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Youssef El-Iklil, Mohammed Karrou, Mohamed Benichou. Salt stress effect on epinasty in relation to ethylene production and water relations in tomato. *Agronomie*, 2000, 20 (4), pp.399-406. 10.1051/agro:2000136 . hal-00886055

HAL Id: hal-00886055

<https://hal.science/hal-00886055>

Submitted on 11 May 2020

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Salt stress effect on epinasty in relation to ethylene production and water relations in tomato

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(Received 15 June 1999; revised 4 April 2000; accepted 20 April 2000)

Abstract – A pool of genetic variability is a prerequisite for any practical approach to improving crop salt tolerance. The objective of this study was to determine how epinasty, a morphological response of tomato to salt stress, can be used as a measure of salt tolerance and how it is related to ethylene production and water relations in tomato. Three *Lycopersicon esculentum* cultivars (Edkawy, Ramy, and Vemar) and one *Lycopersicon sheesmanii* accession were subjected to four levels of salt stress at the roots (0, 50, 100, and 200 mM NaCl). Epinasty increased with increasing levels of salinity depending on genotype, leaf age and duration of the salt stress. Relative ethylene production by tomato petioles also increased with the intensity of salt stress, genotype and leaf age; salt tolerant varieties exhibited less epinasty and a lower relative ethylene production.

epinasty / ethylene / salt stress / tomato

Résumé – Effet du stress salin sur l'épinastie en relation avec la production d'éthylène et les relations hydriques chez la tomate. La recherche de la variabilité génétique est l'approche la plus efficace pour améliorer la tolérance des cultures à la salinité. L'objectif de cette étude est de déterminer comment l'épinastie, une des réponses morphologiques de la tomate au stress salin, peut être utilisée comme critère de tolérance à la salinité. Pour atteindre cet objectif, trois cultivars de *Lycopersicon esculentum* (Edkawy, Ramy et Vemar) et une accession de *Lycopersicon sheesmanii* ont été soumis à 4 niveaux de salinité (0, 50, 100 et 200 mM de NaCl). Les données ont montré que l'épinastie a augmenté avec l'augmentation des niveaux de salinité. Son amplitude a dépendu du génotype, de l'âge de la feuille et de la durée du stress. Parallèlement, la production relative d'éthylène était fonction du niveau du stress salin, du génotype et de l'âge la feuille. Les génotypes tolérants ont manifesté, sous stress salin, une faible épinastie et une faible production relative d'éthylène (% de témoin).

épinastie / éthylène / stress salin / tomate

Communicated by Peter Barraclough (Harpden Herts, UK)

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1. Introduction

Salinity is one of the factors that limits tomato production in the coastal Atlantic area of Morocco. During generations of selection under unstressed environments, cultivated tomato may have lost its salt tolerance character [18] but, this trait can still be found in wild species such as *Lycopersicon sheesmannii* [9, 19]. This character can be incorporated into the cultivated tomato [16].

Salt sensitivity appears to cover a number of maladaptations to this stress, reflected in reduced growth rates, loss of turgor, premature senescence, leaf abscission and petiole epinasty [6, 8, 12]. It is now well established that ACC (1-aminocyclopropane-1-carboxylic acid), the immediate precursor of ethylene, plays an important role in communicating stress from the root to the observed responses in the shoot [3]. Epinasty is a kind of asymmetric petiolar growth related to an accelerating rate of ethylene synthesis [2, 7, 12]. It is induced in tomato under excess water stress [3, 7], or under salt stress [5, 10]. Epinasty is also induced by indoleacetic acid (IAA), dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA) or the rotation of the plant in a horizontal clinostat. Increased endogenous ethylene production appears to be the principal cause of epinasty in these cases [14].

Establishing correlations between salt tolerance and easily identified morphological responses, such as epinasty, could be used as a convenient but indirect selection criterion to help identify genotypes which tolerate salt stress. Accordingly, effects of different levels of salt stress on petiolar epinasty have been determined in relation to ethylene production and water relations of various cultivated tomato genotypes and a wild tomato genotype known for its salt tolerance.

2. Materials and methods

The experiment was carried out in a glass house with automatic ventilation at the Aridoculture Center, INRA-Settat, Morocco. Three tomato cultivars (*L. esculentum* Mill cv. Ramy, Edkawy and

Vemar) and one accession of *L. Sheesmannii* were sown in 500 ml pots filled with 20% soil, 40% peat and 40% vermiculite. Plants were irrigated daily with a nutrient solution consisting of N:224, K:235, P:62, Ca:160, Mg:24, S:32, B:0.27, Mn:0.11, Zn:0.13, Cu:0.03, Mo:0.05 and Fe:1.12 mg·l⁻¹. At the 5-leaf stage, salt treatments were applied by adding 0, 50, 100 and 200 mM NaCl to the nutrient solution. For salinized plants, NaCl was added at 50 mM increments on alternate days until desired concentrations were attained. The experimental design was a "split plot" with three replications. Salinity levels were assigned to the main plots and varieties to the split plots. The latter consisted of 10 plants per plot.

2.1. Measurements

Epinasty was assessed as a change in the angle formed by the petiole and the main stem axis. This was measured with a transparent protractor at 12 h intervals on the 2nd, 3rd and 4th leaf. Epinasty is estimated by the following formula: $E = A_s - A_0$ where A_s and A_0 are the angles of stressed and unstressed plants respectively.

Ethylene production by excised petioles was estimated 36 h after the treatment application. Proximal sections (5 cm) of the 2nd and 4th petiole were enclosed in test tubes capped with a serum stopper. After 30 min, a 0.5 ml gas sample was withdrawn with a gas-tight syringe and ethylene content was determined by a Delsi 330 gas chromatograph fitted with a flame ionization detector and a Poropak Q column (length: 1.5 m). Temperatures were fixed at 80 °C for the oven, 100 °C for the injector and 170 °C for the detector.

Leaf water potential was measured using a Scholander pressure chamber. A terminal leaflet of the 4th leaf was sealed in the chamber and pressure was progressively applied using compressed air until sap appeared at the cut surface. Osmotic potential was measured by thermocouple psychrometry, using a Wescor hygrometer sample chamber and a Wescor Dew Point Microvoltmeter HR-33T calibrated with standard solutions of NaCl. Frozen then thawed leaflet discs from the 4th leaf were placed in the hygrometer chamber.

Turgor potential was calculated as the difference between water potential and osmotic potential. Leaves were sampled between 08:00 and 09:00 h in May, five days after the treatments.

To evaluate the relationship between epinasty, plant growth and tolerance to salt stress, shoot dry matter was measured at the end of the experiment after drying at 70 °C for 36 h.

2.2. Statistical analysis

Analysis of variance, means comparison and correlations were made using Statistical Analysis System software SAS [17].

3. Results

3.1. Water relations

Table I shows that leaf water potential decreased as NaCl concentration increased in the rooting

medium. At 200 mM NaCl, it was reduced 2 to 3 fold compared to the control. The cultivars Ramy and Vemar had the most negative water potentials while values for Sheesmanii and Edkawy were affected less.

Leaf osmotic potential diminished for all cultivars as the level of salinity increased (Tab. I). This diminution reached 0.74 to 1.03 Mpa at the highest level of salinity. Compared to other varieties, osmotic potentials in Sheesmanii were less susceptible to salinity treatment.

Despite the increase in salt concentration, turgor pressure was little changed and statistical analysis showed no significant difference among treatments.

Figure 1 shows correlations between leaf osmotic potential and relative shoot dry matter. The latter decreased with decreasing osmotic potential. Sheesmanii, the tolerant genotype, maintained higher leaf osmotic potentials than Vemar, the susceptible genotype.

Table I. Effect of salinity on water relations (measured in the 4th leaf) of four tomato genotypes. Leaves were sampled 5 days after treatments.

NaCl (mM)		cultivars			
		Ramy	Vemar	Sheesmanii	Edkawy
Leaf Water pot. (LWP)(Mpa)	0 mM	-0.530	-0.620	-0.290	0.370
	50 mM	-0.660	-1.150	-0.570	0.710
	100 mM	-1.020	-1.380	-0.700	0.850
	200 mM	-1.450	-1.610	-0.930	1.200
Leaf Osmotic pot. (LOP)(Mpa)	0 mM	-2.075	-1.878	-1.796	2.040
	50 mM	-2.227	-2.428	-2.062	2.334
	100 mM	-2.341	-2.669	-2.106	2.393
	200 mM	-2.832	-2.909	-2.534	2.854
Leaf Turgor pot. (LTP)(Mpa)	0 mM	1.505	1.258	1.506	1.670
	50 mM	1.567	1.278	1.492	1.624
	100 mM	1.321	1.289	1.406	1.543
	200 mM	1.382	1.299	1.604	1.654
		WP	OP	TP	
LSD 5% salinity:		0.279**	0.131***	ns	
variety:		0.140***	0.303*	ns	
interact.:		*	ns	ns	

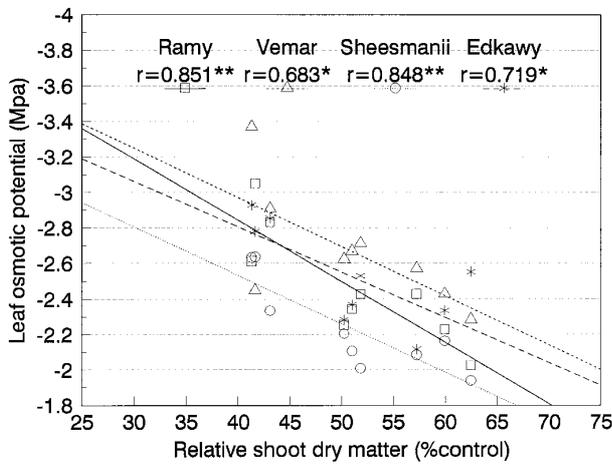


Figure 1. Correlations between leaf osmotic potential and relative shoot dry matter accumulated by four tomato genotypes. Osmotic potential was measured in the 4th leaf 5 days after salt treatment.

3.2. Epinasty

In the absence of salt stress, the difference in petiolar angle among genotypes was minimal. But it increased significantly with increasing levels of salinity (Fig. 2). The genotypic variation was least apparent at 50 mM NaCl. At this concentration, it varied from 0.4° for Sheesmanii to 2.8° for Vemar. A salinity level of 200 mM NaCl was more discriminatory since epinasty varied from 2.7 to 6.8°.

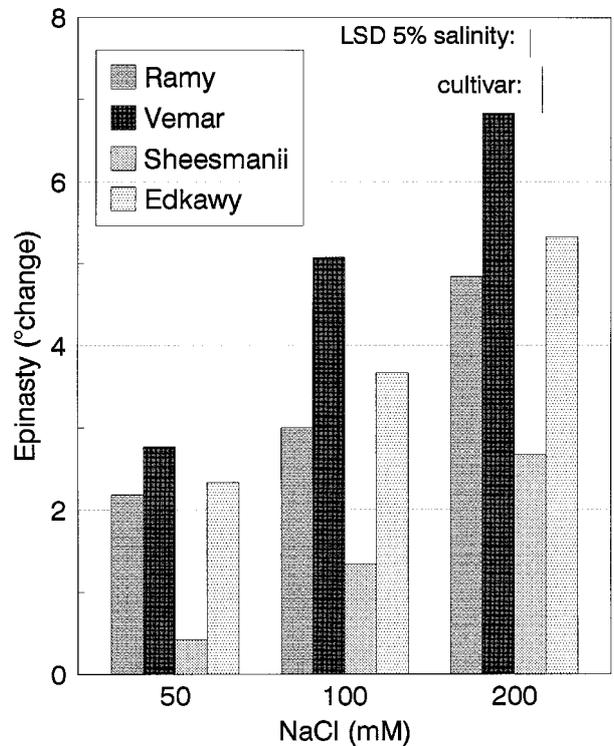


Figure 2. Effect of sodium chloride concentrations on epinasty of four tomato genotypes. Epinasty was expressed as the difference in petiole angles between treated and control plants; thus control values are not presented in the figure. Measures were made on the 4th leaves 36 h after treatment. Vertical bars denote LSD at 0.05 probability level.

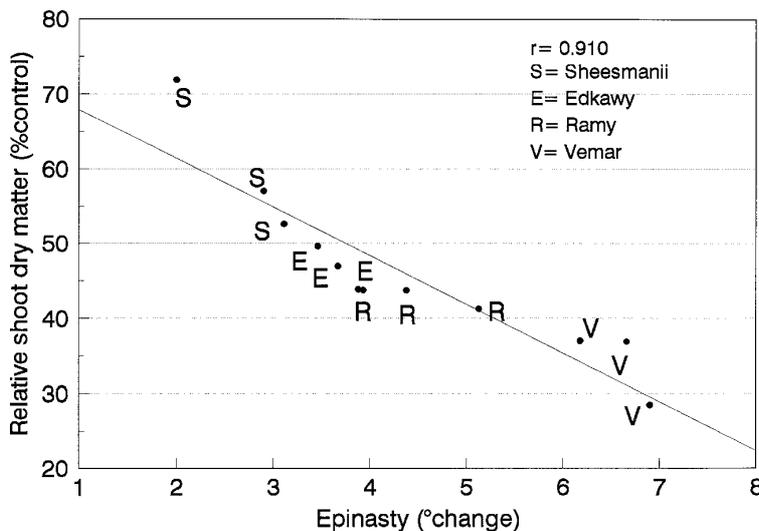


Figure 3. Correlation between epinasty and relative shoot dry matter among four tomato genotypes. Epinasty, in the 4th leaf at 36 h, was expressed as the change in petiole angles of treated (200 mM NaCl) from control (zero NaCl) plants.

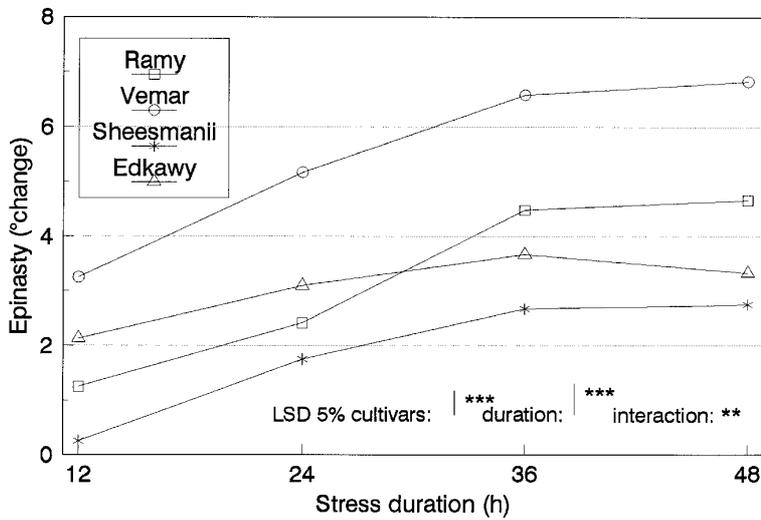


Figure 4. Epinasty as function of stress duration of four tomato genotypes at 200 mM NaCl in the 4th leaf. Vertical bars represent LSD at 0.05 probability level. *** and ** denote significance at $p < 0.001$ and $p < 0.01$ respectively.

The relation between epinasty and dry matter production by different varieties at 200 mM NaCl (Fig. 3) shows a negative relation between epinasty and salt tolerance. The most susceptible variety (Vemar) manifested the highest angle. The most tolerant (Sheesmanii) presented the narrowest angle. Ramy and Edkawy were intermediate.

Epinasty induced by salt stress increased with duration of the stress. Data showed that for successive 12 h intervals, epinasty was low at 12 hours (lower than 1° for all varieties except Vemar) and increased until it reached a maximum at 36 hours (Fig. 4). Epinasty was more pronounced in the 4th leaf than in the 3rd than in the 2nd; the latter being the oldest leaf (Fig. 5). It varied from 2.3 to 4.7° in the 2nd and from 2.7 to 6.8° in the 4th leaf.

3.3. Ethylene

Ethylene production by tomato petioles increased significantly with increasing levels of salinity in the rooting medium (Tab. II). Its magnitude ranged between 1.17 and 2.5 times that of controls.

Ethylene production also differed significantly among varieties. Sheesmanii produced the highest rate of ethylene either under salinity or non-saline conditions. Vemar showed the lowest ethylene rate under non-stress as well as under salt stress conditions. However, the salt induced ethylene,

estimated as a percentage of control rates, was greater for Vemar (142 to 250%) than for Sheesmanii (105 to 117%) in the 4th leaf (Tab. II).

Ethylene production rate also depended on leaf age. It was greater in the 2nd than in the 4th leaf.

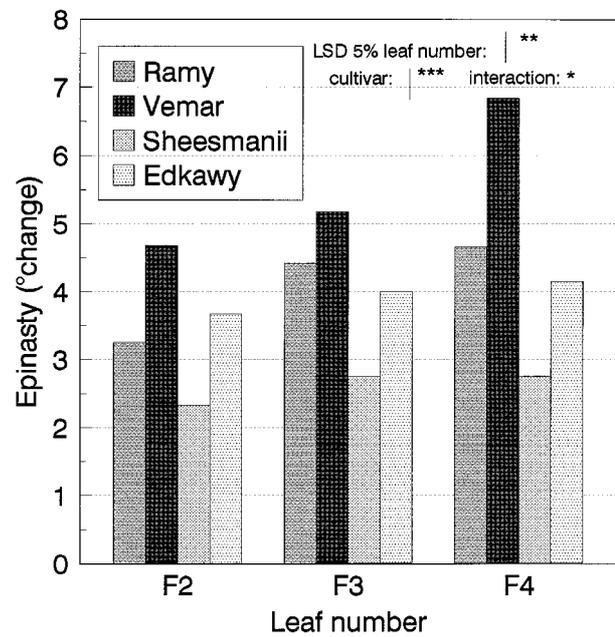


Figure 5. Epinasty as a function of leaf age of four tomato genotypes at 200 mM NaCl 36 h after treatment. F2, F3 and F4 were the 2nd, the 3rd and the 4th leaf respectively. Vertical bars represent LSD at 0.05 probability level. *** and ** denote significance at $p < 0.001$ and $p < 0.01$ respectively.

Table II. Effect of salinity on ethylene production among four tomato genotypes. Ethylene production by excised petioles was estimated for the 2nd and the 4th leaf.

Cultivar	Treatment mM NaCl	Ethylene production			
		Leaf 2		Leaf 4	
		nmol/g fw	%control	nmol/g fw	%control
Ramy	0	6.74	100	6.12	100
	50	7.18	107	6.56	107
	100	8.06	120	7.11	116
	200	10.18	151	8.32	136
Vemar	0	3.78	100	3.11	100
	50	5.17	137	4.42	142
	100	6.25	165	5.31	171
	200	8.43	223	7.77	250
Sheesmanii	0	10.75	100	9.45	100
	50	11.26	105	9.93	105
	100	12.44	116	10.42	110
	200	13.76	127	11.07	117
Edkawy	0	7.79	100	7.11	100
	50	8.22	106	7.54	106
	100	10.00	128	8.06	113
	200	11.22	144	9.28	131
LSD 5%	salinity:	0.391***			
	variety:	0.554***			
	leaf:	0.329**			
	interaction:	ns			

The difference ranged from 0.62 to 2.69 nmol/g fresh weight according to the level of salinity (Tab. II). Except for Vemar, the salt induced ethylene production (% control) was larger in the 2nd than in the 4th leaf for all cultivars.

Figure 6 shows the relation between epinasty, relative ethylene production and osmotic potential in the 4th leaf for all cultivars at 200 mM NaCl. For Vemar, low leaf osmotic potential was accompanied by high relative ethylene production and high epinasty. By contrast, Sheesmanii had a high leaf osmotic potential, a low relative ethylene production and a low epinasty.

4. Discussion

Leaf water and osmotic potentials diminished with increased NaCl concentration; while the turgor pressure stayed constant. In terms of water

potential, Sheesmanii had less negative values (Tab. I). It showed dehydration symptoms more slowly than the other varieties as salt stress became more severe. The maintenance of high water potential in Sheesmanii (Tab. I and Fig. 1) despite the increase of salt stress probably resulted in a greater water absorption capacity and/or a low stomatal conductance [8].

Osmotic potential was related to the accumulation of solutes such as Na⁺, K⁺ and proline (data not shown); indicating that osmotic adjustment had occurred. This allowed turgor to remain constant despite the increased salinity level. We noticed also a negative relationship between leaf osmotic potential and shoot relative dry matter; which has also been observed in cotton [15], and in sorghum [21]. These authors related the growth reduction to the energetic cost required by the osmotic adjustment, particularly when using organic solutes. In fact Sheesmanii which seemed to use inorganic solutes

such as K^+ presented, in comparison with other varieties, a less reduced growth in terms of relative dry matter.

Exposure of tomato plants to salt stress in their root environment was accompanied by epinastic growth for which the magnitude was increased by stress severity (Fig. 2). Our values of epinastic angle which varied from 0.5° to 7° , differed from those reported (5° to 28°) by Jones and El-Abd [10] due probably to the development stage.

Petiole epinasty induced by salinity is probably due to an increase in ethylene synthesis in aerial plant parts [3, 20]. Additional ethylene stimulates epinasty by promoting elongation growth in the adaxial (upper) half of the petiole, while elongation in the abaxial (lower) half is suppressed [14].

This response to salinity depended on the genotype. Vemar and Sheesmanii were the most and least susceptible genotypes respectively (Fig. 3); Ramy and Edkawy were intermediate. Epinasty also depended on the age of the leaf in which measurements were made. The angle was greater in younger leaves. Epinasty depended finally on the time between application of salt stress and measurement (stress duration). The angle being maximal 36 hours after treatment application.

Ethylene produced in tomato petioles following salt stress constraint (3.11 to $13.76 \text{ nmol}\cdot\text{g}^{-1} \text{ fw}$) were higher than values reported for tomato (1 to

$4 \text{ nmol}\cdot\text{g}^{-1}$) by Abou Hadid and El-Beltagy [1], but lower than those reported for wheat (40 to $150 \text{ nmol}\cdot\text{g}^{-1}$) by Narayana et al. [13]. The latter authors think that monocotyledons and dicotyledons could have fundamental differences if ethylene is linked to a process such as leaf abscission which is specific to dicots.

Ethylene production rate was sensitive to the salinity level. This response could have resulted from a change in the activity of enzymes involved in ethylene regulation such as ACC synthase and ACC oxidase and/or availability of their substrates under these salt conditions.

Ethylene production rate depended also on the genotype. A positive correlation ($r = 0.77$) was noticed between ethylene production level and the dry matter accumulated in different varieties. Tolerant genotypes (Sheesmanii, Edkawy) exhibited greater basal endogenous ethylene. Their additional ethylene induced under salt stress (% control in Tab. II) was less marked compared to the sensitive variety (Vemar) and they manifested lower epinasty despite higher absolute levels of ethylene. This suggests that tolerant genotypes have acquired a certain adaptation probably by modulating phytohormones antagonistic to ethylene [7], or by direct alteration of ethylene [11].

Involvement of ethylene in promoting epinasty, shown by several studies [2, 4, 7, 12], was confirmed here for each genotype under different

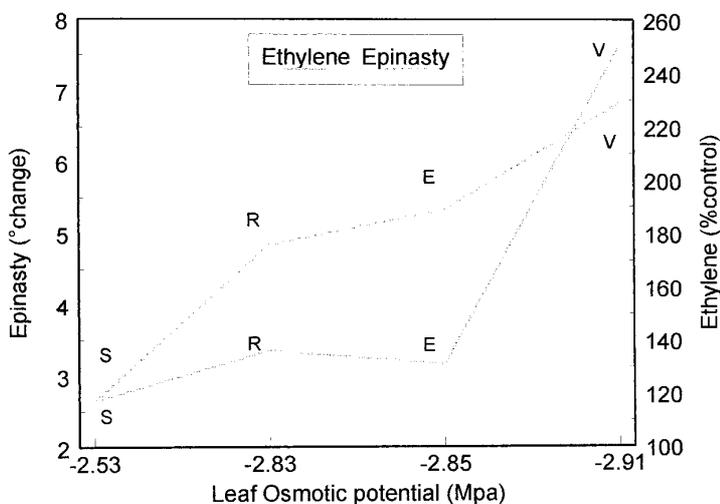


Figure 6. Relationship between epinasty, relative ethylene production and osmotic potential in the 4th leaf at 200 mM NaCl 36 h after treatment. S = Sheesmanii, R = Ramy, E = Edkawy and V = Vemar.

levels of salt stress. But absolute ethylene values of different genotypes at the same level of stress (200 mM NaCl), were negatively correlated ($r = -0.94$) with epinastic angle in the 4th leaf.

Ethylene production rate depended also on the age of the measured petiole. It was greater in the old leaves than in the young ones. The same result was reported by Abou Hadid and El-Beltagy [1]. The low epinasty manifested by old leaves, which had the highest ethylene levels, suggests that cells might be more sensitive to ethylene in young leaves than in old ones.

Figure 6 shows that the susceptible variety (Vemar) produces higher relative ethylene in comparison with the tolerant accession (Sheesmanii), and manifested a higher epinasty.

Data presented in this study indicates that a certain genotypic variability for ethylene and epinasty exists among cultivars depending on salt stress, stress duration and leaf age. But we hesitate to recommend ethylene and/or epinasty as selection criteria for salt tolerance without further investigation of their heritability.

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