

EFFECT OF SALT ON GERMINATION AND EARLY GROWTH IN SUNFLOWER

Osman Y.M.¹, Cremonini R.², Baldini M.¹, Vannozzi G.P.³

¹Dipartimento di Agronomia e gestione dell'Agrosistema, Università di Pisa

²Dipartimento di Scienze Botaniche, Università di Pisa

³Istituto di Produzioni Vegetali, Università di Udine, Italy

SUMMARY

Achenes of eight high oleic content lines of *Helianthus annuus* were germinated in Petri dishes at four osmotic potentials of sodium chloride.

Germination behaviour is analyzed. Seedling growth proved to be more sensitive than germination to salinity stress, cms lines are more affected.

Newly germinated plants show a decrease in size and weight with salinity increment.

Key words: Germination, salt tolerance, oil content, *Helianthus annuus* L.

INTRODUCTION

A large number of investigations has been carried out on crops to elucidate the physiological, biochemical and genetic basis of salinity tolerance.

Soil degradation is becoming a menace phenomenon that can cause soil sterility and starvation for many people. Environmental deterioration may aggravate this phenomenon unless an effective strategy is found.

Soil salinity leads a serious challenge for soil degradation in both developed and developing countries.

Salinity has been recognized as a factor limiting crop productivity, particularly in irrigated areas (Unnikrishnan et al., 1991).

In arid and semiarid regions, insufficient rainfall results in extensive reliance on irrigation and salt concentration in soil solutions creates high osmotic pressure, reducing the availability of soil water in plants and specific ion effects such as toxicity due to high concentration of sodium chloride.

Salt stress has both osmotic and physiological and metabolic components, but plant response to salt stress is affected not only by the salinity as osmotic potential but also by the specific chemical composition of the saline medium (Cramer et al., 1988).

In many studies on salinity, specially those concerned with germination, a single salt solution of sodium chloride had attracted many authors (Ayers, 1952; Ashraf et al., 1983). From many reports it is evident that salinity stress involves many aspects of the life of plants but it is not easy to establish a chain of priorities (Flowers et al., 1977). It is not easy to get a scale of salt tolerance for plants because some species are sensitive to

Correspondence to: Prof. R. Cremonini, Dipartimento di Scienze Botaniche, Via L. Ghini 5, Università degli Studi, I-56100 PISA, Italy

salt conditions in some period of their development and become resistant in other ones (Sayed, 1985).

Since little work has been done on salt tolerance in sunflower, in order to study the mechanism and the nature of salt tolerance we have determined the reactions of our material in terms of germination, initial growth and fresh and dry mass.

The aim of this investigation is to get information both about the utilization of marginal soil for plant production in different environmental conditions and selection of populations or ecotypes able to colonize naturally saline habitats.

MATERIALS AND METHODS

Characteristics of eight lines of *Helianthus annuus* are summarized in Table 1. We have used six restorer lines and two cytoplasmic male sterile lines.

Table 1. Characteristics of eight lines of *Helianthus annuus*

Name	Oil content (%)	Distinctive features	Seed provenance
RHA-344	86.3	restorer-inbred line	USDA-ARS
RHA-345	87.4	restorer-inbred line	USDA-ARS
RHA-346	87.2	restorer-inbred line	USDA-ARS
RHA-347	86.4	restorer-inbred line	USDA-ARS
RHA-348	85.8	restorer-inbred line	USDA-ARS
RHA-355	84.8	restorer-inbred line	USDA-ARS
HA-341	89.6	cms - inbred line	USDA-ARS
HA-350	90.5	cms - inbred line	USDA-ARS

1 - Germination tests.

Achenes were surface-sterilized with 5% sodium hypochloride solution for 10 minutes, and washed in running tap water for 30 minutes. Previous experiments have indicated that they did not have requirement of light for germination and that 6.0 ml of water on NaCl solution added to a Petri dish containing 20 achenes were enough to keep the filter paper moist during the period of germination.

Achenes of the eight lines were sown in 12 mm Petri dishes on filter paper (Watman n.2) soaked with distilled water or various concentrations of sodium chloride (0.062, 0.125, 0.187, 0.250 M, Tab.2). Twenty achenes per dish were used.

The dishes were placed in an incubator in the dark at the temperature of 23°C. Germination was obtained on filter paper wetted with the proper salt solution inside a Petri dishes sealed with laboratory film in order to avoid partial evaporation. For each salt concentration the percentage of germination was tested at 24, 48 and 72 hours from the beginning of the experiments. We consider germination, as usually defined, as radicle emergence.

2 - Growth in salt conditions.

The influence of salt solution was tested at 72 hours by determination of: i) root length, ii) fresh and dry matter of plantlets, iii) water content for each salt concentration.

The experimental data are derived from three different tests carried out.

Table 2 – Sodium chloride molarity and the corresponding osmotic pressure at 23°C

NaCl (Molarity)	Osmotic pressure (Mbars)
0.000	0.00
0.062	-2.89
0.125	-5.73
0.187	-8.56
0.250	-11.46

Table 3. Germination (% , \pm S.E.) of sunflower lines with high oleic acid content in different sodium chloride solutions at 24 (a), 48 (b) and 72 hours (c)

Line		NaCl (M)				
		0.000	0.062	0.125	0.187	0.250
RHA-344	a	95 \pm 0.5	87 \pm 0.3	80 \pm 0.5	46 \pm 1.5	2 \pm 0.3
	b	100	98 \pm 0.3	98 \pm 0.3	92 \pm 0.3	80 \pm 0.2
	c	–	100	100	97 \pm 0.3	96 \pm 0.3
RHA-345	a	75 \pm 0.5	70 \pm 0.9	53 \pm 0.7	32 \pm 1.0	27 \pm 0.5
	b	100	100	98 \pm 0.3	92 \pm 0.3	65 \pm 1.2
	c	–	–	100	98 \pm 0.3	91 \pm 1.0
RHA-346	a	60 \pm 0.5	45 \pm 0.6	40 \pm 0.6	30 \pm 0.6	18 \pm 0.3
	b	88 \pm 0.7	86 \pm 0.7	83 \pm 0.7	85 \pm 0.5	45 \pm 0.3
	c	100	98 \pm 0.5	97 \pm 0.3	91 \pm 0.6	75 \pm 1.4
RHA-347	a	78 \pm 0.7	86 \pm 0.3	85 \pm 0.5	60 \pm 0.8	28 \pm 0.3
	b	96 \pm 0.5	98 \pm 0.3	97 \pm 0.3	92 \pm 0.2	70 \pm 0.8
	c	100	100	100	95 \pm 0.2	91 \pm 0.9
RHA-348	a	90 \pm 0.8	98 \pm 0.3	98 \pm 0.3	98 \pm 0.3	50 \pm 1.2
	b	98 \pm 0.3	100	100	95 \pm 0.1	90 \pm 1.3
	c	100	–	–	95 \pm 0.1	95 \pm 0.5
RHA-355	a	93 \pm 0.3	97 \pm 0.3	97 \pm 0.3	97 \pm 0.3	55 \pm 0.5
	b	97 \pm 0.3	98 \pm 0.3	98 \pm 0.1	100	100
	c	100	100	100	–	–
HA-341	a	63 \pm 0.9	83 \pm 0.7	50 \pm 0.4	33 \pm 0.5	3 \pm 0.3
	b	81 \pm 1.0	90 \pm 0.2	97 \pm 0.3	83 \pm 0.9	58 \pm 0.3
	c	90 \pm 0.5	93 \pm 0.2	98 \pm 0.3	85 \pm 0.5	81 \pm 0.5
HA-350	a	51 \pm 0.5	8 \pm 0.3	8 \pm 0.3	11 \pm 0.9	0
	b	55 \pm 0.5	51 \pm 0.3	60 \pm 2.0	66 \pm 0.9	38 \pm 0.5
	c	65 \pm 0.3	58 \pm 0.3	70 \pm 1.4	77 \pm 0.9	63 \pm 0.5

RESULTS

After 24-hour germination at 0.25 M NaCl, the concentration in the restorer lines varied from 2% in RHA-344 line to 55% in RHA-355G (Table 3). The cms line HA-350 shows values of germination is completely inhibited in the line HA-341. At 48 and 72 hours, some restorer lines evidence high values of germination (RHA-355, RHA-344 and RHA-348), the cms lines 81 and 63% for HA-341 and HA-350 respectively.

At low salt concentration the restorer lines in 48–72 hours reached the values of germination between 91 and 100%; the cms lines give an increase with 0.125 M and 0.187

M NaCl concentrations for HA-341 and HA-350 respectively.

As regards the root length at 72 hours some restorer lines were inhibited by high salt concentration (Table 4; RHA-344, RHA-355, RHA-345 and RHA-347) other restorer lines (RHA-348, RHA-346) showed to be stimulated by intermediate salt concentrations.

Table 4. Root length (mm±E.S.) of eight high oleic *Helianthus annuus* L. lines with different molarity of NaCl at 72 hours

Line	NaCl (M)				
	0.000	0.062	0.125	0.187	0.250
RHA-344	26.5±1.2	19.9±0.6	13.7±0.4	7.5±0.3	4.4±0.6
RHA-355	33.2±2.5	28.6±1.5	18.7±1.1	10.6±0.6	5.1±1.1
RHA-348	9.7±1.8	18.0±0.8	11.6±0.5	6.6±0.4	4.0±0.2
RHA-345	13.1±0.8	13.3±0.5	9.8±0.4	5.7±0.2	3.4±0.1
RHA-346	4.3±0.5	7.9±8.7	8.7±0.4	5.6±0.3	2.9±0.2
RHA-347	10.5±1.2	11.7±0.8	9.2±0.4	5.1±0.2	3.3±0.2
HA-341	7.9±0.7	9.4±0.6	8.8±0.4	5.3±0.2	2.8±0.2
HA-350	9.3±0.8	8.2±0.8	7.5±0.6	6.4±0.6	5.7±0.3

The values of fresh weight, dry weight and water content are summarized in Table 5. The water content in the control solution (0.00 M NaCl) is expressed as 100, the other values are expressed as percentage of the control. In the restorer lines when the molarity of NaCl increased (e.g.: 0.062) two lines RHA-348 and RHA-345 showed an increase of fresh weight and water content. The two cms lines had a different behaviour, while HA-341 increases with the intermediate salt solution, the other, HA-350, showed a continual decrease in weight and water content.

DISCUSSION

A detailed analysis of the results evidences a negative correlation between the increase of salt concentration and germination confirming the previous results (Ungar 1973; Onnis et al., 1987; Forster et al., 1987, 1988; Naidoo and Rughunanan 1990). All restorer lines were able to germinate reaching values between 90 and 100% even with higher concentrated salt solutions. The cms lines evidence a reduced capacity of germination, not directly linkable with the increase of salt concentrations.

The growth of the roots evidences, with stronger salt concentration, correlation, as it happens in some Gramineae (Hapson and Simpson, 1989a, 1989b; Gorham 1990a, 1990b) and in many halophytes (Greenway and Munns, 1980; Naidoo and Rughunanan 1990).

With low salt concentrations the restorer lines show a major growth compared with the cms lines and inside the restorer group it is possible to recognize a class comprising RHA-346, RHA-347 for which the growth is steady or is, in many cases, stimulated. As regards the cms lines, the low salt concentration had no influence on the growth. It was always constant even if reduced compared with the restorer lines.

Table 5. Mean value (\pm S.E.) of fresh matter (a), dry (b) weight (mg) and water content (%),c) of *Helianthus annuus* lines after 72 hours of growth in different NaCl solutions at 23 C

Line		NaCl (M)				
		0.000	0.062	0.125	0.187	0.250
RHA-344	a	75.1 \pm 2.2	69.0 \pm 2.2	62.3 \pm 1.3	54.2 \pm 0.7	44.3 \pm 0.7
	b	21.2 \pm 0.8	22.3 \pm 0.8	23.8 \pm 0.4	26.2 \pm 0.7	26.3 \pm 0.7
	c	100	81	62	40	26
RHA-345	a	85.0 \pm 1.0	94.6 \pm 1.3	72.0 \pm 0.5	54.4 \pm 0.2	45.3 \pm 1.4
	b	31.0 \pm 0.2	34.0 \pm 0.5	30.9 \pm 0.6	28.3 \pm 0.8	26.7 \pm 1.2
	c	100	102	79	53	40
RHA-346	a	59.6 \pm 1.6	63.0 \pm 2.5	66.0 \pm 3.3	57.9 \pm 1.6	51.6 \pm 2.7
	b	33.6 \pm 0.7	30.2 \pm 1.3	32.5 \pm 1.2	32.2 \pm 0.9	30.7 \pm 1.6
	c	100	142	134	103	88
RHA-347	a	59.3 \pm 3.7	60.3 \pm 2.2	53.3 \pm 0.5	42.1 \pm 1.5	39.1
	b	24.1 \pm 1.1	21.8 \pm 1.2	22.0 \pm 0.5	21.1 \pm 0.7	21.9 \pm 1.6
	c	100	120	97	64	54
RHA-348	a	73.0 \pm 3.0	89.0 \pm 3.5	77.0 \pm 0.7	55.0 \pm 2.9	50.0 \pm 0.5
	b	37.0 \pm 1.0	34.0 \pm 1.6	36.0 \pm 1.4	29.0 \pm 1.4	30.0 \pm 1.2
	c	100	166	116	93	69
RHA-355	a	83.0 \pm 4.2	78.0 \pm 2.8	62.0 \pm 0.3	47.6 \pm 1.5	41.0 \pm 2.7
	b	23.0 \pm 1.6	21.0 \pm 1.0	22.0 \pm 0.5	21.0 \pm 0.9	22.0 \pm 1.2
	c	100	104	70	48	33
HA-341	a	53.2 \pm 2.6	63.5 \pm 0.2	64.1 \pm 1.1	52.0 \pm 0.5	38.8 \pm 1.3
	b	21.9 \pm 0.5	21.1 \pm 0.3	23.7 \pm 0.7	22.6 \pm 0.3	20.0 \pm 1.1
	c	100	140	117	91	66
HA-350	a	79.0 \pm 3.8	69.0 \pm 1.6	71.0 \pm 4.7	70.0 \pm 4.5	77.0 \pm 1.9
	b	36.0 \pm 1.2	34.0 \pm 0.3	37.0 \pm 1.2	33.0 \pm 1.2	40.6 \pm 1.0
	c	100	83	79	94	76

The mechanism of salt-temperature interaction at germination is not well understood and explained and the damages to membranes observed in seedling growth studies probably begin at germination stage (Hapson and Simpson 1990). This concept may, at least partially, explain the behaviour of our cms lines; the temperature used was right for the restorer lines but, perhaps, not for the cms ones.

We must also remember that many reports on salinity have been done by exposing plant roots or seeds to uniform salt concentrations in laboratory tests but this exposure differs from the situation in the field. Under field conditions the root of a plant grow in soil which varies in water content, salt concentration and water potential both in space and time.

The relationships between germination and/or development and salt tolerance must be studied with other species too in order to determine whether or not salt tolerance of seeds is generally indicative of how a growing plant will respond to osmotic stress.

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EFFECTOS DE LA SAL SOBRE LA GERMINACION Y CRECIMIENTO TEMPRANO EN GIRASOL

RESUMEN

Los achenios de ocho líneas de alto contenido en ácido oléico de *Helianthus annuus* fueron germinadas en placas de Petri de cuatro potenciales osmóticos de cloruro de sodio. El comportamiento de la germinación es analizado. El crecimiento de las plantulas fué mas sensitivo que la germinación bajo estrés salino, siendo mas afectadas las líneas CMS. Las nuevas plantas germinadas mostraron un descenso en tamaño y peso con el tratamiento salino.

EFFETS DU SEL SUR LA GERMINATION ET LA CROISSANCE PRÉCOCE DU TOURNESOL

RÉSUMÉ:

Les semences de 8 lignées à haute teneur en acide oléique ont été mis à germer en boîte de Pétri en présence de 4 potentiels osmotiques de chlorure de sodium. Le comportement germinatif a été étudié. La croissance des plantules s'est révélée être plus sensible au stress salin que la germination, les lignées CMS y sont plus sensibles. Les plantules nouvellement germées montrent une diminution de la taille et du poids pour des concentrations salines croissantes.