JOURNAL OF BOTANICAL RESEARCH

Volume 1 | Issue 2 | July 2019 | ISSN 2630-5054 (Online)

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Volume 1 Issue 2 • July 2019 • ISSN 2630-5054 (Online)

Journal of Botanical Research

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Volume 1 | Issue 2 | July 2019 | Page 1-24
Journal of Botanical Research

Contents

Article

Review

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research
https://ojs.bilpublishing.com/index.php/jbr

ARTICLE

The Effects of Temperature, Light and Moisture on the Seed Germination of *Siphonostegia chinensis* Benth.

Shuaiqun Fan Kaiyu Qin Jingping Li Qinggui Zhang Fenguo Zhang Yongji Wang *

Shanxi Normal University, Linfen, Shanxi, 041004, China

ARTICLE INFO

Article history
Received: 21 May 2019
Accepted: 3 June 2019
Published Online: 30 July 2019

Keywords:
Seed germination
Siphonostegia chinensis Benth
PEG-6000
Drought dress

ABSTRACT

To explore the optimum temperature, light intensityand water conditions for seed germination of *Siphonostegia chinensis* Benth.,seed germination experiment were carried out under different temperatures (5/15, 10/20, 15/25, 20/30 ℃), different light intensity (14h light/10h darkness, complete darkness) and different concentrations (0%, 5%, 10%, 15%, 20%) of PEG-6000 solution. In terms of concentration, 5% PEG was regarded as the low level, 10% and 15% as the medium level, and 20% as the high level. The results showed that (1) Germination rate, germination potential, and germination index were increased with the rise of temperature. In addition, seed germination was significantly higher under the dark conditions than that with the 14h light/10h darkness. (2) No seed germination occurred when the temperature was below 10/20 ºC at 14h light/10h darkness. (3) Under 14h light/10h darkness, the germination rate, germination potential and germination index first increased and then decreased with the increase of PEG concentration. The low concentration was more beneficial to the seed germination. (4) Under the condition of complete darkness, the germination rate, germination potential and germination index decline with fluctuation with the increase of PEG concentration. Seed germination of *Siphonostegia chinensis* Benth. was inhibited in high concentration of PEG.

1. Introduction

Light intensity has a great impact on seed germination of plants and seedling growth, which is a key environmental factor on population regeneration of plants. The germination of plant seeds is the adaptive response to specific lighting conditions[1]. According to the different responses to light in the seed germination process, the seeds can be divided into positively photoblastic seeds, negatively photoblastic seeds and non-photoblastic seeds [2.3]. The effect of light on plant seed germination may be three types, such as stimulation, inhibition and no significant effect [4.5]. Therefore, the response of seeds to light can be used as a signal factor to indicate the appropriate environment for germination [6.7]. In addition, temperature and water are two key ecological factors for germination, especially for plants in arid and semi-arid regions [8].

Siphonostegia chinensis Benth, a species of Scrophulariaceae, Siphonostegia, is an annual herb, with the height of 30-80 cm. With an opposite leaf arrangement, flowers are arranged opposite on the upper part of stems and

**Corresponding Author:*
Yongji Wang,
Shanxi Normal University, Linfen, Shanxi, 041004, China;
Email: wangyongji126@126.com

 DOI: https://doi.org/10.30564/jrb.v1i2.861

branches, being loose racemes[9]. It has two lips on corolla, with the upper lip slightly purple and the lower lip yellow. It is widespread in China, such as dry mountain slopes and grasslands at 800-3400 m above sea level in the northeast China, central China, southern China, northern China, Inner Mongolia, southwest China. It is also found in Japan, South Korea, and Russia. Its entire parts have high medicinal value. Long-term gathering of wild plants leads to a sharp decline in the number of wild plants. The species have disappeared in some regions[9]. Therefore, the study of optimum germination conditions and growth environments of its seeds is of great value and significance. However, current research on *Siphonostegia chinensis* Benth. has focused on the study of chemical composition and the comparison of plant composition in southern and northern regions. Little research has been reported on the optimum germination and screening of growth conditions for the seeds of *Siphonostegia chinensis* Benth. On the basis of the background, the seeds of *Siphonostegia chinensis* Benth. were used as the primary materials in this study. Data of germination under different conditions (temperature, light, and water) were analyzed through filter paper germination method in Petri dishes to provide reference data for the study of optimum growth environment condition of germination, artificial planting, and introduction, thereby enlarging the application range of *Siphonostegia chinensis* Benth. and improving its economic value.

2. Materials and Methods

2.1 Experimental Materials

The experimental materials in this study were obtained from mature seeds of *Siphonostegia chinensis* Benth. in Huo Mountain (36°43′00″ – 36°25′50″ N, 111°47′15″ - 112°2′46″ E), Huozhou, Shanxi, China in October 2017. The mountain is located in the metamorphic zone formed 2.5 billion years ago. It has a warm temperate semi-humid continental monsoon climate. Its main characteristics are hot and less precipitation in summer, cold and dry in winter. The seeds were treated cleanly and placed in a 5 °C freezer for storage [10.11]. Healthy and full seeds with the uniform size were randomly selected as the experimental subjects in the study.

2.2 Experimental Methods

2.2.1 Seed Morphology Observation and Thousand Grain Weight Determination

Healthy and full seeds with the uniform size were obtained to determine the physical properties of seeds, such the thousand grain weight, length, and width. A total of 250 seeds were randomly selected to weigh on an analysis electronic balance with the readability of 0.001 g[12.13]. The experiment was replicated 4 times. The final mean of 4 sets of data was used as the thousand-seed weight value of *Siphonostegia chinensis* Benth.

2.2.2 Different Temperature and Light Treatment

While performing the experiment, 25 seeds were placed evenly in each disposable petri dish with a diameter of 90 mm and two layers of sterilized filter paper. All Petri dishes were numbered and four temperature gradients5/15℃、10/20℃、15/25℃、20/30℃ were set. In the meantime, two light treatments (14h light/10h darkness and complete darkness) were carried out at each temperature, with 4 repetitions per treatment. During the test, 2 mL of distilled water was added to each Petri dish to keep the filter paper moist in the Petri dish. In a set of repeat experiment, 4 Petri dishes were loaded into transparent zip-lock bags [14.15.16]. Petri dishes in the dark treatment were loaded into the opaque aluminum foil bag. All bags were placed in the monitoring-temperature incubators for culture. All the experiments were set to observe and record the germination situation at the same time every day from the 2nd day of the experimental layout. Radicle breaking through 2 mm of the seed coat was regarded as the germination standard [17.18]. If moldy seeds were found, the seeds were picked out with disinfectant tweezers and recorded in time during the test period, avoiding contaminating other seeds [5.19.20]. If seed germination was not seen for 3-4 consecutive days after the germination period, it was regarded as the end of the germination experiment. The vitality of the seeds was determined after the end of the experiment. The remaining seeds were gently pressed with tweezers under the microscope. If the seeds were hard, they were regarded as active species, and vice versa were non-active seeds. Active seedswere continuously cultured under the corresponding light and temperature conditions [21.22]. The experiment was terminated when the seeds were not germinated for two consecutive days. Finally, data were counted and analyzed to obtain the optimum environmental conditions for stimulating germination [23.24.25].

2.2.3 Different Moisture Gradient Treatment

According to the first round of experimental data, the temperature conditions with the highest germination rate and the fastest germination speed were selected. Under conditions of optimum constant temperature, 14h light/10h darkness and complete darkness, Petri disheswere cultured in different concentrations（0%、5%、10%、15%、20%、25%）of PEG-6000 to simulate water treatment.

DOI: https://doi.org/10.30564/jrb.v1i2.861

After marking the number, the media were cultured in the constant temperature incubator, the germination data are counted at the same time every day. During the test, the media were changed once two days to prevent the change of the medium concentration, seed mildew, or inaccurate experimental results [26.27].

2.3 Determined Indexes

Germination percentage (%) =total number of germinations / number of seeds tested×100%

Germination energy(%)=total number of germination peak / number of seeds tested×100%

Germination index $(GI) = \sum(Gt / Dt)$

Note: *Gt*(daily germination), *Dt*(the corresponding number of days of germination)

2.4 Data Processing

Microsoft Excel was used to perform statistics and calculation for the germination rate, germination potential, and germination index of seeds of *Siphonostegia chinensis* Benth. The variance, standard error and single-variable analysis were conducted with SPSS17.0 software. The significant differences between the factors were compared and the difference map was drawn by Microsoft Excel.

3. Results and Analysis

3.1 Thousand Seeds Weight, The Length and the Width of Seed

Thousand seeds weight 0.077±0.006g, seed length (1.019±0.062cm), seed width0.583±0.037cm.

3.2 The Impact of Light Duration on the Seeds of *Siphonostegia chinensis* Benth. under Different Temperature

The germination rate, germination potential and germination index of the seeds of *Siphonostegia chinensis* Benth. showed obvious difference under the condition of 5/15 °C,10/20 °C,15/25 °C,20/30 °C, 14h light/10h darkness, and complete darkness. Under the condition of 14h light/10h darkness, the seeds of *Siphonostegia chinensis* Benth. were not germinated in the 5/15°Cand 10/20 °Ctemperature range. With the same light, the germination rate, germination potential and germination index increased with the increase of temperature in 15/20 °Cand 20/30 °Ctemperature range. Under different light duration in 15/20 °Cand 20/30 °Ctemperature range, the difference between the germination rate, germination potential and germination index of *Siphonostegia chinensis* Benth. were statistically significant. In addition, the germination rate in the complete darkness was significantly greater than that of 14h light/10h darkness, indicating that high temperature and dark conditions were more suitable for seed germination of *Siphonostegia chinensis* Benth.

3.3 The Impact of Light Duration on the Seeds of *Siphonostegia chinensis* Benth. under Different PEG

Under the light duration of 14h light/10h darkness, the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. increased first and then decreased with the increase of PEG concentration. Among which, each index was the maximum at 5% of PEG concentration; differences of the indexes were statistically significant with 10% and 15% of the PEG concentration. Under the condition of complete darkness, the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. showed a fluctuating trend with the increase of PEG concentration. With the same light condition and 20% of PEG concentration, the seeds of *Siphonostegia chinensis* Benth. were not germinated. It demonstrated the low drought tolerance ability of *Siphonostegia chinensis* Benth.

4. Discussion and Conclusion

Seed germination requires environmental factors such as suitable water, oxygen, temperature or light. The environmental conditions required for different seed germination are various. The effects of different environmental factors diverse from each other, but they are related to each other and affect the life activities of seeds in an integrated way. Different plant seeds have various requirements for temperature conditions. Seedshave active metabolic reactions during germination. Therefore, in a certain temperature range, the germination process stimulates with the increase of temperature, whereas too elevated temperature results in denaturation in some living active substances, such as enzyme degeneration, and then it has a negative effect on germination. The experimental results showed that the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. were on the rise with the increase of temperature. the germination index reached the highest under 20/30 °C, and seed germination indexes of *Siphonostegia chinensis* Benth. under all dark conditions were significantly higher than that of 14h light/10h darkness. Seeds of *Siphonostegia chinensis* Benth. were not germinated at 10/20 °Ctemperature. This result is similar to the study results of Zheng et al. on*Artemisia* sphaerocephala.

 DOI: https://doi.org/10.30564/jrb.v1i2.861

Light is a key factor affecting seed dormancy, which is mainly controlled by the far original red-light absorption photosensitizer (Pfr) and red-light absorption photosensitizer in the seed [28.29]. The effect of light on seed germination is different because of different threshold requirement of original Pfr content to the Pfr/Pr. Quiros et al. proposed that the light cycle demand for the growth of the Lepidium meyenii was not clear. In the origin of Lepidium meyenii, the daytime was less than 13h during the growth, indicating that it may be a short-day or mid-day plant. This study showed that seed germination indexes of *Siphonostegia chinensis* Benth. undercomplete dark conditions were significantly higher than that of 14h light/10h darkness. However, under the condition of drought, the germination rate, germination potential and germination index of *Siphonostegia chinensis* Benth. firstly increased and then decreased at 14h light/10h darkness, and the low concentration was more conducive to the seed germination of *Siphonostegia chinensis* Benth. The germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. under all dark conditions showed a fluctuating decline trend, inhibiting the seed germination.

Moisture is an important factor affecting seed germination. Because the seed coat permeability of each species and the water absorption of seed internal components are quite different, seed germination of each species is of great difference in the water potential of the environment, and the minimum moisture required for seed germination of different plants is also various. Some studies showed that the seeds of some species could not germinate when the concentration of PEG-6000 was higher than 15%. Wild chrysanthemum seeds could not germinate when the concentration of PEG-6000 was greater than 20% [30.31.32]. In this study, it showed that the germination rate, germination potential, and germination index of the seeds of *Siphonostegia chinensis* Benth. firstly increased and then decreased with the rise of PEG concentration and 14h light/10h darkness. The low concentration was more conducive to seed germination of *Siphonostegia chinensis* Benth. The germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. under all dark conditions showed a fluctuating decline trend with the rise of PEG concentration. Under the high concentration and drought condition, the seed germination was inhibited.

In conclusion, suitable water, temperature, and light are key techniques for seed germination of *Siphonostegia chinensis* Benth. This result provides a scientific reference and an effective way for the protection and rational development and utilization of introduction and cultivation of *Siphonostegia chinensis* Benth. as one of wild Traditional Chinese medicine resources Furthermore, there are diverse factors affecting seed germination and seedling growth of *Siphonostegia chinensis* Benth., which need to further study.

Acknowledgements

Thisstudy was supported by the National Natural Science Foundation of China (NSFC: 41801027) and the National Natural Science Foundation of China (NSFC: 3170043).

Supplements

Table1. The impact of light duration on the seeds of *Siphonostegia chinensis* Benth. under different temperature

temperature/℃	Light time/h·d-1	Germination percentage	Germination energy	Germination index
5/15℃	14	0.00±0.00d	0.00±0.00e	0.00±0.00c
10/20℃	14	0.00±0.00d	0.00±0.00e	0.00±0.00c
15/25℃	14	8.25±3.69d	7.00±2.00d	0.26±0.14d
20/30℃	14	28.50±4.73c	15.25±3.60c	0.96±0.16bc
5/15℃	0	0.00±0.00d	0.00±0.00e	0.00±0.00c
10/20℃	0	0.00±0.00d	0.00±0.00e	0.00±0.00c
15/25℃	0	42.00±12.44b	22.00±5.16b	1.24±0.44b
20/30℃	0	67.00±8.87a	40.00±8.64a	2.15±0.36a

Table 2. The impact of light duration on the seeds of *Siphonostegia chinensis* Benth. under different PEG

PEG concentration/%	Light time/h·d^{-1}	Germination percentage	Germination energy	Germination index
0	14	28.50±4.73c	15.25±3.59bcd	0.96±0.16bc
5	14	46.00±15.49b	24.00±9.80b	1.07±0.37b
10	14	39.00±16.13bc	22.00±12.44b	0.98±0.43bc
15	14	11.00±3.83de	7.00±2.00cde	0.28±0.93de
20	14	5.00±3.83e	4.00±3.27de	0.12±0.94e
0	0	67.00±8.87a	40.00±8.64a	2.15±0.36a
5	0	37.00±12.38bc	19.00±8.25bc	0.82±0.29bc
10	0	41.00±11.49bc	19.00±8.25bc	0.95±0.29bc
15	0	26.00±15.49cd	16.00±10.83bcd	0.59±0.37cd
20	0	0.00±0.00e	0.00±0.00e	0.00±0.00e

DOI: https://doi.org/10.30564/jrb.v1i2.861

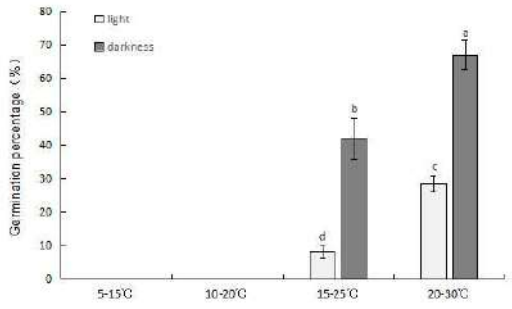

Figure 1. The germination percentage impact of light duration on the seeds of Siuphonostegia chinens is Benth. unedr different temperature.

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

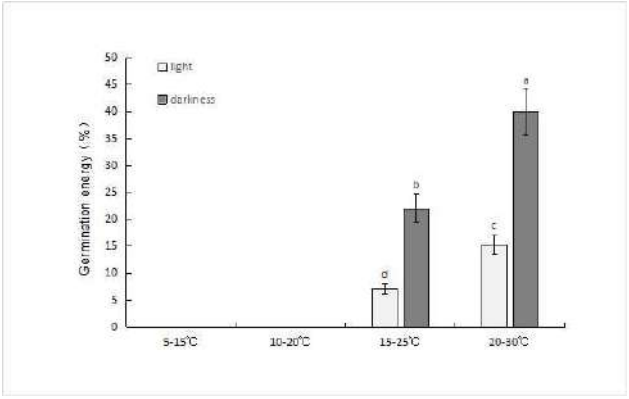

Figure 2. The germination energy impact of light duration on the seeds of Siphonostegia chinens is Benth. under different temperatures.

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

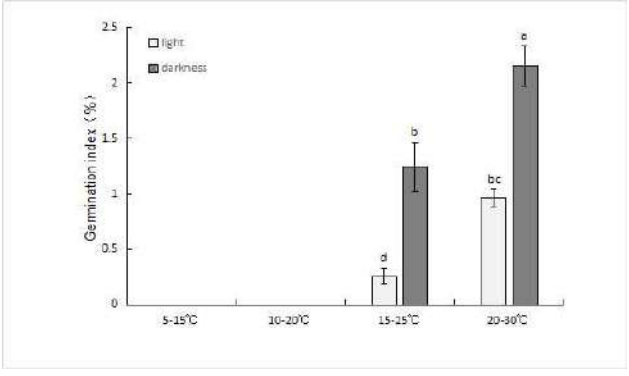

Figure 3. The germination index impact of light duration on the seeds of Siphonostegia chinens is Benth. under different temperatures

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

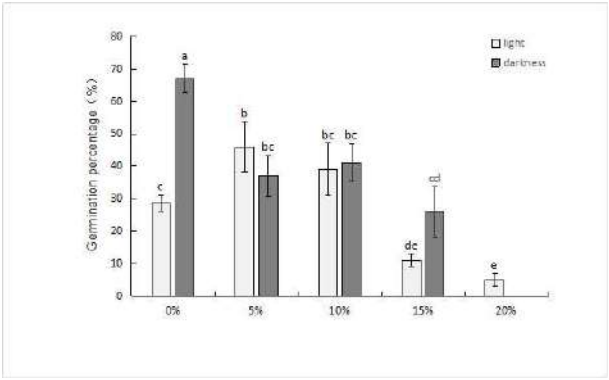

Figure 4. The germination percentage impact of light duration on the seeds of Siphonostegia chinens is Benth. under dfferent PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

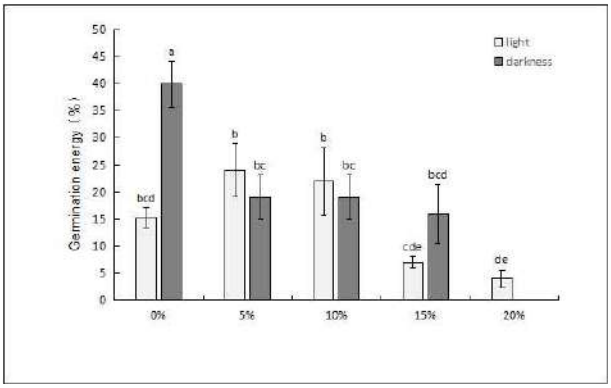

Figure 5. The germination energy impact of light duration on the seeds ofSiphonostegia chinens is Benth. under different PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

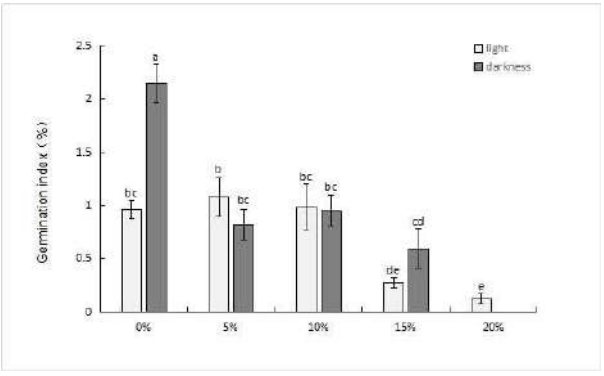

Figure 6. The germination index impact of light duration on the seeds of Siphonostegia chinens is Benth. under different PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

DOI: https://doi.org/10.30564/jrb.v1i2.861

References

[1] Xia, Q.Q., Ando, M. & Seiwa, K.. Interaction of seed size with light quality and temperature regimes as germination cues in 10 temperate pioneer tree species. Functional Ecology, 2016, 30: 866-874.

[2] Zhu, Y.J., Yang, X.J., Baskin, C.C., Baskin, J.M., Dong, M. & Huang, Z.Y.. Effects of amount and frequency of precipitation and sand burial on seed germination, seedling emergence and survival of the dune grass Leymus secalinus in semiarid China. Plant and Soil, 2014, 374: 399-409.

[3] Yang, F., Baskin, J.M., Baskin, C.C., Yang, X.J., Cao, D.C. & Huang, Z.Y.. Effects of germination time on seed morph ratio in a seed-dimorphic species and possible ecological significance. Annals of Botany, 2015, 115: 137-145.

[4] Baskin, J.M. & Baskin, C.C.. Seed germination in cleistogamous species: theoretical considerations and a literature survey of experimental results. Seed Science Research, 2017, 27: 84-98.

[5] Auge, G.A., Blair, L.K., Karediya, A. & Donohue, K.. The autonomous flowering-time pathway pleiotropically regulates seed germination in Arabidopsis thaliana. Annals of Botany, 2018, 121: 183-191.

[6] Bowden, L. & Landais, L.. The impact of light and high light on seed germination and the radicle emergence test. Seed Science and Technology, 2018, 46: 465-471.

[7] Budzen, M., Kornacki, A., Kornarzynski, K. & Sujak, A.. Effect of laser light on germination of Lavatera thuringiaca L. seeds studied by binomial distribution. Seed Science and Technology, 2018, 46: 447-464.

[8] Wang, Y.J., Lai, L.M., Du, H., Jiang, L.H., Wang, F., Zhang, C., Zhuang, P. & Zheng, Y.R.. Phylogeny, habitat together with biological and ecological factors can influence germination of 36 subalpine Rhododendron species from the eastern Tibetan Plateau. Ecology and Evolution, 2018, 8: 3589-3598.

[9] Gao, J., Jin, Y.H., Li, Y.Q., Qian, Y.X., Zhang, C., Wang, R.H. & Qi, Z.C.. The complete chloroplast genome sequence of *Siphonostegia chinensis* Benth. (Orobanchaceae). Mitochondrial DNA Part B-Resources, 2019, 4: 732-733.

[10] Adams, C.A., Baskin, J.M. & Baskin, C.C.. Trait stasis versus adaptation in disjunct relict species: evolutionary changes in seed dormancy-breaking and germination requirements in a subclade of Aristolochia subgenus Siphisia (Piperales). Seed Science Research, 2005, 15: 161-173.

[11] Wang, Y.J., Wang, J.J., Lai, L.M., Jiang, L.H., Zhuang, P., Zhang, L.H., Zheng, Y.R., Baskin, J.M. & Baskin, C.C.. Geographic variation in seed traits within and among forty-two species of Rhododendron (Ericaceae) on the Tibetan plateau: relationships with altitude, habitat, plant height, and phylogeny. Ecology and Evolution, 2014, 4: 1913-1923.

[12] Gonzalez-Zertuche, L., Orozco-Segovia, A., Baskin, C. & Baskin, J.M.. Effects of priming on germination of Buddleja cordata ssp cordata (Loganiaceae) seeds and possible ecological significance. Seed Science and Technology, 2002, 30: 535-548.

[13] Jayasuriya, K.M.G.G., Wijetunga, A.S.T.B., Baskin, J.M. & Baskin, C.C.. Seed dormancy and storage behaviour in tropical Fabaceae: a study of 100 species from Sri Lanka. Seed Science Research, 2013, 23: 257-269.

[14] El Aou-ouad, H., Medrano, H., Lamarti, A. & Gulias, J.. Seed germination at different temperatures and seedling emergence at different depths of Rhamnus spp. Central European Journal of Biology, 2014, 9: 569-578.

[15] Gorai, M., El Aloui, W., Yang, X.J. & Neffati, M.. Toward understanding the ecological role of mucilage in seed germination of a desert shrub Henophyton deserti: interactive effects of temperature, salinity and osmotic stress. Plant and Soil, 2014, 374: 727-738.

[16] Fernandes, T.V., Paolucci, L.N., Carmo, F.M.S., Sperber, C.F. & Campos, R.I.. Seed manipulation by ants: disentangling the effects of ant behaviours on seed germination. Ecological Entomology, 2018, 43: 712-718.

[17] Al-Quraan, N.A., Al-Sharbati, M., Dababneh, Y. & Al-Olabi, M.. Effect of Temperature, Salt and Osmotic Stresses on Seed Germination and Chlorophyll Contents in Lentil (Lens culinaris Medik). International Conference on Agricultural Engineering: New Technologies for Sustainable Agricultural Production and Food Security, 2014, 1054: 47-54.

[18] Aguiar, F.F.A., Kanashiro, S., Giampaoli, P., Modolo, V.A., de Aguiar, J. & Tavares, A.R.. EFFECTS OF LIGHT, TEMPERATURE AND MESOCARP ON SEED GERMINATION OF Euterpe edulis (JUCARA-PALM). Bioscience Journal, 2017, 33: 881-885.

[19] Dias, E.F., Moura, M., Schaefer, H. & Silva, L.. Interactions between temperature, light and chemical promoters trigger seed germination of the rare Azorean lettuce, Lactuca watsoniana (Asteraceae). Seed Science and Technology, 2015, 43: 133-144.

[20] Dewan, S., Vander Mijnsbrugge, K., De Frenne, P., Steenackers, M., Michiels, B. & Verheyen, K.. Maternal temperature during seed maturation affects

DOI: https://doi.org/10.30564/jrb.v1i2.861

seed germination and timing of bud set in seedlings of European black poplar. Forest Ecology and Management, 2018, 410: 126-135.

[21] Baskin, C.C. & Baskin, J.M.. Seed germination ecology of Lesquerella lyrata Rollins (Brassicaceae), a federally threatened winter annual. Natural Areas Journal, 2000, 20: 159-165.

[22] Baskin, C.C. & Baskin, J.M.. Seed germination and propagation of Xyris tennesseensis, a federal endangered wetland species. Wetlands, 2003, 23: 116-124.

[23] Tobe, K., Zhang, L.P. & Omasa, K.. Alleviatory effects of calcium on the toxicity of sodium, potassium and magnesium chlorides to seed germination in three non-halophytes. Seed Science Research, 2003, 13: 47-54.

[24] Lewandrowski, W., Erickson, T.E., Dalziell, E.L. & Stevens, J.C.. Ecological niche and bet-hedging strategies for Triodia (R.Br.) seed germination. Annals of Botany, 2018, 121: 367-375.

[25] Soltani, E., Baskin, C.C., Baskin, J.M., Heshmati, S. & Mirfazeli, M.S.. A meta-analysis of the effects of frugivory (endozoochory) on seed germination: role of seed size and kind of dormancy. Plant Ecology, 2018, 219: 1283-1294.

[26] Awty-Carroll, D., Clifton-Brown, J. & Robson, P.. Using k-NN to analyse images of diverse germination phenotypes and detect single seed germination in Miscanthus sinensis. Plant Methods, 2018, 14.

[27] Cochrane, A.. Salt and waterlogging stress impacts on seed germination and early seedling growth of selected endemic plant species from Western Australia. Plant Ecology, 2018, 219: 633-647.

[28] Tiansawat, P. & Dalling, J.W.. Differential seed germination responses to the ratio of red to far-red light in temperate and tropical species. Plant Ecology, 2013, 214: 751-764.

[29] Zhang, C.H., Willis, C.G., Burghardt, L.T., Qi, W., Liu, K., Souza, P.R.M., Ma, Z. & Du, G.Z.. The community-level effect of light on germination timing in relation to seed mass: a source of regeneration niche differentiation. New Phytologist, 2014, 204: 496-506.

[30] Haghighi, M., Da Silva, J.A.T., Mozafarian, M. & Afifipour, Z.. Can Si and nano-Si alleviate the effect of drought stress induced by PEG in seed germination and seedling growth of tomato? Minerva Biotecnologica, 2013, 25: 17-22.

[31] Abouzaid, E., El-Sayed, E.S.N., Mohamed, E.S.A. & Youssef, M.. Molecular Analysis of Drought Tolerance in Guava Based on In Vitro PEG Evaluation. Tropical Plant Biology, 2016, 9: 73-81.

[32] Bali, S., Nkomo, M. & Klein, A.. The influence of PEG-induced water stress on seed germination, plant growth and photosynthetic metabolism in chia plants. South African Journal of Botany, 2018, 115: 316-316.

 DOI: https://doi.org/10.30564/jrb.v1i2.861

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research
https://ojs.bilpublishing.com/index.php/jbr

ARTICLE

Effects of Exogenous Calcium on Datura Seed Germination under Drought Stress

Kaiyu Qin Shuaiqun Fan Fenguo Zhang Yongji Wang*

Shanxi Normal University, Linfen, Shanxi, 041004, China

ARTICLE INFO

Article history
Received: 21 May 2019
Accepted: 3 June 2019
Published Online: 30 July 2019

Keywords:
Datura
Drought stress
Exogenous calcium
Seed germination

ABSTRACT

With polyethylene glycol (PEG-6000), of 0% (CK), 5%, 10%, 15%, 25% used to simulate drought stress, and CaCl2 concentration 0 (CK), of 15, 20, 25 and 30mmol/L as ion gradient of exogenous calcium, the effects of drought, exogenous calcium and the interaction between the two on the Datura seed germination, so as to explore the optimal application amount of exogenous calcium to ease the suppression of drought stress on Datura seed germination. The results showed that the germination rate, germination potential and germination index of the Datura seeds were significantly lower than those of the control group. Under the normal moisture condition, exogenous calcium of moderate and low concentration had no significant effect on the Datura seed germination, while that of high concentration showed an inhibitory effect on the seed germination. Under drought stress, with the increasing concentration of exogenous calcium, the three indicators of Datura seeds showed a trend of increasing first and then decreasing. When the exogenous calcium had the concentration of 20 mmol/L, all the indicators of seed germination reached the maximum value, while showed a downward trend when exogenous calcium concentration was 25-30 mmol/L, and even increasingly sharp with drought intensifying. Therefore, in the production and utilization of Datura, 20 mmol/L of exogenous calcium can be used to soak seeds before sowing to improve the emergence rate under low and moderate drought conditions.

1. Introduction

Seeds are an important stage in the life history of a plant, and an important time for the study of drought resistance of the plant [1]. The germination rate, germination potential and germination index of the seeds mirror the germination speed, uniformity and the strength potential of seedlings, all of which declined dramatically with the increasing of drought stress intensity [2,3]. Under the action of severe drought stress (over 15%), the seed germination rate was extremely low, implying that even if the seeds were germinated, the growth of the seedlings was significantly inhibited, showing that it is feasible to study the drought resistance of Datura with PEG solution of different osmotic potential gradients to simulate drought stress [3].

33.6 percent of the land area on earth is arid or semi-arid. Years of research data show that among the meteorological disasters in China, drought influences as much as half the national territorial area, more seriously than flood(27.8%) [4]. In the blue book of China's Science and

**Corresponding Author:*
Yongji Wang,
Shanxi Normal University, Linfen, Shanxi, 041004, China;
Email: wangyongji126@126.com

DOI: https://doi.org/10.30564/jrb.v1i2.862

Technology, drought is listed as top of climate disasters in China. Since the beginning of the 21st century, drought occurs every year in China, different in impact scope and severity, but seasonal drought occurs almost yearly [5,6]. Compared with the second half of the 20th century, the frequency of drought has increased significantly, and the average area affected by drought remains almost the same, but the percentage of the disaster area has increased [7,8].

Since Shanxi Province is home to limestone resources [9], plus the requirements of national development, mountain quarrying has become an important economic source in parts of Shanxi Province. However, because of the fact that the quarrying technology is not advanced, the policy provisions are not strict enough, with excessive pursuit of economic benefits, over-exploitation and the like inappropriate actions, the regional ecological environment has been dramatically damaged. There lies a decline in biodiversity, serious soil erosion, environmental pollution, broad distribution of bare rock, etc [10,11]. The exposed quarries and quarrying wasteland will lead to intensified soil erosion, loss of species diversity, waste of resources, reduced vegetation cover, and the like problems [12,13,14]. However, with a further increase in the demand for limestone, the mining scale is expanding year by year, and problems like environmental damage becomes increasingly prominent, so is the difficulty of rectification. The recovery management of limestone after mining is urgent [15]. In the process of ecological restoration, the selected species need to show good adaptability to the soil environment with high calcium.

In recent years, the impact of the mining of such limestone mountains on the environment in Shanxi Province has been studied by many experts and scholars, who try to solve the environmental problems in limestone lands through a scientific method and approach [16,17]. Unfortunately, there has been no mature theoretical and technical standard for this technology. In the restoration project, imported plants like Bermudagrass, Chloris virgata, and alfalfa are adopted. These plants have various problems, such as their ability to adapt to the slope habitat and the distinct climate of the Loess Plateau, their shortcomings of single variety, poor resistance against diseases, population degradation, potential threat to the native species and so on [15,18,19,20,21,22]. However, there is less report on the restoration of limestone mountains with native species.

Datura stramonium Linn. can be seen throughout the country. As a herb or suffruticose therophyte, Datura grows well in places with abundant light. Since it is a heliophyte, it has a strong adaptability to the environment, with low requirements for soil conditions [23,24,25,26]. If there is a piece of soil rich in organic matter and calcareous, Datura can live here quite well. The Chinese herbal medicine field has not only developed a long history, but also has won a good reputation at home and abroad. As one of the traditional Chinese herbal medicines in China, Datura has been recognized and applied for a long time. Its seeds can be used to treat diseases like insomnia and headache, its leaves can be used to treat asthma, its flowers can be used for cough relieving, and furthermore, its flowers also have a positive effect in drugs cessation, especially for the elimination of heroin, which can effectively reduce the relapse of drug addicts [27,28,29,30].The Datura flower is relatively large, with a high ornamental value for a brightly beautiful color, tubular flower buds and a funnel-shaped corolla, so it plays an irreplaceable role in gardening, urban greening construction, environment beautifying, soil conditions improvement, etc. What's more, Datura has volatile oil with complex components, which can be used to make green pesticides, so it is of great value in agriculture and forestry [23,31,32,33,34].

The materials collected in this experiment are located in the limestone area of Huoshan, Linfen City, Shanxi Province, where it is dry, with low precipitation and many calcium ions. Therefore, the effects of different PEG and calcium chloride concentrations as well as the interaction of PEG and calcium chloride on seed germination can be explored[35,36], thereby providing references and a theoretical basis for the reproduction of native Datura and a scientific basis for the germination conditions of Datura seeds. In addition, it can also provide references for the improvement of the ecological adaptability and ecological restoration of Datura.

2. Materials and Method

2.1 Experimental Materials

Collection time of Datura seeds for the test: October 2017; collection location: Huoshan, Shanxi Province.

2.2 Experimental Method

The $CaCl_2$ concentration was designed to be the five gradients of 0, 15, 20, 25 and 30mmol/l, respectively, to simulate exogenous calcium. The PEG — 6000 solution was used to simulate the drought stress, and it was set a total of six osmotic pressure gradients0%, 5%, 10%, 15%, 25%at room temperature (25±2℃). The two factors have a total of 30 treatments, each of which was repeated 4 times.

The seed germination test was carried out on the petri dish paper. Full seeds were selected and placed on the petri dishes which has two layers of filter paper at the bottom. On each petri dish lay 25 seeds, with 2mL of

 DOI: https://doi.org/10.30564/jrb.v1i2.862

pre-configured $CaCl_2$ and PEG solution added into it, and then the petri dishes were placed in a constant temperature incubator of 25 ℃ for germination. Every day, the number of germinated seeds was observed and recorded, and the fact of germ breaking through the seed coat by 2mm was taken as the standard of germination, and the evaporated moisture was supplemented every day.

Seed germination rate (Gr) = the number of germinated seeds in 15 days/ the number of seeds tested × 100%.

Germination potential (Gp) = the number of normally germinated seeds in the first 5 days / the total number of seeds × 100%.

Germination index (Gi) = MDG • PV. Seed vigor index (Vi) = seedling growth potential (seedling fresh quality) × Gi.

In the equations, MDG is the average number of seed germination per day, i.e. the number of germinated seeds at the end of the germination test / the number of days of the germination test; PV is the maximum germination rate of the seed, i.e. the largest germination number on any day during the test /the number of days the maximum value needed.

2.3 Data Processing

All data were processed with SPSS 16 software. The two-way analysis of variance and the least significant difference (LSD) were used to compare the differences between different data sets. The significance level was set as α=5.

3. Results and Analysis

3.1 The Length, Short Diameter, Thickness and Weight of Datura Seed

A mature Datura seed appears to be of a slight brownish or blackish color. The seed is morphologically large, kidney-shaped, with a leathery seed coat of a waxy structure. The umbilicus is triangular and inwardly recessed. The length diameter, short diameter, thickness and TKW of the seed are shown in Table 1.

3.2 Effects of Drought on the Datura Seed Germination

The germination rate (GR), germination potential (GP) and germination index (GI) of the Datura seeds decreased with the increase of drought stress intensity, and the difference between the drought treatments reached a significant level ($P< 0.05$). Based on table 2, it can be known that under normal moisture conditions, the germination rate and germination potential were 64% and 37%, respectively, both of the two indicators significantly reduced when drought stress was 15%, and reached the minimum when drought stress intensity was 25%, 21.8% and 21.6% of the control respectively. The GI of the Datura seeds was 1.47 and decreased gradually with the increase of drought stress intensity and reached the minimum at 25%, 77% of the control, indicating that drought stress seriously affected the Datura seed germination.

3.3 Effects of Exogenous Calcium on Datura Seed Germination

The temperature of the seed germination period of the limestone mountain was simulated, i.e. the temperature of 15/25°C, under which condition the Datura seed germination test was conducted with different $CaCl_2$ concentrations. The results analysis of the germination rate of the Datura seeds are shown in Table 3. With the changing of $CaCl_2$ concentration, the germination rate, germination potential and germination index of the seeds vary sharply, that is, first increase and then decrease. The germination rate varies little among the treatment groups, and the other two indexes reduced to the lowest at 30 mmol/L of $CaCl_2$, which was significantly different from the other control groups. The results showed that exogenous calcium had little effect on the Datura seed germination, but inhibited it when the concentration reached to be 30%.

3.4 Effects of Interaction between Exogenous Calcium and Drought on the Germination Rate of Datura Seed

The two factors analysis of variance on the three indicators of Datura showed that the interaction between exogenous calcium and drought had an effect on the germination rate, germination potential and germination index of Datura seeds, and all the three indicators reached a significant level ($P<0.05$).

According to Table 2, under the same drought stress gradient, the germination rate, germination potential, germination index and vigor index of Datura all rised first and then reduced with the increase of exogenous calcium concentration. When the drought stress was 5%, all the four indexes significantly decreased when the exogenous calcium concentration was 30mmol/L ($P<0.05$). When the drought stress was 10%, the germination rate and germination potential index reached the maximum when the exogenous calcium concentration was 20mmol/L, which were 0.64 and 0.34, respectively, reaching a significant difference ($P<0.05$). With a further

DOI: https://doi.org/10.30564/jrb.v1i2.862

increase in the exogenous calcium concentration, the three indicators began to decline, and the four indicators were significantly lower than the value at 0% when the exogenous calcium concentration was 30 mmol/L. When the drought stress was 15%, the three indexes reached the maximum when the exogenous calcium concentration was 20mmol/L, which was 1.23, 1.29, and 1.14 times those at 0%, respectively. When the exogenous calcium concentration was 30mmol/L, the three indexes decreased sharply, 0.56, 0.76, and 0.59 times those at 0%, respectively.When the drought stress was 25%, the three indicators reached the maximum when the exogenous calcium concentration was 20 mmol/L, with no significant difference from the control group under their own drought gradient, and then reduced with the increase of exogenous calcium concentration, the three indexes were significantly lower than those at 0% of the drought gradient ($P<0.05$).It can be seen that the exogenous calcium of appropriate concentration (20mmol/L) can promote the Datura seed germination under drought stress, especially the moderate drought level (10%, 15%), while that of high concentration (25~20mmol/L) would further inhibit the Datura seed germination under drought stress.

4. Results

Datura has a high medical, agroforestrial and ornamental value. The first important problem that must be faced in the cultivation and application of Datura is that the germination rate of Datura seeds is relatively low. Datura seeds are naturally in dormant state, with a dormancy period of about 6-8 months. The Datura seeds selected for this experiment were collected in early October 2017 and the experiment started in mid-March 2018, so the seeds had been stored for nearly 6 months, and the substances inhibiting germination in the seeds had almost been decomposed. The dormant state had been broken, so no mechanical friction or other processing of the seed was needed during the test.

Calcium is one of the essential elements for the growth of advanced plants. As a signal substance, Ca^{2+} involves in the physiological processes such as plant growth and development, seed dormancy and germination, etc. Also, Calcium is an activator for some enzymes like ATP, hydrolysis, dehydrogenation of succinic acid, etc. The exogenous Ca^{2+} of an appropriate concentration can promote the increase of endogenous free Ca^{2+} content in seeds, while the intracellular free Ca^{2+} and calmodulin are combined to directly or indirectly regulate the activity of relevant enzymes and cell function within the cell, thus to a certain extent reduce the damage of the adversity to the seed and improve the seed germination and vitality. In this study, under normal moisture conditions, 15%-20% of exogenous calcium showed no significant effect on the germination rate, germination potential and germination index of Datura, with differences not reaching a significant level compared with the control group, indicating that exogenous calcium had no positive effect on the Datura seed germination, which varies from the results of previous studies. In the experiment, when the concentration of exogenous calcium ion was 30%, the difference between the treatments reached a significant level, implying that exogenous calcium of high concentration inhibited the Datura seed germination. Exogenous calcium and drought have a significant interactive impact on the Datura seed germination.Under drought stress, exogenous calcium of an appropriate concentration (20%) could promote the Datura seed germination. Under moderate drought conditions (10%, 20%), the promotion was noticeable; severe drought ruined the physiological mechanism of the Datura seed, so exogenous calcium showed no significant effect. Therefore, for the production and utilization of Datura, 20% of exogenous calcium can be applied to soak the seeds before sowing to enhance the germination rate in the moderate arid areas.

Thanks to the weathering effect and some of human activities in the Huoshan Mountain area, a special geological condition---saline-alkali land---formed. The saline-alkali land features calcium ions of a high concentration and drought. Datura seeds have certain tolerance to calcium ions and can germinate normally under the soil conditions in the Huoshan Mountain area. This has a positive effect on improving soil quality of the Huoshan Mountain area.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NSFC: 41801027) and the National Natural Science Foundation of China (NSFC: 31700434).

Supplements

Table 1. The length diameter, short diameter, thickness and TKW of Datura seed

	length diameter (mm)	short diameter (mm)	thickness (mm)	mass of 1000 seeds/g (g)
max	3.49	2.77	1.40	6.472
min	2.77	2.46	1.20	6.151
ave	3.12	2.64	1.31	6.227

 DOI: https://doi.org/10.30564/jrb.v1i2.862

Table 2. Effects of drought on seed germination rate, germination potential and germination index of Datura seed

PEG-6000 %	Germination rate	Germination potential	Vigor index
CK	0.64±0.07^{a}	0.37±0.04^{a}	1.47±0.36^{a}
5	0.58±0.09^{ab}	0.31±0.05^{b}	1.36±0.23^{ab}
10	0.53±0.04^{b}	0.29±0.03^{b}	1.33±0.13^{ab}
15	0.30±0.08^{c}	0.17±0.05^{c}	1.29±0.09^{ab}
25	0.14±0.06^{d}	0.08±0.06^{d}	1.14±0.09^{b}

Note: different letters in the table indicate significant differences in data (P<0.05).

Table 3. Effects of Calcium Chloride on Germination Rate, Germination Potential and Germination Index of Datura Seed

$CaCl_2$mmol/L	Germination percentage	Germination energy	Germination index
CK	0.64±0.07^{a}	0.37±0.04^{ab}	1.47±0.36^{bc}
15	0.63±0.07^{a}	0.40±0.05^{ab}	1.78±0.42^{ab}
20	0.68±0.03^{a}	0.42±0.03^{ab}	1.88±0.27^{a}
25	0.61±0.11^{a}	0.49±0.11^{a}	1.46±0.20^{bc}
30	0.57±0.05^{a}	0.34±0.10^{b}	1.33±0.14^{c}

Note: different letters in the table indicate significant differences in data (P<0.05).

Table 4. Effects of interaction of drought and calcium chloride on seed germination rate, germination potential and germination index of Datura seed

PEG-6000 %	$CaCl_2$mmol/L	Germination rate	Germination potential	Vigor index
0	CK	0.64±0.07^{a}	0.37±0.04^{a}	1.47±0.36^{a}
5	0	0.58±0.09^{ab}	0.31±0.05^{ab}	1.36±0.23^{a}
	15	0.62±0.08^{a}	0.36±0.05^{a}	1.47±0.22^{a}
	20	0.64±0.07^{a}	0.37±0.04^{a}	1.47±0.36^{a}
	25	0.57±0.07^{ab}	0.33±0.06^{ab}	1.35±0.32^{a}
	30	0.53±0.06^{b}	0.30±0.04^{ab}	1.31±0.33^{a}
10	0	0.53±0.04^{b}	0.29±0.03^{ab}	1.61±0.19^{a}
	15	0.56±0.04^{ab}	0.30±0.03^{ab}	1.45±0.22^{a}
	20	0.61±0.05^{a}	0.34±0.05^{a}	1.38±0.08^{ab}
	25	0.52±0.07^{b}	0.31±0.03^{ab}	1.42±0.40^{b}
	30	0.44±0.06^{bc}	0.26±0.02^{b}	1.08±0.32^{b}
15	0	0.30±0.08^{c}	0.17±0.05^{c}	0.92±0.29^{b}
	15	0.33±0.05^{c}	0.18±0.03^{c}	0.68±0.14^{bc}
	20	0.37±0.03^{bc}	0.22±0.02^{bc}	1.05±0.22^{b}
	25	0.28±0.03^{cd}	0.17±0.02^{c}	0.59±0.09^{c}
	30	0.17±0.06^{d}	0.13±0.03^{cd}	0.55±0.17^{c}
25	0	0.14±0.06^{d}	0.08±0.06^{de}	0.40±0.19^{c}
	15	0.17±0.05^{d}	0.11±0.05^{d}	0.47±0.16^{c}
	20	0.19±0.07^{d}	0.12±0.04^{d}	0.46±0.17^{c}
	25	0.10±0.03^{e}	0.08±0.03^{de}	0.36±0.13^{c}
	30	0.04±0.03^{e}	0.04±0.03^{f}	0.15±0.10^{e}

Note: different letters in the table indicate significant differences in data (P<0.05).

References

[1] Wang, Y.J., Wang, J.J., Lai, L.M., Jiang, L.H., Zhuang, P., Zhang, L.H., Zheng, Y.R., Baskin, J.M. & Baskin, C.C.. Geographic variation in seed traits within and among forty-two species of Rhododendron (Ericaceae) on the Tibetan plateau: relationships with altitude, habitat, plant height, and phylogeny. Ecology and Evolution, 2014b, 4: 1913-1923.

[2] Baskin, J.M. & Baskin, C.C.. Pollen limitation and its effect on seed germination. Seed Science Research, 2018, 28: 253-260.

[3] Wang, Y.J., Lai, L.M., Du, H., Jiang, L.H., Wang, F., Zhang, C., Zhuang, P. & Zheng, Y.R.. Phylogeny, habitat together with biological and ecological factors can influence germination of 36 subalpine Rhododendron species from the eastern Tibetan Plateau. Ecology and Evolution, 2018, 8: 3589-3598.

[4] Dong, L.L., Zhang, H.D., Wang, L.Q., Yu, D.S., Yang, F.X., Shi, X.Z., Saleem, H. & Akhtar, M.S.. Irrigation with sediment-laden river water affects the soil texture and composition of organic matter fractions in arid and semi-arid areas of Northwest China. Geoderma, 2018, 328: 10-19.

[5] Zhu, H.X., Fu, B.J., Lv, N., Wang, S. & Hou, J.. Multivariate control of root biomass in a semi-arid grassland on the Loess Plateau, China. Plant and Soil, 2014, 379: 315-324.

[6] Cui, Y.X., Fang, L.C., Guo, X.B., Wang, X., Zhang, Y.J., Li, P.F. & Zhang, X.C.. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. Soil Biology & Biochemistry, 2018, 116: 11-21.

[7] Yu, T.F., Feng, Q., Si, J.H., Xi, H.Y., Li, Z.X. & Chen, A.F.. Hydraulic redistribution of soil water by roots of two desert riparian phreatophytes in northwest China's extremely arid region. Plant and Soil, 2013, 372: 297-308.

[8] Zhang, S.P., Shao, M.G. & Li, D.F.. Prediction of soil moisture scarcity using sequential Gaussian simulation in an arid region of China. Geoderma, 2017, 295: 119-128.

[9] Jiang, A.W., Jiang, D.M., Goodale, E. & Wen, Y.G.. Nest predation on birds that nest in rock cavities in a tropical limestone forest of southern China. Global Ecology and Conservation, 2017, 10: 154-158.

[10] Gao, L., Miao, Z.W., Bai, Z.K., Zhou, X.Y., Zhao, J.K. & Zhu, Y.M.. A case study of ecological restoration at the Xiaoyi Bauxite Mine, Shanxi Province, China. Ecological Engineering, 1998, 11: 221-229.

[11] Miao, Z. & Marrs, R.. Ecological restoration and

DOI: https://doi.org/10.30564/jrb.v1i2.862

land reclamation in open-cast mines in Shanxi Province, China. Journal of Environmental Management, 2000, 59: 205-215.

[12] Shi, Z.L., Wen, A.B., Walling, D.E., Wang, Y.Y. & Chen, J.C.. Exploring particle size selectivity effects during erosion of purple soils in Chongqing municipality, China. Journal of Soils and Sediments, 2017, 17: 1191-1196.

[13] Xi, J., Zhao, X., Wang, X. & Zhang, Z.. Assessing the impact of land use change on soil erosion on the Loess Plateau of China from the end of the 1980s to 2010. Journal of Soil and Water Conservation, 2017, 72: 452-462.

[14] Yu, K.K., Xu, H., Lan, J.H., Sheng, E.G., Liu, B., Wu, H.X., Tan, L.C. & Yeager, K.M.. Climate change and soil erosion in a small alpine lake basin on the Loess Plateau, China. Earth Surface Processes and Landforms, 2017, 42: 1238-1247.

[15] McManamen, C., Nelson, C.R. & Wagner, V.. Timing of seeding after herbicide application influences rates of germination and seedling biomass of native plants used for grassland restoration. Restoration Ecology, 2018, 26: 1137-1148.

[16] Zajc, M., Gosar, A. & Celarc, B.. GPR Study of a Thrust-Fault in an Active Limestone Quarry (SW Slovenia). Journal of Environmental and Engineering Geophysics, 2018, 23: 457-468.

[17] Zhang, S., Lu, P., Cantrell, D., Zaretskiy, Y., Jobe, D. & Agar, S.M.. Improved quantification of the porosity-permeability relationship of limestones using petrographical texture. Petroleum Geoscience, 2018, 24: 440-448.

[18] Birnbaum, C., Bradshaw, L.E., Ruthrof, K.X. & Fontaine, J.B.. Topsoil Stockpiling in Restoration: Impact of Storage Time on Plant Growth and Symbiotic Soil Biota. Ecological Restoration, 2017, 35: 237-245.

[19] Moreno, M., de-Bashan, L.E., Hernandez, J.P., Lopez, B.R. & Bashan, Y.. Success of long-term restoration of degraded arid land using native trees planted 11 years earlier. Plant and Soil, 2017, 421: 83-92.

[20] Wang, F.M., Ding, Y.Z., Sayer, E.J., Li, Q.L., Zou, B., Mo, Q.F., Li, Y.W., Lu, X.L., Tang, J.W., Zhu, W.X. & Li, Z.A.. Tropical forest restoration: Fast resilience of plant biomass contrasts with slow recovery of stable soil C stocks. Functional Ecology, 2017, 31: 2344-2355.

[21] Koziol, L., Schultz, P.A., House, G.L., Bauer, J.T., Middleton, E.L. & Bever, J.D.. The Plant Microbiome and Native Plant Restoration: The Example of Native Mycorrhizal Fungi. Bioscience, 2018, 68: 996-1006.

[22] van Zuidam, J.P., van Leeuwen, C.H.A., Bakker, E.S., Verhoeven, J.T.A., Ijff, S., Peeters, E.T.H.M., van Zuidam, B.G. & Soons, M.B.. Plant functional diversity and nutrient availability can improve restoration of floating fens via facilitation, complementarity and selection effects. Journal of Applied Ecology, 2019, 56: 235-245.

[23] Loddo, D., Sousa, E., Masin, R., Calha, I.M., Zanin, G., Fernandez-Quintanilla, C. & Dorado, J.. Germination response of local Southern European populations of Datura stramonium at a range of constant temperatures. Weed Research, 2014, 54: 356-365.

[24] Camargo, I.D., Nattero, J., Careaga, S.A. & Nunez-Farfan, J.. Flower-level developmental plasticity to nutrient availability in Datura stramonium: implications for the mating system. Annals of Botany, 2017, 120: 603-615.

[25] Cisneros-Silva, A., Castillo, G., Chavez-Pesqueira, M., Bello-Bedoy, R., Camargo, I.D. & Nunez-Farfan, J.. Light limitation reduces tolerance to leaf damage in Datura stramonium. Evolutionary Ecology Research, 2017, 18: 351-362.

[26] Jimenez-Lobato, V., Martinez-Borda, E., Nunez-Farfan, J., Valverde, P.L., Cruz, L.L., Lopez-Velazquez, A., Santos-Gally, R. & Arroyo, J.. Changes in floral biology and inbreeding depression in native and invaded regions of Datura stramonium. Plant Biology, 2018, 20: 214-223.

[27] Andrews, A.E.. The active constituents of the Indian solanaceous plants Datura stramonium, D fastuosa, and D Metel. Journal of the Chemical Society, 1911, 99: 1871-1877.

[28] Ashford, D., Desai, N.N., Allen, A.K., Neuberger, A., Oneill, M.A. & Selvendran, R.R.. Structural Studies of the Carbohydrate Moieties of Lectins from Potato (Solanum-Tuberosum) Tubers and Thornapple (Datura-Stramonium) Seeds. Biochemical Journal, 1982, 201: 199-208.

[29] Boyette, C.D. & Turfitt, L.B.. Factors Influencing Biocontrol of Jimsonweed (Datura-Stramonium L) with the Leaf-Spotting Fungus Alternaria-Crassa. Plant Science, 1988, 56: 261-264.

[30] Arouko, H., Matray, M.D., Braganca, C., Mpaka, J.P., Chinello, L., Castaing, F., Bartou, C. & Poisot, D.. Voluntary poisoning by ingestion of Datura stramonium. Another cause of hospitalization in youth seeking strong sensations. Annales De Medecine Interne, 2003, 154: S46-S50.

[31] Lewis, O.A.M. & Probyn, T.A.. N-15 Incorporation and Glutamine-Synthetase Inhibition Studies of Nitrogen Assimilation in Leaves of the Nitrophile, Datura-Stramonium L. New Phytologist, 1978, 81: 519-526.

 DOI: https://doi.org/10.30564/jrb.v1i2.862

[32] Baselga, J.M., Pigrau, C. & Martinezvazquez, J.M.. Datura-Stramonium - a Flourishing Old Hallucinogenic Agent. Medicina Clinica, 1985, 84: 715-715.

[33] Ballica, R., Ryu, D.D.Y. & Kado, C.I.. Tropane Alkaloid Production in Datura-Stramonium Suspension - Cultures - Elicitor and Precursor Effects. Biotechnology and Bioengineering, 1993, 41: 1075-1081.

[34] Matsuda, K., Aoki, J., Uchida, M.K. & Suzukinishimura, T.. Datura-Stramonium Agglutinin Released Histamine from Rat Peritoneal Mast-Cells That Was Inhibited by Pertussis Toxin, Haptenic Sugar and N - Acetylglucosamine - Specific Lectins - Involvement of Glycoproteins with N-Acetylglucosamine Residues. Japanese Journal of Pharmacology, 1994, 66: 195-204.

[35] Khan, A.A. & Karssen, C.M.. Photoreversible Changes during Germination Prohibitive Imbibition in Peg-6000 Solution Modulating Germination Responses of Chenopodium-Bonus-Henricus L Seeds. Plant Physiology, 1979, 63: 15-15.

[36] Masuda, M., Hata, N., Ombwara, F.K. & Agong, S.G.. Effects of acid scarification, priming with PEG, NaCl or sea water as osmoticum and dehydration on spinach seed germination at 30 degrees C. Journal of the Japanese Society for Horticultural Science, 2005, 74: 134-138.

[37] Anil, V.S., Harmon, A.C. & Rao, K.S.. Spatio-temporal accumulation and activity of calcium-dependent protein kinases during embryogenesis, seed development, and germination in sandalwood. Plant Physiology, 2000, 122: 1035-1043.

[38] Bonilla, I., El-Hamdaoui, A. & Bolanos, L.. Boron and calcium increase Pisum sativum seed germination and seedling development under salt stress. Plant and Soil, 2004, 267: 97-107.

[39] Lucchese, J.R., Lazarotto, M., Fior, C.S., de Sa, L.C. & Brose, C.B.. Analysis of seed vigor and germination of Toona ciliata M. Roem. var. australis. Journal of Seed Science, 2018, 40: 388-395.

[40] Pierce, G.L., Warren, S.L., Mikkelsen, R.L. & Linker, H.M.. Effects of soil calcium and pH on seed germination and subsequent growth of large crabgrass (Digitaria sanguinalis). Weed Technology, 1999, 13: 421-424.

[41] Salahshoor, F. & Kazemi, F.. Effect of calcium on reducing salt stress in seed germination and early growth stage of Festuca ovina L. Plant Soil and Environment, 2016, 62: 460-466.

[42] Sharma, S.S., Sharma, S. & Rai, V.K.. The Effect of Egta, Calcium-Channel Blockers (Lanthanum Chloride and Nifedipine) and Their Interaction with Abscisic-Acid on Seed-Germination of Brassica-Juncea Cv Rlm-198. Annals of Botany, 1992, 70: 295-299.

DOI: https://doi.org/10.30564/jrb.v1i2.862

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research
https://ojs.bilpublishing.com/index.php/jbr

REVIEW

Improved Heat *FT* Induction Leads to Earlier and More Prolific Flowering in Poplar

Abdul Azeez Victor Busov*

School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, United States

ARTICLE INFO

Article history
Received: 11 June 2019
Accepted: 17 July 2019
Published Online: 30 July 2019

Keywords:
Poplar
Heat shock promoter
Flowering Locus T
Flowering
Heat induction

ABSTRACT

Trees have a long juvenile phase before reproductive onset. This makes their breeding and studying floral development difficult. Precocious flowering using FT technology has shown promise. However, transgenic FT overexpression has significant negative pleiotropic effects. Hence, there has been interest in inducible FT expression for flower induction. Previously reported heat inducible expression of FT in poplar successfully induced flowering. However, flowering was sporadic and took up to 6 weeks. Here we report improvements in the protocol, which led to faster and more prolific flowering. Specifically, we increased the once to three times daily heat treatment. The repeated heat inductive treatments led to nearly five times higher FT expression, compared to the single daily treatment. The highly increased FT expression led to significant acceleration and abundance of flowering.

1. Introduction

The long juvenile phase before reproductive onset in perennial trees is a major obstacle in breeding and studies of flower development. To overcome this challenge various methods were developed to induce early flowering such as pruning, girdling, water stress and growth regulator paclobutrazol [1,2]. More recently transgenic up-regulation of floral meristem identity genes like *LEAFY* and *Flowering Locus T* (*FT*) were successfully used to induce early flowering in many trees [3-5]. However, ectopic expression of floral meristem identity genes produces severe pleiotropic phenotypes which renders transgenic plants unusable for breeding purposes and studying gene function. To overcome this problem inducible system driving three FT homologs, two from poplar (FT1 and FT2) and one from Arabidopsis (FT), was developed in poplar [6]. The system employed the heat inducible promoter from soybean heat shock protein and tested in a male (353) and female (717) poplar clones [7]. Surprisingly, among the three FT homologs, the Arabidopsis FT showed best results in inducing flowering in both clones, although the male clone typically produced earlier and more prolific flowers [7]. Although the system was successful, flower inflorescences were sporadic and needed weeks of treatments (3 weeks for the male and 6 weeks for the female clone). The induction protocol employed a daily single 37°C treatment. We hypothesized that this treatment is insufficient to mount sufficient FT expression and thus the lengthy and inefficient flower production. The major objective of this study therefore was to modify the existing protocol to increase the *FT* expression and ac-

**Corresponding Author:*
Victor Busov,
School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, United States;
Email: vbusov@mtu.edu

 DOI: http://doi.org/10.30564/jrb.v1i2.1013

celerate and improve flower abundance.

2. Methodology

We used 353 male and 717 female poplar clones transformed with the Arabidopsis *Flowering Locus T* (*FT*) regulated by a heat-inducible promoter [7]. These clones were kindly provided by Dr. Steven Strauss at Oregon State University.

2.1 Heat Induction Experiment

Approximately 5-6 weeks old plants, grown in a greenhouse (16-hour light, 20-22°C) were used for the heat induction experiment. The 353 and 717 plants were 40-45cm and 45-50cm in height respectively at the time of heat treatment initiation. The plants were transferred to growth chamber (set at 16-hour light, 20°C, except during heat induction period). Heat shock (37°C for 90 min) was administered once daily at same time each day [7]. To increase *FT* expression, we used three cycles of heat induction of 90 min at 3h intervals: First heat cycle 4.30am-6:00am, second 9:00am-10.30am and third cycle 1:30-3:30pm). The rest of the time growth chamber setting was 16h light (4am-8pm), 20°C.

2.2 RNA Isolation and Quantitative Real-time PCR (qRT-PCR) Analysis

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) from leaf samples. 500ng of the total RNA from each sample was used to generate cDNA using an iScript cDNA synthesis kit (BioRad). Selected ACT7 reference genes were validated using GeNorm Software [8]. qRT-PCR analyses were carried out with StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies) using Maxima SYBR Green qPCR master mix (Thermo Scientific Co.), and relative expression values were calculated using the Δ-CT-method, as previously described [9]. A complete list of primers used in RT-PCR analysis is presented in Table 1.

Table 1. List of primers used in this study

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
ACT7	TGGCCGATGCCGAGGATATTCAAC	ATCACCTGCAAACCCAGCCTTCAC
FT	CAGGAATTCATCGTGTCGTG	AGCCACTCTCCCTCTGACAA

3. Results

Using the previously described protocol and transgenic clones, we found similar flower induction as previously described [7]. We used transgenics transformed with Arabidopsis FT, because they showed the best results in both clones. The daily, 90 min of heat treatment (37°C) did induce flowering in both clones but flowering was sporadic and took up to 6 weeks (Figure 1).

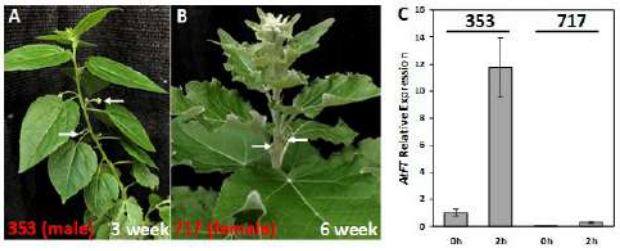

Figure 1. Floral development in transgenic poplar harboring the heat inducible *FT* construct (P_{HSP}::*FT*)

Note: Plants of approximately 40 cm height were exposed to 37°C of 90 min per day for 3-6 weeks, (A) 353 (male) clone initiated flowering after 3 weeks of heat induction (B) 717 (female) clone took more than 6 weeks of heat induction to initiate flowering, White arrows indicates the emerging inflorescences (C) comparison of FT expression at 0h, and 2h after the heat indicative treatments in both genotypes, actin used as internal control to normalize gene expression, error bars ± SE.

Specifically, flowering initiated in 3 weeks in the male 353 genotype and 6 weeks in the female 717 genotype (Figure 1A-B). We studied the *FT* expression in leaf samples at 2h and 4h after heat induction in both clones. We found higher expression in male genotype 353, as compared to female genotype 717 (Figure 1C), which took twice longer to induce floral development. Based on this expression pattern of *FT*, we hypothesized that insufficient *FT* expression may be the limiting factor to induce early flowering. To increase *FT* expression, we used three cycles of heat induction of 90 min at 3h intervals. The repeated heat inductive treatment resulted in an almost five-fold increase in *FT* expression compared to normal once daily heat induction and earlier and more uniform flowering (2-3 weeks after heat induction) (Figure 2 A-H).

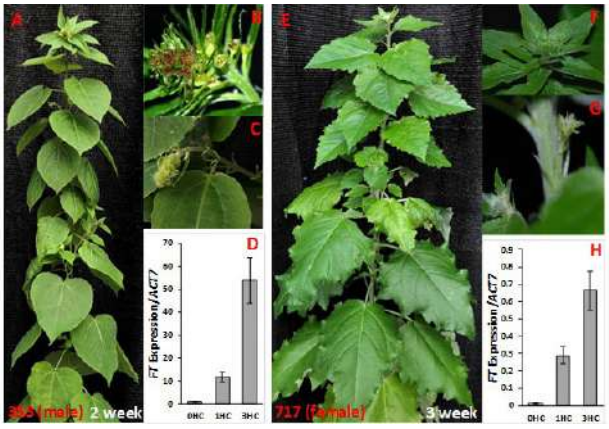

Figure 2. Three cycles of heat induction per day induce much early flowering in transgenic poplar (P_{HSP}::*FT)*

Note: Plants of approximately 40 cm of height were exposed daily three heat cycles (3HC) of 37°C for 90 min with a 2 hour breaks for 2-3 weeks

DOI: http://doi.org/10.30564/jrb.v1i2.1013

(see Methodology for more details). (A) 353 (male) clone start flowering only after 2 weeks of heat treatment (B, C, F, G) close-up of terminal and axillary catkin in 353 and 717 clones (D, H) expression of FT at 0h (OHC), 2h after the one heat cycle (1HC) treatment and 2h after the three heat cycles (3HC) treatment; actin was used as internal control to normalize gene expression; error bars ± SE (E) 717 (female) clone takes less than 3 weeks of heat induction to initiate flowering.

The repeated heat inductive treatment also resulted in a greater number of catkins in both clones (Figure 3 A, B). These results indicate that FT is a limiting factor during heat induction methods used previously, and our method of three cycles of heat induction is a more efficient to induce much earlier and more prolific flowering.

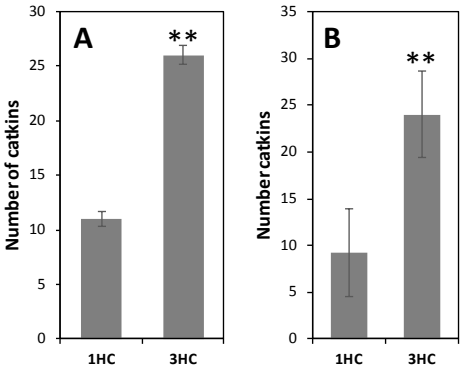

Figure 3. Three cycles of heat induction induce more flowering in transgenic poplar (P_{HSP}::*FT*)

Note: (A) Number of catkins in 353 (male) clone after one heat cycle (1HC), and three heat cycles (3HC) (B) Number of catkins (nascent florescence) developed in 717 (female) clone. Data shown are mean values from 10 plants of each genotype, error bar ± STDEV and asterisks over bar indicate significance differences at P<0.001 with corresponding one heat cycle treatment.

4. Discussion

FT is well studied flowering time gene and ectopic expression induces early flowering across plant species including trees [10,11]. However constitutive upregulation causes major negative pleiotropic effects [10]. Previously developed *FT* inducible system in poplar showed promise to address this challenge but also had some significant limitations [7]. Specifically, up to 6 weeks of the inductive treatments were needed and flowering was sporadic [7]. We show here that the likely cause for these problems was insufficient induction of *FT*. Male genotype 353 flowers twice as fast as compared to the female 717 genotype [7]. *FT* induction at the same time points and conditions was much higher in 353 compared to 717 (Figure 1A-C). Increase in the once to three times of heat induction daily, resulted in almost five-fold expression increase of *FT* in both genotypes. This led to significant acceleration of flowering, particularly in the female 717 clone, which as mentioned earlier has a significantly lower *FT* induction compared to the male 353 clone. In addition to acceleration of flowering, the modified treatment and higher *FT* expression also led to increase in number of catkins (Figure 3A, B). Despite earlier flowering and increased number of catkins, the inflorescence development in the female clone was not complete and catkins aborted within 3-4 weeks after treatment termination. The most likely cause was the much lower expression of *FT* in the female background. Even after the improvement in administrating the heat treatments, *FT* expression was nearly 10-fold lower in the female genotype (Figure 2D, H). The cause for the lower induction of *FT* in the female background is unknown. It could be a result of a position effect due to insertion of the construct in a less active chromatin, overall less effectiveness of the heat induction system in this background or methylation and other chromatin modifications of the transgene. Further investigation of these potential causes will lead to strategies that can overcome the low *FT* induction.

5. Conclusions

We report improvements to the heat induction protocol of *FT* in poplar, which leads to faster and more prolific flowering. The primary cause for the observed improvements is likely the significant increase in *FT* induction, caused by the repeated administration of the inductive treatments. The improvements in the protocol can be also applied to other inducible technologies employing the same promoter.

References

[1] Flachowsky, H., et al., A review on transgenic approaches to accelerate breeding of woody plants. 2009, 128(3): 217-226.
DOI: 10.1111/j.1439-0523.2008.01591.x

[2] Meilan, R.J.N.F., Floral induction in woody angiosperms. 1997, 14(3): 179-202.
DOI: 10.1023/a:1006560603966

[3] Weigel, D. and O. Nilsson, A developmental switch sufficient for flower initiation in diverse plants. Nature, 1995, 377(6549): 495-500.
DOI: 10.1038/377495a0

[4] Klocko, A.L., et al., FT overexpression induces precocious flowering and normal reproductive development in Eucalyptus. Plant Biotechnology Journal, 2016, 14(2): 808-819.
DOI: 10.1111/pbi.12431

[5] Tang, M., et al., An ortholog of LEAFY in Jatropha curcas regulates flowering time and floral organ development. Sci Rep, 2016, 6: 37306.
DOI: 10.1038/srep37306

[6] Borghi, L., Inducible gene expression systems for

plants. Methods Mol Biol, 2010, 655: 65-75.
DOI: 10.1007/978-1-60761-765-5_5

[7] Zhang, H., et al., Precocious flowering in trees: the FLOWERING LOCUS T gene as a research and breeding tool in Populus. J Exp Bot, 2010, 61(10): 2549-60.
DOI: 10.1093/jxb/erq092

[8] Vandesompele, J., et al., Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology, 2002, 3(7): research0034.1.
DOI: 10.1186/gb-2002-3-7-research0034

[9] Azeez, A., et al., A tree ortholog of APETALA1 mediates photoperiodic control of seasonal growth. Curr Biol, 2014, 24(7): 717-24.
DOI: 10.1016/j.cub.2014.02.037

[10] Kardailsky, I., et al., Activation tagging of the floral inducer FT. Science, 1999, 286(5446): 1962-5.
DOI: 10.1126/science.286.5446.1962

[11] Kobayashi, Y., et al., A pair of related genes with antagonistic roles in mediating flowering signals. Science, 1999, 286(5446): 1960-2.
DOI: 10.1126/science.286.5446.1960

DOI: http://doi.org/10.30564/jrb.v1i2.1013

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research
https://ojs.bilpublishing.com/index.php/jbr

ARTICLE

Callus Cultures Of Beans Infected With Virus As A Model For Testing Antiviral Compaunds

Kovalenko Oleksiy* **Kyrychenko Angelina** **Kovalenko Olena**

Zobolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, 03143, Ukraine

ARTICLE INFO

Article history
Received: 17 June 2019
Accepted: 19 July 2019
Published Online: 30 July 2019

Keywords:
Bean common mosaic virus (BCMV)
Bean yellow mosaic virus (BYMV)
Reverse transcriptase polymerase chain reaction (RT-PCR)
Plant tumors
Glycans
Glycolipids
Therapy of virus diseases

ABSTRACT

In the work, bean callus raised from a leaves of *Bean common mosaic virus* infected bean plant was obtained and adapted for the testing of antiviral activity of liposomal glycan-glycolipid complexes. *Ganoderma adspersum* glucans and *Pseudomonas spec*. rhamnolipids were constituents of liposomal compaunds. It has been shown that under the long-term cultivation (up to 3 months) in the presence of a liposomal preparation containing (10-100 mg/l), the virus is eliminated from the tissue. This is evidenced by the absence of 391 bp sequence amplification product established by RT-PCR in the callus tissue, cultured on a medium containing the liposomal complex. The proposed model system is analogous to plant tumors and has obvious advantages over similar systems *in vivo*, since the callus growth is controlled and independent of environmental factors.

1. Introduction

Somatic plant cells, grown under normal conditions, integrate into specialized tissues and organs performing different physiological functions. However, under the influence of certain environmental and anthropogenic factors (injuring, insect and infectious invasions), plants are capable of forming tumors in the form of growths, crowns galls, plant leaf deformations, etc. One of the types of cell neoplasms is tumors induced in plants by the *Agrobacterium tumefaciens* Ti-plasmid [1]. Tumors caused by this pathogen are common in berries, fruit trees, grapes and some field crops and can be modeled *in vitro* in the parenchymal tissues of some vegetable plants and potatoes [2]. In this work, we showed high antitumor and antiviral activity of glycan-containing preparations. Since abnormal plant growth induced by external agents (viruses, bacteria, fungi, insects) as well as by physical and chemical means are actively discussed in literature, the testing of antiviral activity of such substances in callus culture is of particular interest. Thus our earlier studies have shown the possibility of using callus culture (as a species of such tumors) as a model for the selection and study of the mechanism of action of antitumor preparations [2].

This paper deals with tumors formed by meristematic cells that are generated after mechanical wounding of

**Corresponding Author:*
Kovalenko Oleksiy,
Zobolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, 03143, Ukraine;
Email: udajko@ukr.net

 DOI: http://doi.org/10.30564/jrb.v1i2.1252

cambium, and can also be cultured on artificial/synthetic medium with subsequent regeneration of new plants under the plant growth regulators (auxins and kinetins) influence [1]. In our laboratory, it has repeatedly been shown the possibility of eliminating the virus in case of cultivating a virus-infected callus and obtaining healthy and virus-resistant plants-regenerants [3]. The use of such a method of plant recovery is especially relevant for plants with seed transmission of plant viruses as it was shown for beans infected by bean common mosaic virus (BCMV) and bean yellow mosaic virus (BYMV) [4,5].

The purpose of our research was to: develop a callus cell culture (CCC) for BYMV or BCMV infected bean and try to recover it from viral infection using liposomal glycan preparations (LPs) developed in our laboratory [6].

2. Materials and Methods

2.1 Callus Cell Culture

4-8 week-old seedlings of bean plants *P. vulgaris* cv. Red Riding Hood were grown in spring and summer period in a greenhouse with a temperature cycling between 18 and 24°C. The plants were inoculated by rubbing of extracted sap (in cold 0.1 M phosphate buffer, pH 7.2 (1:10 w/vol) onto leaves pre-dusted with carborundum. Bean plants infected with BCMV or BYMV and showing suspected virus symptoms patterns of mosaic, yellowing, mottling or chlorosis were used for callus formation (Figure 1).

Bean leaf and stem internodes used as the starting material were excised from the mother plant and washed with running tap water. Surface sterilization was done within a laminar air flow cabinet by dipping the runner tips in 70% ethanol (2 min) and 3.0 % sodium hypochlorite (NaOCl) for 3-5 min. Afterwards, explants were rinsed several times with sterile distilled water. The explants (leaves and nodal segments sliced into 0.5-1.0 cm^2 pieces) were cultured on Gamborga (GB) nutrient mediums containing kinetin and 2.4-D at a concentration of 0.1 and 1.0 g/l, respectively. The pH was adjusted to 5.5 before adding agar and autoclaving (121 °C, 0,5 MPa, 60 min). The culture vessels containing explants were incubated in a growth chamber under a 16/8 h light/dark cycle at 25 ± 2 °C. During 1-3 months the observations were made on explant development and all calli were screened for the presence of virus by RT-PCR. The culture passage was performed once a month.

2.2 Antiviral Praparations

Liposomal-based glycan preparations (LPs) were obtained according to previously described methodology [6]. In this work, we used two liposomal glycan-glycolipid complexes (GGK-3 and GGK-4), formed on the basis of the water-soluble glucan *Ganoderma adspersum* (GGK-3), and mix of three glycans (*Candida maltose* mannan, *Ganoderma adspersum* glucan, *Tremela mesenterica* glucuronoxylomannan) (GGK-4). Methods of obtaining and properties of polysaccharides were described earlier [7]. Rh-1 and Rh-2 rhamnolipids extracted from *Pseudomonas spec*. PS-17 culture fluid were used for LPs obtaining. Different concentrations of GGK-3 and GGK-4 (10-500 mg/l) in the form of an emulsion were added to the cultural mediums. Mediums without test substances served control in antiviral activity experiments [6].

2.3 Virus Detection and Identification

Bean leaf samples and callus tissues were assayed by the Reverse Transcription and Polymerase Chain Reaction (RT-PCR). Presence of virus RNA in the calli was assayed every transfer. Total RNA was isolated using AmpliSens Ribo-Sorb DNA/RNA extraction kit. PCR test kit AmpliSens Reverta-L-100 was used to generate cDNA according to the manufacturer's instructions. The reaction mixture for the PCR (of 20 µl) contained: 1 × PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 - 50 ng of cDNA, 0,5U Taq polymerase. Primers were used at final concentration of 5 pmol. The amplification was performed in DNA Thermocycler "Tertsyk" TP4-PCR-01.

The primer pairs chosen for BCMV detection amplified DNA fragments comprising 391 bp of 5'- coat protein region [8]. BYMV1f and BYMV2r primer pairs to the site in the coat protein sequence were used in the study for the BYMV detections [9]. Synthesis of primers was made by Biolabtech (Kyiv, Ukraine).

For the specific primers, amplification was used for 35 cycles: denaturation at 94°C for 5 min, 35 cycles of amplification (94°C for 30 s, 60°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 7 min. The PCR fragments were verified in a 1.5% (w/vol) non-denaturing agarose gel after ethidium bromide (0.5 mg/ml) staining. The gel was run at 120 volts and maximum current for 45 min before being viewed under UV light and photographed.

3. Results

In Ukraine fairly high incidence of three virus diseases occurs on bean crop. Soybean mosaic virus (SMV), bean common mosaic and bean yellow mosaic viruses have been found to reduce yield and adversely affect seed quality [4,5]. BYMV is the most destructive disease of bean. The virus is widespread and causes economic damage in susceptible bean cultivars that react with apical bud necrosis, leading to plant death [10]. In addition, the virus is spread by a number

DOI: http://doi.org/10.30564/jrb.v1i2.1252

of aphid species non-persistently as well as being seed and mechanically transmitted. In view of these facts, before studying antiviral activity of liposomal preparation in plant tissue culture, we aimed to develop a suitable model system "virus – CCC", firstly, for BYMV.

To obtain the virus callus BYMV-infected bean plants exhibited mild to severe yellow mosaic symptoms on leaves were used (Figure 1).

Figure 1. Callus derived plants of *Phaseolus vulgaris*, infected with BYMV

Leaf blades were cultured on GB basal medium to study their callus induction ability. The explants formed white friable callus after 2 weeks of culture. Results obtained are illustrated in Figure 2. The results reveal that leaf segments yielded a mass of compact white soft callus with a smooth, wet-looking surface (Figure 2A) that turned pink-yellow over time. In 20-23 days of incubation callus induction rates decreased significantly, callus started to be compact and granular (Figure 2B). The explants turned to yellow in color following two weeks of incubation. With time calli growth was stopped then tissues were dried out, necrotized and wrinkled showing the sign of dying off (Figure 2C). The features of proliferation of callus originally raised from the BYMV-infected bean leaves, as well as the low growth rate of callus tissue, are probably due to the high pathogenicity of the virus in bean plants, which significantly restrained the growth of callus.

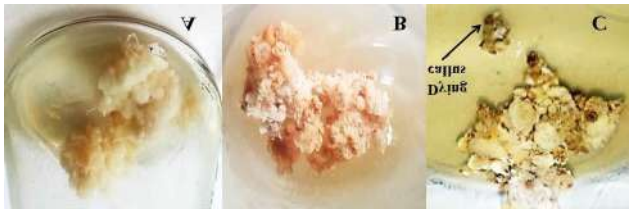

Figure 2. Callus initiation from leaf segments: young calluses after 3 weeks of culture (A); an aging 6 weeks culture (B); later stage of callus incubation (C)

The results obtained indicate that this model system is not suitable for further research on the study of antiviral substances. Therefore, in further work, we focused on the BCMV, as a possible component of the experimental system. According to our observations [4], BCMV is less pathogenic then BYMV and is transmitted at a high frequency through seeds. This circumstance made it possible to obtain virus-infected callus from the 2 sources of explants – leaf and seed germs. Therefore, there was a real opportunity to recovery bean seedling grown from infected seeds. As in the previous case, for callus formation it was selected the bean plants with characteristic symptoms of the BCMV infection – light and dark green systemic mosaic, rugosity, upward and downward leaf curl, stunted growth, leaf roll and malformation of leaves. As a control we used callus raised from a healthy (uninfected) bean plants.

Presence of BCMV in symptomatic plants (Figure 3) was confirmed with polymerase chain reaction (RT-PCR). The amplification of the CP gene from tissues of infected plants with BCMV primers generated a single DNA fragment of the expected size (~391 bp). Amplification did not occur in the control samples (Figure 4).

Figure 3. Callus derived plants of *Phaseolus vulgaris*, infected with BCMV

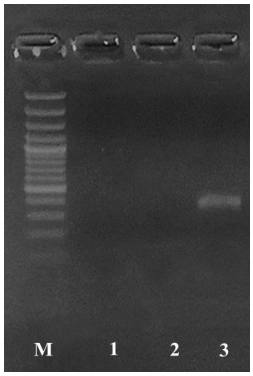

Figure 4. Agarose gel electrophoresis of RT-PCR amplification products

Note: lane 1 (M), 100-bp marker; lane 2, negative control; lane-3, ex-

DOI: http://doi.org/10.30564/jrb.v1i2.1252

tracts from healthy bean; lane-4, extracts from symptomatic bean; expected BCMV band = 391bp

Callus, raised from BCMV-infected bean leaf, seed germ and stem internodes was cultured in a thermostat at 24-25° C for two weeks, and therefore in a luminostat under a photon flux density of 50 µmol/m^2/s, emitted from 'Fluora' fluorescent lamps up to 2.5-3 months. Callus initiation was observed in a week after the explants were placed on media. Generally, the callus tissues grew quite intensively and did not differ in all variants of the experiments (leaf or stem internodes, virus-infected and virus-free) at the beginning. The effective formation of callus was observed in all cases. With time callus proliferated into pale yellow and became compact (Figure 5).

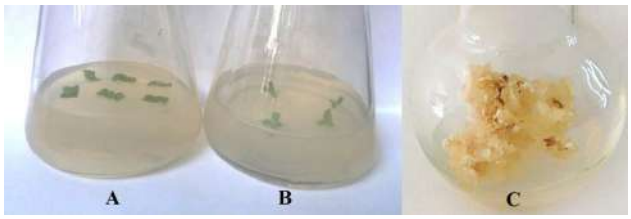

Figure 5. Callus induction on leaf explants (A) and stem internodes (B). Yellow 4-week-old calli initiated from leaf explants. All calluses were raised from BCMV-infected bean

During the experiment, it was found that formation of the derived from different sections of one leaf occurs unevenly: no notable callus formed in a number of explants obtained from infected plant. Eventually explants were died without forming primary callus. In other cases the calluses having translucent pale yellow or white peripheral tissue with greenish inner tissue grew and were not watery in consistency, somewhat fragile, but with a dense inner part. Translucent tissue gradually became dark yellow or orange with brown and relatively compact inner core in later stage. Subsequently, the cell growth stopped, but degenerative processes were not observed.

Antiviral activity of liposomal glycan preparations was measured by inhibitory effects of BCMV replication in cell culture and has been used to evaluate their efficiency *in vitro*. For this purpose we first examined toxicity of LPs to callus cells. According to our previous experiment GGK-3 and GGK-4 application at concentrations in the nutrient medium (500 mg/l) led to severe toxicity development. The date obtained indicates the negative impacts of even at relatively low concentrations of GGK-4 for callus cells and their viability (data not shown). Therefore, in further experiments only GGK-3 was tested at low concentration (100 and 10 mg/l).

Our findings demonstrate that exposure to 10 mg/l of GGK-3 did not decrease the relative growth rate of callus and as a result did not show a clearly pronounced inhibitory activity on callus tissue formation *in vitro* compared with the control (Figure 6). As can be seen from Figure6, morphogenic responses leaf explants exposed to lower concentrations of GGK-3 (Figure 5) and normal (Figure 6) conditions did not differ. The effective callus formation with large cell colonies was observed on leaf explants in all variants of the experiments. The calli grown in both control and experimental groups were composed of translucent and soft inner tissue with white or cream-colored and compact peripheral tissue. This compound at a concentration of 100 mg/l was also slightly toxic. It should also be noted that the most suitable for obtaining the CCC is the explants obtained from the internode and the leaf sections along the central vein.

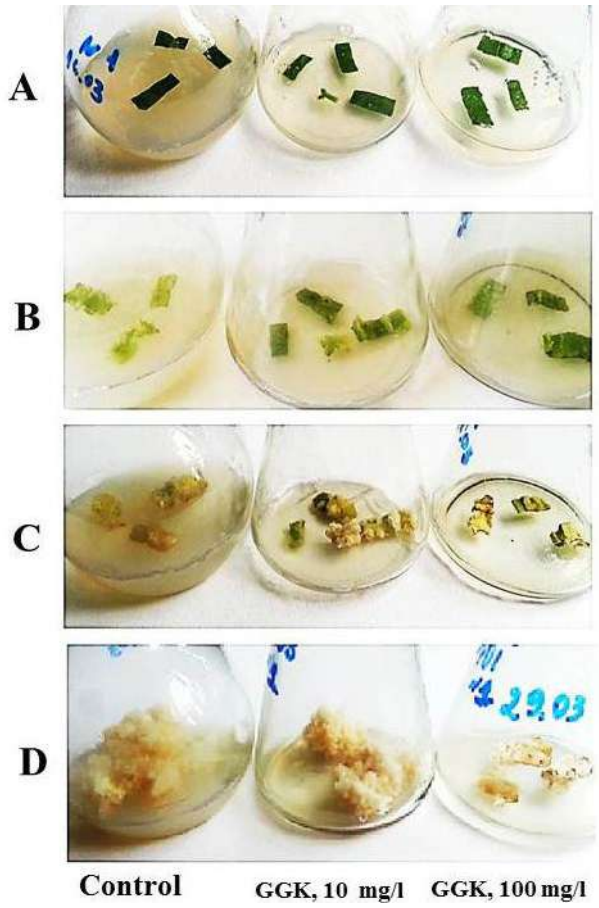

Figure 6. Proliferation of callus obtained from BCMV-infected leaf after 1 day of culture (A), 6 days of culture (B), 13 days of culture (C), 33 days of culture (D)

Thus, in callus tissue growing on the Hamburg B-5 medium with the liposomal glycan-glycolipid complex at a concentration of 10-100 mg/l, probably, a gradual (during 2-3 month) elimination of the virus occurs (Figure 7).

DOI: http://doi.org/10.30564/jrb.v1i2.1252

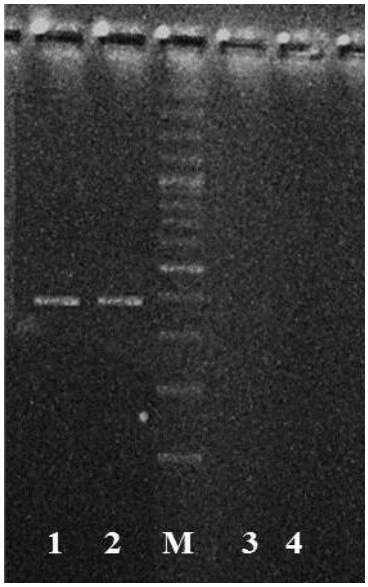

Figure 7. Agarose gel electrophoresis of RT-PCR amplification products

Note: lane 1, starting leaf; lane 2, callus on medium without GGK; lane 3 (M), 100-bp marker; negative control; lane 4, callus on medium with GGK 0,1 mg/l; lane 5, callus on medium with GGK 0,01 mg/l; expected BCMV band = 391bp

These data obtained can be useful for efficient eradication of various viruses from almost all of the most economically important crops and cultivation of virus-free plants as one of the important approaches in novel viral disease control strategy.

4. Discussion

Viruses cause many important plant diseases and are responsible for significant losses in crop. The most unprotected are plants capable of transmitting viruses from generation to generation by seeds or through vegetative propagules. These viruses include Bean yellow mosaic virus and *Bean common mosaic virus* that affect leguminous plants beans [4,5]. Many important horticultural and agronomically important crops are routinely freed of viral contamination using tissue culture procedure combined with chemo- or thermotherapy.

The tissue culture technology is being improved all the time for the mass propagation of plant, but *Fabaceae* plant are lagging behind due to their recalcitrant nature to *in vitro* techniques [11]. By this time the using of environmentally friendly preparations in the tissue culture technology is unknown.

In this work, to obtain the virus-free plant we attempted to combine callus cell culture technique with the using liposomal preparations. For testing antiviral activity of LPs it was selected nutrient media that is most appropriate for bean somatic tissue cultivation. Also it was shown the possibility of virus elimination from callus in the presence of liposomal forms of glycans.

Earlier Shcherbatenko and Oleshchenko reported [3] that under the long-term cultivation, tospoviruses can be eliminated from infected callus. As shown previously, glycans obtained from yeasts and higher *Basidiomycetes* mushrooms, can inhibit viral infections and activate non-specifc defense mechanisms in host plants as well as suppress tumor growth induced by *A. tumefaciens* [2]. In this study liposomal forms of glycans were used at the first time for *in vitro* methods for plant virus eradication. Their high efficacym as a means of controlling plant viral diseases was confirmed on the callus cell culture [6]. In general, our and literature data demonstrate, on the one hand, the nonspecificity of the action of glycans and glycan-containing complexes against pathogens, and, on the other, some analogy of transformed pathogens with undifferentiated (meristematic) plant cells. This makes it possible to use for practical purposes a system with such features of plant cell cultures - as universal models for screening or studying antiviral and antitumor properties of different compounds. The authors hope that further work in this direction will allow to develop a new technology for cultivation of virus-free plants and new effective means against viral, fungal and bacterial diseases.

Conflicts of Interest

The authors indicate no potential conflict of interests regarding the publication of this paper.

Acknowledgments

This work was carried out with funds supplied by the National Academy of Sciences of Ukraine (governmental registration number of work is 0110U001871).

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] J. Gohlke, R. Deeken, Plant responses to *Agrobacterium tumefaciens* and crown gall development, [J]. Front Plant Sci., 2014, 5: 155. https://doi.org/10.3389/fpls.2014.00155

[2] M. Ikeuchi, K. Sugimoto, A. Iwase. Plant callus: mechanisms of induction and repression, [J]. Plant Cell., 2013, 25(9): 3159 – 3173. https://doi.org/10.1105/tpc.113.116053

[3] I.S. Shcherbatenko, L.T. Oleshchenko, Somaclonal variation as a sourse of tomato spotted wilt virus-re-

sistance in plants, [C]. Virus diseases and crop biosecurity, Dordrecht: Springer, 2006: 113-143.

[4] A.N. Kyrychenko, A.G. Kovalenko, Bean common mosaic in the Kiev region: etiology disease and pathoden identivication, [J]. Microbial J., 2018, 80: 96 – 107. (In Ukrainian)

[5] A.M. Kyrychenko, I.O. Antipov, K.V. Hrynchuk. Phylogenetic analysis of Ukrainian BYMV isolates from soybeans and beans, [J]. Cytol Genet., 2017, 51(3): 173 – 178. (In Ukrainian) https://doi.org/10.3103/S0095452717030045

[6] O.G. Kovalenko, V.M. Vasilev, N.I. Adamchuk-Chala, L.V Tytova, E.V. Karpenko, Artifical glycan-glycolipid complexes as antiviral means and effectors of microbial preparations on the base of rhizobia, [J]. Dopov. Nac. acad. nauk Ukr, 2017, 1: 88 – 96 (In Ukrainian) https://doi.org/10.15407/dopovidi2017.01.088

[7] O.G. Kovalenko, S.P. Wasser, Glycans of higher *Basidiomycetes* mushrooms with antiphytoviral properties: isolation, characterization, and biological activity. Fungi and their Applications in the series of Progress in Mycological Research. Eds: Deshmukh S.K., Mishra J.K., Tewari, Japla P., Papp T. Publisher: CRC Press/Taylor & Francis Group, LLC, 2016: 161-200.

[8] I. Antipov, K. Hrynchuk, O. Duplyak, Development PCR systems for identification of mosaic virus ordinary beans (*Bean common mosaic virus*). [J]. Scientific herald of NULES of Ukraine. Series: biology, biotechnology, ecology, 2016, 234: 40-46. (In Ukrainian).

[9] I.O. Antipov, PCR diagnostic of the bean yellow mosaic virus, [J]. Sci. Proc. Nat. Univ. Life and Pryrodokorystuavnnya Ukraine, 2014, 204: 151 – 154.

[10] P.N. Sharma, V. Sharma, A. Sharma, K. Raiput, S.K. Sharma, Identification and molecular characterization of Bean yellow mosaic virus infecting French bean in Himachal Pradesh, [J]. Virus disease, 2015, 26: 315 – 318. https://doi.org/10.1007/s13337-015-0270-z

[11] M.C. Polanco, M.L. Ruiz, Effect of benzylaminopurine on *in vitro* and in vivo root development in lentil (Lens culinaris Medik.), [J]. Plant Cell Rep., 1997, 17: 22 – 26. https://doi.org/10.1007/s002990050345

DOI: http://doi.org/10.30564/jrb.v1i2.1252

Author Guidelines

This document provides some guidelines to authors for submission in order to work towards a seamless submission process. While complete adherence to the following guidelines is not enforced, authors should note that following through with the guidelines will be helpful in expediting the copyediting and proofreading processes, and allow for improved readability during the review process.

Ⅰ. Format

- Program: Microsoft Word (preferred)
- Font: Times New Roman
- Size: 12
- Style: Normal
- Paragraph: Justified
- Required Documents

Ⅱ. Cover Letter

All articles should include a cover letter as a separate document.

The cover letter should include:

- Names and affiliation of author(s)

The corresponding author should be identified.

Eg. Department, University, Province/City/State, Postal Code, Country

- A brief description of the novelty and importance of the findings detailed in the paper

Declaration

v Conflict of Interest

Examples of conflicts of interest include (but are not limited to):

- Research grants
- Honoria
- Employment or consultation
- Project sponsors
- Author’s position on advisory boards or board of directors/management relationships
- Multiple affiliation
- Other financial relationships/support
- Informed Consent

This section confirms that written consent was obtained from all participants prior to the study.

- Ethical Approval

Eg. The paper received the ethical approval of XXX Ethics Committee.

- Trial Registration

Eg. Name of Trial Registry: Trial Registration Number

- Contributorship

The role(s) that each author undertook should be reflected in this section. This section affirms that each credited author has had a significant contribution to the article.

1. Main Manuscript
2. Reference List
3. Supplementary Data/Information

Supplementary figures, small tables, text etc.

As supplementary data/information is not copyedited/proofread, kindly ensure that the section is free from errors, and is presented clearly.

Ⅲ. Abstract

A general introduction to the research topic of the paper should be provided, along with a brief summary of its main results and implications. Kindly ensure the abstract is self-contained and remains readable to a wider audience. The abstract should also be kept to a maximum of 200 words.

Authors should also include 5-8 keywords after the abstract, separated by a semi-colon, avoiding the words already used in the title of the article.

Abstract and keywords should be reflected as font size 14.

Ⅳ. Title

The title should not exceed 50 words. Authors are encouraged to keep their titles succinct and relevant.

Titles should be reflected as font size 26, and in bold type.

Ⅳ. Section Headings

Section headings, sub-headings, and sub-subheadings should be differentiated by font size.

Section Headings: Font size 22, bold type

Sub-Headings: Font size 16, bold type

Sub-Subheadings: Font size 14, bold type

Main Manuscript Outline

Ⅴ. Introduction

The introduction should highlight the significance of the research conducted, in particular, in relation to current state of research in the field. A clear research objective should be conveyed within a single sentence.

Ⅵ. Methodology/Methods

In this section, the methods used to obtain the results in the paper should be clearly elucidated. This allows readers to be able to replicate the study in the future. Authors should ensure that any references made to other research or experiments should be clearly cited.

Ⅶ. Results

In this section, the results of experiments conducted should be detailed. The results should not be discussed at length in

this section. Alternatively, Results and Discussion can also be combined to a single section.

Ⅷ. Discussion

In this section, the results of the experiments conducted can be discussed in detail. Authors should discuss the direct and indirect implications of their findings, and also discuss if the results obtain reflect the current state of research in the field. Applications for the research should be discussed in this section. Suggestions for future research can also be discussed in this section.

Ⅸ. Conclusion

This section offers closure for the paper. An effective conclusion will need to sum up the principal findings of the papers, and its implications for further research.

Ⅹ. References

References should be included as a separate page from the main manuscript. For parts of the manuscript that have referenced a particular source, a superscript (ie. [x]) should be included next to the referenced text.

[x] refers to the allocated number of the source under the Reference List (eg. [1], [2], [3])

In the References section, the corresponding source should be referenced as:

[x] Author(s). Article Title [Publication Type]. Journal Name, Vol. No., Issue No.: Page numbers. (DOI number)

Ⅺ. Glossary of Publication Type

J = Journal/Magazine

M = Monograph/Book

C = (Article) Collection

D = Dissertation/Thesis

P = Patent

S = Standards

N = Newspapers

R = Reports

Kindly note that the order of appearance of the referenced source should follow its order of appearance in the main manuscript.

Graphs, Figures, Tables, and Equations

Graphs, figures and tables should be labelled closely below it and aligned to the center. Each data presentation type should be labelled as Graph, Figure, or Table, and its sequence should be in running order, separate from each other.

Equations should be aligned to the left, and numbered with in running order with its number in parenthesis (aligned right).

Ⅻ. Others

Conflicts of interest, acknowledgements, and publication ethics should also be declared in the final version of the manuscript. Instructions have been provided as its counterpart under Cover Letter.

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research

Aims and Scope

Journal of Botanical Research publishes original research papers that offer a rapid review and publication that freely disseminates research findings in all areas of Botany including Phytogeography, Floristics, Plant Biochemistry, Systematics and more . The Journal focuses on innovations of research methods at all stages and is committed to providing theoretical and practical experience for all those who are involved in these fields.

Journal of Botanical Research aims to discover innovative methods, theories and studies in Botany by publishing original articles, case studies and comprehensive reviews.

The scope of the papers in this journal includes, but is not limited to:

- Floristic
- Plant physiology
- Plant biochemistry
- Plant pathology
- Genetics (including Molecular genetics and Epigenetics)
- Plant taxonomy
- Plant Phylogenetics & Evolution
- Phytogeography
- Population and Conservation Biology
- Plant ecology
- Paleobotany
- Plant anatomy and morphology (including Herbarium-based Research)

Plant associations with other organisms (Plant-microbe interactions, plant-pollinator- interactions, host-pathogen/pest interactions etc.)

Bilingual Publishing Co. (BPC)

Tel:+65 65881289

E-mail:contact@bilpublishing.com

Website:www.bilpublishing.com

About the Publisher

Bilingual Publishing Co. (BPC) is an international publisher of online, open access and scholarly peer-reviewed journals covering a wide range of academic disciplines including science, technology, medicine, engineering,education and social science. Reflecting the latest research from a broad sweep of subjects, our content is accessible worldwide – both in print and online.

BPC aims to provide an analytics as well as platform for information exchange and discussion that help organizations and professionals in advancing society for the betterment of mankind. BPC hopes to be indexed by well-known databases in order to expand its reach to the science community, and eventually grow to be a reputable publisher recognized by scholars and researchers around the world.

BPC adopts the Open Journal Systems, see on http://ojs.s-p.sg

Database Inclusion

National Library, Singapore

Asia & Pacific area Science Citation Index

China National Knowledge Infrastructure

Creative Commons

Google Scholar

Crossref

J-Gate

My Science Work

Bilingual Publishing Co. is a company registered in Singapore in 1984, whose office is at 12 Eu Tong Sen Street, #08-169, Singapore 059819, enjoying a high reputation in Southeast Asian countries, even around the world.

BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Tel:+65 65881289
E-mail:contact@blipublishing.Com
Website:www.Blipublishing.Com

ISSN 2630-5054
02
9 772630 505194