（様式４）

二国間交流事業 共同研究報告書

令和 4 年 4 月 22 日

独立行政法人日本学術振興会理事長 殿

［代表者所属機関・部局］
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［課題番号］
JPJSBP1　　20192301

1. 事　業　名　相手国:　ベルギー　(振興会対応機関:　FWO　)との共同研究

2. 研究課題名

(和文)福島事故後の植物に観察される形態異常の研究：エピジェネティクスが果たす役割は？

(英文)Studying the underlying mechanisms behind the morphological abnormalities observed in plants after the Fukushima accident: does epigenetics play a role?

3. 共同研究全実施期間　2019 年 4 月 1 日 ～ 2022 年 3 月 31 日 (3 年 0 ヶ月)

4. 相手国代表者(所属機関・職・氏名【全て英文】)

Belgian Nuclear Research Centre SCK CEN・Head of Unit・Horemans Nele

5. 委託費総額(返還額を除く)

本事業により執行した委託費総額		4,674,800　円
内訳	1年度目執行経費	2,318,800　円
	2年度目執行経費	2,356,000　円
	3年度目執行経費	－　円

6. 共同研究全実施期間を通じた参加者数(代表者を含む)

日本側参加者等	5 名
相手国側参加者等	5 名

＊ 参加者リスト(様式 B1(1))に表示される合計数を転記してください(途中で不参加となった方も含め、全ての期間で参加した通算の参加者数となります)。

7. 派遣・受入実績

	派遣		受入
	相手国	第三国	
1 年度目	3	0	3(0)
2 年度目	0	0	0(0)
3 年度目	0	0	0(0)
4 年度目			()

＊ 派遣・受入実績(様式 B1(3))に表示される合計数を転記してください。

派遣:本委託費を使用した日本側参加者等の相手国及び相手国以外への渡航実績(延べ人数)。
受入:相手国側参加者等の来日実績(延べ人数)。カッコ内は本委託費で滞在費等を負担した内数。

8. 研究交流実績の概要・成果等

(1)研究交流実績概要（全期間を通じた研究交流の目的・研究交流計画の実施状況等）

The overall goals of the project were to establish an experimental network for long-term radioecological studies and to identify radiation-induced changes occurring at various levels of biological complexity in chronically irradiated plants. The study was conducted on young trees of *Pinus densiflora* (Japanese red pine, a typical conifer species in Japan known for its high radiosensitivity) and *Capsella bursa pastoris* (Shepherd's purse, relative species of the widely used in the radiation effect studies *Arabidopsis thaliana*) growing at the different dose rates in evacuation areas in Fukushima prefecture, and on the plants of these species irradiated in the controlled conditions at the low/intermediate dose rates at the irradiation facility at NIRS and at the high dose rates at the irradiation facility of our partner (SCK CEN).

The project implementation was significantly affected by the COVID-19 situation, as required conducting the joint sampling campaigns in Fukushima and, especially, joint laboratory analyses at partner facilities in Belgium, which became impossible due to travel restrictions in FY2020-2021. Moreover, the partner's work was interrupted by several lockdown periods which led to delays in some laboratory analyses. We have adjusted the initial activity plans to meet the main tasks of the study and to prepare conditions for its continuation after the end of the project period. Despite the COVID-19 restrictions, we were able to fully implement the field observation and sampling program and perform irradiation experiments on pine trees and seeds at NIRS. As such we have set up and maintained an artificial plantation at the Fukushima University campus (Fig. 1) for the long-term observation of radiation effects on the irradiated young pine trees and trees germinated from the irradiated seeds and seeds collected in the evacuation areas. We have collected and shipped to the partner *Capsella* seeds sampled during the springs of 2020 and 2021 in the evacuation and control areas and created a pine tissue archive for further analyses in the partner's labs. The partner carried out epigenetic analyses in a part of the pine tissue samples (will be continued) and is continuing the multi-generation germination/irradiation experiment on *Capsella*. We are preparing articles summarizing the project results for submission to international journals.

Fig. 1. Pine tree plantation at the Fukushima University campus

(2)学術的価値（本研究交流により得られた新たな知見や概念の展開等、学術的成果）

Despite the aforementioned unfavorable COVID-19 situation and the associated delays and changes in research

plans, the project has produced new knowledge important for understanding the mechanisms of radiation effects on plants:

- In field observations, we assessed the frequency of morphological abnormalities (cancellation of the apical dominance) in the studied natural young populations of red pine. At some of our experimental sites, we performed such observations in 2014-2016 (Yoschenko et al., 2016). We found that since then, the proportions of abnormal trees at these sites have decreased: in some trees, apical dominance was restored by preferential growth of one of the lateral branches, which took on the role of the main trunk, as shown in Fig. 2 (white arrows indicate the positions of undeveloped dominant buds). Such reparation occurred in a large part of the transformed trees. For example, in our surveys in May 2019, we found apical dominance recovery in a third of the transformed trees at the experimental site in Tsushima and in half of the transformed trees in Akibadai. In our opinion, such reparation is a very important finding. First, it may explain the apparent difference between the frequencies of apical dominance observed at the same dose rates in pine trees in Fukushima (Yoschenko et al., 2016) and Chornobyl (Yoschenko et al., 2011). Second, the ability of trees to recover apical dominance may indicate an epigenetic mechanism underlying the abnormal transformation. A more reliable conclusion on the role of epigenetic factors will be possible after the completion of molecular analyses at SCK CEN;

Fig. 2. Cancellation of the apical dominance and its restoration in young red pine trees

- Observations on the artificial plantation at the Fukushima University campus confirmed the radiation nature of abnormal transformations in young pine trees. The plantation was formed from four groups of trees (a control group and three groups exposed at the irradiation facility at NIRS to different dose rates for six months prior to planting), and by the groups of small trees germinated from the irradiated seeds and from the seeds collected in the evacuation areas. All trees in the plantation are kept in the same conditions, excluding chemical stressors, drought, damage by animals or birds, etc. We found a statistically significant increase in the frequency of apical dominance cancellation in the trees irradiated at 24 μGy h^{-1} and 41 μGy h^{-1} compared to the control group. In the group of trees irradiated at the highest dose rate, 65 μGy h^{-1}, the frequency of abnormalities decreases, but still remains higher than in the control. Irradiated trees also demonstrate statistically significant suppression of growth: the average height of the trees in the control group now exceeds 90 cm, while in the irradiated groups it ranges from 69 to 75 cm.

Trees sprouted from irradiated seeds showed an increase in the frequency of abnormalities (up to 56% in the highest irradiated group vs. 0% in the control). Finally, the frequency of abnormalities reached 70% in small trees sprouted from seeds collected from abnormal trees in the evacuation zone (Akibadai). The frequency of abnormalities in the groups of trees in the plantation is lower than in the natural populations. A possible explanation is a difference in irradiation scenarios: a six-month period of irradiation for trees in plantations vs. chronic irradiation for trees in evacuation areas. In addition, the trees in the plantations were irradiated in winter, when the radiosensitivity of plants is lower than in other seasons. Finally, new anomalies may also appear after the end of the project period. We will try to establish the relationship between the results of molecular analysis (SCK•CEN) and morphological parameters (apical dominance, height) of individual trees on the plantation;

- Field observations revealed a wide variation of morphological parameters (height, number of stems, etc.) of individual plants of *Capsella* growing within each sampling area, which makes it impossible to detect differences between the populations studied;
- The first results of molecular analyses revealed no significant difference in gene expression between normal, abnormal, and repaired trees from the natural populations of young pine, which may be an important finding for understanding the mechanism of radiation effects in pine. We will continue analyses on the extended set of samples (currently stored at the IER) to verify the preliminary results and draw strong conclusions.

References

Yoschenko, V., Kashparov, V., Melnychuk, M., Levchuk, S., Bondar, Y., Lazarev, N., Yoschenko, M., Farfán, E., Jannik, T., 2011. Chronic irradiation of Scots pine trees (Pinus sylvestris) in the Chernobyl Exclusion Zone: dosimetry and radiobiological effects. Health Physics, 101, 393-408.

Yoschenko, V., Nanba, K., Yoshida, S., Watanabe, Y., Takase, T., Sato, N., Keitoku, K., 2016. Morphological abnormalities in Japanese red pine (Pinus densiflora) at the territories contaminated as a result of the accident at Fukushima Dai-Ichi Nuclear Power Plant. J Environ Radioact, 165, 60-67.

⑶相手国との交流（両国の研究者が協力して学術交流することによって得られた成果）

The partner's researchers visited Fukushima twice during FY 2019. In May, details of the research were discussed at a project kick-off meeting held at the IER, followed by a joint IER – SCK CEN – NIRS sampling campaign in the evacuation areas and at the control sites (Fig. 3). The field observations included the assessment of the local exposure conditions and the determination of morphological parameters of the pine trees in the studied populations. Based on that, the control groups of trees were selected at each site. The soil and pine biomass samples, as well as *Capsella* plants, were collected for further laboratory analyses. In October 2019, a joint sampling campaign was performed on the same pine populations and an artificial pine plantation on the Fukushima University campus. The collected samples were analyzed or processed at the IER facilities, prepared for transportation, or (*Capsella* seeds) shipped to SCK CEN for further analyses.

The IER researchers visited the partner institutes twice during FY2019. In June, Prof. Yoschenko and Prof. Nanba, visited SCK CEN and the University of Antwerp for the elaboration of analytical methods and a cost-efficient sample processing and analyses scheme. Prospects for research into the potential role of species interactions in the ecosystem in shaping the response of pine to radiation exposure were also discussed. In January 2020, Prof. Yoschenko visited the Belgian partner to acquire analytical skills in epigenetic methods and conduct joint laboratory analyses of collected pine tissue samples.

For FY2020 we scheduled two joint sampling campaigns in Fukushima, as well as visits by the IER researchers to SCK CEN for laboratory studies. In the situation of the COVID-19 outbreak, the visit exchanges were postponed

to the project prolongation period in FY2021. However, the situation did not improve, resulting in the cancellation of the visits and adjustments to the initial research plans: all sampling campaigns and field observations were performed by the IER, part of the samples were shipped to SCK CEN while others are pre-processed and stored at the IER, and the discussions of the results were held online and through the e-mail exchange. The opportunity remains that the partner will visit the IER in FY2022 to resume/continue the collaborative study.

Fig. 3. Visits exchange in FY2019

(4)社会的貢献（社会の基盤となる文化の継承と発展、社会生活の質の改善、現代的諸問題の克服と解決に資する等の社会的貢献はどのようにあったか）

In general, this project aims to assess the radiation impact on forest species, which is an important social issue in the context of evaluating the perspectives for the revitalization of forestry in radioactively contaminated areas of Fukushima Prefecture. We focused on the most radiosensitive local tree species, Japanese red pine. Our study quantifies the effect on the development of the young pine trees depending on the level of radiation exposure and thus may help to assess the commercial potential of this species in forestry in the contaminated areas. The project participants, including our foreign partners, presented the project progress and findings in the special programs on NHK, as well as in other media, public dialog meetings, etc.

On the other hand, reciprocal visits within the framework of the project helped familiarize the participants with the nature, culture, and traditions of Japan and Belgium.

(5)若手研究者養成への貢献（若手研究者養成への取り組み、成果）

Due to travel restrictions because of the COVID-19 situation, it was not possible to organize training for the IER young researchers or graduate school students at the counterpart's facilities as planned at the beginning of the project. Instead, the graduate school students, as well as the undergraduate students from Fukushima University visited one of the experimental sites, Tsushima, during the 2020 and 2021 field campaigns to take part in field

observation and sampling. In addition, the partner's PI Prof. Horemans, Dr. Watanabe (QST-NIRS), and Prof. Yoschenko (IER) gave lectures to the graduate school students in the class of Effects of radiation exposures in the 1st semesters of FY2019-2021 (in FY2020 and 2021, the whole class was supervised by Prof. Yoschenko). These lectures presented project results to illustrate current advances and challenges in understanding the mechanisms of radiation effects in plants.

⑹将来発展可能性（本研究交流事業を実施したことにより、今後どの様な発展の可能性が認められるか）

The project partners recognize the benefits of cooperation in studying the radiation effects on plants and intend to continue the joint research begun under this project. In particular, we will continue to observe the development of young pines in an artificial plantation on the Fukushima University campus and will seek opportunities to exchange visits between IER and SCK CEN and conduct joint analyses of samples collected on the plantation as well as those sampled in the Fukushima affected area. We are also considering expanding the scope of collaborative research, for example by exploring the role of plant-fungal interactions as a factor that can modify the stress response in plants, and bringing in new European partners for future research.

⑺その他（上記⑵～⑹以外に得られた成果があれば記述してください）

例：大学間協定の締結、他事業への展開、受賞、産業財産権の出願・取得など

During visits to the partner, we established contacts and had discussions with representatives of Belgian institutions. In particular, we are now exploring the possibility of starting a collaboration with Vrije Universiteit Brussel (VUB), which may include the signing of a formal cooperation document.