PCP
PLANT & CELL PHYSIOLOGY

Letter to the Editor: Gromwell, a Purple Link between Traditional Japanese Culture and Plant Science

Emi Ito[1,2,†,*], Ryosuke Munakata[3,4,†,*] and Kazufumi Yazaki[3]

[1]Institute for Women's Education in Science, Technology, Engineering, Arts, and Mathematics, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo, 112-8610 Japan
[2]Institute for Human Life Science, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo, 112-8610 Japan
[3]Laboratory of Plant Gene Expression, Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, 611–0011 Japan
[4]Japan Science and Technology Agency (JST), PRESTO, 4-1-8, Honcho, Kawaguchi-shi, Saitama, 332-0012 Japan
[†]These authors contributed equally.

*Corresponding authors: Emi Ito, E-mail, nakamura.emi@ocha.ac.jp; Ryosuke Munakata, E-mail, munakata.ryosuke.3z@kyoto-u.ac.jp

(Received 23 February 2023; Accepted 28 April 2023)

Introduction

For hundreds of years, people have traditionally utilized phytochemicals as dyes, spices, medicines and so on (Afendi et al. 2012, Waki et al. 2021). These natural resources are attracting increased attention toward the decarbonization of society through the recommended use of natural products instead of synthetic ones, while continuous efforts to preserve the plants from which they are derived are required to maintain the diversity and quantity of phytochemicals. Gromwell (*Lithospermum erythrorhizon* Sieb. et Zucc., Boraginaceae)—a beloved plant in Japan, also known as 'Murasaki'—is tightly associated with the rich history and tradition of East Asian countries, including Japan, because it contains a pigment and medicinal metabolite group called the shikonins. Unfortunately, this species is threatened by extinction. The purpose of this letter is to raise awareness of this important plant. In doing so, we cover the historical and social implications of gromwell in Japan, and we also introduce the latest research on gromwell and finally discuss how science and society can work together to support endangered plant species of historical and cultural importance.

The History of Gromwell in Japan

'Murasaki' in Japan stands for two things, both the color purple and the common Japanese name of gromwell. *Lithospermum erythrorhizon* is native to Japan, Korea and China, and the roots of this plant are traditionally used as a crude drug and natural purple dye (**Fig. 1A, B**). This plant has a special importance in traditional Japanese culture and history: during the Asuka Dynasty (A.D. 538–644), the 12-cap rank system of the ancient Imperial Court was established, where each title was distinguished by a different color cap. The highest-ranking cap was of a dark purple color produced by the plant's natural dyes. The root surface of *L. erythrorhizon* contains red naphthoquinones, shikonin derivatives (**Fig. 1B, C**), which give a beautiful purple color with a mordant (traditionally the ash of *Camellia japonica* wood) because of its high content of aluminum ions. Besides government officials, only Buddhist monks of the highest rank and members of the Imperial Family were allowed to wear purple-colored cloths stained with this plant pigment (**Fig. 1D**). In addition, the purple dye from gromwell was also used for special Buddhist scripture papers such as 'Koku-Bun-Ji Kyo', onto which letters were written with gold powder. During the Asuka and Nara Dynasty (A.D. 645–793), the roots of *L. erythrorhizon* were subjected to tax by the Imperial Court at Nara (Matsukawa 2021) (**Fig. 1E**). Historical records describe that labor to cultivate this plant was also part of the tax. The Imperial Court also collected gromwell seeds, presumably for cultivation in other areas, and the harvested roots were then paid as tax. The central government needed a constant supply of roots for dyeing cloths for the high-ranking government officials due to the relative susceptibility of this dye to gradually fade in the light.

Substantial scientific research on this plant began in the 1970s, e.g. tissue culture to produce shikonin and its biosynthesis. Shikonin derivatives have been reported to protect root tissues against soil-borne microorganisms due to their antimicrobial properties (Brigham et al. 1999). Due to these properties, the roots of this plant have been used as a crude drug in Asian countries. The most popular example is an ointment called 'Shi-Un-Koh', which is a remedy established by the Japanese medical doctor Seishu Hanaoka (1760–1835) in the Edo era and is still widely available for use on burns, wounds, frostbite, hemorrhoids, etc. In addition, shikonin derivatives exhibit a variety of pharmacological properties, such as anti-inflammatory, tumor-inhibiting, anti-angiogenic and anti-topoisomerase activities (for more information, see review by Yazaki 2017).

In Japan to date, *L. erythrorhizon* is unfortunately listed as an endangered species marked for extinction. In fact, the occurrence of wild-grown *L. erythrorhizon* is extremely rare. The reasons are said to be due to overharvesting and changes in the environment, but a serious concern is the spread of cucumber mosaic virus (CMV), to which this plant species is very susceptible (Izuishi et al. 2020). As such there are ongoing efforts to try to restore this plant species in Japan.

Plant Cell Physiol. 64(6): 567–570 (2023) doi:https://doi.org/10.1093/pcp/pcad038, Advance Access publication on 18 May 2023, available online at https://academic.oup.com/pcp

Fig. 1 *Lithospermum erythrorhizon* and its red pigment in Japanese history and culture. (A) Flowers of *L. erythrorhizon.* (B) Fresh roots of *L. erythrorhizon*. The cut surface of the root is shown. (C) The chemical structure of acetylshikonin as the representative red pigment in *L. erythrorhizon* roots. (D) The costume of a ninth-century high-rank officer at 'Da-Zai-Fu', owned by the Dazaifu Fureai Culture Museum. (E) A piece of historical evidence that *L. erythrorhizon* roots were used as tax to the central court. A wooden document (tablet) called 'Mokkan' was used to tag each transport cargo to the central court. This wooden tablet excavated from 'Da-Zai-Fu' states that 20 roots of *L. erythrorhizon* were obtained from the 'Kasuya' district in the eighth century, owned by the Kyushu Historical Museum; a collection of the Dazaifu Fureai Culture Museum. (F) A volunteer from the Mitaka Gromwell Restoration Project is transferring *L. erythrorhizon* seedlings to an approximately 60-cm-long tube filled with soil. (G) Three-month-old *L. erythrorhizon* seedlings. (H) Two-year-old *L. erythrorhizon* roots. (I) A traditional extraction process of shikonin pigments from *L. erythrorhizon* roots, i.e. crushing dried roots in a stone mortar with a wooden pestle. (J) Typical activity of the Mitaka Gromwell Restoration Project. Classes are held to restore and conserve the traditional techniques for dyeing Japanese silk fabrics with pigments extracted from the dried roots of *L. erythrorhizon*. (K) A volunteer from the Mitaka Gromwell Restoration Project is dipping a silk fabric in the *L. erythrorhizon* root extract. (L) A silk handkerchief dyed with the *L. erythrorhizon* root extract.

Society Initiatives to Revive Gromwell

Not only has *L. erythrorhizon* impacted culture, history and medicine in East Asian countries for many centuries, but it has also recently attracted some new attention. We had a chance to interview a group of non-profit organizations (NPOs) that aim to restore *L. erythrorhizon* in the 'Mitaka-Musashino' area of Tokyo, Japan.

"While the word 'Murasaki' appears in the lyrics of the anthem of a junior high school in Mitaka, no one in the school has seen the actual plant", says Mr. Nishimura, the representative of the Mitaka Gromwell Restoration Project. Mitaka-Musashino is one of the areas where *L. erythrorhizon* was grown since around the eighth century and massively cultivated during the Edo era (1603–1868). However, as the demand for natural dyes and root extracts of *L. erythrorhizon* was superseded by that of chemical dyes during the Meiji period (1868–1912), planting of *L. erythrorhizon* sharply decreased; together with the spread of disease, this led to the loss of *L. erythrorhizon* in this area.

"Considering the impact of growing *L. erythrorhizon* on the current ecosystem, we think it is difficult to restore the natural habitat of *L. erythrorhizon* in the Mitaka-Musashino area", Mr. Nishimura continues. "However, through this project, we are aiming to protect the native *L. erythrorhizon* species surviving in Japan."

Given that the 'Mitaka-Musashino' species is extinct, the 'Amato' species originally from the Nagano prefecture, but propagated in the Kyoto prefecture for > 70 years (**Fig. 1F, G**), is instead being grown according to traditional methods, as indicated in the 'En-Gi-Shiki' (A.D. 927), the ancient Japanese government regulations established during the Heian period (from around the 8th century to the 12th century), for growing *L. erythrorhizon*.

"We also want to conserve the traditions of using *L. erythrorhizon* for everyone's benefit", says Mr. Nishimura. As a result, they regularly hold outreach classes to reproduce traditional techniques to dye Japanese silk fabrics using the roots of this plant, which they harvest and prepare similar to traditional methods (**Fig. 1H, I**) and using local materials, such as *Camellia* ashes and water collected from the 'Oshima' and 'Tama' areas of Tokyo, respectively, to squeeze-extract the pigments from the roots (**Fig. 1J–L**).

Finally, we asked Mr. Nishimura how plant scientists could contribute to the Mitaka Gromwell Restoration Project: "Scientific approaches are always useful to help verify that our techniques are suitable for propagating *L. erythrorhizon*. We would like to compare both traditional and modern techniques to find better ways of growing *L. erythrorhizon* for the benefit of the community."

How Can Plant Scientists Support Gromwell-Related Initiatives?

The wishes of Mr. Nishimura for techniques to help grow *L. erythrorhizon* highlight a scientifically important issue given the susceptibility of this species to natural enemies, such as CMV (Izuishi et al. 2020). This may be overcome by the establishment of a protection system utilizing physical, chemical, biological and cultural controls against the virus and its vector aphids. To this aim, collaborations between researchers and NPOs, such as Mitaka Gromwell Restoration Project, could help rapidly and accurately disseminate new scientific findings on cultivation methods for this species.

Besides its historical and cultural importance, gromwell is also currently valued as a crude drug. Since shikonin derivatives determine the value of plants, a key aim is to develop methods to cultivate *L. erythrorhizon* plants with high shikonin contents. Therefore, recent studies are also focused on understanding how shikonins are produced in *L. erythrorhizon* roots in order to increase the shikonin content. An intriguing trait is that shikonin production occurs specifically under dark conditions (Yazaki 2017). Cultured cell lines and hairy roots, which have been created by dedifferentiation with plant hormones and infection of *Agrobacterium rhizogenes* of *L. erythrorhizon* tissues, respectively, show the dark inducibility of shikonin production (Yazaki 2017). Through various analytical approaches using these model systems, the cellular and molecular mechanisms related to the transcriptional regulation, biosynthesis and apoplast secretion of shikonins have been successively disclosed over the last few decades (Yazaki et al. 2002, Zhao et al. 2015, Tatsumi et al. 2016, 2023, Zhu et al. 2017, Takanashi et al. 2019, Ueoka et al. 2020, Kiyoto et al. 2022). A comprehensive understanding of shikonin production will lead to higher-quality *L. erythrorhizon* plants for use as medicinal and pigment sources. In addition, a mechanistic understanding of other important lipophilic metabolites may also be achieved (Wong 2019, Ichino and Yazaki 2022).

Moreover, research into identifying additional functions for its ingredients could add new values to this medicinal plant and help further promote the restoration of *L. erythrorhizon* plants in Japan. For example, shikonin inhibits the main protease activity of severe acute respiratory syndrome coronavirus 2, which is an attractive target in drug discovery (Jin et al. 2020). Besides shikonin derivatives, *L. erythrorhizon* is capable of producing diverse specialized metabolites, such as benzoquinone derivatives and caffeic acid oligomers, e.g. lithospermic acid B, *epi*-rabdosiin and rosmarinic acid (summarized in Yazaki 2017, Yamamoto et al. 2020). Further biological activity screening and metabolomics may unearth new valuable molecules.

To summarize, it is in fact a serious social concern that *L. erythrorhizon* is now gravely endangered despite its importance in Japanese history, culture and industry for >1,500 years. This situation should be improved by both social and scientific approaches: social activities will help re-familiarize the Japanese public with this species, while scientific achievements will hopefully help promote the cultivation of *L. erythrorhizon* in Japan for both functional and ornamental purposes. One of the goals of plant research is to resolve fundamental problems affecting society and culture, and here we present one such example where multiple scientific research approaches on gromwell could contribute toward achieving this goal.

Data Availability

No new datasets were analyzed or reported in this work.

Funding

Institute for the Future of Human Society of Kyoto University to K.Y., research projects in the humanities, social sciences and interdisciplinary fields for the academic year 2022.

Acknowledgments

We thank Mr. Manabu Nishimura, Ms. Hideko Nakamura and the Mitaka City government office for the interview on the Mitaka Gromwell Restoration Project and for allowing the use of photographs of their activities and of the Mitaka City Osawano Sato Historic Farmhouse. We also thank Mr. Hirokazu Matsukawa (Kyushu Historical Museum) for providing valuable information of 'Da-Zai-Fu' and the wooden documents. *Lithospermum erythrorhizon* seeds and a photo of a root section of *L. erythrorhizon* were kindly provided by Amato Pharmaceutical Products, Ltd., and Mr. Hao Li (Kyoto University), respectively.

Disclosures

The authors have no conflicts of interest to declare.

References

Afendi, F.M., Okada, T., Yamazaki, M., Hirai-Morita, A., Nakamura, Y., Nakamura, K., et al. (2012) KNApSAcK family databases: integrated metabolite–plant species databases for multifaceted plant research. *Plant Cell Physiol.* 53: e1.

Brigham, L.A., Michaels, P.J. and Flores, H.E. (1999) Cell-specific production and antimicrobial activity of naphthoquinones in roots of *Lithospermum erythrorhizon*. *Plant Physiol.* 119: 417–428.

Ichino, T. and Yazaki, K. (2022) Modes of secretion of plant lipophilic metabolites via ABCG transporter-dependent transport and vesicle-mediated trafficking. *Curr. Opin. Plant Biol.* 66: 102184.

Izuishi, Y., Isaka, N., Li, H., Nakanishi, K., Kageyama, J., Ishikawa, K., et al. (2020) Apple latent spherical virus (ALSV)-induced gene silencing in a medicinal plant, *Lithospermum erythrorhizon*. *Sci. Rep.* 10: 1–9.

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., et al. (2020) Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature* 582: 289–293.

Kiyoto, S., Ichino, T., Awano, T. and Yazaki, K. (2022) Improved chemical fixation of lipid-secreting plant cells for transmission electron microscopy. *Microscopy* 71: 206–213.

Matsukawa, H. (2021) The government offices of Dazaifu and Mokkan. *Proc. Jpn. Soc. Study Wooden Doc.* 42: 219–240.

Takanashi, K., Nakagawa, Y., Aburaya, S., Kaminade, K., Aoki, W., Saida-Munakata, Y., et al. (2019) Comparative proteomic analysis of *Lithospermum erythrorhizon* reveals regulation of a variety of metabolic enzymes leading to comprehensive understanding of the shikonin biosynthetic pathway. *Plant Cell Physiol.* 60: 19–28.

Tatsumi, K., Ichino, T., Isaka, N., Sugiyama, A., Okazaki, Y., Higashi, Y., et al. (2023) Excretion of triacylglycerol as a matrix lipid facilitating apoplastic accumulation of a lipophilic metabolite shikonin. *J. Exp. Bot.* 74: 104–117.

Tatsumi, K., Yano, M., Kaminade, K., Sugiyama, A., Sato, M., Toyooka, K., et al. (2016) Characterization of shikonin derivative secretion in *Lithospermum erythrorhizon* hairy roots as a model of lipid-soluble metabolite secretion from plants. *Front. Plant Sci.* 7: 1066.

Ueoka, H., Sasaki, K., Miyawaki, T., Ichino, T., Tatsumi, K., Suzuki, S., et al. (2020) A cytosol-localized geranyl diphosphate synthase from *Lithospermum erythrorhizon* and its molecular evolution. *Plant Physiol.* 182: 1933–1945.

Waki, T., Terashita, M., Fujita, N., Fukuda, K., Kato, M., Negishi, T., et al. (2021) Identification of the genes coding for carthamin synthase, peroxidase homologs that catalyze the final enzymatic step of red pigmentation in safflower (*Carthamus tinctorius* L.). *Plant Cell Physiol.* 62: 1528–1541.

Wong, D.C. (2019) Harnessing integrated omics approaches for plant specialized metabolism research: new insights into shikonin biosynthesis. *Plant Cell Physiol.* 60: 4–6.

Yamamoto, H., Tsukahara, M., Yamano, Y., Wada, A. and Yazaki, K. (2020) Alcohol dehydrogenase activity converts 3′′-hydroxygeranylhydroquinone to an aldehyde intermediate for shikonin and benzoquinone derivatives in *Lithospermum erythrorhizon*. *Plant Cell Physiol.* 61: 1798–1806.

Yazaki, K. (2017) *Lithospermum erythrorhizon* cell cultures: present and future aspects. *Plant Biotechnol.* 34: 131–142.

Yazaki, K., Kunihisa, M., Fujisaki, T. and Sato, F. (2002) Geranyl diphosphate: 4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*: cloning and characterization of a key enzyme in shikonin biosynthesis. *J. Biol. Chem.* 277: 6240–6246.

Zhao, H., Chang, Q.S., Zhang, D.X., Fang, R.J., Wu, F.Y., Wang, X.M., et al. (2015) Overexpression of *LeMYB1* enhances shikonin formation by up-regulating key shikonin biosynthesis-related genes in *Lithospermum erythrorhizon*. *Biol. Plant.* 59: 429–435.

Zhu, Y., Lu, G.H., Bian, Z.W., Wu, F.Y., Pang, Y.J., Wang, X.M., et al. (2017) Involvement of *LeMDR*, an ATP-binding cassette protein gene, in shikonin transport and biosynthesis in *Lithospermum erythrorhizon*. *BMC Plant Biol.* 17: 1–10.