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THE INFLUENCE OF STRESS INDUCED BY NaCl ON MORPHOGENETIC ASPECTS OF THE CALLUS INITIATED FROM IMMATURE MAIZE EMBRYOS

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Summary. The present work is an attempt to establish the most sensitive and tolerant genotypes to the treatment with NaCl depending on the callus weight and the morphogenetic aspects.

Callus resulted from immature embryos of 17 maize genotypes was stressed with NaCl by two treatment variants:

A – The callus was washed with salt solution at various concentrations (5.0 g/l NaCl and 12.5 g/l NaCl) for one hour and cultivated on agar solid medium, without growth regulators, for one month;

B – The callus was cultivated on agar solidified medium with added NaCl (10 mM and 40 mM) for one month. Callus grown on nutrition medium without growth regulators was used as control.

For both treatment variants we analysed the colour, proliferative ability, shape, rhisogenesis (morphological particularities), type of callus formed, number of somatic embryos, embryogenic area and callus weight.

Depending on these parameters we can select the genotypes which have the ability to restart metabolic processes after the salt stress and will be used in the maize breeding programs

Key words: *Zea mays* L., callus tissue, immature embryos, morphological particularities, NaCl

Abbreviations: NaCl – Sodium chloride; MS – Murashige and Skoog (1962) medium $NBMC_0$ (N_6 – macroelements according to Chu et al., 1975); B_5 – microelements according to Gamborg et al. (1968); MS – vitamins according to Murashige and Skoog (1962).

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Introduction

It is well known that a plant can survive under stress conditions by mechanisms of tolerance or stress avoidance (Levitt, 1980).

Stress avoidance enables the reduction of the stress intensity by morphological adaptation.

The assumption of tolerance mechanisms is to endure the stress by altering normal metabolic activity, restarting growth and the development after the removal of the stress factor.

Concerning the tolerance to salts, maize is a moderately tolerant crop, but selection studies for tolerance to NaCl are just at the beginning.

In vitro techniques allow the selection of the variants tolerant to a stress factor by using isolated cells, callus and tissues.

In regard of maize, as it is a recalcitrant species to *in vitro* culture, the selection studies for cellular lines resistant to stress factors are few.

Tuberosa and Lucchese (1990) selected cellular lines tolerant to Basta herbicide and studies concerning the somatic embryogenesis in stress conditions were made by using different substances with an osmotic role: sucrose, mannitol, proline, abscisic acid and gibberellic acid (Emons et al., 1993).

The present paper is concerned with the evolution and morphological characteristics of the callus initiated from immature maize embryos treated for an hour with salt solutions at concentrations of 5,0 g/l NaCl and 12,5 g/l NaCl.

In order to prolong the state of shock caused by the salt stress on the maize callus, we resorted to the addition of solid culture media with NaCl at various concentrations (10 mM NaCl and 40 mM NaCl) and the cultivation of the callus on these media for a month.

In order to select cellular lines resistant or tolerant to NaCl, analysis of the callus at the very first stages, when it is submitted to the selective pressure should be undertaken.

Materials and Methods

We used callus derived from immature embryos of 17 maize genotypes, 4 inbred lines (Lc 3, Lc 15, W 153, A 188), 12 F_1 hybrid combinations derived from the diallelic crossing of the 4 parental forms and one U simple hybrid with a higher oil content in the grain.

For the first treatment variant, the callus was treated with salt solution at various concentrations (S_1= 5,0 g/l NaCl; S_2 = 12,5 g/l NaCl) for one hour and cultivated on solid medium $NBMC_0$ (original formula: N_6 - macroelements, B_5 - microelements,

MS - vitamins) added with 30 g/l sacharose; 7,5 g/l agar; pH = 5,8, without growth hormones, for one month.

The material taken from the initiation medium was cultivated on the same solid medium $NBMC_0$ with 30 g/l succrose added 7,5 g/l agar; pH = 5,8, without growth regulators, for one month.

In the case of the second treatment variant, the callus taken of the initiation medium was cultivated on basic solid medium MS (Murashige-Skoog 1962) with 30 g/l sucrose; 7,0 g/l agar and the following NaCl concentrations: S_3 = 10 mM NaCl; S_4 = 40 mM NaCl, for one month.

For both treatment variants we analysed the following characteristics: colour, proliferative capacity, shape, rhisogenesis (presence or absence of roots, root colour and shape) type of callus formed (nonembryogenic, embryogenic type I, embryogenic type II, rhisogenic) number of somatic embryos and their embryogenic area (for callus of type II) as well as the callus weight (during the treatment period) in three replications (one callus/replication).

The proliferative intensity of the callus was classified into 3 size categories: weakly proliferative callus; proliferative callus; intens proliferative callus).

The shape of the callus was noted: I (irregular); R (round). The weight of the callus was determined by the difference between the initial and the final weight.

Results and Discussions

For the control variant (not treated with NaCl solution) which was the comparison base for both variants of treatment, the analysis of the morphological characteristics showed that the 17 maize genotypes, developed callus from white to yellow-green, of all types, with or without roots.

The callus weight did not show negative values and during this period (one month) no somatic embryos were formed. No necrotic or anthocyanic areas appeared at the callus level, but some callus showed anthocyanic roots.

In the S_1 treatment variant (Table 1), most of the callus were irregular of type I. Only one genotype W 153×Lc 15, formed a completely round and nonembryogenic callus.

As concerns the proliferative ability, the dominant callus were the average proliferative ones. The colour varied a great deal from white to yellow-green, with embryogenic areas, but also with necrotic areas, or callus that at the beginning of the treatment period was yellow and then became necrotic .

At the genotype level, the characteristics were generally the same for all the three replications, except the genotype W 153×A 188.

Concerning the callus weight, we recorded negative values for the genotypes: Lc 15; W 153; Lc 3×A 188; Lc 15×A 188; W 153×Lc 3; A 188×Lc 15; and the lowest value (- 62,6 mg) was for Lc 15.

Table 1. Morphological characteristics of the callus initiated from immature maize embryos in the S_1 variant

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc 3	1	Yellow, green areas	Prolif.	I	Few, white, thin	II	5	9	119.9
	2	Yellow-green	Prolif.	R	Absent	Nonemb.	–	–	16.2
	3	Yellow, green areas	Prolif.	I	Few, white, thin	II	3	6	125.4
Lc 15	1	Brownish yellow	Prolif.	I	Absent	I	–	–	33.7
	2	Brownish yellow	Prolif.	I	Absent	I	–	–	–15.0
	3	Brownish yellow	Prolif.	I	Absent	I	–	–	–62.6
W153	1	Yellow, necrotic areas	Prolif.	I	Absent	I	–	–	78.8
	2	Yellow, necrotic areas	w. prolif.	I	Absent	I	–	–	–0.8
	3	Yellow, necrotic areas	w. prolif.	I	Absent	I	–	–	38.1
A188	1	White yellowish	Prolif.	I	Absent	I	–	–	72.0
	2	White yellowish	Prolif.	I	Absent	I	–	–	7.3
	3	White yellowish	Prolif.	I	Absent	I	–	–	54.7
Lc3xLc15	1	White-yellowish necr	Prolif.	I	Init. diff.	I	–	–	76.8
	2	White-yellowish necr	Prolif.	I	Init. diff.	I	–	–	287.6
	3	White-yellowish necr	Prolif.	I	Init. diff.	I	–	–	73.6
Lc3xW153	1	Yellow	Prolif.	I	Absent	I	–	–	119.3
	2	Yellow	Prolif.	I	Absent	I	–	–	15.6
	3	Yellow-green	Prolif.	I	Few, purple	I	–	–	89.1
Lc3xA188	1	Yellow-necrotic	Prolif.	I	Absent	I	–	–	44.5
	2	Yellow-necrotic	Prolif.	I	Absent	I	–	–	–12.5
	3	Yellow-necrotic	Prolif.	I	Absent	I	–	–	73.0

Table 1. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc15xLc3	1	Yellow	i.prolif	I	Purple, thin	Rhiso	–	–	431.6
	2	Yellow	Prolif	I	White-purple, thin	Rhiso	–	–	50.6
	3	Yellow, green areas	Prolif	I	Absent	Rhiso	–	–	166.7
Lc15xW153	1	Yellow	i.prolif	I	White-purple, thin	Rhiso	–	–	332.6
	2	White	w.prolif	R	Absent	Nonemb.	–	–	6.0
	3	Yellow	w.prolif	I	Absent	I	–	–	60.7
Lc15xA188	1	Yellow	Prolif	I	Absent	I	–	–	84.8
	2	Yellow	Prolif	I	Absent	I	–	–	–28.1
	3	Yellow	Prolif	I	Absent	I	–	–	49.0
W153x Lc3	1	Yellow, green areas	Prolif.	I	Absent	II	2	4	95.8
	2	Yellow-necrotic	Prolif.	I	Absent	I	–	–	–44.9
	3	Yellow, green areas	Prolif.	I	Absent	II	1	3	34.8
W153xLc15	1	White	w.prolif	R	Absent	Nonemb.	–	–	59.4
	2	White-yellowish	Prolif.	R	Absent	Nonemb.	–	–	10.8
	3	White-yellowish	w. prolif.	R	Absent	Nonemb.	–	–	68.0
W153xA188	1	White-yellowish	w. prolif.	I	White, thin	Rhiso.	–	–	83.1
	2	White	i.prolif	I	White, thin	Rhiso.	–	–	44.0
	3	White-green	Prolif.	I	Few, white, thin	I	–	–	69.5
A188xLc3	1	White-yellowish	w. prolif.	I	White-purple, thin	Rhiso.	–	–	72.5
	2	Yellow-green	Prolif.	I	White-purple, thin	Rhiso.	–	–	66.7
	3	White-yellowish	w. prolif.	I	Absent	Nonemb.	–	–	69.0

Table 1. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
A188xLc15	1	Yellow	w. prolif.	I	Absent	I	–	–	71.4
	2	Yellow	w. prolif.	R	Absent	I	–	–	–15.1
	3	Yellow	w. prolif.	I	White-purple	Rhiso.	–	–	73.7
A188xW153	1	White	i.prolif.	I	Few, white, thin	Nonemb.	–	–	130.5
	2	Yellow-green, green areas	Prolif.	I	Absent	II	4	10	141.4
	3	White	Prolif.	I	Few, white, thin	Nonemb.	–	–	88.8
U	1	Yellow	Prolif.	I	Absent	I	–	–	157.3
	2	Yellow, green areas	Prolif.	I	Few, white, thin	II	8	15	85.0
	3	Yellow	i.prolif.	I	Absent	I	–	–	308.5

nonemb. = nonembryogenic; Rhiso. = rhisogenesis; I = iregular; R = round; i. prolif. = intense proliferative; Prolif. = proliferative; w. prolif = weakely proliferative; necr. = necrotic; Init. diff. = initial differentiation

Embryogenic areas with visible somatic embryos were formed by the callus belonging to the following genotypes: Lc 3; W 153×Lc 3; A 188×W 153 and U.

As concerns the somatic embryos number on embryogenic callus type II, we noticed U genotype (8 somatic embryos on 15 sq.mm area).

We can suggest that the treatment of the callus initiated from imature embryos with salted solution (5,0 g/l NaCl), for one hour, stimulated the somatic embryogenesis.

S_2= 12,5 g/l NaCl (Table 2) is the treatment variant that develops all types of callus, most of them without roots and proliferative medium.

Lc 3, Lc 3×A 188, A 188×Lc 3, A 188×W 153 and U genotypes formed embryogenic callus type II with visible somatic embryos. In the hybrid combinations formula, Lc 3 and A 188, were predominant.

As concerns the callus weight, we recorded negative values for 5 genotypes: Lc 15; Lc 15×A 188; W 153×Lc 15; A 188×Lc 3; A 188×Lc 15.

It is notable that an increase of the NaCl concentration up to 12,5 g/l stimulated the growth of roots, the development of the somatic embryos and the proliferative capacity of the callus obtained from immature maize embryos.

The morphological analyses showed that on S_3 = 10 mM NaCl medium variant (Table 3) the proliferation of the callus is inhibited at the beginning and developed yellow-brown or totally brown callus without necrosis. Later on its surface developed white-yellow callus.

There are genotypes which forms embryogenic callus of type II: A 188;

W 153×Lc 15; A 188×Lc 3, and also genotypes, which forms callus of type II with somatic embryos that become necrotic after a few days (Lc 15×Lc 3). Negative values of callus weight were recorded at: Lc 3; Lc 3×W 153 and A 188×W 153. The highest value of callus weight was recorded at A 188 (459,8 mg callus).

The medium variant S_4 = 40 mM NaCl (Table 4) represents for many callus a shock dose which first inhibits its growth and development, and forms brown or necrotic areas. After a few days, some of the callus restarted metabolical processes, followed by roots appearance and even by the development of the white-yellow or yellow callus on the brown areas.

No somatic embryos formed and the callus weight had negative values at the following genotypes: W 153 (- 7,8 mg); Lc 15×Lc 3 (- 10,5 mg).

By prolonging the state of shock caused by salt stress on the maize callus, by adding NaCl to culture media, we can say that, at 10 mM NaCl, the browning of the callus occurs with the inhibition of callus growth and development and then the metabolic processes restart, forming white-yellow callus even on the brown areas.

The 40 mM NaCl represents a shock dose for most of the callus initiated from immature embryos, having the effect of browning or necrosis.

The restarting of metabolic processes after this shock period is slower and somatic embryos are not formed.

Table 2. Morphological characteristics of the callus initiated from immature maize embryos in the S_2 variant

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc 3	1	Yellow, green areas	Prolif.	I	Few, white, thin	II	4	7	103.3
	2	Yellow, green areas	i.prolif.	I	Few, white, thin	II	4	8	33.7
	3	Yellow-green	w.prolif.	I	Absent	I	–	–	75.0
Lc 15	1	Yellow	w.prolif.	R	Absent	I	–	–	60.3
	2	Yellow	w.prolif.	R	Absent	I	–	–	–25.5
	3	Yellow	w.prolif.	R	Absent	I	–	–	–11.9
W153	1	White yellowish, necr.	w.prolif.	I	White, vigorous	Rhiso	–	–	7.1
	2	White yellowish	Prolif.	I	White, vigorous	Rhiso	–	–	27.3
	3	White yellowish, necr.	w.prolif.	I	Absent	I	–	–	43.1
A188	1	White yellowish	Prolif.	I	Absent	I	–	–	98.6
	2	White yellowish	i.prolif.	I	Few, white, thin	I	–	–	21.2
	3	White yellowish	Prolif.	I	Absent	I	–	–	65.1
Lc3xLc15	1	Yellow-green	i.prolif.	I	White-purple, vig.	Rhiso	–	–	224.8
	2	Yellow, necrotic	w.prolif.	I	Absent	I	–	–	18.1
	3	Yellow	w.prolif.	I	Few, white	I	–	–	69.1
Lc3xW153	1	Yellow	Prolif.	I	Absent	I	–	–	80.7
	2	Yellow-green	Prolif.	I	White-purple	Rhiso	–	–	64.6
	3	Yellow, necrotic	w.prolif.	I	Purple	Rhiso	–	–	58.8
Lc3xA188	1	Yellow	Prolif.	I	Few, white, thin	I	–	–	99.8
	2	Yellow, green areas	Prolif.	I	Absent	II	2	10	87.6
	3	Yellow, green areas	i.prolif.	I	Absent	II	2	6	85.6

Table 2. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc15xLc3	1	White	w.prolif	I	Absent	Nonem.	–	–	68.3
	2	Yellow	Prolif	I	White, few	Rhiso	–	–	8.7
	3	Yellow-green	w.prolif	I	White-purple	Rhiso	–	–	78.3
Lc15xW153	1	Yellow	Prolif	I	Few, white	I	–	–	97.4
	2	Yellow	Prolif	I	White, vigorous	Rhiso.	–	–	149.2
	3	Yellow	w.prolif	I	Few, white, thin	I	–	–	38.4
Lc15xA188	1	Yellow	Prolif	I	Absent	I	–	–	82.1
	2	White	w.prolif	R	Absent	Nonem.	–	–	–10.4
	3	White	Prolif	I	Absent	Nonem.	–	–	89.4
W153x Lc3	1	White	i.prolif.	I	White, long	Rhiso.	–	–	605.3
	2	White yellowish	Prolif.	I	Few, white	I	–	–	56.3
	3	White yellowish necr.	Prolif.	I	Absent	I	–	–	44.2
W153xLc15	1	White	i.prolif	I	Absent	Nonemb.	–	–	54.2
	2	White	i.prolif.	I	Absent	Nonemb.	–	–	–18.6
	3	White	i.prolif.	R	Absent	Nonemb.	–	–	120.0
W153xA188	1	White	Prolif.	R	Absent	Nonemb.	–	–	56.4
	2	White	Prolif	R	Absent	Nonemb.	–	–	16.9
	3	White	Prolif.	R	Absent	Nonemb.	–	–	49.6
A188xLc3	1	Yellow	Prolif.	I	Few, white, thin	I	–	–	123.8
	2	Yellow	Prolif.	I	Few, white, thin	I	–	–	–0.7
	3	Yellow, green areas	Prolif.	I	Few, white, thin	II	4	12	150.5

Table 2. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
A188xLc15	1	Yellow	Prolif.	I	Absent	I	–	–	55.9
	2	Yellow	Prolif.	I	Few, white, thin	I	–	–	–0.1
	3	Yellow-green	Prolif.	I	White-purple, thin	I	–	–	87.8
A188xW153	1	White-green	i.prolif.	I	White, vigorous	Rhiso	–	–	370.2
	2	Yellow, green areas	i.prolif.	I	Absent	II	11	18	183.3
	3	White	i.prolif.	I	White, vigorous	Rhiso.	–	–	342.2
U	1	Yellow	Prolif.	I	Absent	I	–	–	77.6
	2	Yellow, green areas	Prolif.	I	Absent	II	2	7	48.9
	3	Yellow	Prolif.	I	Absent	I	–	–	66.3

nonemb. = nonembryogenic; Rhiso. = rhisogenesis; I = irregular; R = round; i. prolif. = intense proliferative; Prolif. = proliferative; w. prolif = weakely proliferative; necr. = necrotic; vig. = vigorouses

Table 3. Morphological characteristics of the callus initiated from immature maize embryos in the S_3 variant

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc 3	1	Yellow-brown→white	w.prolif.	I	Absent	Nonemb.	–	–	89.4
	2	Brownish→yellow	w.prolif.	I	Absent	Nonemb.	–	–	–0.4
	3	Brownish→yellow	w.prolif.	I	Absent	Nonemb.	–	–	83.3
Lc 15	1	Yellow-brown	Prolif.	I	White, thin	Rhiso.	–	–	157.1
	2	Yellow-brown	Prolif.	I	White, thin	Rhiso.	–	–	5.8
	3	Yellow-brown	w.prolif.	R	Initial differentiation	Nonemb.	–	–	77.3
W153	1	Brownish→white-yellowish	w.prolif.	R	Absent	Nonemb.	–	–	109,0
	2	Brownish→white-yellowish	w.prolif.	R	Absent	Nonemb.	–	–	85.3
	3	Brownish→ necrotic	w.prolif.	R	Absent	Nonemb.	–	–	99.6
A188	1	Yellow	i.prolif.	I	White, thin	Rhiso.	–	–	459.8
	2	Yellow	i.prolif.	I	Few, white, thin	I	–	–	104.1
	3	Yellow, green areas	i.prolif.	I	Few, white, thin	II	3	4	190.4
Lc3×Lc15	1	White - yellowish	i.prolif.	R	Few, purple, thin	Nonemb.	–	–	229.6
	2	Yellow- green	i.prolif.	I	Few, purple, thin	I	–	–	167.7
	3	White-yellowish→ necrotic	Prolif.	I	Absent	Nonemb.	–	–	251.0
Lc3×W153	1	Yellow-green	i.prolif.	I	Few, white, vig.	I	–	–	156.2
	2	Yellow-green	Prolif.	I	Absent	I	–	–	–5.0
	3	Yellow-green	i.prolif.	I	Few, white, thin	II	–	–	253.9
Lc3×A188	1	Yellow	w.prolif.	I	Few, purple	I	–	–	110.8
	2	Yellow	i.prolif.	I	Few, white, purple	I	–	–	198.3
	3	Yellow-green	w.prolif.	R	Absent	I	–	–	191.5

Table 3. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc15×Lc3	1	Yellow, green areas	i.prolif	I	Few, white	II	–	–	95.4
	2	Yellow, green areas	i.prolif	I	Few, white,	II	–	–	249.0
	3	Yellow, purple areas	Prolif	I	Absent	I	–	–	68.2
Lc15×W153	1	Brownish→ white	Prolif	I	White, thin	Rhiso	–	–	122.2
	2	Yellow-brown	Prolif	I	White, thin	Rhiso.	–	–	95.9
	3	White→ brownish	w.prolif	R	Absent	Nonemb.	–	–	75.8
Lc15×A188	1	White	i.prolif	R	Absent	Nonemb.	–	–	101.4
	2	White	Prolif	I	White-purple, thin	R-Nonemb	–	–	63.3
	3	White	Prolif	R	Absent	Nonemb.	–	–	90.6
W153× Lc3	1	White-yellowish	i.prolif.	I	Purple, vigorouses	Rhiso.	–	–	397.9
	2	White-yellowish	i.prolif.	I	Purple, vigorouses	Rhiso.	–	–	186.8
	3	White	Prolif.	R	Absent	Nonemb.	–	–	164.7
W153×Lc15	1	Brownish→ yellow-green	Prolif	I	Few, white, thin	I	–	–	168.1
	2	Yellow, green areas	i.prolif.	I	Absent	II	3	5	15.6
	3	Brownish→ yellow	i.prolif.	I	Few, white, thin	I	–	–	153.1
W153×A188	1	White brownish	Prolif.	I	Absent	Nonemb.	–	–	110.5
	2	White-yellowish	Prolif	I	Few, white, thin	Nonemb.	–	–	38.5
	3	White-yellowish	Prolif.	I	Few, white, thin	Nonemb.	–	–	159.1
A188×Lc3	1	Yellow brownish	Prolif.	I	Absent	I	–	–	121.0
	2	Yellow, green areas	i.prolif.	I	Few, white, thin	II	7	14	65.2
	3	Yellow brownish	w.prolif.	I	Absent	I	–	–	76.0

Table 3. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
A188xLc15	1	White-yellowish	Prolif.	I	Few, white	Nonemb.	–	–	132.9
	2	White-yellowish	Prolif.	I	White, thin	R	–	–	55.6
	3	Brownish→ yellow green	w.prolif.	I	Absent	Nonemb.	–	–	101.3
A188xW153	1	Brownish→ white	w.prolif.	I	Absent	Nonemb.	–	–	96.9
	2	Brownish→ white	w.prolif.	I	Absent	Nonemb.	–	–	–2,4
	3	Brownish→ white	w.prolif.	I	Absent	Nonemb.	–	–	76.6
U	1	Brownish→ yellow	w.prolif.	I	Absent	I	–	–	87.9
	2	Yellow	Prolif.	I	Absent	I	–	–	16.9
	3	Brownish→ white-yellowish	w.prolif.	R	Absent	Nonemb.	–	–	86.6

nonemb. = nonembryogenic; Rhiso. = rhysogenesis; I = iregular; R = round; i. prolif. = intense proliferative; Prolif. = proliferative;
w. prolif = weakely proliferative;

Table 4. Morphological characteristics of the callus initiated from immature maize embryos in the S_4 variant

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc 3	1	White-yellowish necrotic areas	Prolif.	I	Absent	I	–	–	106.5
	2	Brownish→yellow	Prolif.	I	Absent	I	–	–	67.9
	3	White-yellowish	w.prolif.	R	Absent	Nonemb.	–	–	101.3
Lc 15	1	Yellow-green	w.prolif.	R	Initial differentiation	Nonemb.	–	–	65.6
	2	Yellow-green	Prolif.	I	Initial differentiation	Nonemb.	–	–	0.2
	3	Yellow-green	w.prolif.	I	White, vig., short	Rhiso.	–	–	107.1
W153	1	Yellow-green	i.prolif.	I	Absent	I	–	–	167.5
	2	Yellow-green	Prolif.	I	Absent	I	–	–	–7.8
	3	Yellow-green	Prolif.	I	Absent	I	–	–	77.2
A188	1	Brownish→yellow	Prolif.	I	Few, White, vig.	I	–	–	94.9
	2	Brownish→yellow	Prolif.	I	Few, white, vig.	I	–	–	29.5
	3	Brownish→yellow	Prolif.	I	Few, white, vig.	I	–	–	106.4
Lc3×Lc15	1	White -yellowish	i.prolif.	I	Few, white, thin	Nonemb.	–	–	117.6
	2	White-yellowish	i.prolif.	I	Few, white, thin	Nonemb.	–	–	79.2
	3	White→ brownish	Prolif.	I	Initial differentiation	Nonemb.	–	–	101.2
Lc3×W153	1	Yellow	w.prolif.	R	Few, white, thin	I	–	–	58.3
	2	Yellow, necrotic areas	w.prolif.	R	Few, white, thin	I	–	–	21.0
	3	Yellow→ necrotic	w.prolif.	R	Absent	I	–	–	37.9
Lc3×A188	1	White-yellowish	i.prolif.	I	Few, white, purple	I	–	–	239.4
	2	Yellow-green	i.prolif.	I	Few, white, purple	I	–	–	138.2
	3	White, green areas	Prolif.	I	Few, white, thin, vig.	I	–	–	157.1

Table 4. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
Lc15×Lc3	1	Yellow-brown	w.prolif	R	Absent	I	–	–	59.0
	2	Yellow, necrotic areas	w.prolif	R	Absent	I	–	–	–10.5
	3	White→brownish	Prolif	I	Absent	I	–	–	59.5
Lc15×W153	1	Yellow-brown	Prolif	I	Few, white, thin, short	I	–	–	92.5
	2	White-yellowish	i.prolif	I	Absent	Nonemb.	–	–	52.1
	3	Yellow→brownish	Prolif	I	Few, white, short, vig.	I	–	–	95.5
Lc15×A188	1	White	i.prolif	I	Absent	Nonemb.	–	–	91.5
	2	White, brownish areas	Prolif	I	Few, short, thin	Nonemb.	–	–	39.0
	3	White, necrotic areas	Prolif	I	Absent	Nonemb.	–	–	40.7
W153× Lc3	1	White	i.prolif.	I	Absent	Nonemb.	–	–	179.3
	2	White	i.prolif.	I	Absent	Nonemb.	–	–	77.7
	3	White→brownish	Prolif.	I	Few, white, short, vig.	Nonemb.	–	–	158.9
W153×Lc15	1	White-yellowish, brown areas	Prolif	R	Absent	Nonemb.	–	–	96.0
	2	White-yellowish, brown areas	w.prolif.	R	Absent	Nonemb	–	–	11.6
	3	White-yellowish, brown areas	w.prolif.	R	Absent	Nonemb.	–	–	101.4
W153×A188	1	White	i.prolif.	I	Few, white, short, vig.	Nonemb.	–	–	106.7
	2	White	i.prolif	I	Few, white, short, vig.	Nonemb.	–	–	58.7
	3	White	i.prolif.	I	Few, white, short, vig.	Nonemb.	–	–	135.2
A188×Lc3	1	White	i.prolif.	I	Absent	Nonemb.	–	–	81.5
	2	Yellow	i.prolif.	I	Few, white, short, vig.	I	–	–	182.1
	3	White	i.prolif.	I	Few, white, short, vig.	Nonemb.	–	–	138.5

Table 4. Continued

Geno-type	Rep	Colour	Prolif. ability	Shape	Rhisogenesis (caract. morph)	Callus type	Somatic embryo number	Embryo area (sq.mm)	Wet weight of the callus (mg)
A188×Lc15	1	White	Prolif.	I	Few, white, short, vig.	Nonemb.	–	–	75.0
	2	White	Prolif.	I	Few, white, short, thin	Nonemb.	–	–	46.3
	3	White-yellowish	i.prolif.	I	Few, white, short, thin	Nonemb.	–	–	90.4
A188×W153	1	White→ brownish	Prolif.	I	Few, white, short, thin	Nonemb.	–	–	127.3
	2	White	i.prolif.	I	Few, white, long, vig.	Nonemb.	–	–	64.4
	3	White, brownish areas	Prolif.	I	Absent	Nonemb.	–	–	98.6
U	1	Yellow-green	Prolif.	I	Absent	I	–	–	207.9
	2	White-yellowish	Prolif.	I	Absent	Nonemb.	–	–	7.2
	3	White-yellowish	i.prolif.	I	Absent	Nonemb.	–	–	126.3

nonemb. = nonembryogenic; Rhiso. = rhysogenesis; I = iregular; R = round; i. prolif. = intense proliferative; Prolif. = proliferative; w. prolif = weakely proliferative; vig. = vigorous.

The reaction of the callus initiated from maize immature embryos to salt stress showed that, for all treatment variants, the genotype is the first factor which determines cellular proliferation.

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