Comparative foliar studies in saline sand – grown and fresh water soil – grown *Trigonella foenum – graecum* Linn. Plants

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Abstract

Trigonella foenum – graecum Linn. is cultivated for commercial purposes on some beaches of Mumbai. The growers dig shallow wells on the beaches which get filled with sea water percolating through the sands during high tide. A project was undertaken to do a comaparative study of various growth parameters of the plants grown under two different conditions of cultivation - 1) grown in sand using saline beach water, 2) grown in soil using tap water. The present paper focuses on the comparative study of the leaves of the two sets of plants studied for their number and size – length, breadth and area, petiole lengths, phyllotaxy and the characteristic of left and right handedness of the experimental plants. Leaf epidermal studies including stomatal indices, were also conducted. Beach well water and the regular fresh water used for cultivation of test plants were analysed for physical and chemical parameters.

All the morphological parameters like number of leaves, length, breadth and area of leaf lamina, size of petioles showed an inhibitory effect of salinity. Shape of epidermal cells and stomatal indices were almost similar for both the saline sand grown and fresh water soil grown plants.

KEYWORDS: *Trigonella foenum – graecum*, salinity, leaf size, phyllotaxy, left and right handedness.

INTRODUCTION

Mumbai being located on the sea coast, has a large number of beaches. Along some of them, notably the Seven Bungalows beach in the north western suburb of Andheri, it was observed that *Trigonella foenum graecum* (locally called *methi*) plants were being cultivated regularly, round the year, in the saline sands of the beach. The week – old plants were harvested at the two – cotyledonary leaf stage, tied into bunches and were sold in the local markets as a leafy vegetable.

The regular soil – grown *Trigonella foenum graecum* plants are a popular and widely consumed leafy vegetable. *Trigonella foenum graecum* is a native of southern Europe and Asia. It is an economic plant grown the world over, especially in parts of central and south Eastern Europe, western Asia, Indian subcontinent and North Africa. It is cultivated both for its culinary and medicinal uses. Seeds are used as a diuretic, carminative and tonic. In addition, the seeds are also significant as astringent, demulcent, emollient and aphrodisiac (Nadkarni, 1976).

After carefully studying the method of local cultivation of *Trigonella foenum graecum* plants in the saline beach sands of Mumbai, it was decided to undertake a detailed comparative scientific study of these two types of plants – sand grown and soil grown. Present paper focuses on the comparative study of the leaves with respect to their number, size, phyllotaxy, the left and right handedness in its population and epidermal structure including stomatal index. In addition, the two types of waters used for growing the test plants were also analysed for their physical and chemical parameters.

MATERIALS AND METHODS

1). Collection of sand and beach water: Sand for cultivation of experimental set of *Trigonella foenum graecum* plants, was collected from the actual site of methi farms on the Seven Bungalows beach, Andheri West, Mumbai – 61. The moist sub layer of beach sand was manually filled in several polythene bags and transported to the experimentation site at Patkar – Varde College, Goregaon West, Mumbai – 62.

Similarly, the water for growing the experimental set of *Trigonella foenum graecum* plants was regularly collected in several jerry cans from the beach wells (term described later) dug on the actual site of methi farms by the beach *methi* cultivators. This water named beach water (by the investigators) was transported to and used to raise the sand – grown plants at the study site in Patkar – Varde College.

Beach wells and beach water: The *methi* cultivators at the Seven Bungalows beach had dug 10 -15 feet deep wells in the sand about 150-200 feet inland from the high tide mark. These were neat, large conical cavities on the beach sand made by patient and consistent removal of sand by spades. A large drum, open from both the sides, was then pushed into the sand in the deepest central part. More sand was removed from the space within the drum which initially became hollow but then gradually got filled up with percolated sea water, especially during high tide. This well was named “beach well” and water collected in the drum at the base of the well was called “beach water” by the investigators.

2). Cultivation of *Trigonella foenum - graecum* plants:

a). Sand – grown plants: The moist sand collected from the beach methi farms was filled in 12 inches diameter polythene bags up to a height of 12 inches. These bags were then put into black polythene bags. *Trigonella foenum graecum* seeds which were soaked in beach water the previous night were sown in many sand – filled bags, at a density of 50 seeds per bag. The bags were then watered lightly with beach water.

b). Soil – grown plants: The second set of bags for the soil – grown plants were prepared in the same manner as described for the sand – grown plants above. The only difference was that these bags were filled with fresh soil + cow dung manure mixture (in the ratio of 3:1). Fresh set of *Trigonella foenum graecum* seeds presoaked overnight in tap water, were sown at the same density of 50 seeds per bag. These bags were watered using ordinary tap water.

3). Study of various leaf parameters:

a). Number of leaves in relation to age: The number of fully opened leaves were noted at different ages for both the sand – grown and soil – grown *Trigonella foenum graecum* plants i.e. the number of days required (after the date of sowing) by a plant to develop a fully opened specific leaf – cotyledonary leaves, prophylls, first trifoliate leaf and so on.

b). Phyllotaxy and left and right handedness: While carefully studying the morphology of the plants their phyllotaxy and left and right handedness too was observed and recorded.

c). Size of the leaves: The lengths and breadths of the cotyledonary leaves at the 7 – days – old cotyledonary stage, were measured in cms. Similarly, the prophylls and the second trifoliate leaves were measured at the vegetative stage for both the sand-grown and soil-grown plants. The petiole lengths too, were measured simultaneously.

d). Leaf areas: areas of the cotyledonary leaves, prophylls and the mature trifoliate leaves were calculated by the graph paper method.

e). Stomatal index: Temporary mountings of the epidermal peelings were studied for the shape of epidermal cells and numbers of stomata and epidermal cells in different areas of vision. Stomatal index was calculated by the formula S/E X 100.

All readings were then subjected to statistical analysis.

4. **Water analyses: Both** the tap water and beach well water were tested for the following parameters: pH (Toshniwal's digital pH meter), conductivity (conductivity meter), salinity (from BNHS Laboratory), alkalinity (titration method), hardness (titration method), total soluble salts (evaporation method), chloride content (Mohr's titration method), nitrate nitrogen (Harper, 1924, Prince, 1945), total phospahate, sulphate (from BNHS Laboratory), phosphate-phosphorus (Bell and Diosys, 1920. Hydroquinone method), iron (Farrar, 1935), calcium and magnesium (EDTA method) and sodium and potassium (Flame photometer).

RESULTS

a). Relation between age and number of leaves: The average number of days taken by the sand – grown seeds to develop the fully opened successive leaves were more as compared to the corresponding figures for the soil – grown seeds. Thus for any specific age the sand – grown plants had fewer number of leaves than the soil – grown plants (refer table 1).

b). Phyllotaxy: The large number of plants observed for their phyllotaxy showed an inconsistent pattern in both the sand – grown and the soil – grown plants. But by and large it was observed to be 1-6 or pentastichous or five ranked phyllotaxy.

c). Left and right handedness: The ratio of left-handed and right-handed plants in both the sand – grown and soil – grown plants of *Trigonella foenum-graecum* L. was 1:1.

d). Leaf measurements – lengths and breadths: Tables 2 and 3 show the length and breadth measurements of various leaves at different stages of growth. All leaves of the

sand – grown plants were shorter and narrower as compared to the corresponding leaves of the soil – grown plants.

e). Leaf areas: Like the lengths and breadths, the leaf areas too were more for the soil – grown plants than the sand – grown ones (refer table 4).

f). Petiole lengths: Petioles of the soil – grown plants were longer than those of the sand – grown plants (refer table 5).

g). Beach and Tap water analyses: Results of physical and chemical analyses of beach well water and the tap water used to grow the two sets of experimental plants are given in table no. 6.

DISCUSSION

Leaves – Appearance, number, length, breadth and area: Regular and careful observations of plants over long durations during the course of the present investigation, proved that that the successive leaves took longer to appear in the sand-grown plants than the soil-grown plants. This delay became evident right from prophyll stage and continued till maturity. Such a delay has also been reported by Gutierrez Boem *et al.* (1994) in *Brassica napus* L. and Parida and Das, 2005.

Salinity had an adverse effect on the total number of leaves that developed per plant which was evident from the present investigation.

It was corroborated by the following reports: in strawberry plants by Awang, Y.B., Atherton, J.G. and Taylor A.J. (1993), in *Triticum aestivum* L. by Prasher, Anju and Varma, S.K. (1992), in *Paulownia elongata* and its clones by Miladinova et.al., (2013), in two **c**ultivars of canola (*Brassica napus* L.) by Homa M. Z. and Ma'ssoumeh B. N., 2011.

Ahmad, et.al., 2005, too have reported a reduction in leaf number in *Pisum sativum* due to salinity but to lesser extent.

In addition to suppressing the leaf number per plant, salinity also affected the leaf areas. This observation too has been reported by many other workers in other genera *e.g.* Soto, Ricardo and Luis, F.Corrales (1987) in *Avicennia germinans* L.; Girdhar, I.K. (1989) in *Helianthus annuus* L.; Pezeshki, S.R. and Pan, S.Z. (1990) in *Oryza sativa* L.; Zhao, K.E.FU, Rana Munns (1992) in *Hordeum vulgare* L.s cv. Clipper and *Atriplex spongiosa;* Knight, S.L., Rogers, R.B., Smith, M.A.L. and Spomer, L.A. (1992) in miniature dwarf tomato Micro-Tom; Awang, Y.B., Atherton, J.G. and Taylor, A.J. (1993) in strawberry plants; Lacerda Claudivan F. *et.al*. (2006), Miladinova et.al., (2013) in *Paulownia elongata* and its clones; Elham Ramezani, *et.al*., in *Echium amoenum* Fisch. & Mey; Cheruth, A.J., *et.al.*,(2008) in *Catharanthus roseus*; Dagar *et. al*. (2004) in *Salvodora persica*; Tabatabaei S.J., *et.al.*, (2007) in olive trees; İlknur Solmaz, *et.al.*, in dihaploid melon lines.

In addition to the average leaf areas, average lengths and breadths of the different leaves and petiole lengths also showed an inhibitory impact of salinity. İlknur Solmaz, *et.al.*, (2011) have reported a decrease in the length and breadth of the leaves in the dihaploid melon lines due to the impact of salinity.

Phyllotaxy: Although there were differences in the phyllotaxy of different plants studied (Priestley and Scott, 1936; Esau, 1943a; Sterling, 1945) by and large it was 1-6 in most of the sand-grown and the soil-grown plants i.e. pentastichous or five ranked phyllotaxy. Salinity had no effect on the phyllotaxy of the plant (Esau, 1953, pg. 343).

Left and right handed plants: A peculiar observation made during the study of phyllotaxy of the plants was that when seen from the apex downwards, the successive leaves appeared either in clockwise direction (called left handed plants by the investigators because when they were seen in lateral view, successive leaves appeared on the left of the previous one) or anticlockwise direction (called right handed plants).

Proportion wise, they appeared in a very precise ratio of 1:1 in both the sand-grown and the soil-grown plants.

Overall findings of the study revealed that salinity had an inhibitory impact on all the foliar parameters studied except phyllotaxy and left and right handedness.

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Table 1: Comparison between the number of days taken to develop the successive leaves in the sand – grown and soil – grown plants of *Trigonella foenum-graecum* L.

Leaves	Number of days taken								% D
	Sand – grown plants				Soil – grown plants				
	Min.	Max.	Avg.	+SD	Min.	Max.	Avg.	+SD	
2c	5.00	10.00	7.00	1.14*	2.00	8.00	5.80	1.21	20.69
P	10.00	20.00	14.30	2.65*	9.00	14.00	10.43	1.25	37.04
1st t	15.00	24.00	19.15	2.80*	10.00	24.00	15.35	2.35	24.74
2nd t	20.00	27.00	22.95	2.13*	15.00	26.00	18.78	2.33	22.26
3rd t	23.00	32.00	29.40	2.78*	17.00	39.00	24.80	4.87	18.53
4th t	30.00	42.00	35.27	4.23*	21.00	48.00	28.43	5.17	24.03
5th t	30.00	50.00	40.88	6.15*	25.00	49.00	31.70	6.11	28.97
6th t	35.00	55.00	46.26	5.98*	27.00	52.00	36.83	6.48	25.60
7th t	37.00	56.00	47.10	4.63*	30.00	54.00	41.00	7.22	14.88
8th t	39.00	59.00	51.75	6.09*	34.00	56.00	42.00	5.53	23.21
9th t	47.00	60.00	52.13	3.87*	37.00	68.00	43.10	5.03	20.93
10th t	44.00	71.00	57.75	7.83*	37.00	71.00	45.63	7.26	26.57
11th t	52.00	82.00	64.33	8.45*	37.00	68.00	46.95	6.56	37.03
12th t	51.00	89.00	67.69	11.37*	42.00	79.00	54.77	9.47	23.60
13th t	68.00	77.00	72.86	3.39*	46.00	79.00	55.97	8.71	30.18
14th t	70.00	84.00	77.25	4.86*	48.00	81.00	56.71	7.52	36.23

2c = two cotyledonary leaves; P = Prophylls; t = Trifoliate leaf; Min. = minimum reading; Max. = maximum reading; Avg. = Average; SD = Standard deviation; %D = Percent difference between the readings of sand – grown and soil – grown plants;
* = significant at $p < 0.05$

Table 2: Comparison of the lengths of various leaves of the sand – grown and soil – grown plants of *Trigonella foenum-graecum* L.

Stage	Leaf	Lengths of the leaves in cms.								%D
		Sand – grown plants				Soil – grown – plants				
		Min.	Max.	Avg.	+_SD	Min.	Max.	Avg.	+_SD	
2c	2c	0.80	1.50	1.11	0.18*	1.30	1.80	1.54	0.13	-28.13
Veg.	P	1.45	2.00	1.66	0.15*	1.90	2.10	1.93	0.06	-14.19
Veg.	t– left	0.95	1.30	1.13	0.09*	1.10	1.40	1.26	0.10	-10.01
Veg.	t-med	1.10	1.85	1.29	0.17*	1.30	1.55	1.41	0.09	-8.96
Veg.	t-right	1.00	1.80	1.16	0.20	1.10	1.45	1.29	0.14	-10.07

Veg. = vegetative stage; t – left = left leaflet of trifoliate leaf; t – median = median leaflet of trifoliate leaf; t – right = right leaflet of trifoliate leaf.

Table 3: Comparison of the breadths of various leaves of the sand – grown and soil – grown plants of *Trigonella foenum-graecum* L.

Stage	Leaf	Breadths of the leaves in cms.								%D
		Sand – grown plants				Soil – grown - plants				
		Min.	Max.	Avg.	+_SD	Min.	Max.	Avg.	+_SD	
2c	2c	0.30	0.60	0.40	0.09*	0.50	0.70	0.59	0.06	-31.91
Veg.	P	1.05	1.50	1.31	0.16*	0.85	1.65	1.50	0.21	-13.22
Veg.	t– left	0.65	1.00	0.80	0.09*	0.85	1.05	0.96	0.08	-16.42
Veg.	t-med	0.85	1.20	1.05	0.10*	1.05	1.40	1.27	0.13	-17.11
Veg.	t-right	0.60	0.95	0.80	0.08*	0.85	1.00	0.95	0.06	-16.12

Table 4: Comparison of the areas of various leaves of the sand – grown and soil – grown plants of Trigonella *foenum-graecum* L.

Leaf	Stg.	Areas of the leaves in sq. mm.								%D
		Sand-grown plants				Soil-grown plants				
		Min.	Max.	Avg.	+SD	Min.	Max.	Avg.	+SD	
2c	2c	39.5	127.0	78.87	22.42*	61.00	123.50	88.59	15.93	-12.33
	P	71.0	98.5	85.10	10.34*	93.50	112.00	102.23	6.43	-20.12
	2t	42.5	131.5	90.00	19.63*	59.50	284.75	111.89	34.12	-24.32
P	P	28.5	122.0	61.08	23.40*	61.50	210.00	133.83	51.78	-119.12
	2t	48.0	108.5	80.57	20.59*	80.50	184.00	137.78	25.80	-71.00
	Veg.	79.5	103.5	90.44	10.57*	93.00	229.00	144.09	41.04	-59.32
IIt-lat	Veg.	35.0	83.0	48.67	12.48*	33.00	128.25	80.36	33.68	-65.12
IIt-med	Veg.	47.75	104.5	66.27	17.90*	53.25	170.50	118.15	50.75	-78.28
IVt-lat	Veg.	32.0	64.5	44.34	9.63*	90.00	168.50	129.88	23.63	-192.92
IVt-med	Veg.	44.0	82.5	60.23	11.31*	125.50	193.50	168.83	24.64	-180.34

Table 5: Comparison of the petiole length of various leaves of the sand – grown and soil – grown plants of *Trigonella foenum-graecum* L.

Leaf	Stage	Petiole lengths in cm.								%D
		Sand-grown plants				Soil-grown plants				
		Min.	Max.	Avg.	+SD	Min.	Max.	Avg.	+SD	
P	Veg.	1.80	3.00	2.45	0.38*	2.40	3.70	3.05	0.33	-19.63
IIt	Veg.	2.00	3.50	2.57	0.42*	1.80	3.80	2.95	0.43	-12.75

Table 6: Beach and Tap water analyses – Results at a glance:

Sr. No.	Parameter	Beach Water	Tap water sample

		Sample	
1.	pH	8.36	7.32
2.	Conductivity	44.8 mmhos/cm	0.098 mmhos/cm
3.	Salinity	34.5 ‰	Nil
4.	Alkalinity – carbonate	40 ppm	Nil
5.	Alkalinity – bicarbonate	252 ppm	Nil
6.	Hardness	160 ppm of $CaCO_3$	48 PPM OF $CaCO_3$
7.	Total soluble salts	3466.33 ppm	275.49 ppm
8.	Chloride content	1550.64 ppm	19.88 ppm
9.	Nitrate nitrogen	6.3875 ppm	1.8375 ppm
10.	Total phosphate	0.025 ppm	Traces
11.	Phosphate – phosphorus	0.0003 ppm	0.0002 ppm
12.	Iron	Nil	Nil
13.	Sulphate	0.5 ppm	Traces