T1978AGRO30

GERMINATION AND HYPOCOTYL GROWTH IN HELIANTHUS ANNUUS (L.) AS INFLUENCED BY DIFFERENTIAL SALT TREATMENTS[1]

By

Richard Hardwick and David Ferguson[2]

Summary

This study was conducted to ascertain if cultivars, inbred lines and F_1 hybrids of Helianthus annuus (L.) exhibited differential responses to increasing salt levels during the germination and initial hypocotyl growth periods. Following a preliminary survey of several cultivars, a factorial cross between three CMS lines and three restorer lines was made to determine if germination and hypocotyl growth in the inbred lines and F_1 hybrids were influenced by salt concentrations. The inbreds and hybrids were subjected to salt levels ranging from 0.0 to 17.5 millimhos per centimeter in increments of 3.5 millimhos per centimeter.

Two measurements were made: the ability to germinate and the elongation of the hypocotyl in a given period of time. The three sterile lines exhibited a greater differential response to salt than the three restorer lines. The differences were large enough to suggest that selection for salt tolerance would be effective in both germination and hypocotyl growth.

These two characters were also examined in terms of the interaction of salt level X inbred line. Also, the analysis of the factorial cross revealed marked influences of the differential salt levels on the combining ability of the inbred lines in both the germination and hypocotyl growth characters studied.

Methods and procedures for screening large numbers of lines and/or populations for salt tolerance are detailed.

Introduction

The purpose of this study was to determine the effect that water and its salts had on the germination and initial hypocotyl growth of Helianthus annuus (L.), and to determine if any of the several cultivars, inbred lines, or F_1 hybrids tested were salt tolerant during these growth periods. Studies of salt tolerance in other crops have been previously conducted leading the authors to believe that some levels of tolerance might be found in H. annuus.

---

[1] Work conducted under the auspices of David & Sons, Inc., Fresno, California, and submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science, California State University at Fresno.

[2] Chief Chemist, Producers Cotton Oil Company, Fresno, California, and Agricultural Research Manager, David & Sons, Inc., Fresno, California.

Materials and Methods

Oilseed Cultivar Survey

Seed of ten cultivars of oilseed sunflower, H. annuus, was obtained from several sources. The cultivars tested were Luch, VNIIMK 8931-66, Record, Peredovik-66, Outlook, Sterile Majok, Issanka, Guayacan, Kraznodarets, and Sputnik-71. The methods and materials used to test these cultivars were the same as those used to test the confectionery types.

Confectionery Inbreds and Hybrids

Three cytoplasmic male sterile lines and three resotrer lines of H. annuus were used in a factorial cross. The inbreds and their hybrid progeny were subjected to a regimen of saline solutions during germination and initial hypocotyl growth. The inbred male lines used were: RHA 280, RHA 282, and RHA 293. The inbred female lines used were: CMS HA 288, CMS HA 292, and CMS HA 285. One hundred seeds from each group were randomly selected and placed in a towel "sandwich" (Figure 1) (Myhill and Konzak) and each sandwich was then randomly placed in one of six polyethylene containers containing a specific salt treatment solution. Each treatment solution helf fifteen towel sandwiches and 1500 seeds. This configuration provided a quick and economical means of evaluating several thousand seeds at one time.

The sandwiches were prepared by folding a 15x229 centimeter (cm) portion of the No. 281 Roll Towel (Fort Howard Paper Company) in half along the width. This fold was then pushed down towards the ends of the towel and refolded about 5 cm from the ends. This folding provided a bed on which the seeds were placed. The overall width was 8 cm, with the bed located about 2-1/2 cm from the top. Therefore, when the seeds were inserted into the sandwich they were sufficiently higher than the top of the treatment solution. This placement prevented the seeds from becoming waterlogged. The lower portion of the sandwich provided the necessary wicking action to provide the seeds with their salt water treatment solution. Each towel portion was then folded longitudinally every 23 cm, and each 23 cm section was placed next to each other with alternative folds. Each sandwich then contained ten folds where ten seeds per fold were inserted.

The water used to prepare the various salt treatment solutions was passed through a mixed bed ion exchange column in order to deionize it. The salts used to make the stock sea water solution were of reagent grade. The chemical composition of the stock sea water solution is shown in Table 1, which approximates the chemical composition of sea water.

The salt concentration of each of the treatment solutions was established by diluting the stock sea water solution and was measured by taking electrical conductivity measurements on a Solu Bridge. The final salt solution prepared had electrical conductivities (E.C.'s) of 0.0 (control), 3.5, 7.0, 10.5, and 17.5 millimhos per centimeter (mmho/cm). The 0.02 millimolar (mM) calcium sulfate that was added to the control did not change the electrical conductivity significantly from zero (calculated 0.002 mmho/cm).

Prior to the addition of seed to the sandwiches and salt solutions to the containers, the containers were decontaminated by washing them with a 30% sodium

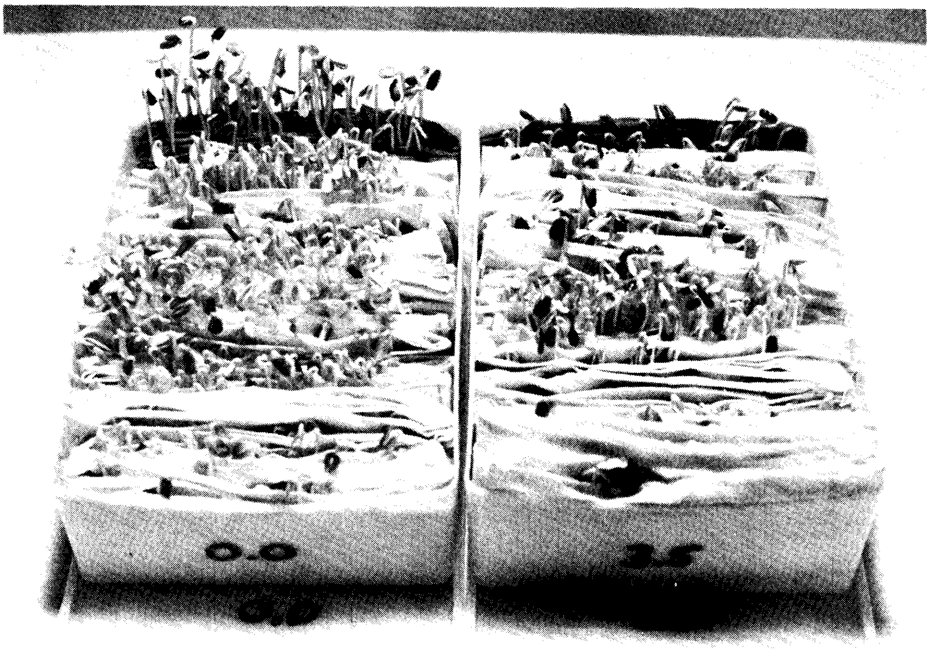

Figure 1. Toweling Used to Hold Seeds.

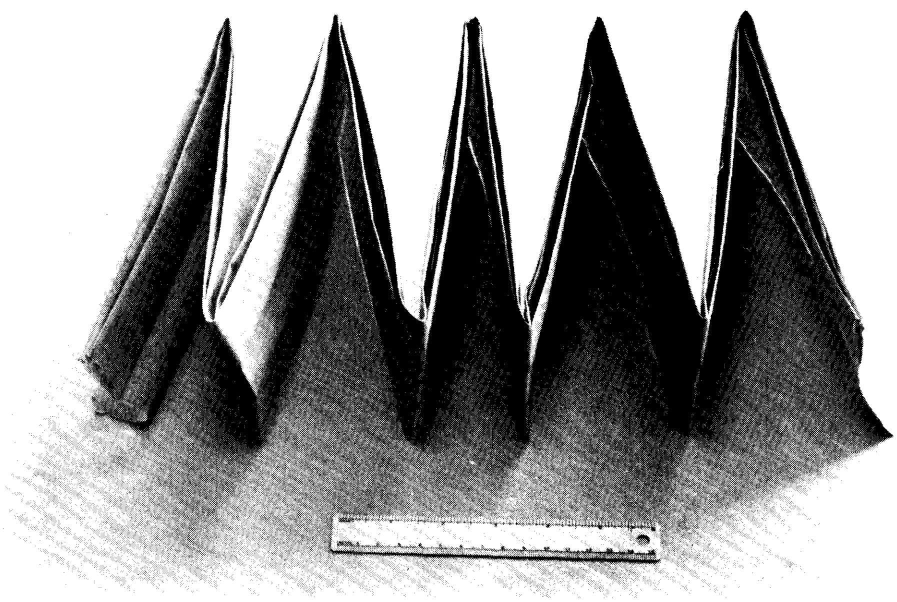

Figure 2. Germinated Sunflowers at 0.0 (on left) and 3.5 mmho/cm.

hypoclorite solution. The sunflower seeds were treated with Arasan 70 dust at a 3 oz./100 lb. seed rate.

TABLE 1. Chemical Composition of Stock Salt Treatment Solution

Salt	Grams per liter
NaCl	26.518
$MgCl_2$	2.447
$CaSO_4$	3.305
$CaCl_2$	1.141
KCl	0.725
$NaHCO_3$	0.202
KBr	0.083

Source: Sverdrup, H.U., 1942. The Oceans. Prentice-Hall, Inc., Englewood Cliffs, p. 186.

200 milliliters of salt treatment solution were added to each of the treatment containers. The lids were fastened to the containers, and the containers were placed in the dark at room temperature of approximately 20°C to ensure proper germinating conditions. Germination was determined when any portion of the seed coat was penetrated by the hypocotyl (Mayer and Poljakoff-Mayber).

Observations were made daily, but no results were recorded until the tenth day, at which time the germination period was terminated. Figure 2 pictures the heights of the germinated seedlings at 0.0 and 3.5 millimhos/centimeter.

Results and Discussion

Oilseed Cultivar Survey

Ten common open-pollinated cultivars of H. annuus were subjected to a regimen of salt solutions to ascertain if a differential response in germination could be found. This was ascertained. Table 2 shows the germination percentages of these cultivars in the various salt treatment solutions.

Figure 3 indicates these germination results graphically as a function of salt concentration. These ten cultivars were separated into three groups according to their overall germination performance. These groups are represented in Figure 3. In Figure 3, it can be noted that the three cultivars, Sterile Majok, Issanka, and Guayacan were able to germinate at essentially the same percentage independent of salt concentration. Sterile Majok had the least response to salt of the three. Issanka andGuayacan had termination percentages higher at electrical conductivities (E.C.'s) of 3.5, 7.0, and 10.5 mmho/cm than at 0.0.

The second grouping of cultivars, Record, Outlook, Luch, and Peredovik-66, performed essentially the same with each other, as can be seen in Figure 3b, but differently from the cultivars noted in Figures 3a and 3c. Here, two cultivars, Luch and Peredovik, did well initially in the control solution, but germinated rather poorly as the salt concentration increased. Record and Outlook both improved from the controlled situation to E.C. 3.5, but showed poorer germination performance at the higher salt concentrations.

TABLE 2. Germination results of ten oilseed cultivars at five salt concentrations (percentage germination).

Cultivar	Electrical Conductivity (mmho/cm)				
	0.0	3.5	7.0	10.5	14.0
Sterile Majok	96	93	96	95	96
Issanka	71	94	89	90	79
Guayacan	82	92	84	87	73
Record	50	80	73	71	67
Outlook	72	81	78	63	52
Luch	90	70	77	58	49
Peredovik-66	90	81	77	44	47
VNIIMK 8931-66	39	47	62	19	33
Kraznodarets	77	65	69	33	30
Sputnik-71	66	70	70	46	14

The cultivars, VNIIMK 8931-66, Kraznodarets, and Sputnik-71 (Fig. 3c) performed poorer in overall germination than the other two groups that are shown in Figures 3a and 3b. Here, Sputnik-71 and VNIIMK 8931-66 improved from the controlled solution to E.C. 3.5, but dropped off drastically as the salt concentration increased.

These ten cultivars germinated essentially the same at E.C.'s of 0.0, 3.5, and 7.0 mmho/cm. There were differences in germination as the salt concentrations increased to 10.5 and 14.0 mmho/cm. The differential responses to salt among the oilseed cultivars suggest further investigation.

Confectionery Inbreds and Hybrids

A set of confectionery inbred lines and F_1 hybrids were studied in a factorial cross to determine if germination and hypocotyl growth were influenced by salt concentration. In the process of choosing a medium to do a combining ability study, convectioneries were examined because of their hull thickness variations. Tables 3 and 4 show the germination of the various sunflower inbreds and F_1 hybrids in six different salt treatment solutions. Tables 5 and 6 show the hypocotyl lengths of these lines in the same salt solutions.

Inbreds

The performance of germination and hypocotyl growth in the inbred lines is indicated graphically in Figures 4a and 5a, respectively, for the male lines, and in Figures 4b and 5b for the female lines.

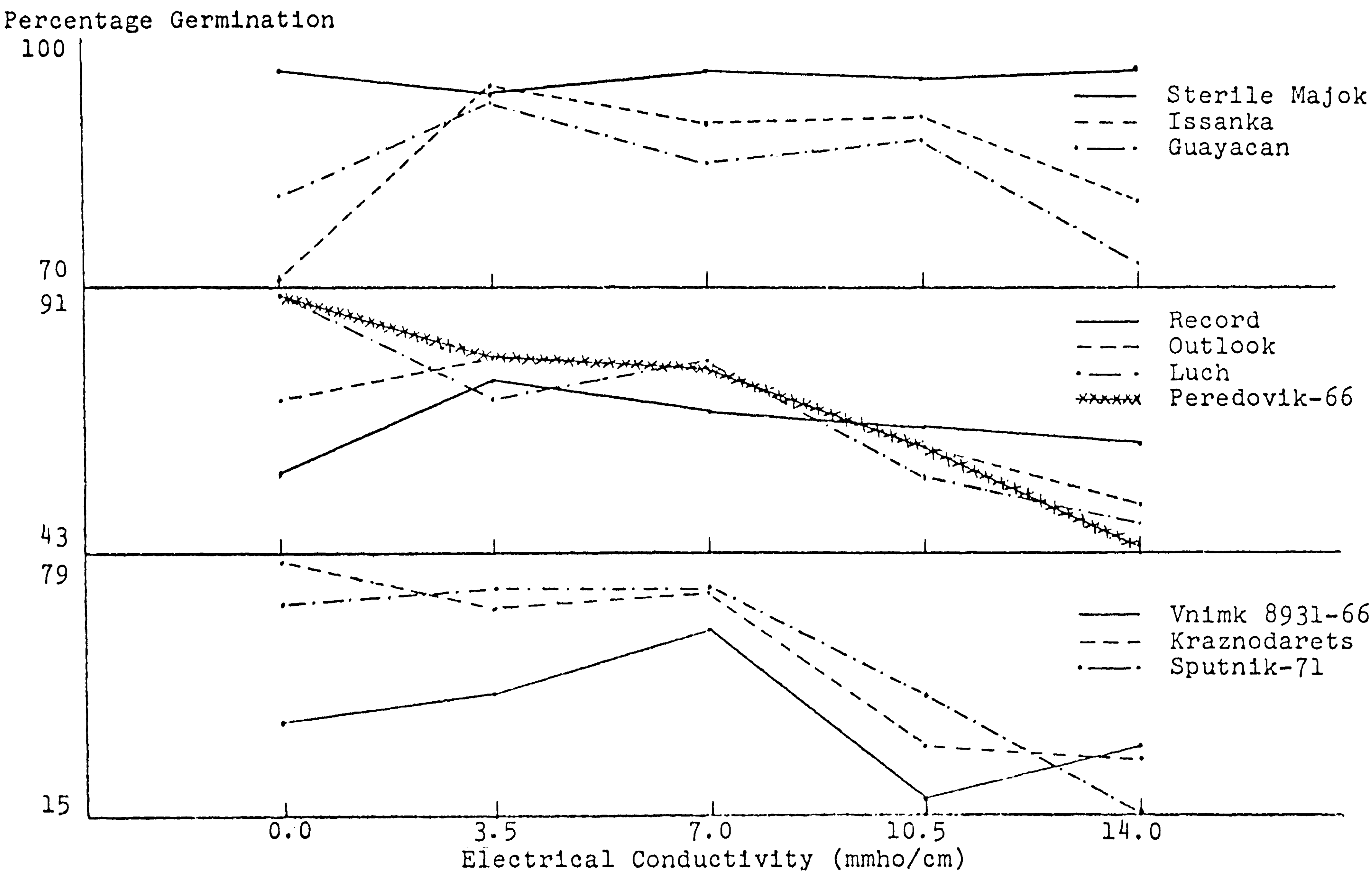

Figure 3. Germination of Oilseed Cultivars versus Salt Treatments. (a) Top: Sterile Majok, Issanka, Guayacan; (b) Middle: Record, Outlook, Luch, Peredovik-66; (c) Bottom: Vnimk 8931-66, Kraznodarets, Sputnik-71.

TABLE 3. Germination results of inbred lines at six salt concentrations (percentage germination)*

Inbred	Electrical Conductivity (mmho/cm)						
	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMS HA 288	93	90	91	90	90	86	90
CMS HA 292	99	96	99	97	99	88	96
CMS HA 285	65	73	77	69	55	53	65
RHA 280	99	99	100	94	99	97	98
RHA 282	92	97	90	88	87	64	86
RHA 293	90	91	85	68	79	77	82
$\bar{x}$	90	91	90	84	85	78	86

* Means of ten replications.
L.S.D. Inbreds 5.27
L.S.D. Treatments 5.27

TABLE 4. Germination results of F_1 hybrids at six salt concentrations (percentage germination)*

Inbred	Electrical Conductivity (mmho/cm)						
	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMSHA288xRHA280	99	100	96	96	96	98	98
CMSHA288xRHA282	99	100	97	98	98	99	99
CMSHA288xRHA293	60	70	57	60	69	49	61
CMSHA292xRHA280	82	80	56	69	76	51	69
CMSHA292xRHA282	83	88	79	90	85	78	84
CMSHA292xRHA293	97	96	76	91	82	68	85
CMSHA285xRHA280	100	100	96	100	98	98	99
CMSHA285xRHA282	92	96	96	95	96	91	94
CMSHA285xRHA293	79	87	76	85	92	90	85
$\bar{\bar{x}}$	88	91	81	87	88	80	86

* Means of ten replications
L.S.D. F_1 Hybrids 4.03
L.S.D. Treatments 3.55

TABLE 5. Hypocotyl Lengths of inbred lines at six salt concentrations (mm)*

Inbred	Electrical Conductivity (mmho/cm)						
	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMS HA 288	21.3	17.6	15.1	11.3	10.1	4.4	13.3
CMS HA 292	36.3	29.4	22.9	18.0	13.1	6.6	21.1
CMS HA 285	9.8	16.5	11.6	8.3	6.0	2.7	9.2
RHA 280	38.0	34.7	34.7	13.2	9.4	5.0	22.5
RHA 282	20.6	16.0	10.4	8.8	4.6	1.6	10.4
RHA 293	36.8	33.5	26.8	14.3	13.4	7.8	22.1
$\bar{x}$	27.1	24.6	20.3	12.3	9.4	4.7	16.4

* Means of ten replications.
L.S.D. Inbreds 1.72
L.S.D. Treatments 1.72

TABLE 6. Hypocotyl lengths of F_1 hybrid at six salt concentrations (mm)*

Inbred	Electrical Conductivity (mmho/cm)						
	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMSHA288xRHA280	60.2	62.2	38.1	19.3	23.7	12.7	36.0
CMSHA288xRHA282	48.6	43.3	32.8	16.7	13.8	6.8	27.0
CMSHA288xRHA293	24.4	24.0	18.8	13.4	10.2	3.1	15.7
CMSHA292xRHA280	17.7	15.4	10.4	8.1	4.6	1.8	9.7
CMSHA292xRHA282	16.9	19.9	17.7	11.9	7.2	2.9	12.8
CMSHA292xRHA293	22.2	25.6	17.3	17.0	9.9	2.5	15.8
CMSHA285xRHA280	51.9	51.2	42.3	30.9	20.0	7.3	33.9
CMSHA285xRHA282	30.2	31.4	21.5	19.7	10.8	5.2	19.8
CMSHA285xRHA293	53.2	30.7	29.9	17.8	16.6	13.3	26.9
$\bar{x}$	36.1	33.7	25.4	17.2	13.0	6.2	22.0

* Means of ten replications.
L.S.D. F_1 hybrids 2.46
L.S.D. Treatments 2.00

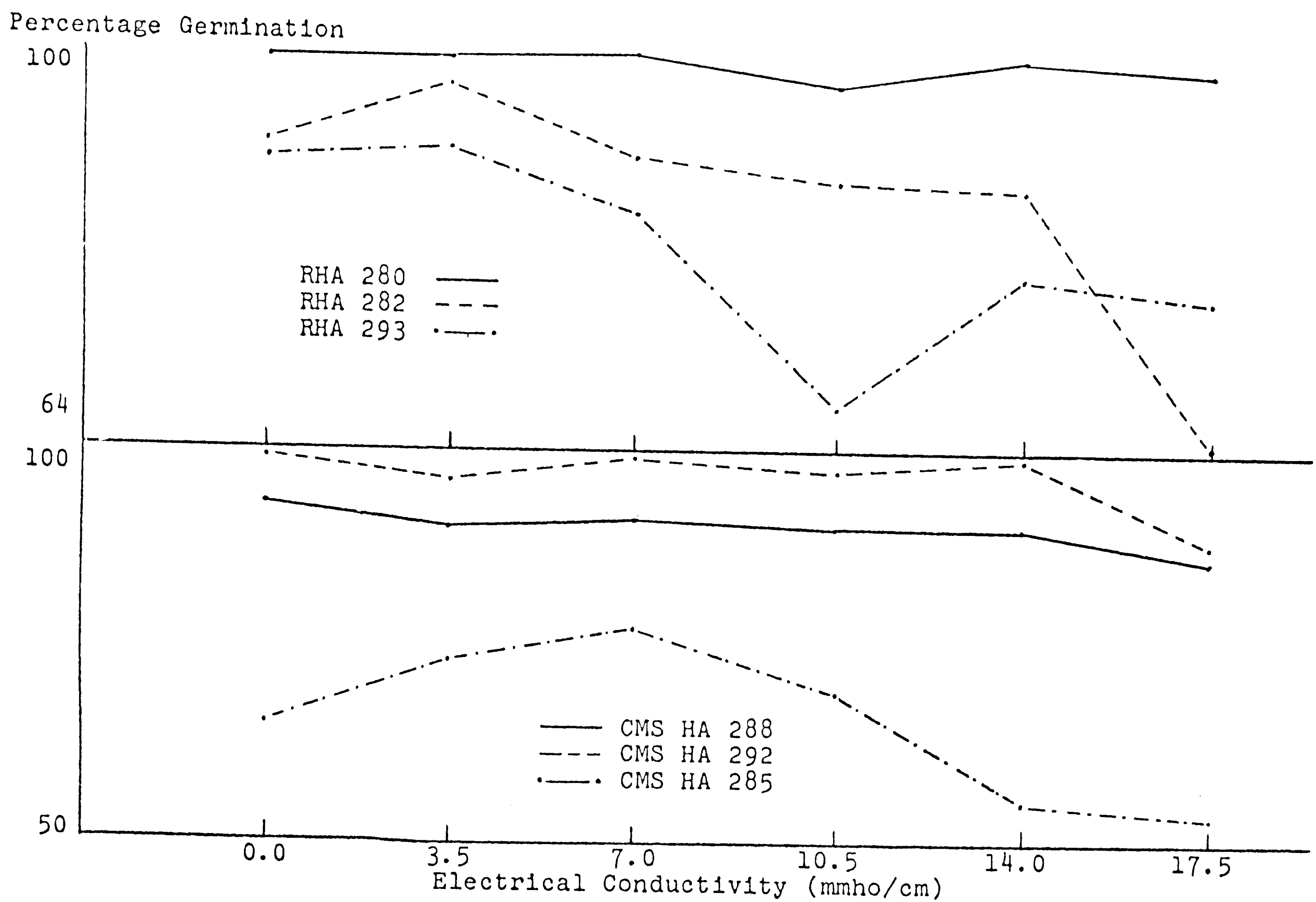

Figure 4. Germination of Inbred Lines versus Salt Treatments. (a) Top: Males; (b) Bottom: Females.

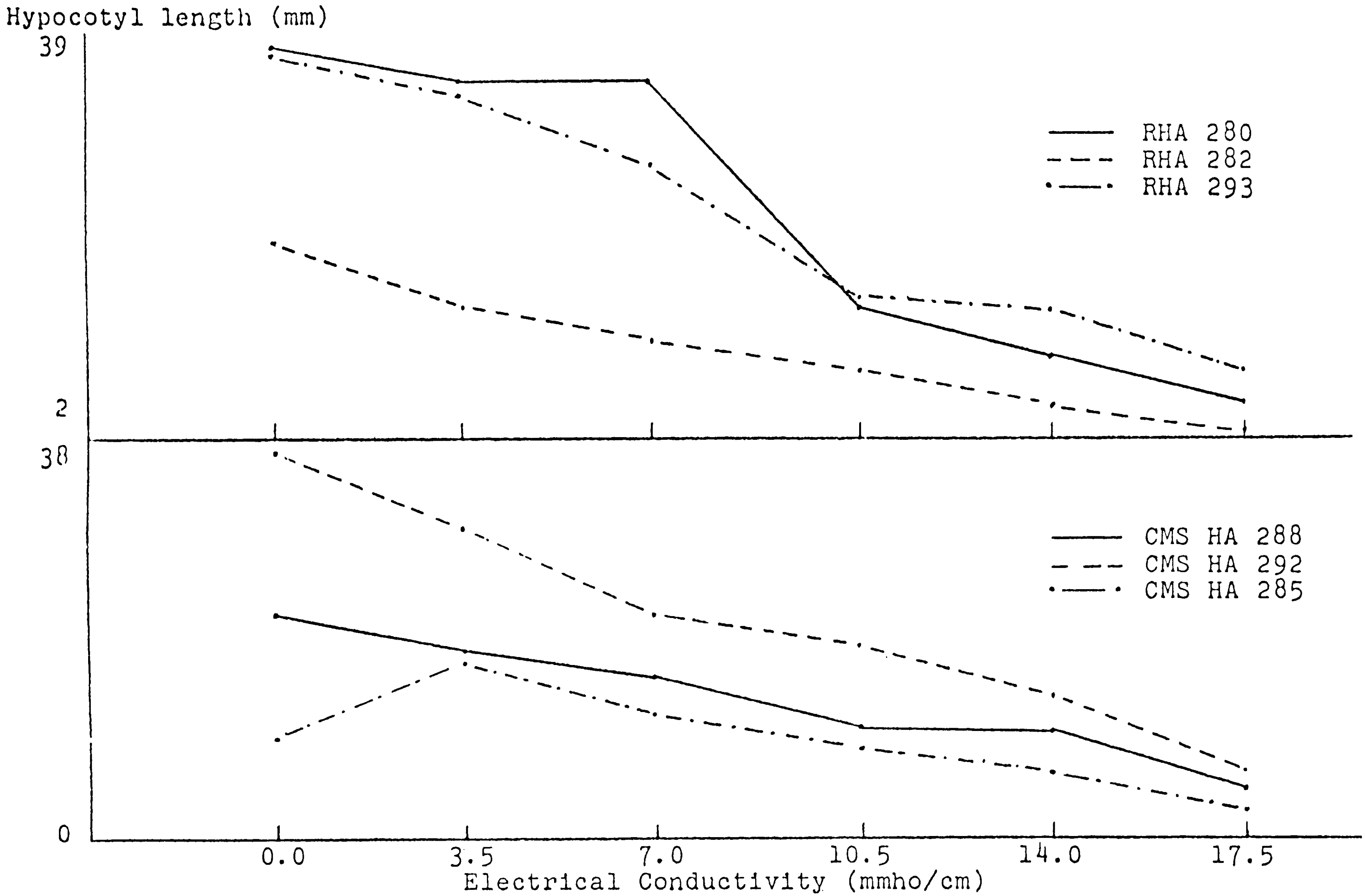

Figure 5 . Hypocotyl Lengths of Inbred Lines versus Salt Treatments. (a) Top: Males; (b) Bottom: Females.

1. Treatments

In the germination performance of the inbred lines, it was determined that there was a significant influence from a linear standpoint; i.e., there was a lowering of germination performance as salt concentrations increased. All lines performed, on the average, the same at salt levels of electrical conductivities of 0.0, 3.5, and 7.0 millimhos per centimeter, but performed significantly poorer at E.C.'s of 10.5 and 14.0, and still poorer at 17.5. The average germination percentage at 0.0, 3.5, and 7.0 was 91% and at 17.5 it was 78%.

It was also determined in the hypocotyl growth study of the inbred lines that there was a significant influence from a linear standpoint. In other words, salt concentration was inversely associated with hypocotyl growth in the inbred lines. The average hypocotyl length at E.C. 0.0 was 27.1 mm and at 17.5 it was 4.7 mm.

2. Inbreds

Figure 4 shows the average difference in response to salt within the male lines was not as great as the average difference between the female groups. In Figure 4a it can be seen that RHA 280 responded the least of the three males as salt concentrations increased. RHA 282 and RHA 293 performed identically over all salt concentrations. The overall range of performance for the male inbred lines was from 64% to 100% germination across salt concentrations with an average of 89% germination.

Figure 4b shows that CMS HA 292 performed significantly different from both CMS HA 288 and CMS HA 285. CMS HA 285 performed at a significantly poorer rate than the other two female lines, but nonetheless, exhibited an increase in germination performance at E.C. 3.5 and 7.0. The range of germination performance of the female inbred lines was 53% to 99% across salt concentrations, with an average of 84% germination. The average females performed somewhat poorer than the average males over these concentrations.

The graph on hypocotyl lengths, Figure 5, shows that two of the male lines, RHA 280 and RHA 293, performed essentially the same over all salt concentrations and RHA 282 performed significantly poorer. The female lines performed likewise, with CMS HA 292 performing significantly better. When comparing the males to the females, the average male hypocotyl length was 18.3 mm while that for the females was 14.5 mm, a significant difference.

3. Inbred x Treatment Interaction

This interaction is significant. It indicates that a differential response of the inbred lines to the various salt solutions occurred. The differential response was greater among females than among males. This interaction suggests selection at the salt concentration desired.

Hybrids

The performance in germination and hyopcotyl growth in the F_1 hybrids is indicated in Tables 4 and 6, respectively.

1. Treatments, Hybrids

In the germination of the F_1 hybrids, it was determined that there was a significant influence from the linear component, i.e., there was a lowering of germination performance as salt concentrations increased. CMS HA 288 x RHA 280, CMS HA 288 x RHA 282, and CMS HA 285 x RHA 280 appear not to have responded differently from each other as the salt concentration increased. But they responded significantly better than CMS HA 285 x RHA 282. CMS HA 288 x RHA 293 performed significantly the poorest. These situations may be a basis for a selection to be made for salt tolerance in the F_1 hybrids. It is important to note that CMS HA 292 crosses did not perform as well as the CMS HA 285 crosses.

It was also determined, in hypocotyl growth performance of the hybrids, that there was a significant influence from the linear component. In other words, there was a lowering of hypocotyl lengths as the salt concentration increased. All hybrids reacted the same at E.C.'s of 0.0 and 3.5 mmho/cm. At all other salt treatment solutions, there was a significant difference exhibited with respect to hybrid hypocotyl growth. The hypocotyl lengths averaged 36.1 mm at E.C. 0.0 and 9.3 mm at 17.5.

2. General and Specific Combining Ability of Inbreds in Hybrid Combination.

Tables 7 and 8 represent the germination performance of inbred lines in hybrid combination and hypocotyl lengths of the inbred lines in hybrid combination at six salt concentrations. Figures 6 and 7 represent these figures graphically. A significance found in both characters can be attributed to the general combining ability (GCA) of males, the general combining ability (GCA) of females, and in their mutual specific combining ability (SCA). It is important to note here that the CMS HA 285 inbreds in hybrid combination performed significantly better than the CMS HA 292 inbreds in hybrid combination in both characters examined.

TABLE 7. Average Germination Performance of Inbred Lines in Hybrid Combination (GCA)*

	Electrical Conductivity (mmho/cm)						
Inbred	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMS HA 288	86	90	83	85	88	82	86
CMS HA 292	87	88	70	83	81	66	79
CMS HA 285	90	94	89	93	95	93	92
RHA 280	94	93	83	88	90	82	88
RHA 282	91	95	91	94	93	89	92
RHA 293	79	84	70	79	81	69	77
$\bar{x}$	88	91	81	87	88	80	86

* Means of ten replications.
L.S.D. Inbreds in hybrid combination 4.03
L.S.D. Treatments 3.55

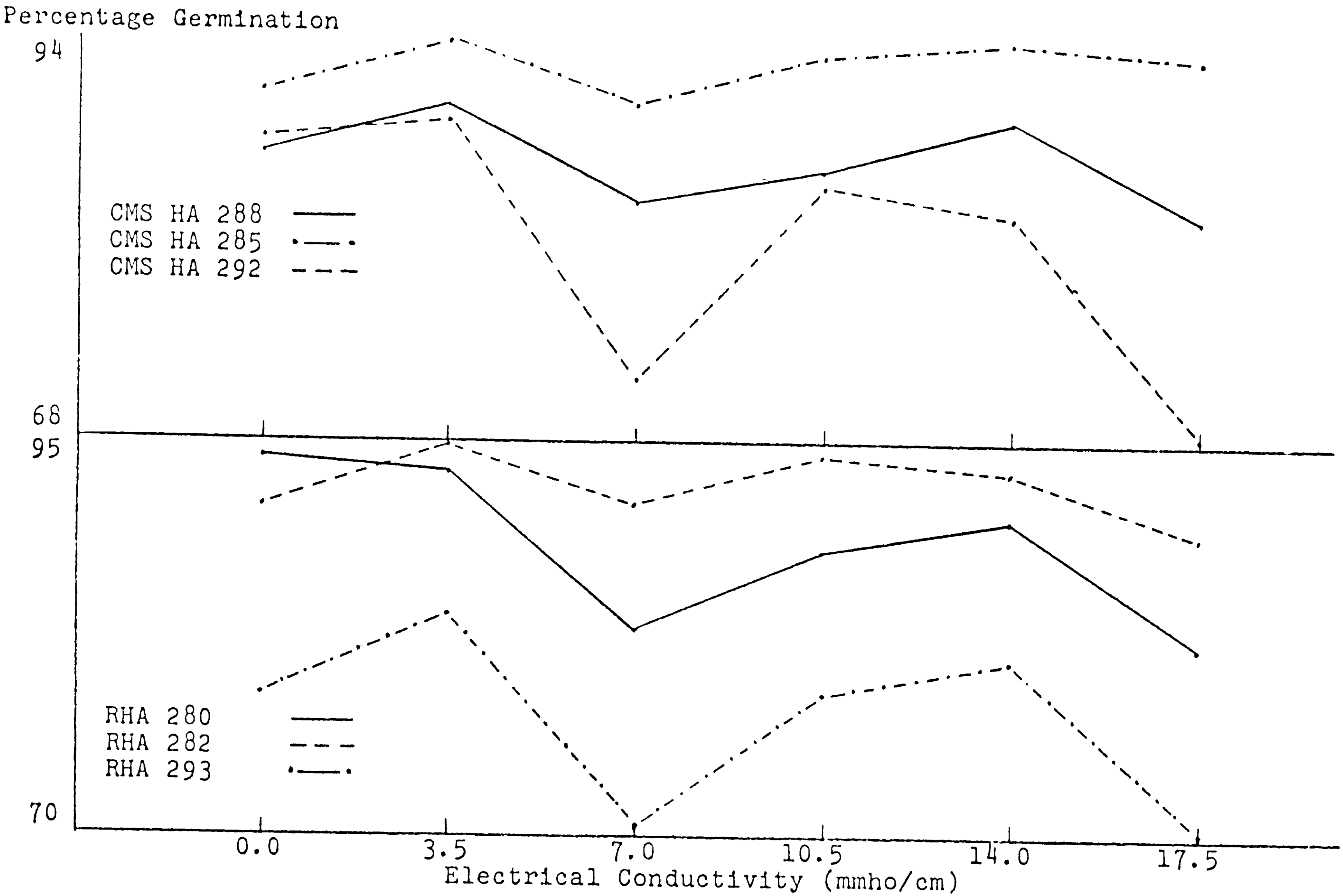

Figure 6 . Germination Performance of Inbred Lines in Hybrid Combination versus Salt Treatments. (a) Top: Females, (b) Bottom: Males.

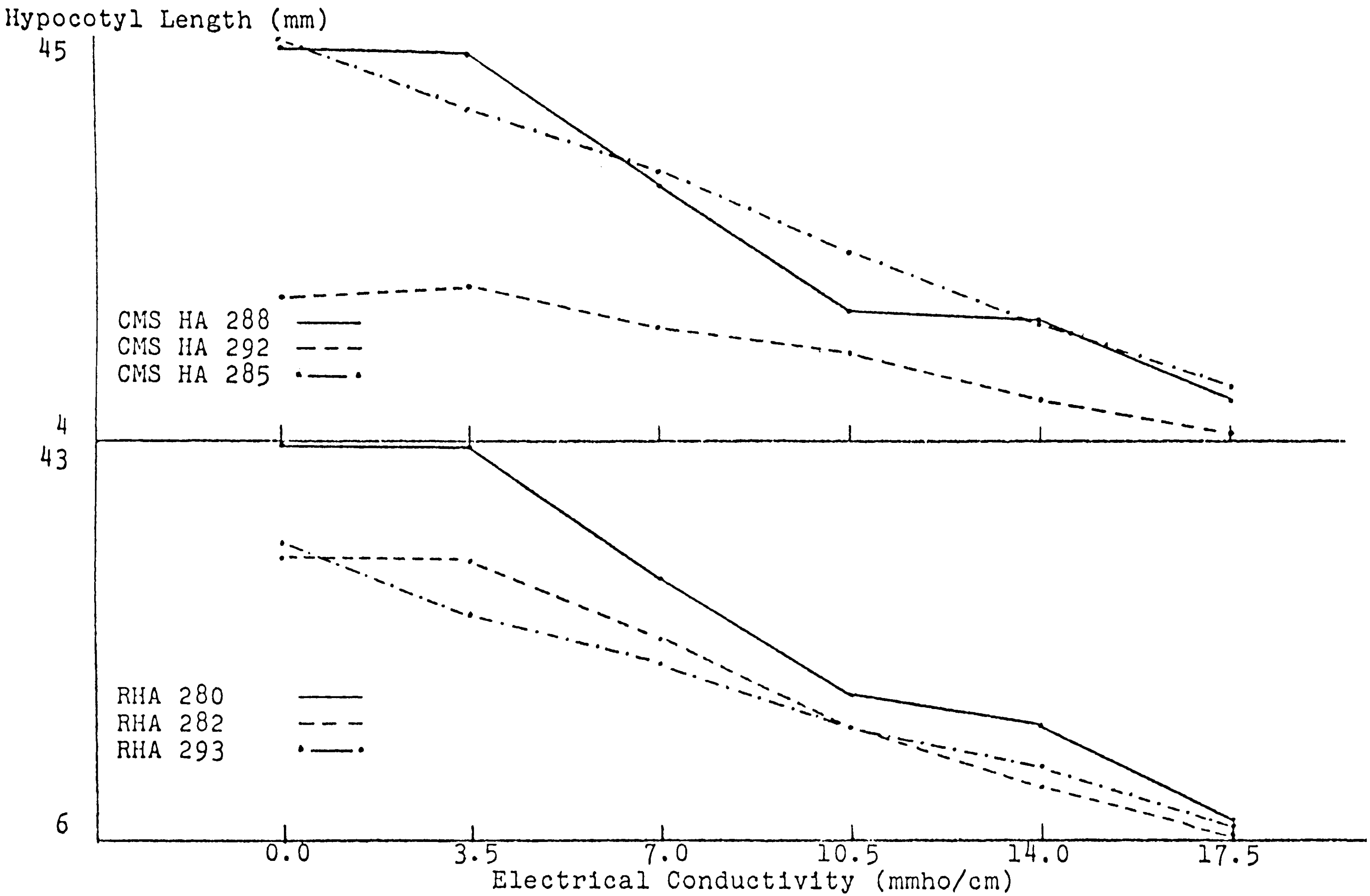

Figure 7 . Hypocotyl Lengths of Inbred Lines in Hybrid Combination versus Salt Treatments. (a) Top: Females, (b) Bottom: Males.

TABLE 8. Average Hypocotyl Lengths of Inbred Lines in Hybrid Combination (GCA)*

Inbred	Electrical Conductivity (mmho/cm)						
	0.0	3.5	7.0	10.5	14.0	17.5	$\bar{x}$
CMS HA 288	44.4	43.2	29.9	16.5	15.9	7.5	26.2
CMS HA 292	18.9	20.3	15.1	12.3	7.2	2.4	12.7
CMS HA 285	45.1	37.8	31.2	22.8	15.8	8.6	26.9
RHA 280	43.3	42.9	30.3	19.4	16.1	7.3	26.6
RHA 282	31.9	31.5	24.0	16.1	10.6	5.0	19.9
RHA 293	33.3	26.8	22.0	16.1	12.2	6.3	19.5
$\bar{x}$	36.1	33.7	25.4	17.2	13.0	6.2	22.0

* Means of ten replications
L.S.D. Inbreds in hybrid combination 2.46
L.S.D. Treatments 2.00

2. Hybrids x Treatment Interaction

The interaction is significant indicating a differential response of the hybrids to the various salt colutions. When this interaction was partitioned to measure the GCA males x treatments, GCA females x treatments, and SCA x treatments, it was noted that the combining ability ranking varied in response to the solution concentrations.

The GCA males x treatments interaction in the germination experiment was not significant. This germination response is assumed to be influenced by the hull which is completely female tissue. The fact that the inbred males themselves exhibited less of a response than the females to the salt concentrations lends credence to this assumption.

From a hypocotyl growth standpoint, the general combining ability of the males as well as the females, and the specific combining ability of them mutually, all play a significant role. Hypocotyl length measurements were derived after the emgryos had pierced their hulls, hence it would appear that the male would play a somewhat equal role as the female in growth of the hybrid hypocotyl. Here, the barrier to the embryo, the hull, had been overcome, and the actual growth of the hybrid tissue was determined by the combination of the male and female gametes.

Conclusion

There are several things to be noted in this study:

1. Heritability was not computed due to the small sample size. This character does, however, appear to have a reasonably high heritability to respond to selection.

2. Hull thickness will mask the contribution of the male gamete for germination. The male gamete does, however, make a significant contribution to hypocotyl growth.

3. Large population screening of sunflower seed for germination and hypocotyl growth is possible by the methods described and should result in establishing a tolerance for this species to increasingly salty environments.

Literature Cited

1. AYERS, A.D., 1953. Germination and emergence of several varieties of barley in salinized soil cultures. Agronomy Journal. 45:68-71.

2. DEWEY, D.R., 1960. Salt tolerance of twenty-five strains of Agronpyron. Agronomy Journal. 52:631-635.

3. DEWEY, D.R., 1972. Breeding crested-wheatgrass for salt tolerance. Crop Science. 2:403-407.

4. DONOVAN, T.J., and A.D. DAY, 1969. Some effects of high salinity on germination and emergence of barley (*Hordeum vulgare* L. emend. Lam.). Agronomy Journal 61:236-238.

5. EPSTEIN, E., 1972. *Mineral Nutrition of Plants: Principles and Perspectives*. John Wiley and Sons, Inc., New York, 412 pp.

6. HUNT, O.J., 1965. Salt tolerance in intermediate wheatgrass. Crop Science. 5:407-409.

7. MAYER, A., and A. POLJAKOFF-MAYBER, 1975. *The Germination of Seeds*. Pergamon Press. Oxford. 192 pp.

8. MYHILL, R.R., and C. KONZAK, 1967. A new technique for culturing and measuring barley seedlings. Crop Science. 7:275-276.