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Effect of Nitrogen Forms on Tomato Growth  
and Fruit Quality and Improving N<sub>2</sub>-Fixation  
on Yield of Chickpeas and Fava beans

By

Jihad Muhammad Abbadi

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By

**Jihad Muhammad Abbadi**

B.Sc.: Al-Quds University, Palestine

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*Effect of Nitrogen Forms on Tomato Growth and Fruit  
Quality and Improving N<sub>2</sub>- Fixation on Yield of  
Chickpeas and Fava Beans*

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2001-2002

## DECLARATION

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:  .....

Jihad Muhammad Said Abbadi

Date: ...3/8/2002.....

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## ABSTRACT

On a molar basis; plants take up nitrogen more than any other nutrient, and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are the main forms available for uptake under field conditions. Numerous studies have characterised the influence of various combinations of these N forms on growth, nutrition and physiology of plants. Generally  $\text{NH}_4^+$  supplied at high proportions has a suppressive effect on growth and yield of tomato. In order to explain this phenomenon, several hypotheses have been considered in earlier work such as reduced cation content and  $\text{NH}_4^+$  accumulation. The tolerance of tomato plants towards salt stress is influenced by the form of N nutrition.  $\text{NH}_4^+$  may reduce the contents of cations and sodium in particular, while nitrate supply may restrict the uptake of chloride. Using continuous fertigation to minimize pH effects and to maintain constant salt concentration, the influence of N forms ( $\text{NO}_3^-$  vs  $\text{NH}_4^+$ ) at two salt levels on yield and chemical quality of tomato (*Lycopersicon esculentum* L. cv. Daniella) fruits was evaluated.

Tomato cv. Daniella seedlings were planted individually in 10L pots filed with 8L perlite on 25, January 2000 and cultivated in a greenhouse under natural environmental conditions. Plants were continuously irrigated with solutions (200mL  $\text{h}^{-1}$ ) containing 10mM total N ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ), 6mM K, 1.5mM Ca, 1mM Mg, 1mM P, all trace nutrients and the nitrification inhibitor DMPP (1% of total N). Eight treatments (5 replicates each) were used consisting of 4 treatment (0, 10, 20, 40%  $\text{NH}_4^+$ ) and 2 salt levels (0, 40mM NaCl). Plants were irrigated using 100%  $\text{NO}_3^-$  solution without NaCl for 7 days after planing (DAP) using half strength and full strength in the second week. Salt treatments were initiated when the first fruit truss appeared by increasing the NaCl supply by 5mM each day up to 40mM. Total fruit yield (green and mature) to harvest (84 DAP) was recorded, but for fruit analysis fruits were picked, washed and blended three days after they reached the brake point (onset of colour change). This homogenate was used to measure pH, EC and °Brix, and, after hot water extraction, to analyse contents of minerals and carboxyates by ion chromatography (IC). After centrifuging the homogenate; glucose and fructose in the clear supernatant were analysed by IC. Pigments were measured according to Nagata and Yamashita (1992).

Exposure to 40mM NaCl generally reduced yield. Particularly when grown without  $\text{NH}_4^+$  addition. A lower yield of salt-stressed plants is well documented and often associated with reduced fruit size, this leads to a concentration effect. Which in this case results in higher °Brix, and EC values and pigment contents. Higher °Brix values of salt-stressed tomato fruits are often discussed in terms of sugar contents, but in this study the salt treatment increased the °Brix values on average by a factor of 1.35, while glucose and fructose values were not substantially changed. This indicates that the increase in °Brix was caused by other constituents, of which the mineral ions and carboxylates are most likely. Indeed the salt treatment substantially increased the  $\text{Na}^+$  and  $\text{Cl}^-$  contents of the fruit, and contents of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  to some extent. The Ca/K ratio was low and most salt-stressed plants grown with 40mM NaCl suffered from blossom end rot (BER). Plants grown without NaCl showed less BER, particularly those treatments that gave lowest yields, but a dependence on the  $\text{NH}_4^+$  supply were not apparent. The main cation in the tomato fruit is  $\text{K}^+$ , which also serves as an inorganic osmoticum. The  $\text{K}^+$  contents of fruit not exposed to salt were unaffected by the N form, indicating that the well-known reduction of  $\text{K}^+$  contents in vegetative parts due to  $\text{NH}_4^+$  supply did not affect  $\text{K}^+$  translocation into the fruits. Interestingly, in the presence of 40mM NaCl, contents of  $\text{K}^+$  and malate of the fruits were even higher, but both parameters decreased in response to  $\text{NH}_4^+$  supply in agreement with previous results. Citrate present at high concentration ( $700\text{-}800\text{mg kg}^{-1}$ ) was not affected. 40mM NaCl in solution clearly increased the  $\text{Na}^+$  and  $\text{Cl}^-$  contents of the fruits, while the form of N supply induced no obvious trend. Little is known about the influence of N form and NaCl stress, exposure to salt stress increased the  $\text{Cl}^-$  contents of the fruits by  $4.5\text{mmol kg}^{-1}$  when averaged over the N forms, while the Na contents increased only by  $2.6\text{mmol kg}^{-1}$ . This shows the preferred translocation of  $\text{Cl}^-$  into the fruits and underlines the function of chloride as an inorganic osmoticum in salt-stressed tomato fruits. The very low  $\text{NO}_3^-$  content might be explained by the limited phloem mobility of this ion. The  $\text{NO}_3^-$  contents were also unaffected by the treatment, indicating that well-known antagonism between  $\text{NO}_3^-$  and  $\text{Cl}^-$  and the reduction of  $\text{NO}_3^-$  contents by increasing  $\text{NH}_4^+$  supply as found in vegetative tissues does not apply to tomato fruits. Contents of  $\text{SO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  were not effected by either treatment. The homogenate pH was only marginally reduced by the salt treatment (4.1 vs 4.2) and not influenced by the  $\text{NH}_4^+$  supply.

The legume-*Rhizobium* symbiosis is known to be the most efficient system for biological N<sub>2</sub>-fixation and provides an important source of protein for human consumption. Roots of *Cicer arietinum* (chickpea), and *Vicia faba* (fava beans) are nodulated with *Rhizobium cicer*. The free-living N<sub>2</sub>-fixing rhizobacteria of the genus *Azospirillum* live in close association with plants, and may promote plant growth and yield under appropriate conditions. By evaluating data accumulated over the past 20 years from field inoculation experiments, it can be concluded that these bacteria are capable of increasing the yield of important crops grown in various soils and climatic regions. Positive effects of *Azospirillum* inoculation are mainly attributed to improved root development and to the subsequent increase in the rate of water and mineral uptake. Available evidence indicates that excretion of plant-growth promoting substances by the bacteria is at least partly responsible for these effects.

The effect of coinoculation of legumes with *Rhizobium* and *Azospirillum* has received increasing attention in recent years. Positive effects of combined inoculation have been reported for several legumes. The increase in dry-matter production and nitrogen content of dually inoculated plants may attribute to early nodulation, increased number of nodules, higher N<sub>2</sub>-fixation rates and a general improvement of root development. Dual inoculation with *Rhizobium* and *Azospirillum* can either stimulate or inhibit nodule formation and growth in a given symbiotic system, depending on the concentration and timing of inoculation.

In the present study, attempts were made to evaluate the effects of the inoculation of chickpeas (*Cicer arietinum* L.), and fava bean (*Vicia Faba* L.) with *Azospirillum brasilense* strain Cd on nodulation, N<sub>2</sub>-fixation, growth, and yield of both legumes in the presence of native *Rhizobium* bacteria in the soil were studied under different growth conditions. Two greenhouse experiments were conducted at Al-Quds university (October 1998- April 1999), using both legumes for each experiment at an *Azospirillum* concentration of  $5 \times 10^7$  CFU ml<sup>-1</sup>, in a mixture of washed sand and Terra Rosa soil; typical of the region of Abu-Dies contained native *Rhizobium* bacteria. Three field experiments were conducted in Bani-Naim, Ya'bad, and Arrabeh (January-June); 1999, 2000, 2001 respectively, using Chickpea (*Cicer arietinum* L; cv Bulgarian and Jordan), two experiments with fava beans (*Vicia faba* L, local cultivar) were conducted in Ya'bad and Arrabeh in the same periods as in chickpea experiments. *Azospirillum* was inoculated using peat-based inoculum of  $5 \times 10^7$  CFU/ seed. Experimental areas were infected with native *Rhizobium* and not

irrigated, except for rainfall. In greenhouse experiments with both legumes, inoculation with *Azospirillum brasilense* significantly enhanced nodulation by native rhizobia and improved root and shoot development, when compared with non-inoculated controls. In field experiments, inoculation of chickpeas and fava beans with *Azospirillum brasilense* peat-based inoculum also resulted in a significant increase in nodulation, root and shoot growth, and crop yield as compared with non-inoculated controls.

Results of this study show that inoculation with *Azospirillum* for naturally nodulated chickpea and fava beans should increase yield of inoculated plants over the control. This is attributed to enhance root development, and the improved root metabolism, in consequence with improving water and mineral uptake, along with improving earlier and total nodulation and N<sub>2</sub>-fixation by plants and general growth development under limited water and nitrogen availability. It may also lead to a decrease in the need for N-fertilization, lowering costs of production and reducing the negative impact on the environment.

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## LIST OF ABRIVIATIONS

ATCC	American Type Culture Collection
ATPase	Adenosine Triphosphatase
BER	Blossom End Rot
Car	Carotene
Cd	<i>Cynodon dactylon</i>
CFU	Colony Forming Unit
Ch a	Chlorophyll (a)
Ch b	Chlorophyll (b)
cv	Cultivar
d.a.s	Day after sowing
DAP	Day After Planting
EC	Electric Conductivity
GA <sub>3</sub>	Gibberellins
GDH	Glutamate Dehydrogenase
HATS	High Affinity Transport System
HPLC	High Performance Liquid Chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IC	Ion Chromatograph
LATS	Low Affinity Transport System
LWC	Leaf Water Content
Lyc	Lycopene
O.D	Optical Density
PAD	Pulsed Amperometric Detection
PGRs	Plant growth regulators
RDM	Root Dry Matter
rev	Revolution
rpm	Round per minute
SPAD	Single Photon Attenuation Technique
W.U.E	Water Use Efficiency

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