ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2019, 8(1), pp. 61 - 68, DOI:10.18036/aubtdc.427128

THE EFFECT OF INTER-PLANTAL COMPETITION ON *IN VITRO* SEED GERMINATION AND SEEDLING GROWTH IN FLAX (*LINUM USITATISSIMUM* L.)

Ramazan BEYAZ [1,*], Mustafa YILDIZ [2]

[1] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Kırşehir Ahi Evran University, Bağbaşı, Kırşehir, Turkey

[2] Department of Field Crops, Faculty of Agriculture, Ankara University, 06110-Dışkapı, Ankara, Turkey

ABSTRACT

This study was carried out to investigate the relationship between in vitro competition and stress, and their effects on seedling growth of three flax (Linum usitatissimum) cultivars ('Madaras', 'Clarck', and '1886 Sel.'). Competition among plants was achieved by changing the distances they sown. Four different distances were used as '0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm. The results showed that encouraging seedlings for competition by decreasing the distances among seeds sown per Magenta vessel elevated chlorophyll contents (chl. a, b and total chl.), seedling height and root length significantly up to a threshold from where stress initiated and significant decreases in all parameters were observed. In term of chlorophyll contents (chl. a, b and total chl.), seedling height and root length, the highest results (1540.1, 581.9, and 1253.7 µg/g tissue, 4.54 and 7.77 cm, respectively) were obtained from '1.5x1.5' cm distance in cv. 'Madaras'. For cv. 'Clarck', the highest values in chlorophyll contents (chl. a, b and total chl.), seedling height and root length were recorded as 1860.6, 652.6, and 1429.0 µg/g tissue, 4.58 and 6.79 cm, respectively in '1.5x1.5' cm distance application. The highest results with respect to chlorophyll contents (chl. a, b and total chl.), seedling height and root length were aigain recorded at '1.5x1.5' cm distance application in cv. '1886 Sel.' as 1782.6, 552.0, and 1138.7 µg/g tissue, 4.62 and 6.45 cm, respectively. As a consequence, the application of '1.5x1.5' cm distance seems to best for all cultivars. This study showed that competition among in vitro-grown seedlings which can be the source of explants may be used to increase the success of tissue culture studies.

Keywords: *In vitro* competition, Flax, Seed germination, Seedling growth

BİTKİLER ARASI REKABETİN KETENDE (*LINUM USITATISSIMUM* L.) *IN VITRO* ÇİMLENME VE FİDE GELİŞİMİ ÜZERİNE ETKİSİ

ÖZET

Bu çalışma, *in vitro* rekabet ve stress arasındaki ilişkileri ve bunların üç keten (*Linum usitatissimum*) çeşidinde fide gelişimi üzerine etkilerini araştırmak için yapılmıştır. Bitkiler arasındaki rekabet, tohumların ekildiği mesafeler değiştirilerek sağlanmıştır. '0.5x0.5', '1.0x1.0', '1.5x1.5' ve '2.0x2.0' cm olarak dört farklı mesafe kullanılmıştır. Sonuçlar, fidelerin Magenta kaplarına ekilen tohumlar arasındaki mesafenin daraltılması ile elde edilen rekabete özendirilmesinin klorofil kapsamları (klorofil a, klorofil b ve toplam klorofil), fide boyu ve kök uzunluğunu stresin başladığı ve bütün parametrelerde önemli düşüşlerin gözlendiği belli bir noktaya kadar önemli derecede artırdığını göstermiştir. 'Madaras' çeşidinde klorofil içerikleri (klrofil a, klorfil b ve toplam klorfil), fide boyu ve kök uzunluğu bakımından en yüksek sonuçlar (1540.1, 581.9, ve 1253.7 µg/g tissue, 4.54 and 7.77 cm) '1.5x1.5' cm mesafesinden elde edilmiştir. 'Clarck' çeşidi için, klorofil (klorofil a, klorofil b ve toplam klorofil) içerikleri, fide boyu ve kök uzunluğunda en yüksek değerler sırasıyla 1860.6, 652.6, and 1429.0 µg/g tissue, 4.58 ve 6.79 cm olarak 1.5x1.5 cm mesafe uygulamasında kaydedilmiştir. '1886 Sel.' çeşidinde klorofil (klorofil a, klorofil b ve toplam klorofil) içerikleri, fide boyu ve kök uzunluğu bakımından en yüksek değerler sırasıyla 1860.6, 652.6, and 1429.0 µg/g tissue, 4.58 ve 6.79 cm olarak yine '1.5x1.5' cm mesafe uygulamasında kayıt edilmiştir. Sonuç olarak, '1.5x1.5' cm mesafe uygulaması tüm çeşitler için en iyisi olarak görünmektedir. Bu çalışma, eksplant kaynağı olarak kullanılacak *in vitro* yetiştirilen fideler arasındaki rekabetin doku kültürü çalışmalarının başarısını artırmak için kullanılabileceğini göstermiştir.

Anahtar Kelimeler: *In vitro* rekabet, Keten, Tohum çimlenmesi, Fide gelişimi

*Corresponding Author: ramazanbeyaz@gmail.com
Received: 25.05.2018 Accepted: 07.01.2019

1. INTRODUCTION

Plants compete each other in the environment where they live for water, nutrients and light. The competition among plants in field conditions reported by many studies [1, 2]. However, to our knowledge, there are no studies that evaluated competition among seedlings that are grown *in vitro*. Yıldız et al. [3] reported that seedlings which will be used as explant sources should be grown *in vitro* rather than in greenhouses for high-frequency shoot regeneration. A linear increase has indicated in fruit yield when plant density is increased [4, 5]. Daşgan and Abak [6] have stated that early and total yield per pepper plant decreased by increasing plant density which is probably caused by higher interplant competition due to less spacing. The highest planting density causes yield reduction in bean due to the high competition among plants to uptakes minerals and water [7]. Root diameter increased to uptake more water under high density by the high competition among plants [8]. For field conditions, the effect of plant density and competition reported by research studies, however, there are limited reports for *in vitro* conditions.

Flax (*Linum usitatissimum* L.) is an economically and agriculturally important species from the family Linaceae. The genus *Linum* includes more than 200 species of annual and perennial herbaceous plants, disseminated in temperate and subtropical areas all over the world. The most economically important within these species is the cultural flax *(Linum usitatissimum* L.). There are two distinguishable groups within *L. usitatissimum*, fibre flax ('Madaras', 'Clarck', and '1886 Sel.' cultivars are including in this group) and oilseed flax (linseed). In Europe however, the term "flax" is used for varieties grown for fibre production. These two groups differ much in agronomic characters. Linseed has reduced height, more branching and a later harvest time compared with fibre flax; moreover these two groups differ much in their behavior *in vitro*. Moreover, flax has medicinal uses as an anticancer agent, and it is also used in livestock and human feeding [9]. It is also one of the model systems for genetic engineering due to having a small nuclear genome [10, 11, 12].

Tissue culture studies in plants aim to get a high shoot regeneration frequency, which is required for a useful gene transfer as well as a primary goal. As long as there are no efficient and reliable regeneration protocols in plant tissue culture, there is a lack of efforts to produce transgenic plants. The success of gene transformation studies depends on the ability to create adventitious shoot regeneration of explants [13, 14]. The health of explants is a primary point in the determination of regeneration capacity. Explant viability, age and the tissue source for explants are crucial to obtain high shoot regeneration frequency [15]. The shoot regeneration capacity of explants should be increased as much as possible to obtain a high rate of transgenic plants. High frequency shoot regeneration of explants highly depends on where explants are isolated from. Yıldız et al. [16] have reported that the best regeneration capacity of flax hypocotyls was recorded from explants isolated from *in vitro*-grown seedlings. On the contrary, explants from green house-grown seedlings caused dramatic decreases in regeneration capacity.

The aim of this study was to examine the effect of *in vitro* competition and stress among seedlings on the growth of three flax cultivars. Competition among seedlings was achieved by sowing seeds at 4 different distances ('0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm) in a Magenta vessel. Increasing the seed density in unit area decreased the source of water and nutrients per seed and seedling. This was the reason for competition and stress among seedlings *in vitro*.

2. MATERIALS AND METHODS

2.1. Plant Material, Seed Sterilization and Germination

The seeds of flax cultivars ('Madaras', 'Clarck' and '1886 Sel.') were used as material. Cultivar seeds were obtained from the Northern Crop Science Laboratories in North Dakota, USA. The seeds were surface-sterilized in 40% commercial bleach (ACE-Turkey, containing 5% NaOCl) at 10ºC for 20 min

under continuous stirring, followed by 3 washes in sterile distilled water at 10ºC using the protocol described by Yildiz and Er [15]. The sterilized-seeds were transferred to germinate on medium consisted of mineral salts and vitamins of Murashige and Skoog [17], 3% (w/v) sucrose and 0.7% (w/v) agar. The pH of the medium was adjusted to 5.6-5.8 with 1M NaOH or 1M HCl before the addition of agar and autoclaving. All cultures were incubated at 24ºC under cool white fluorescent light (27 mmol photons m^{-2} s^{-1}) with a 16/8- h light/dark photoperiod. The seeds were sown in a Magenta vessel with dimension of 15x15 cm at '0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm distances (Figure 1). The shoot and root lengths, chlorophyll contents (chl. a, chl. b and total chl) were recorded 2 weeks after study initiation.

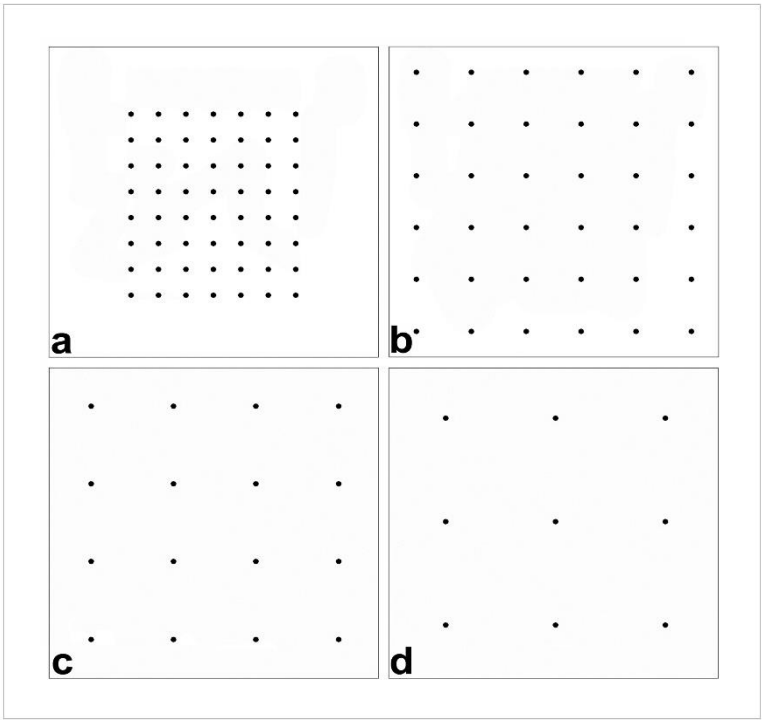

Figure 1. Schematic plan of seeds sown at different distances in Magenta vessel. (a) '0.5x0.5' cm, (b) '1.0x1.0' cm, (c) '1.5x1.5' cm and (d) '2.0x2.0' cm.

2.2. Chlorophyll Content

The protocol proposed by Curtis and Shetty [18] was used to determine the chlorophyll a, chlorophyll b and total chlorophyll contents of the plants on which grown at different distances ('0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm) under *in vitro* condititons. By following the protocol, 50 mg of green material was put into 3 ml of methanol and kept at 23°C in darkness for 2 hours to allow the chlorophyll in the green material to dissolve into the methanol. Then, the optic density (OD) of 1.5 ml of the liquid part (the chlorophyll-containing methanol) was determined at 650 and 665 nm using spectrophotometry, and the chlorophyll a, chlorophyll b and total chlorophyll amounts were determined in "µg chlorophyll/g of fresh tissue".

2.3. Statistical Analysis

The experiments were carried out as two parallels with three replications. One magenta vessel was considered as the unit of each replication. The number of seeds per replication changed considering the distance. Statistical analysis was conducted using "IBM SPSS Statistics 22". The differences between the means in the experiments and control were evaluated for significance by using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

In this study, *in vitro*-grown seedlings of three flax cultivars ('Madaras', '1886 Sel.' and 'Clarck') grown at different distances ('0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm) were compared regarding seedling height, root length and chlorophyll (a, b and total) contents. The results got lower at distances below and above '1.5x1.5' cm. Increasing seed and seedling density in a Magenta vessel caused stress at '0.5x0.5' and '1.0x1.0' cm distances or relief at '2.0x2.0' cm distance. There are 56, 36, 16 and 9 seeds per Magenta vessel at '0.5x0.5', '1.0x1.0', '1.5x1.5' and '2.0x2.0' cm distances, respectively.

Seedling height, root length, chlorophyll (a, b and total) content of seedlings of three flax (*Linum usitatissimum* L.) cultivars ('Madaras', 'Clarck', and '1886 Sel.') grown under *in vitro* conditions were presented in Table 1. It was observed that there were statistically significant differences in all characteristics studied among the seedlings grown at different distances. The highest values for seedling length, root length and photosynthetic activity as chlorophyll (a, b and total) contents were recorded as 4.54 and 7.77 cm, 1540.1, 581.9 and 1253.7 µg/g tissue, respectively, from the seedlings grown at '1.5x1.5' cm distance (Table 1.) for cv. 'Madaras'. Similarly, In cv. 'Clarck', the highest values (4.58 and 6.79 cm, 1860.6, 652.6 and 1429.0 µg/g tissue, rescpectively) for seedling height, root length and photosynthetic activity as chlorophyll a, chlorophyll b and total chlorophyll content were noticed at '1.5x1.5' cm distances. In addition, compered to other distances ('0.5x0.5', '1.0x1.0', and '2.0x2.0' cm), the maximum values were obtained from '1.5x1.5' cm distances with 4.62 and 6.45 cm, 1782.6, 814.8 and 1440.6 µg/g tissue, respectively, for seedling length, root length and photosynthetic activity as chlorophyll a, chlorophyll b and total chlorophyll content in '1886 Sel.'.

Yildiz et al. [19] noted that the success of tissue culture studies for related genotypes could be increased not only by determination of correct concentrations and combinations of auxins and cytokinins in growth media but also by evaluating competition among the cultivated explants. It was also reported that shoot regeneration frequency of *Lathyrus chrysanthus* Boiss. cotyledon node explants could be increased by encouraging explants into a spacing competition. Spacing competition at higher densities gave rise to a decrease in water uptake from the environment and consequently, a reduced mobilization of plant growth regulators which prevent cell division and cell elongation [20]. Shoot and root lengths of seedlings grown at '1.5x1.5' cm distance in all cultivars were higher than that of other distances which were supposed to be caused by competition among seedlings (Figure 2 and Figure 3). At a '1.5x1.5' cm distance, seedling length and root length were recorded as 4.54 and 7.77 cm in 'Madaras'; 4.58 and 6.79 cm in 'Clarck'; 4.62 and 6.45 cm in '1886 Sel.' (Table 1.). In general, the results of this study showed that spacing competition causes increases in shoot and root lengths in tree cultivars up to a certain threshold (1.5 cm distance), except for one distance ('2.0x2.0' cm). Similar results were founded by Yildiz et al. [19] in *Linum usitatissimum* L. and Yildiz [21] in three flax (*Linum usitatissimum* L.) cultivars.

Our findings were confirmed by Posada-Perez et al. [22] who reported that germination rate of somatic embryos of papaya (*Carica papaya* L.) was low at the density of 300 mg FWt since inadequate amount of water, sucrose and nutrients available in the culture medium and by Romero et al. [23] who stated that reducing plant density was because of reducing competition between protocorms of orchid *Chloraea gavilu* Lindl..

Figure 2. Development of seedlings grown at different distances (a) '0.5x0.5' cm, (b) '1.0x1.0' cm, (c) '1.5x1.5' cm and (d) '2.0x2.0' cm

Photosynthetic activity as chlorophyll a, chlorophyll b and total chlorophyll content which was an indicator of seedling health and vitality, was also higher at a distance of '1.5x1.5' cm. At other distances, the seedlings seemed to slow down their photosynthetic activity due to stress. Yildiz [20] stated that *in vitro* spacing competition causes an increase in total chlorophyll contents in three flax (*Linum usitatissimum* L.) cultivars. Moreover, Aycan et al. [20] reported that there is a positive effect of spacing competition among explants that was observed in total chlorophyll contents of *Lathyrus chrysanthus* Boiss. regenerated shoots. Yildiz [21] stated that, with decreasing distance among explants, chlorophyll contents increase up to a certain point ('1.5x1.5' cm distance) in comparison to the controls in all cultivars.

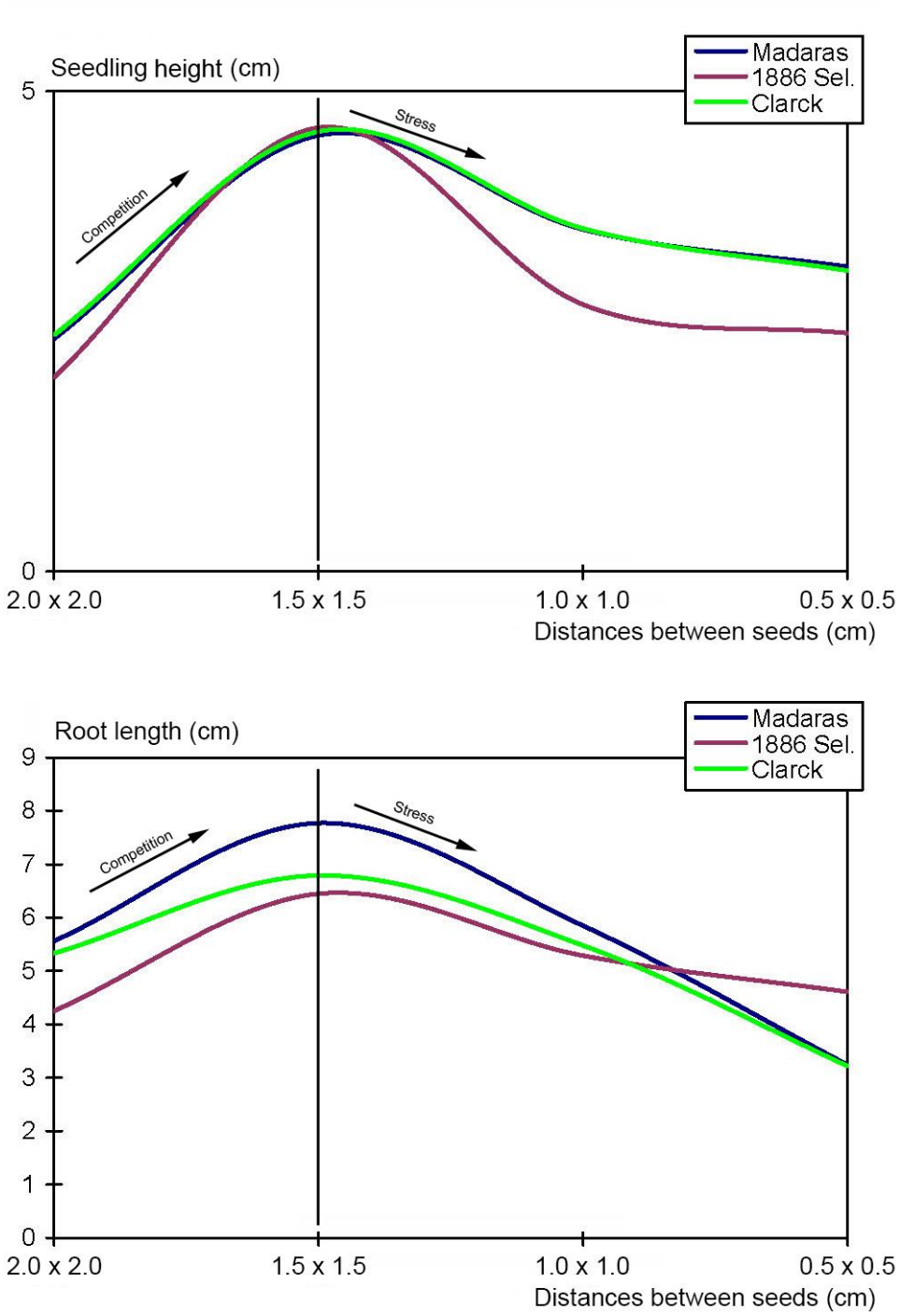

Figure 3. Competition-stress curve of cultivars 'Madaras', 'Clarck' and '1886 Sel.' with respect to seedling and root lengths

Table 1. Influence of seed and seedling density on seedling length, root length, chlorophyll a, chlorophyll b and total chlorophyll contents of seedlings of three flax cultivars

Cultivar	Distances between seeds cultured (cm)	Seedling height (cm)	Root length (cm)	Chlorophyll a content (µg/g tissue)	Chlorophyll b content (µg/g tissue)	Total chlorophyll content (µg/g tissue)
'Madaras'	'0.5x0.5'	3.17 b	3.24 c	952.8 c	310.6 c	613.4 c
	'1.0x1.0'	3.56 b	5.85 b	1152.4 b	378.9 b	889.5 b
	'1.5x1.5'	4.54 a	7.77 a	1540.1 a	581.9 a	1253.7 a
	'2.0x2.0'	2.42 c	5.56 b	1142.5 b	365.8 b	960.3 c
'Clarck'	'0.5x0.5'	3.13 b	3.22 c	919.4 c	314.9 c	713.0 c
	'1.0x1.0'	3.57 b	5.48 b	1637.0 b	480.1 b	1203.5 b
	'1.5x1.5'	4.58 a	6.79 a	1860.6 a	652.6 a	1429.0 a
	'2.0x2.0'	2.46 c	5.33 b	1507.0 b	455.8 a	1070.2 b
'1886 Sel.'	'0.5x0.5'	2.48 b	4.61 bc	879.2 c	308.6 c	681.2 c
	'1.0x1.0'	2.78 b	5.29 b	1266.1 b	552.0 b	1138.7 b
	'1.5x1.5'	4.62 a	6.45 a	1782.6 a	814.8 a	1440.6 a
	'2.0x2.0'	2.02 b	4.25 c	1204.4 b	477.6 bc	1026.8 b

*: Means followed by same letter(s) show siginificant differences at $p<0.05$.

4. CONCLUSION

Decreasing results in high seedling density (in '0.5x0.5' cm and '1.0x1.0' cm spacing density) were resulted from decreasing amount of water and nutrient per seedling. On the other hand, decreasing results were again noted at low seedling density due to lack of competition among seedlings. There was no reason to compete with each other at low seedling density conditions as in '2.0x2.0' cm. There were already too much water and nutrient in the surrounding area. Consequently, determining appropriate seedling density for related plant which will be used in tissue culture studies, are extremely improtant to get vital and well-grown explant source. The health and vitality of an explant source is a prerequisite for the success of further *in vitro* studies. Therefore, this study showed that encouraging *in vitro*-grown seedlings for competition increased their vitality and growth in flax (*Linum usitatissimum* L.).

REFERENCES

[1] Wilson JB. Shoot competition and root competition. J Appl Ecol 1988; 25: 279-296.

[2] McPhee CS, Aarssen LW. The seperation of above- and below-ground competion in plants. A review and critique of methodology. Plant Ecol 2001; 152: 119-136.

[3] Yıldız M, Özcan, S, Er C. The effect of different explant sources on adventitious shoot regeneration in flax (*Linum usitatissimum* L.). Turk J. Biol 2002; 26: 37-40.

[4] Jolliffe PA, Gaye MM. Dynamics of growth and yield component responses of bell peppers (*Capsicum annuum* L.) to row covers and population density. Scientia Hortic 1995; 62: 153-164.

[5] Morgade L, Willey R. Effect of plant population and nitrogen fertilizer on yield and efficiency of maize/bean intercropping. Pesq Agropec Bras Brasilia 2003; 38: 1257-1264.

[6] Daşgan HY, Abak K. Effects of plant density and number of shoots on yield and fruit characteristics of peppers grown in glasshouses. Turk J Agric For 2003; 27: 29-35.

[7] Abubaker S. Effect of plant density on flowering date, yield and quality attribute of bush beans (*Phaseolus vulgaris* L.) under center pivot irrigation system. Am J Agr Biol Sci 2008; 3: 666-668.

[8] Asghari MT, Daneshian J, Farahani AA. Effects of drought stress and planting density on quantity and morphological characteristics of chicory (*Cichorium intybus* L.). Asian J Agr Sci 2009; 1: 12-14.

[9] Tawfik RS, Badr A, Sammour R, Ibrahim U, Matter M, Sakr M. Improvement of Flax Drought Tolerance Using Gene Transfer. Plant Tiss Cult Biotech 2016; 26(2): 197-207.

[10] Basiran N, Armitage P, Scott RJ, Draper J. Genetic transformation of flax (*Linum usitatissimum*) by *Agrobacterium tumefaciens*: regeneration of transformed shoots via a callus phase. Plant Cell Rep 1987; 6: 396-399.

[11] Dong JZ, Mc Hughen A. Patterns of transformation intensity on flax hypocotyls inoculated with *Agrobacterium tumefaciens*. Plant Cell Rep 1991; 10: 555-560.

[12] Millam S, Davidson D, Powell W. The use of flax (*Linum usitatissimum*) as a model system for studies on organogenesis *in vitro*: the effect of different carbohydrates. Plant Cell Tiss Org 1992; 28: 163-166.

[13] Jordan MC, Mc Hughen A. Transformed calllus does not necessarily regenerate transformed shoots. Plant Cell Rep 1988; 7: 285-287.

[14] Dong JZ, Mc Hughen A. An improved procedure for production of transgenic flax plants using *Agrobacterium tumefaciens*. Plant Sci 1993; 88: 61-71.

[15] Yıldız M, Er C. The effect of sodium hypochlorite solutions on *in vitro* seedling growth and shoot regeneration of flax (*Linum usitatissimum*). Naturwissenschaften 2002; 89: 259-261.

[16] Yıldız M, Özcan S, Er C. The effect of different explant sources on adventitious shoot regeneration in flax (*Linum usitatissimum* L.). Turk J Biol 2002; 26: 37-40.

[17] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 1962; 15: 473-479.

[18] Curtis OF, Shetty K. Growth medium effects on vitrification, total phenolics, chlorophyll, and water content of *in vitro* propagated oregano clones. Acta Horticulture 1996; 426: 498-503.

[19] Yildiz M, Sağlik Ç, Telci C, Erkiliç EG. The effect of *in vitro* competition on shoot regeneration from hypocotyl explants of *Linum usitatissimum*. Turk J Bot 2011; 35:211-218.

[20] Aycan M, Kayan M, Yildiz M. The effect of *in vitro* spacing competition on shoot regeneration from cotyledon node explants of *Lathyrus chrysanthus* Boiss. Aust J Crop Sci 2014; 8(7):1019-1023.

[21] Yildiz M. Evaluation of the effect of *in vitro* stress and competition on tissue culture response of flax. Biol Plantarum 2011; 55(3): 541-544.

[22] Posada-Perez L, Montesinos YP, Guerra DG, Daniels D. Complete germination of papaya (*Carica papaya* L. cv. `Maradol Roja´) somatic embryos using temporary immersion system type RITA® and phloroglucinol in semi-solid culture medium. In Vitro Cell Dev Biol 2011; 53: 505-513.

[23] Romero C, Cuba-Diaz M, Silva R. *In vitro* culture of *Chloraea gavilu* Lindl., an endemic terrestrial orchid from Chile. Plant Biosyst 2018; 152: 612-620.