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INVESTIGATIONS ON THE GROWTH ABNORMALITIES OF *MEDICAGO SATIVA* L. UNDER FIELD CONDITIONS

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فحوص النموات الشاذة على البرسيم الحجازي النامي تحت الظروف الحقلية

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تمت دراسات النموات الشاذة في الحقول المنزرعة بالبرسيم الحجازي في منطقة الخرج الزراعية، جنوب شرقي الرياض .

وقد سجلت ملاحظات لفروق معنوية بين النباتات العادية والشاذة خاصة في أعداد وأطوال الفروع القاعدية (المسرطنات)، مساحة الورقة، الوزن الرطب والجاف والبروتينات وأيضاً اليخضور كما أظهرت النباتات المريضة انخفاضاً معنوياً في كلورفيل أ، وتغير في أنماط البروتينات، مما يشير إلى وجود مسبب فيروسي للتغيرات التي طرأت على المعايير المدروسة.

Key words: Growth Abnormalities, *Medicago sativa* L., Alfalfa.

ABSTRACT

Morphological growth abnormalities were pursued and studied in field-grown alfalfa (*Medicago sativa* L.) in Al-Kharj agricultural area, south east of Riyadh. Significant differences between normal and abnormal plants were observed in branch length, number, leaf area, plant dry weight, and protein as well as chlorophyll content. Diseased plants showed significant decrease in chlorophyll a, and changes in protein profiles, which indicates the involvement of viral etiology.

INTRODUCTION

Plant growth under natural conditions is usually characterized by homogeneity, consistency and normal morphology. However, growth abnormalities in field grown plants could be manifested due to one or more external or internal factors. These include genetic changes or reassortments, invasion of disease-causing agents, and imbalance in nutrition and other ecological stimulii.

Growth abnormalities in alfalfa (*Medicago sativa* L.) could arise also from strain biofactors and environmental conditions, e.g. salinity. In addition to that viral infections of field grown alfalfa plants usually lead to decrease in forage yield [1], [2], [3], even as a symptomatic infection in lucerne [3], [4]. Deviation from normal growth of alfalfa include: mosaic, yellowing, chlorosis, deformations and others [4].

Observations of field-grown alfalfa were focused firstly, on clustering of lateral vegetative branches emerging toward the top. This was the first peculiar abnormal phenomenon in the investigated fields of alfalfa. Secondly, the upper buds shifted their development to vegetative instead of floral differentiation. Thirdly, the patterns of plant growth were inconsistent, and finally chlorosis, yellow mosaic dwarfing and deformations were common.

No field observations on alfalfa growth abnormalities in agricultural areas were carried out in Saudi Arabia which urged us to perform this study.

MATERIALS AND METHODS

A field of alfalfa grown under normal conventional agricultural practices was chosen for this investigation. Ten plants and soil samples were collected in October, 1992 from the healthy and abnormal plants. Samples of alfalfa plants were picked up randomly, kept in polyethylene bags and brought immediately to the laboratory for further investigations.

Assorted plant samples were used for morphological, physiological and microbiological analysis. Plants were assorted into 3 categories: (H) for normal healthy and (D_1 & (D_2) for abnormal diseased plants. Differentiation between these samples was based primarily according to their morphological and symptomatic differences.

The investigated morphological parameters include branch numbers and length, leaf numbers and areas, and the numbers of nodes, buds and inflorescence. Leaf area was measured using L1-COR automatic leaf area-meter (L1-COR, Lincoln, Nebraska, USA).

Physiological estimates included: fresh and dry weights and the contents of chlorophyll a b and total chlorophyll, extraction and estimation was carried out as described by [5].

Microbiological investigations included the search for endogenous bacteria, fungi and viruses in one of the most affected diseased plants (D_2). Leaves (5 gm) were surface sterilized in mercuric chloride solution, washed several times by sterile distilled water, thereafter, leaves, were mixed with 200 ml of 0.1 M phosphate buffer, (pH 7.2) and ground in a sterile mortar and pestle under aseptic conditions. The extract was used for detection of bacteria, using nutrient agar and for fungi using Czapek's medium with rose bengal. For detection of viruses the plant extract was artificially inoculated on carborundum-dusted leaves of tested plants according to [6]. Test plants included:

Phaseolus vulgaris, Datura metel, Beta vulgaris, Vigna sinensis, Chenopodium album, Brassica oleracea var. Captitata, Lycopersicum esculentum, Cucumis sativus, Solanum melanogena and *Medicago sativa.*

Protein determination: The soluble protein was extracted by homogenizing 1g of leaves in 5 ml 0.2 M Tris/HC1, pH 7.2), in a polyethylene tube overnight at 4°C. The crude extract was centrifuged at 4000 rpm for 5 minutes and the supernatant was used for protein determination.

Protein content was determined according to the procedure by [7].

Electrophoresis: Total protein was extracted by homogenizing 1g of leaves in 5 ml 0.2 M Tris/HC1 buffer, (pH 7.2), containing 2% (w/v) sodium dodecyl sulphate (SDS) and 20% (w/v) sucrose, in a polyethylene tube overnight at 4°C. The crude extract was centrifuged at 4000 rpm for 5 minutes and the supernatant was used for electrophoresis.

Discontinuous vertical polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by [8]. Protein bands were made visible by staining in coomassie blue dissolved in ethanol: acetic acid: water (100:15:85) and destaining was carried out in a mixture of the same solvents.

Statistical analysis was performed as described for one-way analysis of variance by [9], and when the F-value was significant, Duncun's New Multiple-Range Test was used to compare each growth parameter mean with every other parameter mean.

RESULTS

Symptomatically of field collected samples and the artificially inoculated test plants are shown in Table 1. Morphological differences between healthy and diseased plants are shown in plate 1 (a-d). Plate 2 shows apical branching of old alfalfa, plate 3 shows primary leaves of *Phaseolus vulgaris* seedlings artificially inoculated by juice extract from (D_2) plants showing (left & right) yellow and necrotic lesions. No bacterial or fungal growth appears on the plates after inoculation by the aseptically ground leaves.

The estimates of shoot fresh and dry weights of alfalfa plants indicate a significant reduction for the abnormal (D_1) compared with the normal (H) and abnormal (D_2).

Data from Table 2 shows that morphological differences exist between the abnormal and normal plants. Branch numbers increased in the abnormal plants (D_1 and D_2). However, there is a high significant difference between (D1 and H), at 10% L.S.D. level of significance.

Regarding the branch length (Table 2) there was a decrease in the abnormal (D_1) compared with (D_2) and (H). The results show differences in node and leaf numbers between the normal and abnormal plants. The set of (D_2) plants showed increase in both parameters compared with the set of (D_1) and (H) plants. Concerning inflorescence numbers, no significant difference was found. However, leaf area was affected in both diseased plant sets which was more reduced in (D_1) than (D_2). On the other hand, leaf node and bud numbers in (D_2) exceeded their corresponding nodes and buds in both sets of (D_1) and (H) plants. The lat-

(a) (b) (c) (d)

Plate 1: (a) Healthy (H); (b): Diseased (D_1) with basal branching and chlorosis; (c): Diseased (D_1) with apical branching and (d): Diseased with yellows (D_2).

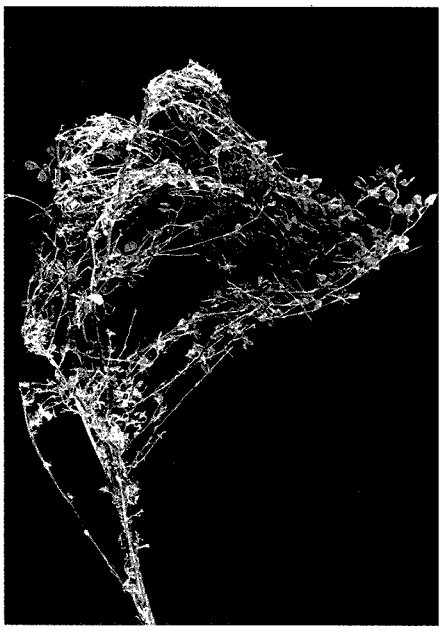

Plate 2: Old alfalfa plant of the preceding season with dense apical branching.

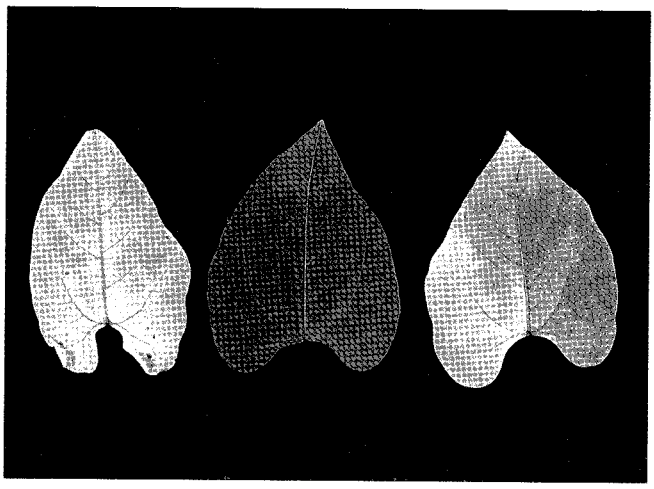

Plate 3: Aritifically inoculated primary leaves of *Phaseolus vulgaris* inoculated with juice from (D_2) and showing albinism (left and right) and small local lesions (left) compared with healthy (middle).

Table 1

Symptoms and host-range of naturally infected *Medicago sativa* L. of an inoculated plants (N.I.) and the artificially inocuated plants (M.I.) by a juice from (D2) alfalfa plants.

Plant	Source	Symptoms
Medicago sativa (old)	N.I. (D_1 set)	dense basal branching, reduced areas of leaflets, chlorosis and stunting. In old plants dense branching crowded near the apex
" " (old)	N.I. (D_2 set)	Yellow
" " seedlings	M.I. ----------	Not developed upto 2 months
Phaseolus vulgaris	"	Local lesions, chlorosis and defoliation
Vigna sinensis	"	Nil
Brassica rapa var. Oleracea	"	severe stunting and albinism
Capsicum annum	"	Nil
Datura metel	"	Nil
Lycopersicum esculentum	"	Nil
Cucumis sativa	"	Nil
Chenopadium album	"	Nil
Solanum melanogena	"	Nil
Beta vulgaris	"	Nil

Table 2

Morphological and physiological parameters used to study the differences between healthy (H) and diseased (D1) and (D2) plants means (M.), standard deviation (S.D.) and least significant differences (L.S.D.) at 10% level are also presented.

Parameters	**Variants (M. and (± S.D.)**						**L.S.D. at 10%**
	H		**D_1**		**D_2**		
	M	**SD**	**M**	**SD**	**M**	**SD**	
Branch number	5.4	1.5	30.6	4.0	8.0	1.5	10
Branch length (cm)	32.6	3.0	17.8	3.0	29.7	5.0	6.5
Leaf area per branch (cm)	32.1	10	5.7	1.6	24.24	10	6.3
Leaf number/branch	8.3	0.3	7.2	0.7	10.7	3.0	1.25
Node number	8.3	0.3	7.2	0.7	10.7	0.5	1.25
Buds number	2.7	1.0	2.2	0.9	4.6	0.7	1.6
Inflorescence number	0.3	0.6	0.75	0.6	0.67	0.5	4.2
Branch fresh weight (g)	1.40	0.5	0.3	0.11	1.8	0.6	0.8
Branch dry weight (g)	0.46	0.1	0.08	0.0	0.46	0.1	0.17
Total protein (mg/g)	**46**		**46**		**62**		
Chlorophyll-a (mg/g)	1.82	0.4	0.56	0.02	0.62	0.04	0.3
Chlorophyll-b (mg/g)	1.11	0.01	1.04	0.01	1.02	0.01	0.3
Total chlorophyll (mg/g)	**2.93**		**1.60**		**1.64**		

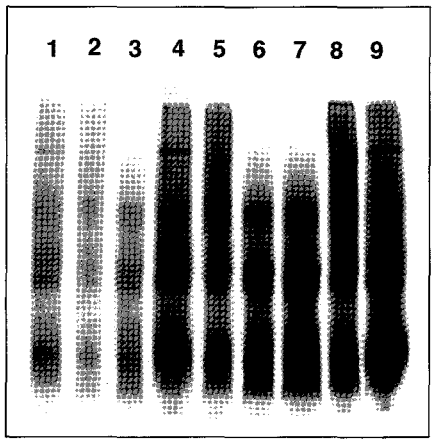

Plate 4: Zymogram patterns of leaf proteins on 17% SDS-PAGE under non-reducing conditions. Tracks 1, 4 and 7 healthy plant.
2, 5 and 8 diseased (D_1) plant
3, 6 and 9 diseased (D_2) plant

Samples loaded was:
20 ul in tracks 1, 2 and 3;
40 ul in tracks 4, 5 and 6;
60 ul in tracks 7, 8 and 9.

ter data were similar with those of the fresh and dry weights for branches of the corresponding plant sets.

The diseased agent(s) significantly decreased chlorophyll a, and total chlorophyll's, but with no effect on chlorophyll b. Soluble protein content was higher in the second abnormal set (D_2) compared with the first abnormal (D_1) and the normal plants (H). The results presented (Plate 4) showed that each extract was resolved into several bands. The protein patterns of the diseased plants (D_1 and D_2) appeared to be identical while that of the healthy plants had an additional band of high molecular weight.

DISCUSSION

Alfalfa is a major forage crop in arid and semi-arid zones including Saudi Arabia. Field observations of growth abnormalities of alfalfa plants in AlKharj agricultural area were accumulated year after year.

Deviation is a major alfalfa growth from normal included: mosaic yellowing, chlorosis, deformation, dwarfing and other manifestations. These observations were similar to those mentioned by [4]. Clustering of lateral vegetative branches toward the top was a peculiar abnormal phenomenon in the investigated fields of alfalfa.

The obtained results from this study indicate clear distinct morphological and physiological alterations existing between different normal and abnormal plants. This study shows that the abnormal alfalfa plants exhibit considerable variations morphologically especially branching length, number and area of leaves as well as vegetative and floral bud differentiations.

It appears, however, that these differences could be as a result of some biotic factors since all of the collected samples were taken from one field.

Growth abnormalities in field grown plants are usually caused by one or more of the external factors. These include genetic changes or reassortments, invasion of disease agents and the imbalance in nutrition and other ecological stimulii. Growth abnormalities of alfalfa plants in AlKharj fields could be due to viral infections as shown from the result of the artificially inoculated test plants and the exclusion of bacterial and fungal etiologies. It is well known that viral infections of alfalfa could lead to decrease in forage yield [1], [2], [3].

The sporadic randomly distributed field infections of growth abnormalities in alfalfa may be caused by seed-transmitted viruses [10, [11], [12] and secondary spread by aphid species [13]. Viral infections often minimize forage even though these infections are symptomatic [4, [3].

The changes in the protein and chlorophyll estimates as well as the protein constituents could be the result of the invading pathogen.

Results (Table 2) could indicate the involvement of viral etiology in the occurring phenomenon. However, confirmation of viral infections, needs further investigation with more host range, serology and electron-microscopy.

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