**Effect of an endomycorrhiza (*Glomus intraradices*) on the water balance and salt tolerance of peppers in the Arava**

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**Abstract**

This report summarizes an experiment on peppers of the ‘Celica’ variety, testing effects of the presence of the endomycorrhiza, *Glomus intraradices*, at two levels of fertilization and three levels of irrigation. The basic motivation for the study is to improve the growth and fertilizer regime based on current knowledge that indicates improved water uptake of water and nutrients by plants inoculated with mycorrhizae in general, and with *G. intraradices* in particular. The three levels of irrigation in the experiment were 50%, 75% and 100% of the irrigation level recommended by the Agricultural Extension Service, based on pan evaporation data and a coefficient that varies according to the stage of growth. The fertilizer treatments were full fertilization according to the accepted practice and low phosphorus fertilization. The experiment took place in a 25-mesh nethouse at the Ya’ir Station during the 2007/8 season. The experiment was performed in two fields. Each field received a different fertilization treatment, and included four replicates of combinations of irrigation dose and presence or absence of mycorrhiza. The measured variables were total yield and percentage yield for export. The data were collected during the season; in this report we present only the cumulative results from the end of the growing season. The data were analyzed by ANOVA using a General Linear Model (GLM). The effect of the level of phosphorus fertilization was highly significant – full fertilization was better than low phosphorus fertilization. In the low phosphorus treatment there was no effect of the irrigation dose, while a significantly higher yield was obtained in the presence of mycorrhiza. In the full fertilization treatment there were significant effects of both mycorrhiza and irrigation dose – in both cases a higher yield was obtained in the presence of mycorrhiza and when irrigated at 100% of the recommended level. There was no interaction between the irrigation dose and the presence of mycorrhiza – one variable cannot be used to compensate for the other. Another interesting observation was the very high variability under low phosphorus fertilization. This variability simply reflects the great lack of uniformity that was observed throughout the growing season. From a financial point of view, it seems possible to increase revenue following addition of mycorrhiza and an increased yield of approximately 315,000 NIS in return for an investment of 6,750 NIS on an average 45-dunam pepper farm.

**Introduction**

This report relates to Objective No. 3 of the research concerning the optimal irrigation dose for growing peppers of the ‘Celica’ variety at two levels of fertilization (full and low phosporus), and in the presence or absence of a mycorrhizal fungus. The motivation for the study was two-fold. On one hand, the increasing lack of water for farmers in the Arava, and in particular the continual decrease in water quality, and on the other hand, the common natural symbiosis between mycorrhizal (AM) fungi and higher plants, a symbiosis that contributes to improved nutrition and growth of the plant. Soil fungi penetrate the root tissue of the plant and form mycorrhiza (in Latin: myco – fungus, rhiza – root). The fungal hyphae grow inside and outside of the plant root and apparently increase the surface area of the root, thus improving processes of water and solute uptake. In addition, the fungus contributes to changing the hormonal balance, improving the soil structure, and also provides resistance to pathogens that attack the root (such as *Fusarium, Pythium, Phytophthora* and nematodes).

Improving the water balance and resistance of the plant to drought and salinity has been described for geraniums. Plants that were inoculated with endomycorrhiza were more resistant to drought conditions in the soil and to salinity than plants that were not treated with mycorrhiza (Sweatt and Davies, 1984). Last year, the effect of mycorrhizae on plant tolerance to water or salt stress was examined at the Ya’ir Station in the Arava. A great improvement was observed in the development and growth of mycorrhizal plants that looked as developed as the control plants (high level of phosphorus) regardless of the irrigation dose. The results of previous experiments indicate that pepper plants irrigated with saline water in the presence of mycorrhiza are more tolerant of drought and/or salinity that develop in the root system. These facts distinguish the mycorrhizal system not only as a source of improved uptake of nutrients from soil, but as an attractive system that can facilitate and promote savings of significant amounts of water in this unique region of the country, where most of the agricultural produce is intended for export and water sources are diminishing.

**Materials and Methods**

Three factors were examined in this experiment: (1) the amount of phosphorus fertilization with two levels: 1. full fertilization and 2. low phosphorus fertilization. (2) irrigation dose relative to the recommended dose with three levels: 1. 100, 2. 75 and 3. 50% (Table 1). (3) mycorrhiza with two levels: 1. with mycorrhiza and 2. without mycorrhiza. The experiment took place in an American nethouse (25 mesh) at the Ya’ir Station. The area of land: 20 m X 25 m; soil sterilization: methyl bromide. The experiment is three-factorial with 12 treatments replicated four times. Each equivalent plot was 4 m in length and in each planting bed there were two rows of plants for a total of 20 plants per treatment. The 100% irrigation dose was determined according to the recommendations of the Agricultural Extension Service that define the irrigation coefficients relative to pan evaporation data and growth stage. Evaporation values are presented on the Arava R&D website (<http://yair.arava.co.il/climatic/mclm.htm?nojump>). Full fertilization is continuous fertilization with a solute ratio of 7:3:7 while the low phosphorus treatment is the same solution with 20% phosphorus content.

The seedlings were prepared in a nursery with an organic growing regime; inoculation of the seedlings with a mycorrhizal fungus for the relevant treatments was conducted at the nursery stage, by adding inoculum of the mycorrhizal fungus into the planting hole.

The data collected were total yield and percentage yield for export. These data were collected throughout the growing season. This report presents the cumulative data for the entire season. The experiment was set up in a split-plot design, where fertilizer treatments were the whole-plot treatment as shown in Figure 1.

Table 1: Planned and actual irrigation doses in the different treatments

|  |  |  |
| --- | --- | --- |
| Fertilizer | Planned irrigation | Actual irrigation (m3 per dunam) |
| Low phosphorus | 100% | 717.2 |
| 75% | 550.4 |
| 50% | 350.3 |
| Full fertilization | 100% | 710.3 |
| 75% | 537.9 |
| 50% | 348.9 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 100% | 50% | 75% | 100% | 75% | 50% | 100% | 50% | 75% | 50% | 100% | 75% |
| Low phosphorus | **M+** | **M-** | **M+** | **M-** | **M-** | **M+** | **M-** | **M+** | **M+** | **M-** | **M+** | **M-** |
| **M-** | **M+** | **M-** | **M+** | **M+** | **M-** | **M+** | **M-** | **M-** | **M+** | **M-** | **M+** |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 75% | 100% | 50% | 100% | 50% | 75% | 50% | 75% | 100% | 100% | 75% | 50% |
| Full fertilization | **M-** | **M+** | **M+** | **M+** | **M-** | **M-** | **M+** | **M-** | **M+** | **M-** | **M+** | **M-** |
| **M+** | **M-** | **M-** | **M-** | **M+** | **M+** | **M-** | **M+** | **M-** | **M+** | **M-** | **M+** |

Figure 1: Experimental setup in the nethouse

Legend: The percentage numbers indicate the irrigation dose relative to the recommended dose. M+ and M- indicate with and without mycorrhiza, respectively

For the statistical analysis, the three variables were coded by numbers as follows:

* Phosphorus (F): 1 – full fertilization, 0 – low phosphorus fertilization
* Water dose (W): 100%, 75% and 50% (see Table 1 for actual irrigation doses)
* Mycorrhiza (M): 1 – with mycorrhiza, 0 – without mycorrhiza

**Results and Analysis**

Before we begin an in-depth description of the analysis of the results we must consider the correlations among the values obtained for general yield, percentage yield for export, and yield for export (Pearson Correlations). Table 2 shows significant linear correlations among the three measurements. We note the difference in the coefficient of determination of the correlation between total yield and yield for export (0.97) and that of the correlation between total yield and percentage yield for export (0.66). This difference will be examined separately in the statistical analyses with respect to the total yield and with respect to the percentage yield for export.

Table 2: Pearson Coefficient of Determination among total yield, percentage yield for export and yield for export (kg per dunam).

A graphical representation of the distribution of yield for export as a function of total yield, differentiating between full fertilization and low phosphorus fertilization, is presented in Figure 2. This figure clearly shows the linear dependence obtained between yield for export and total yield with a coefficient of determination of 97%.

Figure 2: Distribution of yield (kg per dunam) for export as a function of total yield, according to fertilizer treatment.

From this point onwards we conducted ANOVA according to two models: (1) a model including the fertilization variable and (2) Separate ANOVAs for each level of fertilization.

A general glance at total yield, percentage yield for export and yield for export is presented in Figures 3, 4 and 5, respectively. The error bars are 95% confidence intervals for each mean (95% confidence interval is the interval in which there is a 95% probability that the treatment mean is found). It is interesting to note that the confidence intervals of the low phosphorus treatment are wider than those of the full fertilization treatment – a fact that sits well with the relatively high lack of uniformity that was observed in these treatments relative to the full fertilization treatments. The significance letters appearing next to each treatment are the result of one-way ANOVA and should be treated with caution.

The remainder of this report is the statistical analysis of these observations.

Figure 3: Total yield as a function of treatment, including confidence intervals (95%) of the means (with and without mycorrhiza, percentages are irrigation treatments).

Figure 4: Percentage yield for export as a function of treatment, including confidence intervals (95%) of the means (with and without mycorrhiza, percentages are irrigation treatments).

Figure 5: Yield for export as a function of treatment, including confidence intervals (95%) of the means (with and without mycorrhiza, percentages are irrigation treatments).

**ANOVA including levels of fertilization**

The tested linear model is as follows:

**Y = F W(F) M(F) W\*M**

The terms W(F) and M(F) reflect the fact that the irrigation and mycorrhiza treatments are nested in the fertilizer plots. They also include the interactions between these variables.

The analysis was conducted using the Multivariate GLM model.

The ANOVA results are presented in Table 3. Values that are non-significant at the 5% level are highlighted in green (the abbreviation ‘Sig.’ indicates the significance of the result: if the value obtained is lower than the accepted threshold value of 5% (or 0.05), then the effect of the variable is considered significant, in contrast to values with a significance (Sig.) value above 0.05. The smaller the value, the higher the level of significance, or the effect, of the variable).

The overall mean values of full fertilization and low phosphorus fertilization including the coefficient of variation and confidence interval of each mean are presented in Table 4a – a 95% confidence interval is the interval based on the results of the experiment and under the assumption of normal distribution of the measure variable, in which there is a 95% probability of finding the real, unknown mean. Table 4b presents the differences among yield, percentage yield for export and yield for export at two levels of fertilization. For each of the measured variables, the value obtained for full fertilization was outside of the confidence interval for low phosphorus fertilization. The differences between full fertilization and low phosphorus fertilization are 2,532 kg for total yield, 10.3% for percentage yield for export and 1,487 kg for yield for export. These three differences are highly significant – these data are equivalent to the significance presented for ANOVA with respect to fertilization (Table 3).

**Conclusions – fertilization level**

The effect of fertilization level (full or low phosphorus) was highly significant for all three measured variables. The interaction between irrigation dose and mycorrhiza (W\*M) is not significant at the 95% level for any of the three variables – one factor cannot be used to predict another factor. The effect of mycorrhiza was significant for all three measured variables – yields with mycorrhiza are higher than those without mycorrhiza. The effect of irrigation dose is significant in the context of total yield and yield for export – these yields are higher under a higher irrigation dose. There is no significant effect of the irrigation dose on the percentage yield for export. The R2 values are relatively high – the model adequately describes the observations.

Table 3: ANOVA with a split-plot model

Table 4a: Overall mean values for full fertilization and low phosphorus fertilization including standard errors and confidence intervals for these means

Table 4b: Comparison of mean yield values between two fertilization levels

**ANOVA per fertilization level**

After making conclusions about the significant effect of fertilization we performed ANOVA ignoring this variable, with separate analyses for each fertilization level.

The tested linear model was as follows:

**Y = W M W\*M**

**Low phosphorus**

The results of the ANOVA are presented in Table 5. Non-significant values at the 5% level are highlighted in green. Tables 6a and 6b present means and the comparison between the two mycorrhizal treatments under low phosphorus fertilization.

Table 5: ANOVA for the low phosphorus experiment

Table 6a: Mean yield values with and without mycorrhizaunder low phosphorus fertilization, including standard errors and confidence intervals for these means.

Table 6b: The difference between mean yield values in the two mycorrhizal treatments (“With Mycorrhiza” minus “Without Mycorrhiza”) under low phosphorus fertilization.

**Conclusions for low phosphorus**

The overall yield with mycorrhiza was 2,990 kg higher than the overall yield without mycorrhiza. The respective difference for yield for export is 2,832 kg. The difference in percentage yield for export is 30.1%. These three differences are highly significant.

The ANOVA of low phosphorus fertilization data shows a lack of statistical significance of irrigation dose in this treatment (low phosphorus) – the lack of fertilization appears to be the main limiting factor that masks the effect of irrigation dose.

The interaction between irrigation dose and mycorrhiza is not significant – a direct result of the non-significant effect of irrigation. The only factor that affects yield is addition or non-addition of mycorrhiza (M=1 or 0, respectively). The fact that the irrigation dose is not significant in this experiment, but was significant in the overall analysis (Table 3) indicates that we can expect a significant effect of irrigation dose under the full fertilization treatments.

The linear model describes the observations less well than when fertilization was included in the model – this correlates with the fact that differences between the different plots in the low phosphorus treatment were highly evident.

**Full Fertilization**

The ANOVA results are presented in Table 7. Values that are non-significant at the 5% level are highlighted in green. Tables 8a and 8b present the mean yield values of the irrigation doses and the comparison between them under full fertilization. Tables 9a and 9b present mean yield values of mycorrhiza treatments and the comparison between them under full fertilization.

Table 7: ANOVA of the full fertilization experiment, R2=0.661

Table 8a: Mean yield values of irrigation doses under full fertilization

Table 8b: The difference in mean yield values following different irrigation doses under full fertilization

Table 9a: Mean yield values of mycorrhiza treatments under full fertilization

Table 9b: The differences in mean yield values between mycorrhiza treatments under full fertilization

**Conclusions for full fertilization**

Concerning irrigation, there is a significant difference for actual yield (total and export) between irrigation at 100% of the recommended dose compared to the 75% and 50% doses. There is no significant difference between 75% and 50%. When percentage yield for export is tested, there is no significant difference between the 100% irrigation dose and the 75% dose but both differ from the 50% dose. Concerning mycorrhiza, there is a significant difference between addition and non-addition of the fungus for all the tested variables.

The effect of irrigation dose (W) and mycorrhiza treatment (M) is highly significant and noticeable under full fertilization. The interaction between irrigation and mycorrhiza (W\*M) is not significant – increasing the irrigation dose cannot compensate for a lack of mycorrhiza. The ability of the linear model to explain the percent yield for export is not adequate.

**The effect of the fertilization and irrigation regime on the soil solution**

Using pumps buried in the different treatments at depths of 15 and 30 cm we monitored variation in the salt content of the soil solution under the different treatments. The salt content of the soil solution is expressed by the electrical conductivity (EC) of the soil solution.

The graphs in Figure 6 present the electrical conductivity of the soil solution at two depths – 15 and 30 cm – in the two fertilization treatments (full fertilization and low phosphorus) and two irrigation doses (100% and 50%) relative to pan evaporation data. We note that the 50% irrigation dose was planned in such a way that it would not drop below 2.5 mm per day. The salt content in the dripper water is also presented in all the graphs.

1. The difference between 100% and 50% irrigation is clear: at 100% irrigation, the salt content of the soil solution remains relatively stable throughout the growing season while it varies, rising and falling along the season when the irrigation dose is only 50% of the pan evaporation data. The conclusion is that the 100% irrigation dose serves not only the plant’s needs but also prevents salts from entering the root zone.
2. There were no noticeable differences in electrical conductivity between 15 cm depth and 30 cm depth.
3. The rise in salt content of the soil solution at 50% irrigation was smaller under low phosphorus than under full fertilization. Coupling this with the differences in yield and vegetative growth between the two fertilization levels, we can explain the differences: plants that received full fertilization developed well and their water consumption was higher than that of plants receiving low phosphorus fertilization, therefore they consumed most of the irrigation water and thus no water remained to prevent salts entering the root zone. The opposite is true for plants receiving the low phosphorus fertilization, where some of the irrigation water served to prevent salts from entering the root zone.
4. The most significant observation in this experiment was the variability in salt content of the soil solution during the growing season at 50% irrigation (under both fertilization treatments): an increase in salt content until late December, then a decrease in salt content until mid-February and an increase through the late winter and spring until the end of the growing season. The increase in salt content indicates provision of excess nutrients relative to the uptake capacity of the plant or a lack of adequate flushing. In this context we note that the pan evaporation data for the winter period (December to February) were low, thus most of the time the plot was irrigated at a dose of 2.5 mm per day despite low evaporation (and consumption). At this stage, a large proportion of the irrigation water served to prevent salts from entering the root zone, thus the salt content of the soil solution decreased until mid-February. During late winter and spring, both evaporation and water uptake by the plants began to increase, thus salts once again began to accumulate in the root zone.

Figure 6: Electrical conductivity (EC) of dripper water and at depths of 15 and 30 cm in the soil under fertilization treatments and at two irrigation doses – 50% and 100%.

**General Conclusions**

* Fertilization treatments have a significant effect on yield – yield under full fertilization is higher than under low phosphorus fertilization
* At all levels of analysis there are no significant interactions between irrigation dose and addition or non-addition of mycorrhiza
* Under low phosphorus fertilization:
  + The only significant effect is that of the mycorrhiza – addition of mycorrhiza increased yield
  + There is no effect of irrigation dose under low phosphorus fertilization
* Full fertilization:
  + There is an effect of irrigation dose (100% is better than the other two doses which do not differ from each other)
  + There is an effect of mycorrhiza – addition of mycorrhiza increased yield
  + Irrigation cannot be used to compensate for a lack of mycorrhiza

**A word on economics:** If we consider the numbers obtained in this experiment as a real situation – the increase in yield for export following addition of mycorrhiza under full fertilization is equivalent to 1,399 kg per dunam. We will multiply this number by 5 NIS per kg as a reasonable estimate and we will receive an increase in revenue in the order of 6,700 NIS per dunam. Multiplying by 45 dunams per farm we have a substantial addition of approx. 315,000 NIS per farm in return for an investment of approx. 150 NIS per dunam or 6,750 NIS for inoculating an area of 45 dunam with the mycorrhiza.

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