, or after transport to other organs and tissues. Plant development is believed to be controlled by a tight network of interaction among several different classes of such PGRs [24]. In addition, [8] reports that the role of stress as one of the principal causes for a cell or tissue to change its pre-existing somatic program, reprogramming itself to express the embryogenic pathway.

[25] defined stress as potentially adverse environmental conditions (stressors) that affect plant growth and development and trigger a wide range of responses, from altered gene expression and modification in cellular metabolism to change in growth regulator and crop yield. *In vitro* culture of tomato has been successfully exploited for selection of tolerant cell lines for various biotic and abiotic stress under laboratory conditions [24]. Establishment of an efficient tissue culture protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement [22]. Tissue culture technique has emerged as a feasible and cost-effective alternative tool for developing stress tolerant plants in recent years [4]. This technique can operate under controlled conditions with limited space and time [13].

Thidiazuron (TDZ) has gained a considerable attention during past decades due to its efficient role in plant cell and tissue culture, is widely applied in plant *in vitro* or *in vivo* that influences a number of parameters in plants. Wide array of physiological responses were observed in responses to TDZ applications in different plant species [6].

Many studies showed that TDZ replaces purines based cytokinins in *in vitro* and this is confirmed via purine metabolism inhibitors like diaminopurine (DAP) halted TDZ stimulated somatic embryogenesis in geranium and peanut [33], and was emerged as an effective bio-regulant in cell and tissue cultures in wide array of plant species [32, 31, 16, 15]. TDZ treatment promoted accumulation of mineral ions which induced process of regeneration. Somatic embryogenesis was induced by incorporation of zinc, copper or sodium into the culture media of carrots. Thus, it was inferred that application of TDZ enhanced accumulative minerals or other metabolites and predisposes the explant to stress [6]. [17] suggest a possible close connection or an overlapping between embryogenesis and stress response pathway; [35] proposed that the physiological response to stress conditions could depend on two main factors, the physiological state of cells and the levels (time and intensity) of stress conditions, the cell will die, but if there i slow levels of stress, the cells could induces adaptation conditions. The introduction of a given genotype in *in vitro* selection programs depends on its aptitude to *in vitro* culture, particularly to callus induction and embryogenic callus production [19].

The aim of this work is to study the *in vitro* variability of six genotypes of tomato widely grown in Algeria in response to salt stress in the presence of TDZ as plant growth regulator whish predispose tissue to accept other inductive stimuli. In this context four increasing levels of NaCl combined with one concentration of this PGR in the medium of [34] was used as an efficient protocol. This research was conducted in order to evaluate the variability of morphogenetic responses *in vitro* of theses genotypes under stress conditions.

MATERIALS AND METHODS

Plants material

Tomato seeds (*Lycopersicon esculentum* Mill.) of six genotypes widely grown in Algeria were selected to undertaken this study including: AGORA (F1), TOP48 (F1), Aicha, Heinz1350, Rio-Grande (RG) and Saint-Pierre (STP).

Seed germination and sterilization

Tomato seeds were soaked in tap water for 24 hours for maximum germination according [26]. After soaking duration surface sterilization of seeds was done by washed with water containing a few drops of tween-20 for 3 minutes and then rinsed with tap water for 30 min before the disinfecting agent. This consists in immersion in ethanol at 70 ° for two seconds and then in a sodium hypochlorite solution (1 to 2%) for 15 to 20 minutes. The latter immersion is followed by several rinses with sterilized distilled water for 10 min each. The sterilized seeds of each variety were transferred in sterilized Petri plates contained a basal MS medium Murashige and Skoog (MS) and incubated in the dark for six days, after there are transferred to the growth room (16/8 light/dark) at 25°C ± 2°C. Germinated seedlings aged of 21 days old was served as explants source for tissue culture.

Concentrations screening of TDZ and Na Cl.

One concentration of Thidiazuron was used, the choice of this concentration to accomplished this experiment since, in experiments conducted by [9] whish showed that medium supplemented with **3 mg/L** of TDZ gave the highest percentage of callogenesis and callus formation compared with other plant growth regulator (PGR).

While for the gradual concentrations of NaCl selected, [2] has indicated that under artificial stress conditions, plants are suddenly exposed to height saline concentration (e.g. 100 or 200mM of NaCl) or in increasing steps (25, 50, 75 mM Na Cl). [34] proposed this method of selection as a stepwise long-term treatments, in which cultures are exposed to stress with gradual increase in concentration of selecting agent. These increasing concentration were used in previously experiments; we cited those conducted by [5].

Culture media and sterilization

Murashige and Skoog medium was taken as basic culture medium. Four different Treatments media MT0, MT1, MT2 and MT3 were prepared and adjusted to pH 5.8, filled into culture bottles and sterilized by autoclaving at 121°C for 20 min using Steam sterilizer-blue autoclave (Raypa ; Model : AH75N). Media previously sterilized while still hot were under laminar flow hood and allowed to cool, plant growth regulator Thidiazuron (TDZ, originated from Sigma-Aldrich) was added to each medium using a sterilizing filter with a pore diameter of 0.2 µm after cooling of the culture media. For the different media 25 ml was poured into each Petri dishes. The different media used are:

MT0 = MS nutrient medium + 0 mM NaCl + 3 mg/l TDZ. Medium used as control.

MT1 = MS nutrient medium + 25 mM NaCl + 3 mg/l TDZ.

MT2 = MS nutrient medium + 50 mM NaCl + 3 mg/l TDZ.

MT3 = MS nutrient medium + 75 mM NaCl + 3 mg/l TDZ.

Explants preparation and conditions culture

Hypocotyls segments and cotyledons fragments of 21 days old *in vitro* plants are used as explants and excised under aseptic conditions with a sterile scalpel. 10 pieces per type of explants for each treatment and each variety were cultured separately and placed in different Petri dishes, repeated three times. A total of 1440 explants were treated. Cotyledons were excised, sectioned transversely into four parts (size: 5 mm × 5 mm) and plated with adaxial side up on the different media. All cultures were sealed with parafilm and maintained at 24 ± 2°C under 16 h photoperiod with a photosynthetic photon flux density provided by cool white fluorescent lamps (18W/33, Philips) with a relative humidity of 65%.

Parameters evaluation

Coloration and discrete zone of the cell cluster were used as a criterion to select embryogenic calluses. Embryogenic calluses presented nodular features and a smooth surface, while non embryogenic calluses were drought, friable and translucent [17].

[28] indicated that embryogenic and non embryogenic tissues from *Coffea arabica* calluses are discernible based on coloration; embryogenic calluses are brown (Phenolized) and hard, while non embryogenic are pale and friable; moreover, this correlation between calluses appearance and embryogenic competence has been confirmed by histological studies.

Experimental design and data analysis

The factorial experiment (6×4×2) was designed in a randomized completely block design (RCBD) with three

replicates. Cultures were randomly disposed on the plane of the growth chamber racks and daily controlled. All data were analyzed statistically using the Statistical Analysis program Statistica 6.1 version (Stat Soft, Inc. France). Data were recorded for the Mean ± SD (standard deviation). Before analysis all data were normalized by a transformation using Log_{10} (x + 1). Transformed data were subjected to factorial ANOVA followed by Newman-keuls post-hoc test, the mean and standard deviation values were calculated and compared by multiple range data. P value at 0.05 was used to declare statistical significance.

RESULTS

For each of the characteristics evaluated, the descriptive statistical analysis including six genotypes, four media and two types of explants was obtained after eight weeks of culture and presented in **Table 1** which shows Means ± SD based on the average of data collected among tri-replicates for all the morphogenetic parameters analyzed.

The induced calli exhibited morphological variation and two types of calli were observed: calli were friable **Fig2 (C)** and calli were compact and globular structures **Fig2 (D)**. The results indicated in **Table 1** showed that callus formation could be enhanced by combining intermediates concentration of Chloride sodium (NaCl) and Thidiazuron as Cytokinins PGRs and suppressed by combining higher level of NaCl in the medium. We observed that morphogenetic potential depended to the genotype nature, media treatments and explant origin.

An increase in NaCl concentration from 25 to 50 mM in media culture increased callus induction especially embryogenic callus for all genotypes except **Saint-Pierre** variety which appears as sensible material. Whereas the callus formation was decreased when NaCl concentration up to 75 mM. **Figure 1.**

Analysis of variance **(Table 2)** showed significant variation among the genotypes, media treatments, explant types and interactions for all the parameters **(Table 2)**. According to the F-value, a high genotypic variability was found for embryogenic callus (F = 18.263) followed by number of shoots (**SHT**) and non embryogenic callus (**NEC**). For the media effects on different morphogenetic formation, a highly variation was observed for embryogenic callus (F = 40.709) followed by **NEC**. Maximum explant variation was observed for non embryogenic callus (F= 116.421). In addition, analysis of this variance model indicates the presence of interactions among genotypes × media × explant, which explains the variation in classification and factor interactions. Genotypes and media (G×M) interacted significantly (P<0.01) for all characters except for leafy shoots parameter, similar results was reported for the other interactions (G×M×E) and (G×E). However, no significance interaction was found in shoot formation for media and explant (M×E) interaction, and interacted significantly for leafy shoots at P<0.05, this last was only significantly influenced by this interaction (F = 0.824) and medium has more impact compared to explant factor.

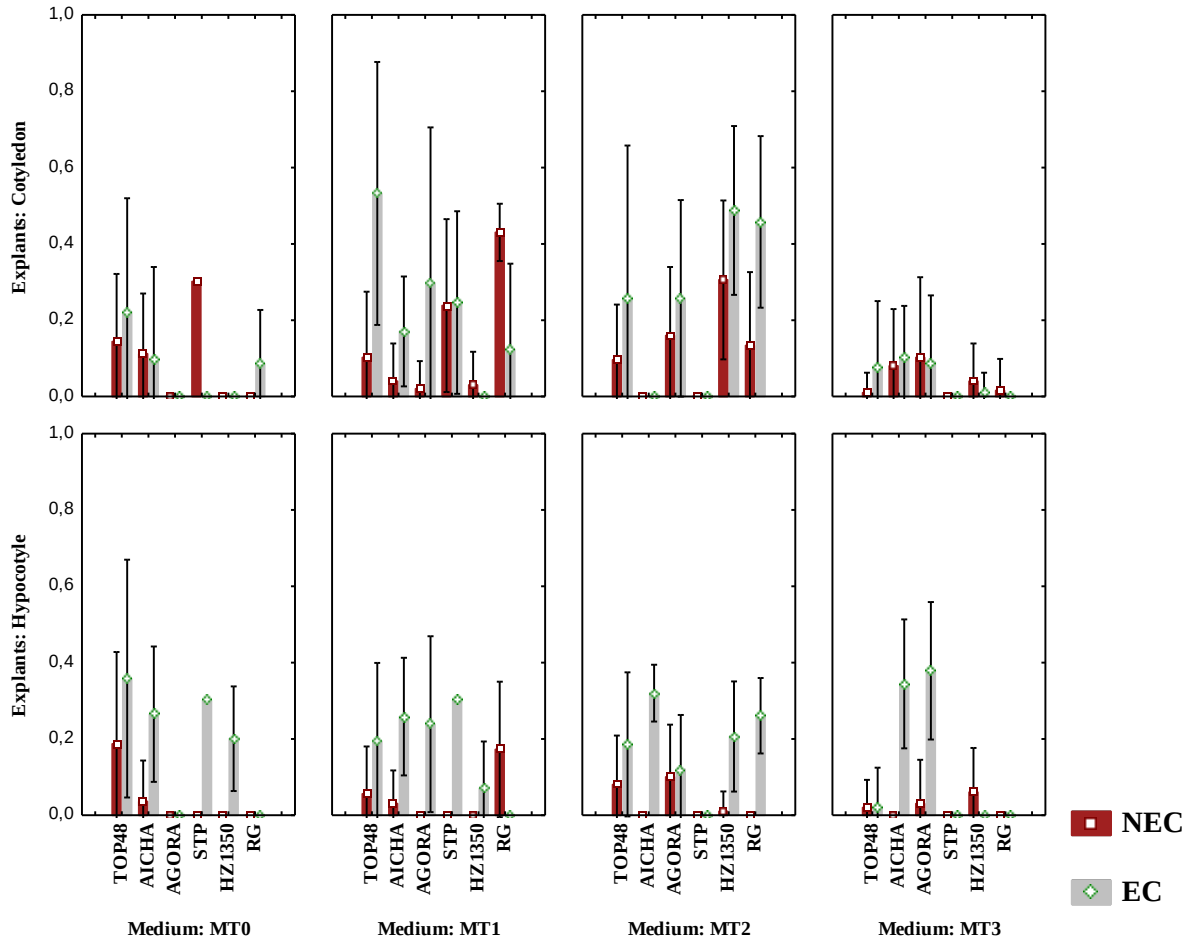

Figure 1. Effect of Genotypes nature, Explant type and Media composition on callus induction (NEC) and embryos production (EC) for all Varieties.

Figure 2. Organogenesis and Somatic Embryogenesis obtained 8 weeks after culture : **A**) Shoots regeneration from Cotyledon callus obtained on MT2 (**Heinz 1350**); **B**) Somatic embryo germination from hypocotyls explants in MT3 (**AGORA F1**); **C**) White and compact callus and shoot growth from hypocotyls of in MT0 (**TOP48 F1**) ; **D**) Callus induction from cotyledons explant in MT1 medium with brown and hard structure (**TOP48 F1**); **E**) Germination of embryogenic callus from cotyledons explant in MT3 (**TOP48 F1**); **F**) Somatic embryo in cotyledonary stage obtained from Hypocotyls in MT1 medium (**TOP48 F1**) .

This could be explained by the fact that the leafy shoots was more influenced by the media and explants tested than by genotypes. Therefore, evaluates of these morphogenetic parameters were affected by genotypes, media treatments, explants and interactions, suggests that they possess ample influence on the morphogenetic potential under *in vitro* culture conditions.

Newman-keuls test (**Table 3**) indicates different groups for all factors, results showed a variation in classification of genotypes, media treatment and explant according each morphogenetic parameter tested. These data suggested the presence of genotypic variability among the genotype tested which allows the screening of the best genotypes in order to enhance this variability. Indeed, the hybrid **TOP48 F1** appears as the best and the only genotype for embryogenic callus with means value of **0.230** and indicated the same group with **Rio-Grande** for non embryogenic callus with two means respectively **0.086** and **0.094**, in addition the two genotypes **AGORA F1** and **AICHA** are also interesting for embryos production and indicate the same group. For shoot formation, **Rio-Grande** shows one group **0.027** compared with the rest of genotypes. Regarding medium effects, it appears that **MT1** supplemented with 25 mM of NaCl says the best medium for all types' formation and records the following values **0.202**; **0.093** and **0.012** respectively for embryogenic callus (**EC**), non embryogenic callus (**NEC**) and shoot regeneration (**SHT**). In addition, medium added with 50 mM of Chloride sodium **MT2,** revealed the same group with **MT1** and found as the most discriminating medium for embryos production (**EC**) with a higher values of **0.212** and induces important proliferation of callus in medium**,** which leads us to think that the use of moderate levels of NaCl at 25 and 50 mM combined with Thidiazuron (**TDZ)** in media culture improve callus induction and especially embryogenic callus production (**EC**). Both treatments **MT1** and **MT2** indicated one group for shoot formation (**SHT**). However, increasing concentration of NaCl to 75 mM (**MT3**) in the medium, drastically affect all morphogenetic formation and was classified as the third group for callus induction with 0.085 for (**EC**) and 0.025 (**NEC**) respectively.

DISCUSSION

Two categories of inductive conditions which allow differentiated cells to develop into competent dedifferentiated cells are now recognized, there are: plant growth regulators (PGRs) (internal and / or external cellular level) and stress factors (osmotic shock, culture medium dehydration, water stress, heavy metal ions, alterations of culture medium pH, heat or cool shock treatment, hypoxia, antibiotics, ultraviolet radiation and mechanical or chemical treatment [29, 27, 18, 20].

In vitro culture of plant cells, tissues or organs on a medium containing selective agents offers the opportunity to select and regenerate plants with desirable characteristics [4]. The technique has also been effectively utilized to induce tolerance which includes the use of some selective agents that permit the preferential survival and growth of desired phenotype [34]. The selecting agents usually employed for *in vitro* selection include NaCl (for salt-tolerance), PEG or manitol (for drought-tolerance). The explants are exposed to a broad range of these selective agent added to the culture medium. Only the explants capable of sustaining such environment survive in the long run and are selected. The tissue culture induces variation in regenerated plants, called soma clonal variation [36]. Methods of selection (long-treatment with increasing gradual concentrations and shock treatment with high concentration) are based on the induction of genetic variation among cells, tissues and / or organs in cultured and regenerated plants [30]. *In vitro* culture of tomato has been successfully exploited for selection of tolerant cell lines for various biotic and abiotic stresses under laboratory conditions, as it requires comparatively less effort and fewer resource's than selection of tomato genotypes under field conditions [24].

The investigation described in this paper aim to evaluate the variability of tomato explants in response to the effects of NaCl in the presence of TDZ. In several studies various types of explants have used such as hypocotyl, cotyledon, stem, and leaf but the use of cotyledon and hypocotyl as explants for *in vitro* plant regeneration has received significant attention, theses explants possess a high capacity for shoot regeneration, somatic embryogenesis and protoplast culture [23]. Callus induction and regeneration capacity of a cell is restricted by many factors like genotype and *in vitro* culture conditions like nutrients, hormone composition and type of explants [12]. For this investigation, the different parameters evaluated indicates a variable responses on all media combination, we suggested that morphogenetic responses varied with nature of genotype, media treatments and explant origin.

Table 1. Morphogenetic parameters average evaluated among genotypes.

Varieties	Media	Explants	N^{br}. NEC	EC	N^{br}. Shoots	N^{br}. LSH
TOP48 (F1)	MT0	Cotyledon	**0.53 ± 0.73**	**1.33 ± 2.69**	0	0
		Hypocotyl	**0.93 ± 1.98**	**2.33 ± 4.42**	**0.5 ± 1.30**	**0.3 ± 1.20**
	MT1	Cotyledon	0.4 ± 0.77	**4.00 ± 5.16**	0.03 ± 0.18	0.06 ± 0.36
		Hypocotyl	**0.48 ± 0.48**	0.8 ± 1.21	0.03 ± 0.18	0
	MT2	Cotyledon	**0.33 ± 0.54**	**1.53 ± 3.12**	0	0
		Hypocotyl	0.26 ± 0.44	0.7 ± 0.79	**0.4 ± 0.4**	0
	MT3	Cotyledon	0.03 ± 0.18	0.33 ± 0.80	0	0
		Hypocotyl	0.06 ± 0.25	0.10 ± 0.54	0	0
AICHA	MT0	Cotyledon	**0.40 ± 0.62**	0.60 ± 1.61	0	0
		Hypocotyl	0.13 ± 0.43	**1.03 ± 1.12**	0.13 ± 0.50	0.13 ± 0.73
	MT1	Cotyledon	0.13 ± 0.34	0.56 ± 0.50	0	0
		Hypocotyl	0.10 ± 0.30	0.93 ± 0.69	**0.18 ± 0.18**	0
	MT2	Cotyledon	0	0	0	0
		Hypocotyl	0	**1.13 ± 0.57**	0.06 ± 0.36	0
	MT3	Cotyledon	0.3 ± 0.65	0.33 ± 0.47	0	0
		Hypocotyl	0	**1.56 ± 2.59**	0.03 ± 0.18	0.06 ± 0.36
AGORA (F1)	MT0	Cotyledon	0	0	0	0
		Hypocotyl	0	0	0	0
	MT1	Cotyledon	0.06 ± 0.25	**2.43 ± 4.13**	0.03 ± 0.18	0
		Hypocotyl	0	**1.00 ± 1.01**	0.03 ± 0.18	0
	MT2	Cotyledon	**0.6 ±0.85**	**1.26 ± 1.94**	0	0
		Hypocotyl	0.33±0.47	0.40±0.49	0	0
	MT3	Cotyledon	0.50± 1.19	0.36±0.76	0.03±0.18	0.06±0.36
		Hypocotyl	0.13±0.57	**1.73±2.09**	0.03±0.18	0
Heinz 1350	MT0	Cotyledon	0	0	0	0
		Hypocotyl	0	0.66±0.47	0.13±0.43	0
	MT1	Cotyledon	0.1±0.30	0	0	0
		Hypocotyl	0	0.23±0.43	**0.23±0.43**	0
	MT2	Cotyledon	**1.3±1.31**	**2.56±2.34**	0.3±0.95	**0.3±0.80**
		Hypocotyl	0.03±0.18	0.7±0.53	0.23±0.56	0
	MT3	Cotyledon	0.13± 0.34	0.03±0.18	0	0
		Hypocotyl	0.2±0.40	0	0	0
Rio-Grande	MT0	Cotyledon	0	0.3 ± 0.53	0	0
		Hypocotyl	0	0	0	0
	MT1	Cotyledon	**1.73 ± 0.44**	0.60 ± 1.3	0	0
		Hypocotyl	**0.63 ± 0.76**	0	**0.46 ± 0.77**	0
	MT2	Cotyledon	**0.53 ± 0.86**	**2.26 ± 1.61**	0	0
		Hypocotyl	0	0.86 ± 0.34	**0.36 ± 0.66**	0
	MT3	Cotyledon	0.06 ± 0.36	0	0	0
		Hypocotyl	0	0	0	0
Saint-Pierre	MT0	Cotyledon	**1.00 ± 0.00**	0	0	0
		Hypocotyl	0	**1.00 ± 0.00**	**0.56 ± 1.16**	0
	MT1	Cotyledon	**1.00 ± 1.08**	**1.10 ± 1.39**	0	0
		Hypocotyl	0	**1.00 ± 0.00**	0	0
	MT2	Cotyledon	0	0	0	0
		Hypocotyl	0	0	0	0
	MT3	Cotyledon	0	0	0	0
		Hypocotyl	0	0	0	0

The bold values show the highest frequency of morphogenetic character induction. Values are means ± standard deviation.

Table 2. Factorial ANOVA analysis of the effect of Genotypes, Media Treatments and Explants on morphogenetic parameters tested.

Source of variation		NEC			EC		SHT		LSHT	
		df	F	P	F	P	F	P	F	P
Genotypes (G)		5	8.462	0.000^{***}	**18.263**	0.000^{***}	8.653	0.000^{***}	1.21	0.310^{ns}
Media (M)		3	18.899	0.000^{***}	**40.709**	0.000^{***}	5.095	0.001^{***}	0.678	0.565^{ns}
Explants (E)		1	**116.421**	0.000^{***}	4.937	0.026^{**}	11.365	0.000^{***}	0.083	0.772^{ns}
Interactions	**G×M**	15	31.680	0.000^{***}	28.751	0.000^{***}	4.465	0.000^{***}	1.502	0.096^{ns}
	G×E	5	10.393	0.000^{***}	23.464	0.000^{***}	12.485	0.000^{***}	1.608	0.154^{ns}
	M×E	3	7.768	0.000^{***}	22.534	0.000^{***}	2.465	0.060^{ns}	2.824	0.037^{**}
	G×M×E	15	13.106	0.000^{***}	9.184	0.000^{***}	4.851	0.000^{***}	1.452	0.115^{ns}

EC, embryogenic callus; NEC, non embryogenic callus; SHT, shoot; LHST leafy shoot, ^{ns} no significance difference; *** $P \leq 0.01$; ** $P \leq 0.05$; F-value, coefficient of Fisher-Snedecor (test at level 5 %).

Table 3. Determination of homogeneous groups according to the genotype nature, media treatments and explant origin.

Source of variation		NEC	EC	SHT
Genotypes	TOP48 F1	0.086 a	0.230 a	0.000 b
	AGORA F1	0.051 c	0.172 b	0.005 b
	Saint-Pierre	0.067 b	0.106 c	0.000 b
	HEINZ	0.055 b	0.121 c	0.007 b
	Rio-Grande	0.094 a	0.116 c	0.027 a
	AICHA	0.037 c	0.194 b	0.008 b
Media	MT0	0.064 b	0.127 b	0.002 b
	MT1	0.093 a	0.202 a	0.012 a
	MT2	0.073 b	0.212 a	0.014 a
	MT3	0.029 c	0.085 c	0.002 b
Explants	Hypocotyls	0.032 b	0.167 a	0.012 a
	Cotyledons	0.098 a	0.146 b	0.003 b

Different letter within columns indicate significant differences ($P < 0.05$) among genotypes, media and explants by the Newman-keuls multiple range test.

[21] reported that for a couple of reasons, the internal hormone content appears to be an inadequate marker of embryogenic potential. First, the internal hormone levels are extremely variable in competent cells of different genotypes and species. Second, in the same experiment, very few differences were observed between competent and non-competent genotypes. For these reasons, other factors must be involved in determining the competence of explants. Based on the wide variation of inducers [8] indicates that Somatic embryogenesis cannot be defined as a specific response to one or more exogenously applied PGRs. On the contrary, these observations indicate that stress plays a critical role as an embryogenic stimulus. [3] reported that exogenous application of phytohormones has been proposed as a pragmatic approach to cope with salt stress. In the same way, [14] indicated that plant treated exogenously with phytohormones revealed prompt and transitory variations in genome wide transcripts profiles.

CONCLUSION

As general conclusion, we can suggest that morphogenetic variations in tomato under *in vitro* culture conditions with combined effect of salt stress with Thidiazuron as plant growth regulator are somehow related to the increasing levels of Chloride sodium (Na Cl) added to the different media, the moderate concentrations varied between 25 and 50 mM appears as an embryogenic stimulus and indicates a variations among the different genotypes treated. we observed the influences of Na Cl on morphogenetic variability and the most variations were increased in these two media, furthermore embryos production was stimulated by the presence of Na Cl in the media, which implies the contribution of this compounds in the improvement of callus production, somatic embryogenesis induction and selection of genotypes with high capacity for tissue culture under stress conditions. These media can be used to explore variability for salt tolerance and used as a stimulus for somatic embryogenesis.

Understanding the role of individual Na Cl or its crosstalk with phytohormones would be seen in the future studies whether changes on genetic and molecular levels for better insight.

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