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Patterns of dry biomass accumulation and nutrient uptake by okra (*Abelmoschus esculentus* (L.) Moench.) under different rates of nitrogen application

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Abstract

The effect of nitrogen application on biomass accumulation and nutrient uptake by okra (*Abelmoschus esculentus* (L.) Moench.) grown in an unheated greenhouse was determined. Nitrogen (N) was applied in the form of a liquid feed at 0 (N-0), 150 (N-150) and 300 (N-300) ppm while other nutrients were maintained at a constant level. The accumulation of dry matter in the aerial plant parts (DMAe) and nutrient uptake (N, P, K, Ca, Mg) by the aerial plant parts (NUe) were recorded throughout the growth period. The data were analysed with the aid of logistic equations to determine the maximum daily DMAe and total NUe in relation to DMAe. Both DMAe and NUe follow a sigmoid curve, accurately described by a logistic equation. From the results it was found that during growth there was a period when DMAe occurred at an intense rate. The time of onset of this period and its duration varied with N application. Maximum daily DMAe (RDMAe) as well as maximum daily NUe (RNUe) occurred when 50% of the maximum DMAe and NUe had been achieved, irrespective of N application. The maximum daily uptake of N (RN-NUe) and K (RK-NUe) by the plant occurred about 2-6 days earlier than the RDMAe, irrespective of N-level. In addition, although the uptake of P (P-NUe) was low, maximum daily P-NUe also occurred prior to the maximum daily DMAe. However, the maximum daily uptake of the other ions that were measured (Ca and Mg) was less clearly defined.

Key words: Logistic equation, dry matter accumulation, crop growth rate, nutrient uptake, rate of nutrient uptake.

Abbreviations: DMAe - dry matter accumulation in aerial plant parts; RDMAe - rate of dry matter accumulation in aerial plant parts; NUe - nutrient uptake in aerial plant parts; N-NUe - nitrogen uptake in aerial plant parts; RN-NUe - rate of nitrogen uptake in aerial plant parts; K-NUE - potassium uptake in aerial plant parts; RK-NUE - rate of potassium uptake in aerial plant parts; P-NUe - phosphorus uptake in aerial plant parts; RP-NUe - rate of phosphorus uptake in aerial plant parts; RCa-NUE - rate of calcium uptake in aerial plant parts; Mg-NUe -, magnesium uptake in aerial plant parts; RMg-NUe - rate of magnesium uptake in aerial plant parts.

Introduction

Okra (Abelmoschus esculentus [L.] Moench. syn. Hibiscus esculentus L.) is widely cultivated within tropical and subtropical regions, including the Mediterranean Basin, for its immature pods, which are consumed either fresh or after processing (dried, canned or frozen) (Doymaz 2005; Duzyaman 2009). In Greece, okra is cultivated throughout the summer, both as an irrigated and a non-irrigated crop (Passam and Rekoumi 2009). Fertilizer is applied mainly in the form of a base dressing followed by additional side dressings of N throughout the period of active plant growth (Lamont 1999). Increasing concern about damage to the environment caused by the excessive use of synthetic fertilizers necessitates the implementation of rational fertilizer application programmes for agricultural crops (Addiscott et al. 1991). Apart from knowing the nutrient status of the soil, to devise a rational fertilizer application programme for okra it is necessary to determine: (a) the maximum uptake of individual nutrients that corresponds to maximum yield, (b) the rate of uptake of the nutrients throughout the biological cycle of the crop, so as to determine the stage of maximum uptake, and (c)

the distribution of nutrients among the tissues of the plant so as to evaluate the amounts of nutrients removed during harvest (Moustakas and Ntzanis 2005). The nutrient requirements of okra may be determined from the quantities of nutrients removed by the plants throughout their growth cycle in relation to the total biomass yield (i.e. pods harvested and plant parts remaining at the end of harvest). Under tropical and subtropical conditions, the requirements of okra for N, P_2O_5 and K_2O are reported to be 79, 32 and 89 kg ha⁻¹ respectively for a yield of 20 t ha⁻¹ (IFA 2000), although these amounts vary with factors such as: cultivar, plant density, soil type, whether the crop is irrigated or not, the climate and other environmental conditions (Lamont 1999). Despite its importance as a vegetable crop, there are currently no data available in the literature concerning the rates of nutrient uptake in relation to biomass production. Moreover, current fertilization practices are largely based on empirical methods, which frequently result in the overapplication of nutrients (Passam and Rekoumi 2009), with consequent wastage and damage to the environment

(Addiscott et al. 1991). Therefore the present study was undertaken paper was to determine the uptake of the major nutrients (N, K, P, Ca, Mg) in relation to biomass production, under different N applications with the aim of establishing fertilizer application guidelines for this crop.

Theoretical approach

The application of mathematical models to growth has developed to the stage where today it may even be considered a special branch of plant physiology. The growth of a crop passes through three consecutive phases which all together form a quantitative relationship between production/yield and time. This relationship is described by a sigmoid growth curve.

The logistic equation:
$$W = \left\lfloor \frac{\alpha}{1 + e^{(b-ct)}} \right\rfloor$$
 (Hunt 1982) (1)

is a mathematical expression that satisfactorily describes the sigmoid growth curve of plants, where *W* is the value of a plant variable (biomass production or nutrient uptake) for time *t* after transplantation, α is the maximum value of biomass or nutrient uptake, *b* is the initial biomass or nutrient uptake, *c* is an accumulation or uptake constant that is determined by calculation and *e* the natural logarithm. The logistic equation expresses production in relation to time, so the rate of growth, i.e. the increase in production per unit time, can be calculated from the first derivative of equation (1) and is equivalent to:

$$\frac{dW}{dt} = \frac{abe^{(b-ct)}}{(1+e^{(b-ct)})^2}$$
(2)

From half the maximum value of the result of equation (1) a point of inflexion on the curve is derived that corresponds to time *t* at which the growth rate is maximum. In the second derivative of equation (1):

$$\frac{dW^2}{d^2t} = \frac{ace^{(b-ct)}(e^{(b-ct)}-1)}{(1+e^{(b-ct)})^3}$$

(3)

The value *t* at which the second derivative is equal to zero corresponds to the time at which growth rate is maximal.

Results

Dry matter accumulation (DMAe)

In the analysis that follows, the DMAe during the growth period follows a sigmoid curve which can be accurately described by the logistic equation (1) where W is the accumulation of DMAe at time t (days from transplantation), a is the maximum value of DMAe, b the initial DMAe, and c an accumulation constant calculated using Rosenbrock's method (Machura and Mulawa 1973). The correlation coefficient r between predicted and observed values was very high (Table 1) and ensured the statistical integrity of the curve. Figure 1 shows the accumulation curves for the aerial

plant parts at different N levels derived from the application of the logistic equation to the data. DMAe followed a sigmoid curve, irrespective of the N-level, and was relatively slow until 26 DAT, indicating the slow adaptation of the plants to their new environment. At maturation 37.8, 39.5 and 44.5 g plant⁻¹ accumulated in N-0, N-150 and N-300 treatments, respectively (Fig. 1). The period between transplantation and maturation was characterized by rapid DMAe the onset and duration of which differed between N treatments. Specifically: for N-0, this period of rapid DMAe occurred 36-61 DAT (80% DMAe was achieved, corresponding to 83% of DMAe at maturity); for N-150, rapid DMAe occurred 36-59 DAT (81% DMAe was achieved, corresponding to 87% of DMAe at maturity); for N-300, DMAe occurred 34-60 DAT (80% DMAe, corresponding to 87% of DMAe at maturity). DMAe per unit time is calculated from equation (2) and expresses the rate of growth, i.e. the daily DMAe in the okra plants (RDMAe). The maximum RDMAe for N-0, N-150 and N-300 was 19.4, 19.7 and 22.3 g plant⁻¹ d⁻¹ respectively, and occurred at 49, 46 and 46 DAT respectively (Fig. 1). As DmAe reached a maximum, the RDMAe decreased to zero. Figure 2 shows that the maximum growth rate, i.e. RDMAe, is achieved at time t where the second factor of equation (3) tends to zero and DMAe is at half its maximum at N-0. The same pattern was observed for the other N treatments (not presented here).

Nutrient uptake (NUe)

The NUe of okra plants followed a sigmoid curve described by logistic equation (1) where W is replaced by U which represents nutrient uptake (g plant⁻¹) at time t (days from transplantation), a is maximum nutrient uptake (g plant⁻¹), b the initial nutrient uptake (g plant⁻¹) and c a nutrient uptake constant. The NUe curves for okra at different N levels resulting from the application of the logistic equation to the nutrient uptake data are shown in Fig. 3, 5, 6, 7, 8.

Nitrogen Uptake (N-NUe)

The pattern of N-NUe in relation to N application is shown in Fig. 3. A period of rapid change in N-NUe was observed the onset and duration of which differed between the N treatments. For the N-0 treatment, this rapid change occurred between 34-56 DAT and accounted for 71% of N-NUe, corresponding to 81% of total N-NUe at maturity. The maximum daily uptake of N-NUe (RN-NUe) in this treatment (0.25 g plant⁻¹) was observed 44 DAT. For N-150, rapid change occurred at 31-56 DAT and accounted for 73% of N-NUe, corresponding to 76% of total N-NUe at maturity.

The maximum daily uptake (RN-NUe) in this treatment (0.32 g plant⁻¹) was observed on 44 DAT. For N-300, rapid change in N-NUe was observed 30-56 DAT and accounted for 82% of N-NUe, corresponding to 88% of total N-NUe at maturity. The RN-NUe in this treatment (0.40 g plant⁻¹) was observed 42 DAT at. The duration of rapid uptake in N-NUe was 22, 25 and 26 days for N-0, N-150 and N-300 respectively, indicating that higher N rates increased this period. From Fig. 4 it may be observed that the maximum rate of N uptake rate, i.e. RN-NUe, was achieved at time *t* where the second factor of equation (3) tended to zero and N-NUe was observed for the other nutrients, irrespective of N treatment (data not presented).

Table 1. Computation parameters *a*, *b*, *c* from the logistic equation $W = \left[\frac{\alpha}{1+e^{(b-ct)}}\right]^*$, using non-linear regression and

correlation coefficient <i>r</i> between observed and predicted values under different rates of Nitrogen application.												
	<i>N-0</i>				N-150				N-300			
Variable	а	b	С	r	а	b	С	r	а	b	С	r
DMAe	38.79	6.02	0.12	0.999	39.48	6.91	0.149	0.999	44.5	6.05	0.131	0.999
N-NUe	0.51	5.34	0.12	0.999	0.646	4.68	0.105	0.999	0.10	5.7	0.134	0.999
K-NUe	0.53	8.196	0.19	0.999	0.663	6.08	0.138	0.999	0.62	4.63	0.115	0.999
P-NUe	0.076	6.82	0.162	0.999	0.073	10.14	0.254	0.999	0.08	10.19	0.254	0.999
Ca-NUe	0.128	4.93	0.095	0.999	0.116	7.1	0.154	0.999	0.11	7.18	0.163	0.999
Mg-NUe	0.034	3.71	0.072	0.999	0.038	5.38	0.112	0.999	0.04	0.69	0.131	0.999

*W is the value of a plant variable (biomass production or nutrient uptake) for time t after transplantation, α is the maximum value of biomass or nutrient uptake, b is the initial biomass or nutrient uptake, and c is an accumulation or uptake constant that is determined by calculation.



Fig 1. Dry matter accumulation and growth rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application.

Potassium Uptake (K-NUe)

The pattern of K-NUe in relation to N application is shown in Fig. 5. A period of rapid change in K-NUe was observed in all N treatments at 34-56 DAT (N-0), 33–56 DAT (N-150) and 28–56DAT (N-300), when 83, 76 and 78% respectively of K-NUe (corresponding to 91, 81 and 87% total K-NUe) occurred. The maximum daily uptake of K (RK-NUe) in N-0, N-150 and N-300 was observed on 44, 44 and 42 DAT and amounted to 0.51, 0.60 and 0.81 g plant⁻¹ respectively.

Phosphorous Uptake (P-NUe)

The pattern of P-NUe in relation to N application is shown in Fig. 6. A period of rapid change in P-NUe was observed in all N treatments at 32-48 DAT (N-0), 33–51 DAT (N-150) and 32–50 DAT (N-300), when 80, 85 and 92% respectively of P-NUe (corresponding to 88, 76 and 85% of total P-NUe) occurred. The maximum daily uptake of P (RP-NUe) in N-0, N-150 and N-300 occurred at 42, 40 and 40 DAT and amounted to 0.04, 0.04 and 0.044 g plant⁻¹ respectively. There was virtually no difference in the daily uptake of P

throughout the experimental period between the different N applications.

Calcium Uptake (Ca-NUe)

The pattern of Ca-NUe in relation to N application is shown in Fig. 7. A period of rapid change in Ca-NUe was observed in all N treatments at 38-63 DAT (N-0), 35–56 DAT (N-150) and 33–54 DAT (N-300), when 78, 82 and 85 of Ca-NUe respectively occurred, corresponding to 72, 82 and 83 of the total Ca-NUe. The maximum daily uptake of Ca (RCa-NUe) in N-0, N-150 and N-300 was observed on 52, 46 and 44 DAT and amounted to 0.13, 0.12 and 0.12 g plant⁻¹ respectively. There was virtually no difference in the daily uptake of Ca-NUe throughout the experimental period between the different N applications.

Magnesium Uptake (Mg-NUe)

The pattern of Mg-NUe in relation to N application is shown in Fig. 8. A period of rapid change in Mg-NUe was observed in all N treatments at 35-68 DAT (N-0), 32–60 DAT (N-150) and 29–56 DAT (N-300), when 78, 82 and 85% of Mg-NUe



Fig 2. Dry matter accumulation curve, crop growth rate curve $(1^{st}$ derivative) and crop growth acceleration curve $(2^{nd}$ derivative), in aerial plant parts of okra, from transplantation to maturity, in N-0 treatment.



Fig 3. Nitrogen uptake and nitrogen uptake rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application.

respectively occurred, corresponding to 72, 82 and 83% of the total Mg-NUe. The maximum daily uptake of Mg (RMg-NUe) in N-0, N-150 and N-300 was observed on 51, 48 and 43 DAT and amounted to 0.02, 0.02 and 0.02 g plant⁻¹ respectively. There was virtually no difference in the daily uptake of Mg-NUe throughout the experimental period between the different N applications.

Discussion

Although N application is known to have a positive effect on okra growth and yield (Dutta and Naik 2009), the effect of N concentration on the rate and magnitude of dry matter and nutrient accumulation has not been previously studied, so recommended fertilizer rates are often empirical and frequently excessive (Lamont 1999; Manga and Mohammed 2006, Passam and Rekoumi 2009). From the data of the present paper, it may be concluded that the pattern of dry matter accumulation in okra is very similar to that of faba bean and pea (Herdina and Silsbury 1990), soybean (Warembourg and Fernandez 1985), lupin (Farrington et al. 1977), cowpea (Piha and Munns 1987), corn (Katsadonis et al. 1997a) and tobacco (Sifola et al. 2003; Moustakas and Ntzanis 2005). Moreover, the whole growth curve fits well to a logistic function, as observed for flue-cured tobacco (Moustakas and Ntzanis 2005). The period of maximum N



Fig 4. Nitrogen uptake curve, nitrogen uptake rate curve (1st derivative) and nitrogen acceleration curve (2nd derivative), in aerial plant parts of okra, from transplantation to maturity, in N-0 treatment.



Fig 5. Potassium uptake and potassium uptake rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application

uptake corresponded to the stage of rapid shoot growth, foliage formation and the early stages flowering and pod production (Passam et al. 1998). Flowers form singly at the nodes and excessive N application is not only wasteful and harmful to the environment, but also leads to excessive foliar growth at the expense of flower formation (Lamont 1999). Knowledge of the pattern of N uptake is therefore important to establish balanced plant growth and flower induction. Whereas in legume crops (e.g. faba bean and pea) the N that is taken up during the rapid accumulation stage is largely transferred to the developing seeds (Herdina and Silsbury 1990), okra pods for fresh consumption are harvested at a very immature stage, i.e. before seed filling is complete. Comparing the pattern of uptake of the various ions, it is clear that during okra growth there was a period of rapid change in nutrient uptake, irrespective of N level, the onset

and duration of which varied with the specific ion. Thus, the onset of the rapid phase of nutrient uptake of K, Ca and Mg (similar to N) occurred slightly earlier at increasing N application rates, whereas rapid accumulation of P occurred at 32-33DAT irrespective of N rate. Additionally the duration of rapid P uptake (16-18 days) was shorter than that of N, K Ca (21-28 days) and Mg (27-33 days). Rapid ion accumulation coincided with intense DMAe and both maximum RDMAe and RNUe occurred when 50% of the maximum plant DMAe had been achieved, irrespective of N application. The maximum RN-NUe and maximum RK-NUe of the plant occurred about 2-6 days earlier than the maximum RDMAe. Consequently during this period, the soil must have sufficient nutrients available to supply the plant's needs. Although the P-NUe was low, maximum RP-NUe of this ion also occurred prior to the maximum RDMAe.



Fig 6. Phosphorus uptake and phosphorus uptake rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application.



Fig 7. Calcium uptake and calcium uptake rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application.

However, the maximum daily uptake of the other ions that were measured (Ca and Mg) was less clearly defined. Previous studies of the ion composition of okra refer primarily to the pods at the time of commercial maturity (Khalifa et al. 2009) and have not generally been concerned with specific ion uptake rates. Siemonsma (1982) reported that the mineral content of the total cumulative biomass was high in young plants and stabilized from the age of 11 weeks, approximately the same time at which maximum Nue was observed in the present paper. Under the climatic conditions of tropical Africa, the nutrient content of the whole plant was 1.9% N, 0.2% P, 1.2% K, 1.6% Ca and 0.7% Mg (Prabhakar et al. 2009), indicating a higher Ca and Mg content in relation to P than that observed here (Fig. 6-8). Fertilizer use efficiency, however, varies with agroclimatic conditions and is reckoned under a tropical climate to be about 50% for N (Prabhakar et al. 2009). Nitrogen use and plant biomass yield are also affected by plant population (Manga and Mohammed 2006) and the preceding crop (Olasantan 1998). Overall, the pattern of nutrient uptake for okra followed a similar pattern to that reported for the whole plant of flue tobacco (Moustakas and Ntzanis 2005) and Ca in corn (Katsadonis et al. 1997b).

Materials and methods

Pot experiments

The experiment was carried out in a greenhouse at the Agricultural University of Athens (latitude 37°58'55" and longitude 23°32'14", 30 m above sea level) using okra (*Abelmoschus esculentus* (L.) Moench.) cv. Boyiatiou, a major commercial cultivar in Greece (Koutsos 2009). Seeds were sown in trays of a commercial peat-based compost KTS2 (Klasmann-Deilmann GmbH, Geeste, Germany) in March. When the plants had reached a height of about 20-25 cm with 5-6 leaves (9 April), they were transplanted to 11 L pots containing a 1:1 (v/v) mixture of peat and perlite (P4-Perloflor, Isocon A.E., Athens, Greece). The pots were placed in an unheated glasshouse according to a completely



Fig 8. Magnesium uptake and Magnesium uptake rate curves in aerial plant parts of okra, from transplantation to maturity, under different rates of nitrogen application.

randomized design with 3 N treatments and 26 replicates per treatment, i.e. 78 plants in total. The quantities of K and P were the same for all treatments and only the amount of N varied. Fertilizer was applied once a week in the form of a liquid feed with the concentrations of nutrients (ppm) as follows: (1) 0N:150P:150K, (2) 150N:150P:150K and (3) 300N:150P:150K. The plants were trained as single stems on vertical cordons. Pods were harvested regularly at the stage of commercial maturity (4-7 cm in length). Sampling started five weeks after transplantation (15 May) and continued for seven weeks.

Plant analysis

On each sampling date three plants per treatment were randomly selected, cut at soil level and separated into stems and leaves. After weighing, the plant parts were dried at 70°C until constant weight in order to calculate the dry biomass. The dry tissues were then ground to a particle size of <250 mesh. A portion of the ground samples was incinerated at 550°C and the ash dissolved in concentrated HNO₃. This solution was used for the determination of Ca, Mg, P and K. Calcium and Mg were determined by atomic absorption spectrometry using an acetylene-N2O flame for Ca and acetylene-O2 flame for Mg. Potassium was determined by flame photometry, P by the ascorbic acid method of Murphy-Riley and total N by Kjeldahl digestion. In each case, the analytical methods used were those described by Page (1982). The dry weight of leaves and stems was determined for each sampling date and expressed as g plant⁻¹. The amounts of N, K, P, Ca, and Mg taken up and distributed within the aerial plant parts were calculated by multiplying the dry weight by the corresponding elemental concentration and expressed as g plant⁻¹. Dry matter accumulation in the aerial plant parts is the sum of dry matter accumulation in leaves plus the dry matter accumulation in stems (DMAe = DML + DMS). Nutrient uptake by the aerial plant parts is equal to the sum of nutrient uptake by leaves plus the nutrient uptake by stems (NUe=NUL+NUS).

Statistical analysis

The logistic equation (1) was applied to DMAe, NUe, N-NUe, K-NUe, P-NUe, Ca-NUe, Mg-NUe, using STATISTICATM version 5.0 (StatSoft 1998). Additionally, data were tested by non linear regression for adaptation to a logistic equation and the estimation of parameters a, b, and c.

Conclusion

On the basis of DMAe, a N level of 300 ppm is indicated for okra with a split application of 1/3 N (base dressing) 2–3 days prior to transplanting and 2/3 N (side dressing) 20–30 DAT in order to fulfil the plant's requirements.

References

- Addiscott TM, Whitmore AP, Powlson DS (1991) Farming, Fertilizers and the Nitrate Problem. CAB international, Wallingford, U.K., 170 pp.
- Farrington P, Greenwood EAN, Titmanis ZV, Trinick MJ, Smith DW (1977) Fixation accumulation and distribution of nitrogen in a crop of *Lupinus angustifolious*. Aust J Agr Res 28:237-248.
- Doymaz I. (2005) Drying characteristics and kinetics of okra. J Food Eng 69:275-279.
- Dutta OP, Naik LB (2009) Seed production technology. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 227-246.
- Duzyaman E (2009) Okra in Turkey domestic landraces. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 323-346.
- Herdina, Silsbury JH (1990) Growth nitrogen accumulation and partitioning, and N₂ fixation in faba bean (*Vicia faba* cv. Fiord) and pea (*Pisum sativum* cv. Early Dun). Field Crops Res 24:173-188.
- Hunt R (1982) Plant Growth Curves. Edward Arnold, Ltd. London.

- IFA (2000) Okra (*Abelmoscus esculentus*, L). In "World Fertilizer Use Manual", IFA Publ.
- Katsadonis N, Evangelidis G, Bladenopoulou S, Simonis A (1997a) Nutrition of corn plant: I. Uptake and distribution of N, P, K in plant part of three corn hybrids. Geot Sci issues 3:17-24.
- Katsadonis N, Evangelidis G, Bladenopoulou S, Simonis A (1997 b) Nutrition of corn plant: II. Uptake and distribution of Ca, Mg, Mn, Zn, Fe, B, and Cu in plant part of three corn hybrids. Geot Sci issues 3:25-31.
- Khalifa AHA, Dhankhar BS, Mishra JP (2009) Quality, nutrition, and culinary uses. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 87101.
- Koutsos TV (2009) Greek okra cultivars sustainable agriculture systems. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 361-373.
- Lamont WJ (1999) Okra a versatile vegetable crop. HortTechnology 9 (2):179-184.
- Machura M, Mulawa A (1973) Rosenbrock function minimization. Algorithm 450. Comm. ACM 16: 482-483.
- Manga AA, Mohammed SG (2006) Effect of plant population and nitrogen levels on growth and yield of okra [Abelmoschus esculentus (L.) Moench.]. Adv Hort Sci 20: 137-139.
- Moustakas NK, Ntzanis H (2005) Dry matter accumulation and nutrient uptake in flue-cured tobacco (*Nicotiana tabacum* L.). Field Crops Res 94:1-13
- Olasantan FO (1998) Effects of preceding maize (*Zea mays*) and cowpea (*Vigna unguiculata*) in sole cropping and intercropping on growth, yield and nitrogen requirement of okra (*Abelmoschus esculentus*). J Agric Sci 131: 293-298.

- Page AL (1982) Chemical and microbiological properties. *In*: Page AL (Ed.) Methods of Soil Analysis, Part 2. Agronomy 9, ASA, Inc., Madison, WI.
- Passam HC, Akoumianakis K, Saragiannidi A (1998) The effect of time of sowing on the production of okra (*Hibiscus esculentus* L.) seed in the Mediterranean region. Plant Var. Seeds 11: 145-150.
- Passam HC, Rekoumi K (2009) Okra in Greece production and constraints. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 347-359.
- Piha MI, Munns DN (1987) Nitrogen fixation capacity of fied grown bean compared to other legumes. Agro J 79:690-696.
- Prabhakar M, Hebbar SS, Gayathri M (2009) Production technology. *In:* Dhankhar BS and Singh R. (eds) Okra Handbook, HNB Publishing, N.Y. pp. 159-174.
- Siemonsma JS (1982) La culture du Gombo (*Abelmoschus* spp.) legume-fruit tropical (avec reference speciale a la Cote d'Ivoire). WUA dissertation no. 905.
- Sifola MI, Cuocolo B, Postiglione L (2003) Dry matter accumulation, leaf development and stem elongation in tobacco plants grown under different regimes of nitrogen fertilization and irrifation. Agrochimica vol XLVII-N:40-53.
- StatSoft Inc (1998) STATISTICA for Windows. StatSoft Inc., Tulsa, OK.
- Wittembourg FR, Fernandez MP (1976) Distribution and remobilization of symbiotically fixed nitrogen in soybean (*Glycine max*). Physiol Plant 65:281-286