Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 3 (1): 103-109, 2009
ISSN 1819-3412

Evaluation of Water Stress and Nitrogen Fertilizer Effects on Relative Water Content, Membrane Stability Index, Chlorophyll and Some Other Traits of Lentils (*Lens culinaris* L.) Under Hydroponics Conditions

[1]M. Salehpour, [1]A. Ebadi, [2]M. Izadi and [3]Sh. Jamaati-e-Somarin
[1]Department of Agronomy and Plant Breeding, Faculty of Agriculture,
University of Mohaghegh Ardabili, Ardabil, Iran
[2]Faculty of Agriculture, Shiraz University, Shiraz, Iran
[3]Young Researchers Club, Department of Agronomy, Faculty of Agriculture,
Islamic Azad University, Ardabil Branch, Ardabil, Iran

Abstract: In order to investigate the response of Lentil cultivar Gachsaran to nitrogen and water stress under hydroponics conditions, a factorial experiment based on randomized complete block design was conducted with three replications using Hoagland nutrient solution without nitrogen, at the growth chamber in 2007, Ardebil, Iran. Factors were four water stress levels (0, -0.6, -0.9 and -1.2 MPa) prepared with polyethylene glycol (PEG) and three nitrogen levels (0, 0.5 and 1.0 Mmol). Results showed that all traits were decreased with increasing stress. Both treatments had significant effects on Relative Water Content (RWC), Leaf Chlorophyll Content (LCC), Leaf Area (LA), length, height and dry weight of stem and Total Biomass (TB) and their interaction effects on LCC (before and after stress), LA ($p<0.05$) and on length and dry weight of stem ($p<0.01$). The most values of RWC, LCC, LA, length and dry weight of stem and total biomass were obtained using 0.5 Mmol nitrogen is sufficient and higher values is not recommended because of its preventive effect on nitrogen fixation in plant.

Key words: RWC, MSI, LA, Lentil

INTRODUCTION

Seed legumes are important sources of vegetable proteins for human and animals and are main crops in agriculture (Dita *et al*., 2006) and because of their ability to nitrogen fixation (Howieson and Ballard, 2004). Lentils (*Lens culinaris* L.) is a member of legumes family and does nitrogen fixation in presence of Rhizobium leguminosarum (Schmidtke *et al*., 2004). Because of high content of protein in legumes, demand for nitrogen is higher than other nutrient elements. So, its deficiency is very critical for plant, because in the lack of this element, chloroplast, chlorophyll and many of metabolic enzymes is disordered. There are two nitrogen sources for legumes including mineral and fixed nitrogen which have been shown to be fixed in Lentil of about 78% by ^{15}N method (Maskey *et al*., 2001). Moreover, water stress is one of the most important and prevalent environmental stresses that limits farming (Rosales-Sernaa *et al*., 2004). Drought stress decreases number, size, area and chlorophyll content of the leaf, height and biomass of the plant which could be attributed to vegetative growth decrease such as low production of new leaves, high leaf senescence, decrease in mean leaf size, decrease in plant height and etc. (Pagter *et al*., 2005). Also, drought stress has adverse effects on nodulation and nitrogen fixation (Athar and Johnson, 1996). The highest fixation is done while soil nitrogen content is low and soil humidity and temperature is favorite. It had been found that drought stress resulted in

Corresponding Author: Shahzad Jamaati-e-Somarin, Young Researchers Club, Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Ardabil Branch, Ardabil, Iran
Tel: +989141594490

low nodulation and nitrogen fixation in bean up to 25-30% than control (Verdoy *et al*., 2004). Based on the time of nitrogen application and root zone conditions, nitrogen can cause early maturing or idiomatically, green-drying of plant under drought circumstances and consequently, decrease of yield. The reason is that water consumption by plant becomes faster and stored carbohydrates, decreases (Sadras, 2002). Perhaps, for this reason the, most farmers stop or decrease nitrogen utilization in drought circumstances. Yang *et al*. (2000, 2001) reports that increase of nitrogen led to yield increment. Usually in case of desired nitrogen fixation, there is no require to nitrogen application. However, symbiosis between seed legumes and rhizobium have been improved to the large extent, but is sensitive to environmental conditions and stresses, it will extinct. In this condition, nitrogen applications can improve plant resistance and yield against stresses. Antolin *et al*. (1995) and Solan *et al*. (1990) have reported same results in alfalfa and soybean and stated that plants which received nitrate, were more tolerant to dehydration against nitrogen fixator plants. This improvement in plant growth relates to more produced aerial parts such as crown, stem and leaf area and more nitrogen fixation in roots. Generally, environmental factors such as temperature call not be controlled by farmers but nutrient deficit and weak nitrogen fixation can be improved by infection, fertilization irrigation and other management methods (Lindmann and Glovir, 2003). Many researchers showed that legumes which fed by nitrate or ammonium, had better growth than those relying on nitrogen fixation (Silsbury *et al*., 1986; Sprent and Thomas, 1984). This is because of effect of nitrogen on lengthening the stem, increase of number and area of the leaves increase of LA which may arising from increase of number or area of the leaves caused by nitrogen utilization. Response of plant to drought stress pertain to water deficit and comprises of shot-term and long-term reactions. RWC, LCC, MSI, TB and plant vegetative growth such as number and area of the leaf, plant height and etc are used ass criterions representing plant tolerance to drought stress. Costa-Fraca *et al*. (2000) reported that RWC in bean leaves was reduced under water deficit. High values of leaf RWC in tolerant cultivars may attributable to lower water losing from stomata and/or higher water absorption by roots as a result of special mechanism exerted by plants (Jiyang and Hung, 2001). LCC in one of key factors determining photosynthesis rate and dry matter production, as well (Ghosh *et al*., 2004). Also, MSI is a criterion representing plant resistance against water deficit and its value decreases in this condition (Saneoka *et al*., 2004). As we know, nutrient elements are taken up by plant as solution, thus, water deficit indirectly causes less absorption of this element and consequently reduces plant growth and TB. Oweis *et al*. (2004) indicating grain yield and biomass loss of lens under drought conditions and they showed that above mentioned traits were improved as a result of irrigation.

The aim of this study was evaluation of effects of drought stress and nitrogen fertilizer on RWC, MSI, LCC, LA, stem length, stem dry weight and TB and to determine how much nitrogen is required to the plant especially in the low rate nitrogen fixation under water deficit conditions.

MATERIALS AND METHODS

In order to investigate the drought stress and nitrogen fertilizer effects on some traits of lens cultivar Gachsaran such as RWC, MSI, LCC, LA, stem length, stem dry weight and TB, a factorial experiment based on randomized complete block design as hydroponics was carried out with three university of mohaghegh Ardabili, Ardabil, Iran, in 2007. Factors were: drought levels (0, -0.6, 0.9 and -1.2 MPa using PEG) and nitrogen levels (0, 0.5 and 1.0 Mmol) stable aeration system for root respiration was used. Seeds were inoculated (infected) with RLV 73 which contained nitrogen fixator bacteria and then were sown in the sand. There after, seeding were transferred into 2 L pails involving nutrient solution. Photoperiod was adjusted as 16 h light and 8 h dark plants placed in the holes of the pails and their roots contacted with solution. This solution renewed each 12 days. Reduced water was recompensed daily with distilled water. Hoagland nutrient solution (Table 1) was used (Mehravaran, 2003).

Table 1: Elements and their rates used in the Hoagland solution

Chemical name	Concentration	Chemical name	Concentration
EDTA	0.78 mM	Na_2MoO_4	0.50 M μ
H_3BO_4	46.20 M μ	KH_2PO_4	0.25 mM
$MnCl_2$	0.14 M μ	$MgSO_4.7H_2O$	1.00 mM
$ZnSO_4$	0.76 M μ	$CaSO_4$	2.50 mM
$CuSO_4.2H_2O$	0.32 M μ	K_2SO_4	1.25 mM
$FeSO_4$	0.71 mM		

After establishment and favorite growth of seedlings, nitrogen treatments were exerted. PEG treatment was done after words. At the end of treatment sampling was made. RWC was measured as follows (Rosales-Sernaa *et al*., 2004):

$$RWC = \left[\frac{FW - DW}{TW - DW}\right] \times 100$$

FW, DW and TW were: fresh, dry and turgid weight, respectively. Sampling was done from newly-expanded leaves. Leaves immediately weighed and then immerged in distilled water for 5 h. Thereafter, turgid weight was obtained and finally, dry weight was measured after 24 h at the oven of 75°C. LCC was measured using chlorophyll meter (Conicaminalta-SPAD, Tokyo, Japan) (Rosales-Sernaa *et al*., 2004). Measurement was done at two staged: 1- After nitrogen treatment, 2- After drought stress treatment to calculation of LA. Some leaves were choose and their area was measured using leaf area meter (Scanman, USA), There after were dried at the oven of 75°C for 48 h finally, leaves were weighed and comparing leaf area and leaf weight, total leaf area for whole plant was gained. In order to measuring MSI, newly full expanded leaves were washed 3 timed with distilled water to removing surface electrolytes. In control treatment tubes 20 mL of distilled water and in stress one, 20 mL of PEG (M = 6000) were used. Samples were immersed in 40°C for 24 h and then there were washed three times. Then both tubes were filled with distilled water and samples were placed in, for nix 24 h until reaching temperature of the environment. Electrical conductivity (EC) was measured as Mm $hoss^{-1}$. Samples were autoclaved for 25 min in 115°C and the second EC were measured MSI was calculated ad follows (Saneoka *et al*., 2004):

$$IP = \frac{1 - \frac{T_1}{T_2}}{1 - \frac{C_1}{C_2}} \times 100, \quad MSI = 1 - IP$$

where, IP is injury percent, T_1, T_2 is the EC of the stress treatment before and after autoclaving, respectively, C_1, C_2 is the EC of the control treatment before and after autoclaving, respectively. Length and dry weight of stem and TB were measured. Analysis of variances and mean comparisons were done using SAS software. Graphs were drawn by Excel program.

RESULTS AND DISCUSSION

RWC and MSI

Results showed that with increasing drought stress, RWC significantly ($p<0.01$) was increased and reached from 94.75% (control) to 76.74% (Table 2). Nitrogen had significant effect, as well and the most RWE was obtained at 0.5 Mmol nitrogen. Turkan *et al*. (2005) stated that water deficit obviously decreased RWC in bean and their findings is in accordance with present results. Also, MSI was decreased ($p<0.05$) under stress and reached to 73.09 from 81.39% in control (Table 2) but nitrogen had no significant effect on it. Confronting with drought causes to increase in gene transcriptions which oxidant cell wall lipids and consequently destroy the wall (Saneoka *et al*., 2004).

Table 2: Mean comparison of measured traits under drought stress and nitrogen

Treatments		Relative water content (RWC)	Cell membrane stability index (MSI)	Total biomass (g plant^{-1})	Shoot length (cm)	Shoot dry weight (g)
Drought	0.0	75.94^{a}	81.39^{a}	3.63^{a}	48.33^{a}	1.69^{a}
stress levels	-0.6	82.31^{b}	80.76^{a}	3.36^{b}	47.29^{b}	0.85^{b}
(MPa)	-0.9	76.33^{c}	79.46^{a}	2.43^{c}	40.23^{c}	0.67^{c}
	-1.2	76.74^{c}	73.09^{b}	2.12^{d}	32.14^{d}	0.50^{c}
Nitrogen levels	0.0	81.35^{b}	78.88^{a}	2.83^{c}	36.58^{c}	0.73^{c}
(Mm)	0.5	84.27^{a}	78.70^{a}	2.95^{a}	45.70^{a}	1.12^{a}
	1.0	81.97^{b}	78.44^{a}	2.88^{b}	43.44^{b}	0.93^{b}

*No. with same words in each column, have no significant differences to each other

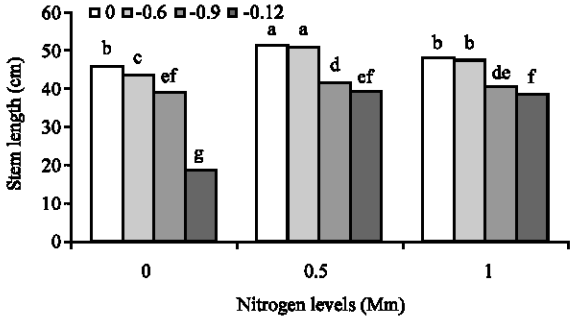

Fig. 1: Effects of different nitrogen levels on stem length under various root water potentials

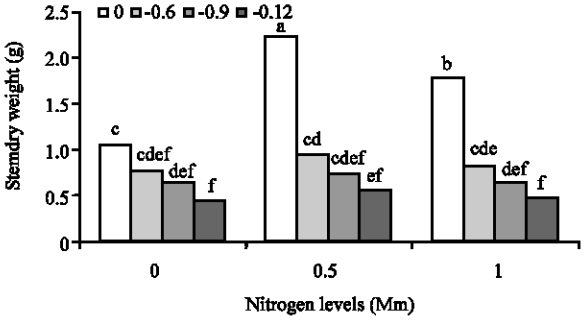

Fig. 2: Effects of different nitrogen levels on stem dry weight under various root water potentials

Length and Dry Weight of Stem and TB

Results showed that length and dry weight of stem and TB was decreased under drought stress so that, its rate was observed of 2.12 g plant $^{-1}$. Length and dry weight of stem were measured of 32.14 cm and 0.50 g, respectively (Table 2). Drought and nitrogen treatments has significant ($p<0.01$) effects on measured traits and had significant interaction effects on stem length (Fig. 1) and stem dry weight (Fig. 2) ($p<0.01$). Water has large effect on plant growth, biomass accumulation and nitrogen fixation and in case of water deficit, above mentioned aspects, decrease (Tomas *et al*., 2004). Athar and Johnson (1996) showed that reduction of water potential from -0.3 to -1.0 MPa, reduced biomass of 65%.

In this study, increase of nitrogen up to 0.5 Mmol, increased length and dry weight of stem and TB but in 1.0 Mmol nitrogen measured traits were decreased. This may because of either inactivation of nitrogen fixation in roots or insufficient values of nitrogen in absence of fixation.

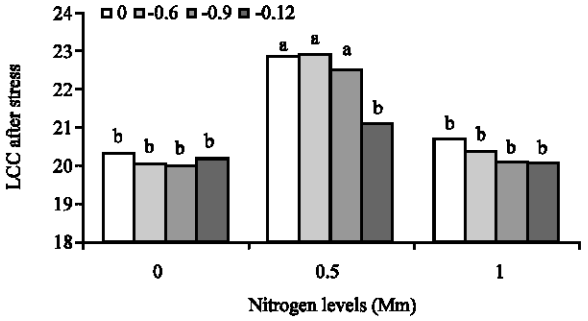

Fig. 3: Effects of different nitrogen levels on LCC under various root water potentials

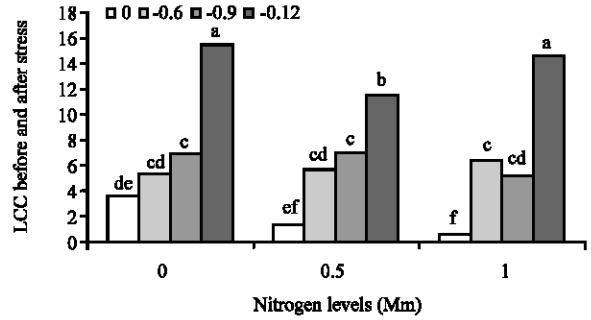

Fig. 4: Effect of different nitrogen levels on LCC after and before stress in various root water potentials

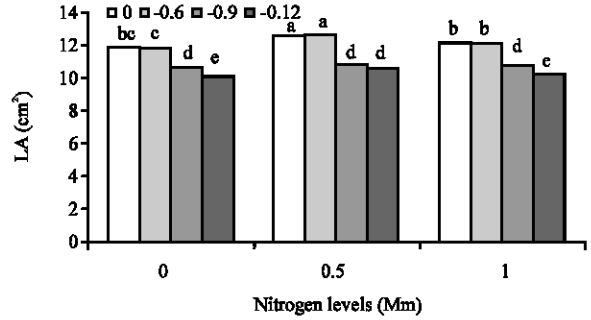

Fig. 5: Effects of different nitrogen levels on LA in various root water potentials

LCC and LA

Both drought stress and nitrogen treatment had significant ($p<0.05$) on LCC and LA. Their interaction effects on LCC after drought stress (Fig. 3), LCC after and before drought stress (Fig. 4) and LA (Fig. 5) were significant ($p<0.05$).

The most difference between LCC before and after stress in severe drought conditions (-1.2 MPa) was obtained of 13.855 mg g^{-1} leaf dry weight, whereas, this value in control treatment was 1.877 mg g^{-1} leaf dry weight usually, water stress accelerates leaf senescence which has been seen in chickpea and lentil (Davies *et al*., 1999). The most LCC was got subjecting to 0.5 Mmol nitrogen under water stress (Table 3). Leaf production and expansion is very sensitive to available water and the reason is that cell division and growth need to water (Pagter *et al*., 2005).

Table 3: Mean comparisons of measured traits under drought stress and nitrogen levels

Treatments		Chlorophyll content before stress	Chlorophyll content after stress	Chlorophyll content difference before and after stress	Leaf area (LA) (cm^2 $plant^{-1}$)
Drought	0	21.28a	19.41a	1.877c	12.228a
stress levels	-0.6	21.11ab	15.23b	5.877b	12.162a
(MPa)	-0.9	20.84ab	14.44b	6.40b	10.723b
	-1.2	20.44b	6.58c	13.855a	10.291c
Nitrogen levels	0	20.15b	12.30b	7.85a	11.108c
(Mm)	0.5	22.32a	15.91a	6.40b	11.635a
	1	20.29b	13.54c	6.75ab	11.310b

*No. with same words in each column, have no significant differences to each other

Brevedan and Egli (2003) reported that LCC and LA of soybean were decreased under water deficit RWC, LCC and stem biomass were decreased under the same conditions (Rosales-Sernaa *et al*., 2004).

CONCLUSIONS

Generally it was seen that whit increasing water deficit, all traits were decreased. The most RWC, LCC, LA stem nitrogen. So, it can be said that lentil has less demand than other legumes and it seems that 0.5 Mmol nitrogen is sufficient for plant growth and excessive values mighty have unflavored effects because of reducing nitrogen fixation by plant and being insufficient for total plant requirement for this element. Therefore, adequate rates of nitrogen under water stress conditions noticing fixation capability, is recommended.

ACKNOWLEDGMENTS

This study was supported by the Central Laboratory of Agricultural Faculty, University of Mohaghegh Ardabili. Valuable experimental support by Aziz Jamaati-e-Somarin and Asad golizadeh is greatly appreciated. This study was extracted from M.Sc. Thesis of Mitra Salehpour.

REFERENCES

Antolin, M.C., J. Yoller and M. Sachez-Diaz, 1995. Effects of temporary drought on nitrate-fed and nitrogen-fixation alfalfa plants. Plants Sci., 107: 159-165.

Athar, M. and D.A. Johnson, 1996. Nodulation biomass production and nitrogen fixation in alfalfa under drought. J. Plant Nutr., 19: 185-199.

Brevedan, R.E. and D.B. Egli, 2003. Short periods of water stress during seed filling, leaf senescence and yield of soybean. Crop Sci., 43: 2083-2088.

Costa-Fraca, M.G., A.T. Pham, C. Pimentel, R.O. Pereyra-Rossiello and Y. Zuily-Fodil *et al*., 2000. Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environ. Exp. Bot., 43: 227-237.

Davies, S., N.C. Turner, K.H.M. Siddique, L. Leport and J. Plummer, 1999. Seed growth of desi and kabuli chickpea (*Cicer arietinum*) in a short season Mediterranean-type environment. Aust. J. Exp. Agric., 39: 181-188.

Dita, M.A., N. Rispail, E. Prats, D. Rubiales and K.B. Singh, 2006. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica, 147: 1-24.

Ghosh, P.K., K.K. Ajay, M.C. Bandyopadhyay, K.G. Manna and A.K. Mandal *et al*., 2004. Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping system in vertisols of semi-arid tropics. Dry matter yield, nodulation. Chlorophyll content and enzyme activity. Bioresour. Technol., 95: 85-93.

Howieson, J. and R. Ballard, 2004. Optimizing the legume symbiosis in stressful and competitive environments within southern Australia: Some contemporary thoughts. Soil Biol. Biochem., 36: 1261-1273.

Jiyang, Y.M. and B. Hung, 2001. Osmotic adjustment and root growth associated with drought preconditioning enhanced heat tolerance in Kentucky bluegrass. Crop Sci., 41: 1168-1173.

Lindmann, C. and R. Glovir, 2003. Nitrogen fixation by legumes. University new Mexsico State. http://www.cahe.nmsu.edu/pubs/_a/a-129.pdf.

Maskey, S.L., S. Bhattarai, M.B. Peoples and D.F. Herridge, 2001. On-farm measurements of nitrogen fixation by winter and summer legumes in the hill and terai region of Nepal. Field Crops Res., 70: 209-221.

Mehravaran, H., 2003. Techniques and Job Making in Hydroponics and Soils Cultures. 2nd Edn., Jehad Daneshgahi of Uremia Press, Uremia, Iran, ISBN: 964-6035-22-2.

Oweis, T., A. Hachum and M. Pala, 2004. Lentil production under supplemental irrigation in a Mediterranean environment. Agric. Water Manage., 68: 251-265.

Pagter, M., C. Bragato and H. Brix, 2005. Tolerance and physiological responses of phragmites Australia to water deficit. Aquatic Bot., 81: 285-299.

Rosales-Sernaa, R., J. Kohashi-Shibataa, J. A. Acosta-Gallegosb, C. Trejo-Lo Peza and J. Ortiz-Cereceresc *et al*., 2004. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. Field Crops Res., 85: 203-211.

Sadras, V., 2002. Interaction between rainfall and nitrogen fertilization of wheat in environments prone to terminal drought: Economic and environmental risk analysis. Field Crops Res., 77: 201-215.

Saneoka, H., R.E.A. Moghaieb, G.S. Premachandra and K. Fujita, 2004. Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relation in *Agrostis palustris* hunds. Environ. Exp. Bot., 52: 131-138.

Schmidtke, K., A. Neumann, C. Hof and R. Rauber, 2004. Soil and atmospheric nitrogen uptake by lentil (*Lens culinaris* Medic.) and barley (*Hordeum vulgare* ssp. *Nudum* L.) as monocrops and intercrops. Field Crops Res., 87: 245-256.

Silsbury, J.H., D.W. Catchpoole and W. Wallace, 1986. Effects of nitrate and ammonium on nitrogenase activity of swards of subterranean clover (*Trifoliwn subterraneum* L.). Aust. J. Plant Physiol., 13: 257-273.

Solan, R.J., R.P. Patterson and T.E Carter J., 1990. Field drought tolerance of soybean plant introduction. Crop Sci., 30: 118-123.

Sprent, J.I. and R.J. Thomas, 1984. Nitrogen nutrition of seedling grain legumes some taxonomic, morphological and physiological constraints. Plant Cell Environ., 7: 637-645.

Tomas, M.J. Robertson, S. Fukai and M.B. Peoples, 2004. The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mungbean. Field Crops Res., 86: 67-80.

Turkan, I., B. Melike, F. Ozdemir and H. Koca, 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci., 168: 223-231.

Verdoy, D., M.M. Lucas, E. Manrique, A.A. Covarrubias and M.R. Defelipe *et al*., 2004. Differential organ-specific response to salt stress and water deficit in nodulated bean (*Phaseolus vulgaris*). Plant Cell Environ., 27: 757-767.

Yang, J., J. Zahang, Z. Huang, Q. Zhu and L. Wang, 2000. Remobilization of carbon reserves is improved by controlled soil drying during grain filling of wheat. Crop Sci., 40: 1645-1655.

Yang, J., J. Zahang, Z. Huang, Q. Zhu and L. Liu, 2001. Water deficit induced senescence and its relationship to the remobilization of ore stored carbon in wheat during grain filling. Agron. J., 93: 196-206.