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Nutrition Media Optimization Study in Micropropagation: *Phormium tenax* (New Zealand Flax)

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ABSTRACT

Phormium tenax (New Zealand Flax) is a plant used in many sectors such as the flax industry, paper, medicine, cosmetics, landscaping and even recently the food industry, thanks to its fibrous structure. It has been aimed to determine the environments that can be used in micropropagation of *Phormium tenax* (New Zealand Flax), which is one of the most imported ornamental plants, which is frequently used in landscapes in Turkey and the world in recent years. For this purpose, widely used MS, DKW, WPM, GAMBORG B5 and MS-Mod nutrient media prepared considering the soil requirements of the plant were used. In *Phormium tenax* media trials, the number of shoots per explant (1.53 shoots/explant) was seen in MS-Mod media with 1,00 mg L-1 BAP, 0,01 mg L-1 IBA. In addition, since MS-Mod medium gave better results in other parameters, it was determined that it was suitable for micropropagation. According to the results obtained in the research, it has been determined that the MS-Mod medium with lower nitrogen content for the *Phormium tenax* (New Zealand Flax) plant is both economical and environmentally friendly.

Keywords*:* *Phormium tenax*, New Zealand Flax, micropropagation, Tissue Culture, clonal propagation, plant media.

Mikroçoğaltımda *Phormium Tenax* (Yeni Zelanda Keteni) Besi Ortamı Optimizasyon Çalışması

ÖZET

Phormium tenax (Yeni Zelanda Keteni) lifli yapısı sayesinde keten endüstrisi başta olmak üzere, kağıt, ilaç, kozmetik, peyzaj ve hatta yakın zamanda gıda endüstrisi gibi bir çok sektörde kullanılan bir bitkidir. Son yıllarda Türkiye'de ve Dünya'da dış mekânda sık kullanılan ve bu nedenle en çok ithal edilen süs bitkilerinden biri olan *Phormium tenax* (Yeni Zelanda Keteni) bitkisinin mikroçoğaltımda kullanılabilecek ortamların belirlenmesi hedeflenmiştir. Bu amaçla yaygın olarak kullanılan MS, DKW, WPM, GAMBORG B5 ve bitkinin toprak istekleri dikkate alınarak hazırlanan MS-Mod besin ortamları kullanılmıştır. *Phormium tenax* ortam denemelerinde, eksplant başına sürgün sayısı (1,53 sürgün/eksplant) 1,00 mg L-1 BAP, 0,01 mg L-1 IBA ile MS-Mod ortamında görülmüştür. Ayrıca diğer parametrelerde de MS-Mod ortamı daha iyi sonuçlar verdiğinden mikroçoğaltım için uygun olduğu belirlenmiştir. Araştırmada elde edilen sonuçlara göre *Phormium tenax* (Yeni Zelanda Keteni) bitkisi için daha düşük azot içeriğine sahip MS-Mod ortamının hem ekonomik hem de çevreci olduğu tespit edilmiştir.

Anahtar Kelimeler: *Phormium tenax*, Yeni Zelanda Keteni, mikroçoğaltım, doku kültürü, klonal çoğaltım, besi ortamı.

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1 Introduction

Phormium tenax (New Zealand Flax) is an important evergreen fiber plant belonging to the *Hemerocallidaceae* family. It is native to New Zealand and Norfolk Island. Adaptable to all kinds of terrain conditions, *P. tenax* naturally spreads up to 1,200 m above sea level. It generally adapts to equatorial, oceanic, subtropical and tropical climates (Furtado, 2022). With the presence of more than one color in its leaves, it provides a striking and colorful visual appearance among seasonal flowers and flowering shrub species (Rouinsard, 2021).

New Zealand flax was first used by the natives for hunting and fishing, and later as medicine. It has been reported that the gel and nectar obtained from the plant are used in the treatment of boils, skin wounds and even toothache. It is known that the root juice of the plant is used as a disinfectant, and the leaves are used to bind broken bones. It is an excellent adhesive and also a very good antiseptic. Studies have also been conducted on the use of flax gel as a standard thickener for cosmetic products. *Phormium tenax* is widely used in the flax industry due to its fibrous structure.

P. tenax seed has a very rich oil content (5-16% oleic acid, 75-89% linoleic acid, 3-11% palmitic acid and 1-3% stearic acid). Unlike other flaxseeds, *Phormium tenax* seed oil is rich in omega-6 fatty acid linoleic acid. It has been stated that the oil from linseed is rich enough to be compared with sunflower and safflower, and the first class of edible vegetable oil compared to oil from rapeseed and soybean (Jebrell, 2021). In addition, the amount of 57.5% holocellulose in *Phormium tenax* is very close to the amount of pine (68%) and eucalyptus (59.8%) used in the paper industry. For this reason, it has a high potential for use in paper production. When Phormium tenax is processed as linen in the industry, ornaments made from materials produced from by-products, hard packaging, small containers, and furniture such as chairs and tables used in cafes and patisseries can also be used as anti-mildew thanks to its antioxidant feature. (Furtado, 2022) The most widely used method in ornamental plant production is vegetative production (Düzer, 2010). Tissue culture techniques are used for faster, disease-free and standard plant production compared to traditional methods. (Hamidi Birecikli, 2018). This technique is useful for the rapid propagation of a wide variety of ornamental plants. (Rouinsard, 2021). The production of a new plant, tissue or various secondary metabolites from a cell, tissue or organ taken from the plant in aseptic and controlled conditions in an artificial nutrient medium is called tissue culture (Babaoğlu, 2001).

Phormium tenax is difficult to grow by vegetative methods outside of its natural distribution area. In recent years, it is one of the most imported ornamental plants, which is frequently used in outdoor rock gardens and pool garden designs in Turkey and the world. It is used as a background plant in the landscape, especially because of its variegated leaves (Jebrell, 2021). This study, it was aimed to develop tissue culture and micropropagation protocols for *Phormium tenax* plant. For this purpose, the effect of the number of shoots per explant and the average growth performance of growing shoots on plant reproduction were investigated in different nutrient media.

2 Research Methodology

In this study, *Phormium tenax* plant in the Sakarya University of Applied Science, Faculty of Agriculture Plant Tissue Culture Laboratory was used. Differently preferred nutrient media and their contents in the study are given in Table 1. The media to be used were sterilized in an autoclave at 121°C for 20 minutes (1 atm) with liquid steam pressure.
The nutrient media were selected according to the plant and soil requirements. In order to create micropropagation, 1,00 mg L-1 BAP and 0,01 mg L-1 IBA were added to each medium, taking into

account the literature. Explants from the donor plant were first washed in running water for 30 minutes and treated with antifungals for 15 minutes. followed by surface sterilization with 25% volume of sodium hypochlorite (ACE) for 20 minutes. Finally, it was washed with sterile distilled water for 5 minutes and 3 times and inoculated in the determined nutrient media. It was cultured at 24 ± 20°C for a period of 30 days with a photoperiod of 16 hours. At the end of the regeneration period, the number of shoots per explant (shoot/explant), stem length (mm), shoot length (mm), stem diameter (mm) and the number of leaves per explant (leaf/explant) were determined. determined. The effects of media on these characteristics were determined statistically. All plant trials were subjected to Duncan multiple comparison and analysis of variance in SPSS statistical program. All plant trials were subjected to Duncan multiple comparison and analysis of variance in SPSS 20,0 statistical program.

Table 1: *Nutrient media and ingredients (mg L-1).*

Ingredients	**MS** (Murashige, 1962)	**MS-modified** (Babalı, 2020)	**DKW** (Driver, 1984)	**WPM** (Lloyd, 1981)	**GAMBORG-B5** (Gamborg, 1968)
NH4NO3	1650	500	1416	400	150
KNO3	1900	2000	-	-	-
Ca(NO3.4H2O)	-	1200	1968	386	-
K2SO4	-	-	1559	990	2885
MgSO4	181	370	740	180	122
CaCl2	333	-	147	72	113
KH2PO4	170	170	259	170	150
FeSO4.7H2O	27,8	33,80	42,25	27,8	27,8
Na2EDTA	37,26	45,40	56,75	37,26	37,26
Na2MoO4	0,25	0,39	0,40	0,25	0,20
CuSO4.5H2O	0,25	0,25	0,25	0,25	0,12
H3BO3	6,2	4,8	12,4	6,2	3,1
Zn(NO3)7H2O	16,9	17	26,7	8,6	6,6
MnSO4.2H2O	8,6	33,5	33,8	22,3	8,45
NiSO4.6H2O	5	5	5	5	-
Glycine	2	2	2	2	1
Nikotinik acid	0,5	1	1	0,5	0,5
Thiamine HCl	0,1	2	2	1	1
My-inositol	1	1	1	1	1
L-glutamine	1	-	1	-	-

3 Results and Discussion

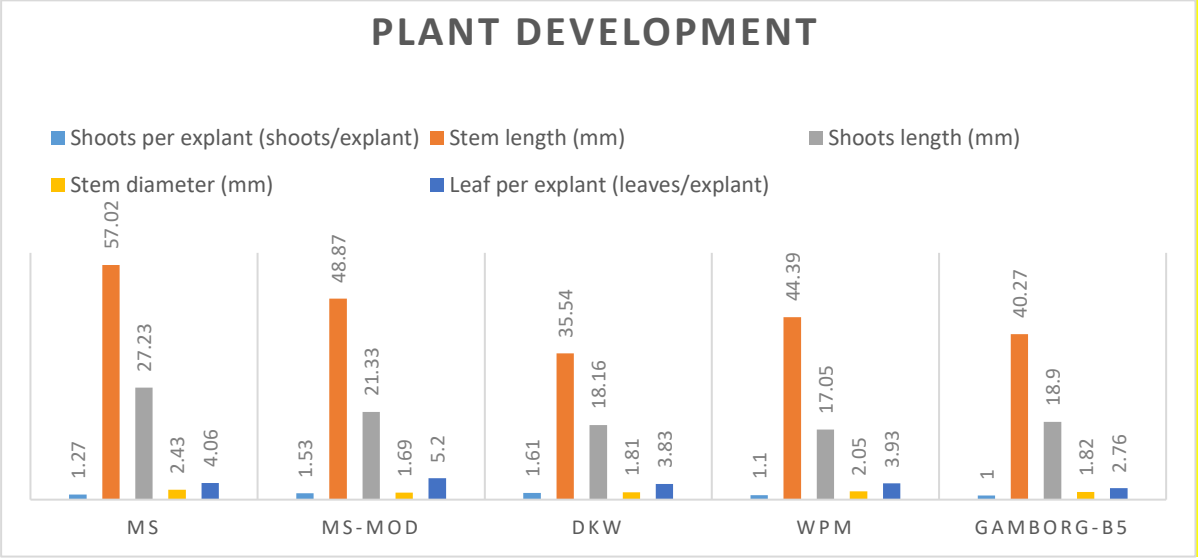
In this study, MS-Mod nutrient medium modified according to the soil requirements of the plants was used, taking into account the MS medium components that are generally used in the literature. In addition, 1,00 mg L-1 BAP and 0,01 mg L-1 IBA plant growth regulators were added to the nutrient medium for plant propogate.

Table 2: *Phormium tenax shoot per explant development.*

Plant media	Shoots per explant (shoots/explant)	Stem length (mm)	Shoot length (mm)	Stem diameter (mm)	Leaf per explant (leaves/explant)
MS	1,27 [ab]	57,02 [a]	27,23	2,43	4,06 [ab]
MS-Mod	1,53 [a]	48,87 [ab]	21,33	1,69	5,20 [a]
DKW	1,61 [a]	35,54 [c]	18,16	1,81	3,83 [bc]
WPM	1,10 [ab]	44,39 [bc]	17,05	2,05	3,93 [abc]
GAMBORG-B5	1,00 [b]	40,27 [bc]	18,90	1,82	2,76 [c]
	*	*	N.S.	N.S.	*

a-c: The difference between the samples in the same column is statistically significant (P<0,05). NS: not significant. There is no statistical difference between the ones denoted by the same letter.

In Table 2, shoots per explant differed between 1,00 and 1,61 mm. The maximum number of shoots per explant was obtained from DKW, MS-Mod, MS and WPM media (1,61 mm, 1,53 mm, 1,27 mm, 1,10 mm, respectively). It was determined that the lowest results in terms of shoot number per explant were obtained from GAMBORG-B5 medium (1,00 mm). The highest stem length per explant was 57,02 mm from MS medium. MS and MS-Mod medium gave the same results for stem length per explant (57,02 and 48,87 mm, respectively). In this study, shoot lengths varied between 17,05 mm and 27,23 mm. Detected from 1,81 mm to 2,43 mm. It has been determined that there is no statistical difference in these two characters. Leaf per explant has been measured between 2,76 and 5,20. There was no difference in the number of leaves per explant between MS-Mod, MS and WPM media. There was no statistically significant difference between media in terms of shoot length and stem diameter. It was determined that there was no significant difference in the number of leaves between the MS-Mod medium and the DKW medium, with a maximum of 6,25 pieces of plant leaves from the DKW medium. The most suitable growth medium for *Phormium tenax* was determined as MS-Mod medium.



Rigg and Watson (1945), in their study with *Phormium tenax*, determined that the plant grows better in an environment with low nitrogen content and high potassium and sulfur content. The most suitable MS-Mod medium in this study has low nitrogen content and high potassium content. In this respect, it can be thought that nitrogen used at low rates contributes to development. According to Battistini et al.

(2003) stated that the best growth in *P. tenax in vitro* liquid culture was MS medium containing 4.00 mg L-1 BAP. A high amount of BAP was not used in our study. Plant growth was also observed at low bap amount. Zhu et al. (2009) observed in their micropropagation study that the most ideal growth was achieved in MS+6-BA 0.50 mg/L+KT 2.00 mg/L+NAA 0.20 mg/L medium. In our study, only BAP was used as cytokinin and IBA was used as auxin. Vegetative growth was also observed in the presence of bap only. In addition, it was determined that the MS-Mod medium with low nitrogen content was more efficient than the MS medium with the amount of cytokinin and auxin used.

4 Conclusion

It was concluded that the nutrients needed for the development of the plant were determined and the environments prepared accordingly were more successful. In *Phormium tenax* media trials, the best results were obtained in MS-Mod media containing 1,00 mg L-1 BAP, 0.01 mg L-1 IBA, including all parameters examined. Thus, it shows that it is important to meet the natural requirements of the plant in tissue culture. Accordingly, the media prepared specifically for the plant species in micropropagation provide a great advantage in obtaining both economic and environmentally friendly and more successful results.

5 Declarations

There is no conflict of competing interest in this study.

6 Author contributions

Neslihan BABALI provided laboratory studies, analyzes and writing of the article. The planning, execution and evaluation of the research were provided by Taki DEMİR. The authors have read and approved the final version of the article.

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All studies in this research were carried out in the Sakarya University of Applied Science, Faculty of Agriculture Plant Tissue Culture Laboratory was used.

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