Commagene Journal of Biology

Acemi (2021) *Comm. J. Biol.* 5(1), 1-6.
DOI: 10.31594/commagene.827909

e-ISSN 2602-456X

Research Article / Araştırma Makalesi

Effect of Medium Composition on *in vitro* Seed Germination and Plant Development in Kentucky Bluegrass (*Poa pratensis* L. cv. Evora)

Arda ACEMİ

Kocaeli University, Faculty of Arts and Sciences, Department of Biology, İzmit, Kocaeli, Turkey
ORCID ID: Arda ACEMİ: https://orcid.org/0000-0003-0270-8507

Received: 18.11.2020 **Accepted:** 12.01.2021 **Published online:** 31.03.2021 **Issue published:** 30.06.2021

Abstract: In the current study, the effects of De Greef & Jacobs (DG), Linsmaier & Skoog (LS), Murashige & Skoog (MS), and Schenk & Hildebrandt (SH) media were tested on seed germination and plant development in *Poa pratensis* cv. Evora. The highest germination rate (83±2.74%) was found on SH medium, whereas LS medium gave the lowest (46±4.18%) germination rate. The statistically same leaf numbers were recorded from SH (2.10±0.27) and DG (2.12±0.18) media. SH and DG media gave 4.28±0.28 cm and 4.16±0.31 cm mean leaf lengths, respectively. SH medium gave the maximum mean root number (3.09±0.26). However, the LS medium gave the lowest mean root number (1.84±0.10). The longest roots (1.43±0.19 cm) were observed in the plants grown in SH medium. However, DG medium had the minimum mean root length (0.81±0.08 cm). In conclusion, SH medium should be preferred over the other medium tested in *in vitro* tissue culture studies on the species to increase the biomass production yield. The efficacy of SH medium in the *in vitro* propagation of *P. pratensis* could be increased using growth promoters.

Keywords: Macroelements; microelements; tissue culture; turfgrass; vitamins.

Besiyeri Bileşiminin Çayır Salkımotunda (*Poa pratensis* L. cv. Evora) *in vitro* Tohum Çimlenmesi ve Bitki Gelişimi Üzerindeki Etkisi

Öz: Bu çalışmada, *Poa pratensis* cv. Evora'da De Greef & Jacobs (DG), Linsmaier & Skoog (LS), Murashige & Skoog (MS) ve Schenk & Hildebrandt (SH) besiyerlerinin tohum çimlenmesi ve bitki gelişimi üzerindeki etkileri test edilmiştir. En yüksek çimlenme oranı (%83±2.74) SH ortamında bulunurken, LS ortamı en düşük (%46±4.18) çimlenme oranını vermiştir. Ortalama yaprak sayıları SH (2.10±0.27) ve DG (2.12±0.18) ortamlarından istatistiksel olarak aynı kaydedilmiştir. Ortalama yaprak uzunlukları SH ve DG ortamlarında sırasıyla 4.28±0.28 cm ve 4.16±0.31 cm olarak ölçülmüştür. Maksimum ortalama kök sayısı (3.09±0.26) SH ortamından elde edilmiştir. Bununla birlikte, en düşük ortalama kök sayısı (1.84±0.10) LS ortamında kaydedilmiştir. En uzun kökler (1.43±0.19 cm) SH ortamında yetiştirilen bitkilerde gözlenmiştir. Bununla birlikte, DG ortamı minimum ortalama kök uzunluğunu (0.81±0.08 cm) vermiştir. Sonuç olarak, biyokütle üretimini artırmak için bu tür üzerinde yapılan *in vitro* doku kültürü çalışmalarında test edilen diğer besiyerlerine kıyasla SH besiyeri tercih edilmelidir. SH ortamının *P. pratensis*'in *in vitro* gelişimindeki etkinliği büyüme destekleyicileri kullanılarak artırılabilir.

Anahtar kelimeler: Makroelementler; mikroelementler; doku kültürü; çim; vitaminler.

1. Introduction

Kentucky bluegrass (*Poa pratensis* L.) is a perennial and dense turf-producing cool-season grass, which is generally used after getting mixed with perennial ryegrass (*Lolium perenne* L.) to obtain a more stabilized soil and disease-resistant turf with better color and quality (Walker et al., 2007). The species is native to Europe and Asia but has been cultivated for sports and ornamental use in many regions with a temperate climate (Casler, 2006). It grows well on limestone-originated, medium-textured, and well-drained soils. However, it can adapt to poorly-drained and heavy-textured soils. The species can also tolerate severe droughts; although, it prefers humid areas with temperatures between 15 and 32°C (Bush, 2002). These advantages of *P. pratensis* make it an attractive option among other grass species used for forage and lawn production. Like other turfgrasses, the primary propagation method of *P. pratensis* is seed sowing since the vegetative propagation from rhizomes is labor-intensive for such plants.

Plant tissue culture is primarily used to process *in vitro* plant material utilizing meristems and organs that can be differentiated under controlled environmental conditions. The technique is used alongside other plant biotechnology applications such as molecular breeding and disease-resistant plants' production through genetic engineering applications. The optimization of tissue culture medium for *in vitro* plant development plays a significant role in forage and turfgrass biotechnology (Esmaeili et al., 2018). Components in tissue culture media have a vital function in the germination of plant seeds. Every plant tissue culture medium has specific formulations consisted of macro- and microelements, vitamins, and other components such as amino acids at different ratios (Acemi et al., 2018). The success of the *in vitro* propagation study is highly dependent on tissue culture medium composition. Therefore, different medium compositions should be tested to select the most favorable culture medium that meets the plant's macro- and microelement requirements in *in vitro* culture. The seeds' poor germination, the comparatively modest

*Corresponding author: arda.acemi@kocaeli.edu.tr

growth rate of the seedlings, and the lowest yield of *P. pratensis* among other *Poa* species pose difficulties in the turf establishment using the species (Giolo et al., 2017; Akdeniz et al., 2018). Thus, this study aims to determine the plant's requirements for its efficient seed germination and development. In addition, a better understanding of the plant's developmental physiology is provided by exploring the optimum *in vitro* seed germination medium for *P. pratensis*.

2. Material and Methods

2.1. Seed source and disinfection

The seeds of commercially available *P. pratensis* L. cv. Evora (DLF Seeds Ltd., Denmark) were supplied by a local dealer (Sekoya Tohumculuk Ziraat San. & Tic. A. Ş.) in Turkey and kept in a dry and dark place. Seeds' disinfection and sowing were performed in a laminar flow cabinet. One hundred seeds were placed into pouches (4×4 cm) made from filter papers and were disinfected by gently shaking for 8 min in 1% (w/v) sodium hypochlorite (NaOCl) solution. The NaOCl residues on the seeds were removed by soaking the pouch into sterile distilled water several times. The pouch with seeds was then placed onto a sterile platform made of filter papers to take excess water on the seeds.

2.2. Media preparation, seeds' sowing, and culture conditions

Four culture media with different formulations were employed to test their effects on seeds' germination and development of the species. The media described by De Greef & Jacobs (1979) (DG), Linsmaier & Skoog (1965) (LS), Murashige & Skoog (1962) (MS), and Schenk & Hildebrandt (1972) (SH) were prepared and supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} agar and their pH was set to 5.7 using 1 N NaOH or 1 N HCl before autoclaving at 121°C under a pressure of 118 kPa for 20 min. The media formulations are given in Table 1. The paper pouches were opened with a sterile blade and forceps and the disinfected seeds were then sown onto the media. The cultures were incubated in a plant growth chamber with a 16-h photoperiod at 23±1°C and under the illumination of 60 µmol m^{-2} s^{-1} photosynthetic photon flux density.

Table 1. Comparison of the compositions of the culture media tested on *in vitro* development of *P. pratensis*.

Content	Quantity in medium (mg L^{-1})			
Macroelements	**DG**	**LS**	**MS**	**SH**
$CaCl_2$	226.50	332.02	332.02	151.00
KNO_3	2000.00	1900.00	1900.00	2500.00
$MgSO_4$	244.33	180.54	180.54	195.05
NaH_2PO_4	250.00			
KH_2PO_4		170.00	170.00	
$(NH_4)H_2PO_4$				300.00
$(NH_4)_2SO_4$	400.00			
NH_4NO_3		1650.00	1650.00	
KCl	600.00			
Microelements				
$CoCl_2.6H_2O$	0.0025	0.025	0.025	0.10
$CuSO_4.5H_2O$	0.0025	0.025	0.025	0.20
FeNaEDTA	36.7	36.7	36.7	19.8
H_3BO_3	10.62	6.2	6.2	5.00
KI	1.58	0.83	0.83	1.00
$MnSO_4.H_2O$	1.68	16.9	16.9	10.00
$Na_2MoO_4.2H_2O$	0.0025	0.25	0.25	0.10
$ZnSO_4.7H_2O$	1.06	8.6	8.6	1.00
Vitamins				
myo-inositol	100.0	100.0	100.0	1000.0
Nicotinic acid	1.0		0.5	5.0
Pyridoxine HCl	1.0		0.5	0.5
Thiamine HCl	10.0	0.4	0.1	5.0
Amino acids				
Glycine			2.0	

2.3. Data collection and statistical analysis

One hundred seeds (20 seeds per vessel) were sown onto each medium. Each repeat consisted of one culture vessel. The number of germinated seeds and mean leaf and root numbers and lengths were calculated at the end of the incubation period of 30 d. Data were given as mean±standard deviation (SD). Means were compared using Duncan's multiple range test at a significance level of $P< 0.05$. IBM SPSS Statistics 22 software was used for statistical analysis. The data were normalized to 0–1 interval and hierarchical clustering analysis was performed according to the Euclidean distance and unweighted pair group method with arithmetic mean (UPGMA). BioVinci data visualization software, version 1.1.5, was used to create the clustering heatmap.

3. Results and Discussion

The general appearances of the cultures at the end of the incubation period are given in Figure 1. The percentage of germinated seeds greatly varied among the media tested (Fig. 2). SH medium had the highest (83±2.74%) germination percentage, whereas the seeds cultured on LS medium had the lowest (46±4.18%) germination rate. The results showed that the SH medium gave 80%, 30%, and 22% higher germination rates than LS, MS, and DG media, respectively. Statistically, the same germination rates were found from MS and DG media. Seed germination in many species is affected by soil's chemical and physical properties and by several environmental conditions in nature (Borawska-Jarmułowicz et al., 2017; Benvenuti & Mazzoncini, 2018). However, environmental conditions

such as light intensity, photoperiod, temperature, and relative humidity remain stable in tissue culture studies allowing researchers to test the effects of culture medium composition, simulating soil's chemical components. Therefore, culture medium components such as macro- and microelements and supplements such as plant growth regulators are decisive in seed germination success in such studies (Acemi & Özen, 2019). The current study is solely based on testing the effects of medium compositions that means the differences in all developmental parameters are culture medium-specific. Therefore, the highest germination rate obtained from SH medium might be attributed to its rich KNO_3, PO_4, and vitamin content since the other media contain these components at lower levels. Especially, considering that the difference between LS and MS media is only vitamin-sourced and the better germination performance in MS than LS medium, it can be concluded that the vitamin composition of culture medium plays a vital role in the seed germination of *P. pratensis*. On the other hand, faster seed germination in *P. pratensis* after priming the seeds with KNO_3 solution has been reported by Pill and Korengel (1997). Ervin et al., (2017) stated that ammonium tends to be oxidized in time and leads to the decreased availability of P for plants following soil acidification that is an unfavorable condition for bluegrass. The same researchers also concluded that without using P- or K-containing fertilizers, only sulfates of ammonium and Fe reduce the growth and spread of *P. annua* (Ervin et al., 2017). Therefore, the better seed germination performance of SH and DG than MS and LS media might be explained by their lesser NH_4 but higher P contents. Furthermore, ammonium phosphate may increase nutrient availability with a lesser salt effect. In this context, $NH_4H_2PO_4$ has been previously shown to induce nutrient availability in *Hypericum* × *moserianum* (Pizzeghello et al., 2019).

Mean leaf numbers developed from per seed are shown in Figure 3. The highest number of leaves (2.12±0.18) was found in the DG medium, whereas LS medium gave the lowest (1.13±0.04) result. MS and LS media gave statistically the same results, while the same statistical result was found between SH and DG media. Therefore, the DG and SH media were found better for leaf development than the other media tested. A similar trend with mean leaf numbers was also found in the mean leaf lengths (Fig. 4). SH and DG media gave statistically the same results, while the same statistical relationship was found between MS and LS media. The most elongated leaves (4.28±0.28 cm) were measured from the plants developed on SH medium, while LS medium gave the lowest (2.32±0.26 cm) mean leaf lengths. Therefore, better leaf elongation performance in *P. pratensis* was found in the SH and DG media than the other media tested. In addition to the above-discussed differences among macroelement compositions of the media tested, the LS medium's drawback in supplying leaf growth for *P. pratensis* may be due to the lack of vitamins such as pyridoxine and nicotinic acid in its formulation. The exogenously provided non-phosphorylated B_6 vitamers have been shown to reduce singlet oxygen accumulation in plants (Vanderschuren et al., 2013). Also, Colinas et al. (2016) showed that balancing B_6 vitamers is vital for the metabolism and plant development in *Arabidopsis*. Vitamin B has many beneficial roles in plants, such as activation of plant disease resistance (Ahn et al., 2005), alleviating the effects of several environmental stresses, and regulating post-embryonic root development (Chen & Xiong, 2005) since they are cofactors required by numerous enzymes. For instance, nicotinic acid has been found to alleviate the effects of salt stress in *Allium cepa* (Ali, 2002) and *Ricinus communis* (Hussein et al., 2014). Although LS medium includes thiamine (vitamin B_1) as vitamin B, it seems that the lack of nicotinic acid (vitamin B_3) and pyridoxine (vitamin B_6) is the main disadvantage of LS medium in comparison with other media tested in this study.

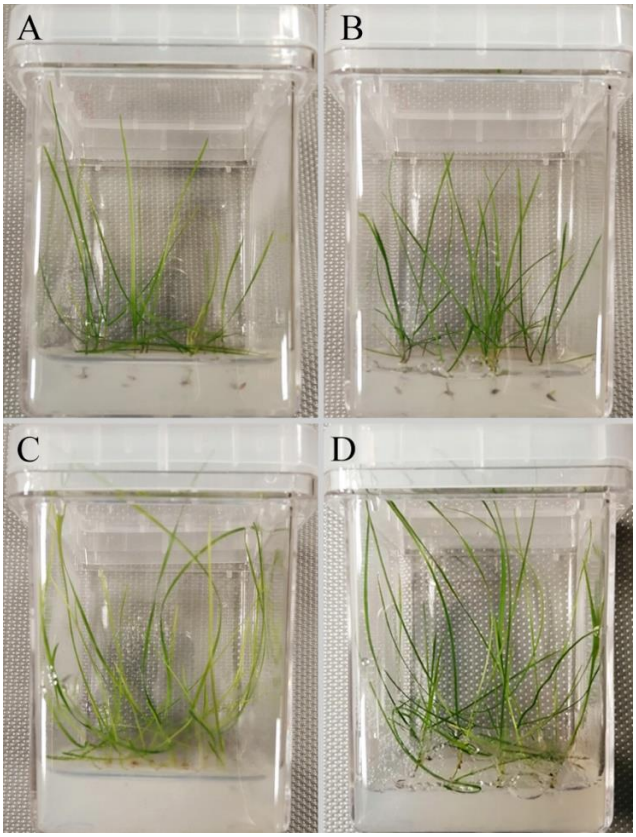

Figure 1. The appearance of the cultures at the end of the incubation period. Seeds germinated in LS (A), MS (B), DG (C), and SH media (D).

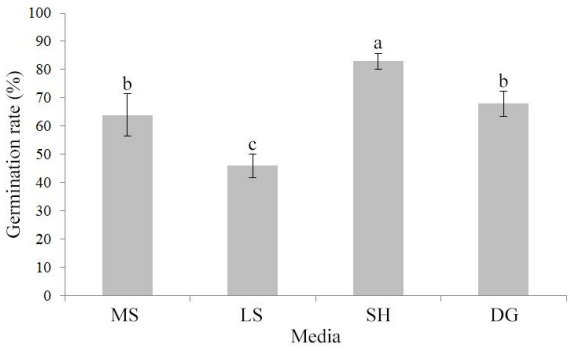

Figure 2. Effect of culture medium on *in vitro* germination of *P. pratensis* seeds. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

The root development performance of *P. pratensis* in the media tested is shown in Fig. 5. The highest number of roots (3.09±0.26) was found in the SH medium, whereas the LS medium gave the lowest (1.84±0.1) mean root

number. MS and DG media gave statistically the same results. The root elongation was found better in the SH medium than the other media tested (Fig. 6). However, MS and LS media gave statistically the same results. The longest roots (1.43±0.19 cm) were measured from the plants developed on SH medium, while DG medium gave the lowest (0.81±0.08 cm) mean root lengths. These results suggested that SH medium better induces the rhizogenesis in *P. pratensis* than the other media tested. The turfgrass roots can release phytosiderophores under Fe- or Zn-deficient conditions that serve as natural chelating agents to extract micronutrients such as iron manganese, copper, and zinc from the soil (Ueno et al., 2007). However, a significant portion of the differentiation among the media's effects might come mostly from the macroelement and vitamin compositions since they cover relatively more space in a medium's formulation than microelements. Nevertheless, it should be noted that some microelements such as molybdenum (Mo) and zinc (Zn) are required for the biosynthesis of abscisic acid and indole-3 butyric acid that modulate the growth of primary and lateral roots (Kaiser et al., 2005). Furthermore, the role of another microelement, cobalt, in lateral root formation has been demonstrated in *Oryza sativa* (Hsu et al., 2013). Therefore, even microelements are required in low concentration, the disadvantage of DG medium in root elongation might stem from its minimal Mo and Co content. Another significant difference among the media tested is their *myo*-inositol contents. SH medium has the highest amount of *myo*-inositol that is ten times higher than those of the other media tested. *Myo*-inositol plays a significant role in many biosynthetic pathways of stress-molecule production and cell wall formation in plants (Loewus & Murthy, 2000). Also, it takes part in phosphate storage, cell communication, and transportation of plant hormones (Luo et al., 2011). In a tissue culture study, *myo*-inositol has been found to enhance the shoot growth and root development in the *Malus domestica* and *Pyrus communis* explants. The authors stated that higher levels of *myo*-inositol could be required to improve the explants' morphogenetic ability (Toma et al., 2012).

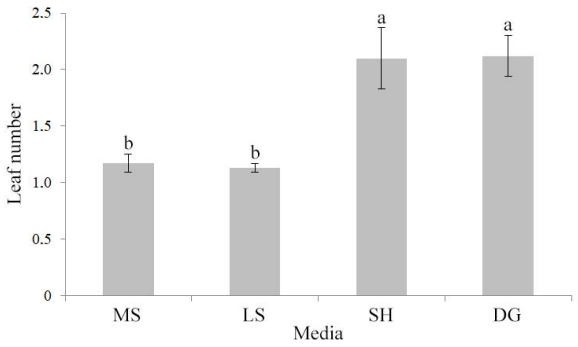

Figure 3. Effect of culture medium on *in vitro* leaf production in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

The developmental data-based hierarchical clustering heatmap shows the relationship among the media tested (Fig. 7). All the media tested were grouped into two main clusters. SH and DG media were found in the same cluster, while MS and LS media were placed in another cluster suggesting that the media's effects in the same cluster were more similar than the other media. The normalized data having the red color better developmental results in *P. pratensis*, while the data represented by blue color shows the lower developmental performance.

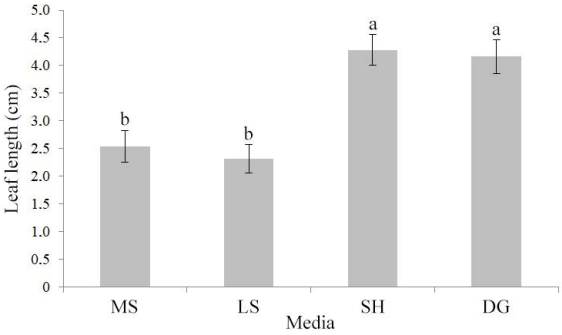

Figure 4. Effect of culture medium on *in vitro* leaf elongation in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

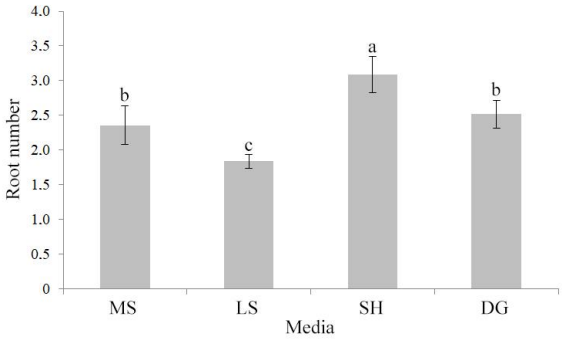

Figure 5. Effect of culture medium on *in vitro* root production in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

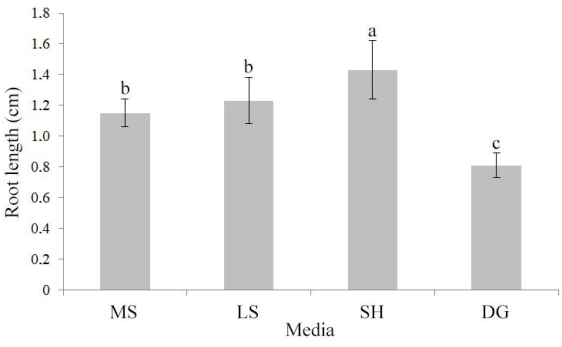

Figure 6. Effect of culture medium on *in vitro* root elongation in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

4. Conclusion

In the present study, influences of different culture media on *in vitro* seed germination and plant development in *P. pratensis* L. were evaluated based on the developmental parameters. In conclusion, SH media should be preferred

over the other medium to achieve a higher seed germination rate and better plant development in *P. pratensis*. The findings of this study would contribute to the improvement studies on the forage and grass species. Also, the effects of growth promoters should be tested to increase the efficiency of the medium.

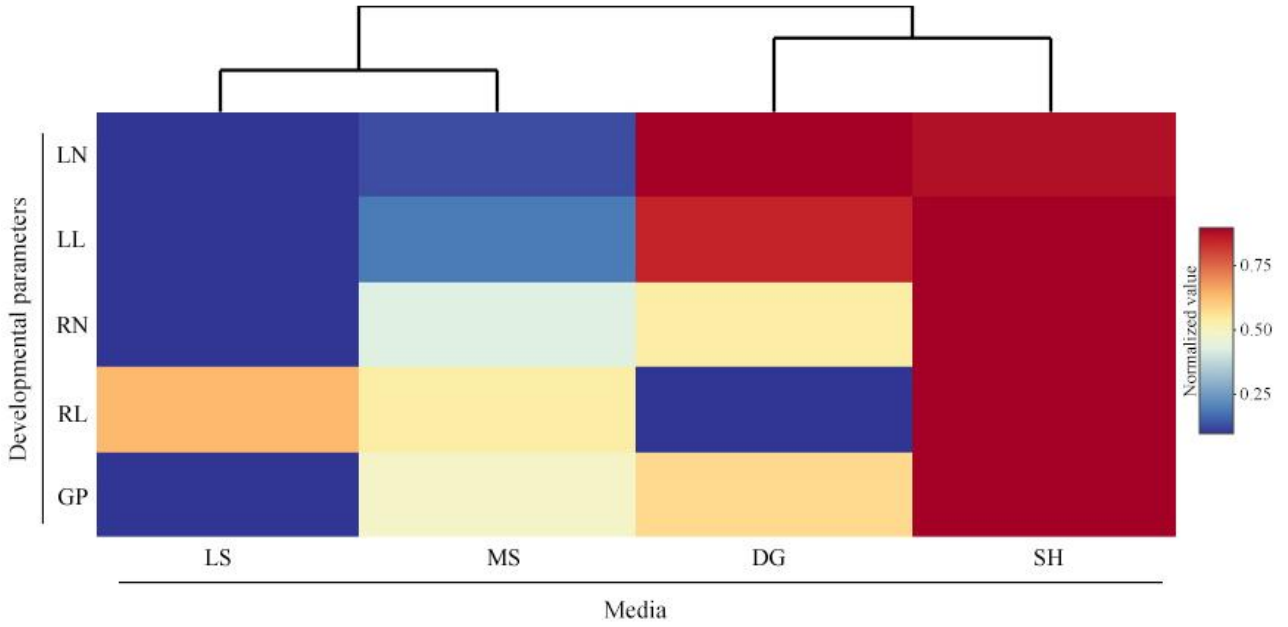

Figure 7. Hierarchical clustering heatmap-based comparison of the developmental data. Leaf number (LN), Leaf length (LL), Root number (RN), Root length (RL), Germination percentage (GP)

Acknowledgements: The author would like to thank Duhan Tırlı and Serdar Yıldız for their help with the inoculation process of the cultures used in this paper.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

References

Acemi, A., & Özen, F. (2019). Optimization of *in vitro* asymbiotic seed germination protocol for *Serapias vomeracea*. *The EuroBiotech Journal*, 3(3), 143-151. https://doi.org/10.2478/ebtj-2019-0017

Acemi, A., Bayrak, B., Çakır, M., Demiryürek, E., Gün, E., El Gueddari, N. E., & Özen, F. (2018). Comparative analysis of the effects of chitosan and common plant growth regulators on *in vitro* propagation of *Ipomoea purpurea* (L.) Roth from nodal explants. *In Vitro Cellular & Developmental Biology - Plant*, 54(5), 537–544. https://doi.org/10.1007/s11627-018-9915-0

Ahn, I., Kim, S., & Lee, Y. (2005). Vitamin B_1 functions as an activator of plant disease resistance. *Plant Physiology*, 138(3), 1505–1515. https://doi.org/10.1104/pp.104.058693

Akdeniz, H., Hosaflıoğlu, İ., & Keskin, B. (2018). Impact of different sowing rates and cutting times on quality properties of Kentucky Bluegrass (*Poa pratensis* L. cv. Geronimo). *Iğdır University Journal of the Institute of Science and Technology*, 8(1), 301-308. https://doi.org/10.21597/jist.407889

Ali, R.M. (2002). Effect of nicotinic acid and nicotinamide adenine dinucleotide on growth and content of oil, glycerol and ricinine alkaloids of salinity stressed *Ricinus communis* L. *Phyton (Horn, Austria)*, 42(2), 269–277.

Benvenuti, S., & Mazzoncini, M. (2018). Soil physics involvement in the germination ecology of buried weed seeds. *Plants*, 8(1), 7. https://doi.org/10.3390/plants8010007

Borawska-Jarmułowicz, B., Mastalerczuk, G., Gozdowski, D., Małuszyńska, E., & Szydłowska, A. (2017). The sensitivity of *Lolium perenne* and *Poa pratensis* to salinity and drought during the seed germination and under different photoperiod conditions. *Zemdirbyste-Agriculture*, 104(1), 71–78. https://doi.org/10.13080/z-a.2017.104.010

Bush, T. (2002). *USDA NRCS Plant Fact Sheet - Kentucky Bluegrass Poa pratensis L.* Retrieved from https://plants.usda.gov/factsheet/pdf/fs_popr.pdf

Casler, M.D. (2006). Perennial grasses for turf, sport and amenity uses: Evolution of form, function and fitness for human benefit. *Journal of Agricultural Science*, 144(3), 189–203. https://doi.org/10.1017/S0021859606006137

Chen, H., & Xiong, L. (2005). Pyridoxine is required for post-embryonic root development and tolerance to osmotic and oxidative stresses. *The Plant Journal*, 44(3), 396–408. https://doi.org/10.1111/j.1365-313x.2005.02538.x

Colinas, M., Eisenhut, M., Tohge, T., Pesquera, M., Fernie, A.R., Weber, A.P., & Fitzpatrick, T.B. (2016). Balancing of B_6 vitamers is essential for plant development and metabolism in *Arabidopsis*. *The Plant Cell*, 28(2), 439–453. https://doi.org/10.1105/tpc.15.01033

De Greef, W., & Jacobs, M. (1979). *In vitro* culture of the sugarbeet: Description of a cell line with high regeneration capacity. *Plant Science Letters*, 17(1), 55–61. https://doi.org/10.1016/0304-4211(79)90109-3

Ervin, E.H., Reams, N., Zhang, X., Boyd, A., & Askew, S. (2017). An integrated nutritional and chemical approach to *Poa annua* suppression in creeping Bentgrass Greens. *Crop Science*, 57(2), 567–572. https://doi.org/10.2135/cropsci2016.05.0308

Esmaeili, S., Salehi, H., & Khosh-Khui, M. (2018). Direct and indirect *in vitro* plant regeneration of two commercial cultivars of perennial ryegrass. *Advances in Horticultural Science*, 32(2), 273–280. https://doi.org/10.13128/ahs-20654

Giolo, M., Dalla Montà, A., Barolo, E., Ferrari, F., Masin, R., & Macolino, S. (2017). High-temperature effects on seed germination of fourteen Kentucky bluegrass (*Poa pratensis* L.) cultivars. *Agronomy Research*, 15(1), 123–132.

Hsu, Y.Y., Chao, Y., & Kao, C.H. (2013). Cobalt chloride-induced lateral root formation in rice: The role of heme oxygenase. *Journal of Plant Physiology*, 170(12), 1075–1081. https://doi.org/10.1016/j.jplph.2013.03.004

Hussein, M.M., Faham, S.Y., & Alva, A.K. (2014). Role of foliar application of nicotinic acid and tryptophan on onion plants response to salinity stress. *Journal of Agricultural Science*, 6(8), 41-51. https://doi.org/10.5539/jas.v6n8p41

Kaiser, B.N., Gridley, K.L., Ngaire Brady, J., Phillips, T., & Tyerman, S.D. (2005). The role of molybdenum in agricultural plant production. *Annals of Botany*, 96(5), 745–754. https://doi.org/10.1093/aob/mci226

Linsmaier, E.M., & Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum*, 18(1), 100–127. https://doi.org/10.1111/j.1399-3054.1965.tb06874.x

Loewus, F.A., & Murthy, P.P. (2000). *Myo*-inositol metabolism in plants. *Plant Science*, 150(1), 1–19. https://doi.org/10.1016/s0168-9452(99)00150-8

Luo, Y., Qin, G., Zhang, J., Liang, Y., Song, Y., Zhao, ……,& Qu, L. (2011). D-*myo*-Inositol-3-Phosphate affects phosphatidylinositol-mediated endomembrane function in *Arabidopsis* and is essential for auxin-regulated embryogenesis. *The Plant Cell*, 23(4), 1352–1372. https://doi.org/10.1105/tpc.111.083337

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Pill, W.G., & Korengel, T.K. (1997). Seed priming advances the germination of Kentucky Bluegrass (*Poa pratensis* L.). *Journal of Turfgrass Management*, 2(1), 27–43. https://doi.org/10.1300/j099v02n01_03

Pizzeghello, D., Schiavon, M., Maretto, L., Stevanato, P., Ertani, A., Altissimo, A., & Nardi, S. (2019). Short-term application of polymer-coated mono-ammonium phosphate in a calcareous soil affects the pools of available phosphorus and the growth of *Hypericum* × *moserianum* (L.). *Frontiers in Sustainable Food Systems*, 3. https://doi.org/10.3389/fsufs.2019.00004

Schenk, R.U., & Hildebrandt, A.C. (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Canadian Journal of Botany*, 50(1), 199–204. https://doi.org/10.1139/b72-026

Toma, R.S., Danial, G.N., & Habash, A.H. (2012). *In vitro* morphogenetic response of Apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.) to the elevated levels of copper and *myo*-inositol. *Acta Agrobotanica*, 65(3), 43–48. https://doi.org/10.5586/aa.2012.005

Ueno, D., Rombolà, A.D., Iwashita, T., Nomoto, K., & Ma, J.F. (2007). Identification of two novel phytosiderophores secreted by perennial grasses. *New Phytologist*, 174(2), 304–310. https://doi.org/10.1111/j.1469-8137.2007.02056.x

Vanderschuren, H., Boycheva, S., Li, K., Szydlowski, N., Gruissem, W., & Fitzpatrick, T.B. (2013). Strategies for vitamin B_6 biofortification of plants: A dual role as a micronutrient and a stress protectant. *Frontiers in Plant Science*, 4. https://doi.org/10.3389/fpls.2013.00143

Walker, K.S., Bigelow, C.A., Smith, D.R., Van Scoyoc, G.E., & Reicher, Z.J. (2007). Aboveground responses of cool-season lawn species to nitrogen rates and application timings. *Crop Science*, 47(3), 1225–1236. https://doi.org/10.2135/cropsci2006.09.0595