The Utilization of Ferns as a Model Organism for Studying Natural Polyploidization Concept in Genetics Course

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Abstract: The Genetics lecture in the Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang (FMIPA UM) has its own characteristics distinguishing it from Genetics lecture at the other universities. One of characteristics is there are research projects designed by students utilizing various model organisms. Natural polyploidization is one of the concepts learned through the research project. Fern is used as one of model organisms in the research project. The aim of this study was to describe the utilization of ferns as a model organism in Genetics I course in FMIPA UM. The study was conducted with a qualitative approach. The data were collected from January 2016 to September 2016 by interview, observation, and documentation techniques. The data were analyzed using an interactive model of Miles and Huberman. The validity of data were re-tested using an extension of participation, persistence of observation, and triangulation. The research results showed that through research project activities, the students (1) were able to learn the natural polyploidization concept contextually; (2) easier to understand the natural polyploidization concept; and (3) were trained to be a real researcher.

Keywords: fern, genetics course, model organism

Genetics lecture at FMIPA UM has characteristics distinguishing it from Genetics lecture at the other universities that are the approach and the kind of learning activities (Fauzi et al, 2016). The Genetics lecture in FMIPA UM adopts the content approach, not the historical approach as in the other universities (Khairil, 2009). The lecture materials discussed at FMIPA UM hierarchically structured into seven great concepts, that are the Understanding of Genetics, Genetics Material, the Reproduction of Genetics Material, the Work of Genetics Material, the Change of Genetics Material, Population Genetics, and Genetic Engineering (Genetics I FMIPA UM Learning Plans, 2016; Genetics II FMIPA UM Learning Plans, 2016). Then, there are two main activates in Genetics lecture at FMIPA UM that are theoretical lectures and practical lectures. Furthermore, the practical activity consists of classical and project researches (Khairil, 2009; Fauzi et al, 2016).

The existence of project research is one of characteristics in Genetics lecture at FMIPA UM. The research project is the research activities conducted by Genetics students for one semester. In the activity, students are required to find a research idea until communicate research report with the guide of project assistant (Fauzi et al, 2016). Various genetics concepts learned through research project activities. One of the concepts is the natural polyploidization.

Polyploidization is the increase in genome size caused by the inheritance of an additional set (or sets) of chromosomes (Otto, 2007). There are two main mechanisms of polyploidization, autopolyploidy and allopolyploidy. (Krebs et al, 2013). In autopolyploidy, the duplicated sets of chromosomes originate from the same or a closely related individual, while in allopolyploidy the duplicated sets of chromosomes originate from the hybridization of two different species (Tamarin, 2001). One common effect generated from polyploidization is an increase of cell size

(Snustad & Simmons, 2012). The increasing of the cells size by polyploidization can cause the increasing of body size in some multicellular organisms (Kliman, 2016).

Polyploidy is a common phenomenon occurring in plants, but rarely occurs in animals (Snustad & Simmons, 2012). It is estimated that about 30 to 80% of all plant species are polyploid species (Tamarin, 2001; Meyers & Levin, 2006; Otto, 2007). The high frequency of polyploidization occurrence in plants is also related to the role of this phenomenon in evolution and speciation of the plants (Ranney, 2006). The occurrences of polyploid species in plants can arise spontaneously in nature through a variety of mechanisms, including an error of mitosis and meiosis and the fusion of unreduced gametes (2n) (Comai, 2005). The groups of plants that contribute the largest number of polyploid species are angiosperms and ferns (Tamarin, 2001; Meyers & Levin, 2006).

A fern is a member of a group of vascular plants that reproduce via spores, have neither seeds nor flowers, and undergo an alternation of generations, the gametophyte and sporophyte (Christenhusz & Chase, 2014). Related to the polyploidy speciation frequency, ferns occupied the top of the list, beating angiosperms (Otto & Whitton, 2000; Wood et al, 2009). Still related with polyploidy incidence, some previous studies also indicated that in highlands that have a lower temperature, the ploidy level of ferns tend to be greater (Zubaidah, 1998; Setyawati, 2000). This is an agreement with the surveys presented in previous study by Weiss-Schneeweiss et al (2013) which concluded the increasing of altitude causing the increasing of polyploidy frequency.

In Genetics lecture at FMIPA UM, ferns are one of model organism in studying natural polyploidization through the project research activities. Various benefits can apparently be obtained from the research project activities. However, the research that studying the benefits obtained from the activity has not been done. Moreover, even though it provides many benefits, ferns also have never been used as a model organism in project activates in Genetics lecture at the other universities. Therefore, the study that describes the process of ferns utilization as model organism in Genetics lecture need to be done. The aim of this study was to describe the utilization of ferns as a model organism in Genetics I lecture in FMIPA UM. The research questions in this study, are (1) How the process of utilizing the ferns as a model organism in studying the natural polyploidization in Genetics lecture at FMIPA UM?; (2) What the benefits of utilizing the ferns as a model organism in studying the natural polyploidization in Genetics lecture at FMIPA UM?; and (3) What the obstacles during the utilizing the ferns as a model organism in studying the natural polyploidization in Genetics lecture at FMIPA UM?

METHODS

The study was conducted with a qualitative approach. The data were collected from January 2016 to September 2016 by interview, observation, and documentation techniques. The data were analyzed using an interactive model of Miles and Huberman. The validity of data was re-tested using an extension of participation, persistence of observation, and triangulation.

RESULTS AND DISCUSSION

General Process

The project research of the utilizing of ferns as model organism in studying the natural polyploidization concept is held in Genetics 1 course. Same with the other research projects, this research is also held for one semester (Khairil, 2009). The project activities were mostly conducted in Genetics Laboratory, the third floor of Biology Building, FMIPA UM. In the

activity, students were trained to find the idea or the problem of research, arrange a research design and research procedures, collect and interpret data, prepare a research report, until communicate the results (Fauzi et al, 2016). During the research project, students were also assisted by project assistant who guides their research.

A series of ferns project activities began by dividing the project group into 16 groups in the first week of Genetics I course. In this week, at each Genetics Class, the project assistant was dividing the project group randomly. Then, one of project group at each class were randomly selected as a ferns group. There were five Genetics classes at the semester, therefore, there were five ferns groups in this semester.

In the second week, ferns project groups met their project assistant. The assistant that guide the ferns project in this semester was Ika Sukma, S. Pd. At the meeting, the assistant project explained an overview of the project activates that will be carried out by ferns project groups for one semester. After that, the project assistant also commanded the ferns groups to search for some scientific publications studying ferns polyploidization. Based on the interview with Ika Sukma, S. Pd, the task was intended to make the students understand about the characteristics of the ferns to be used in their research and knowing the chromosome basic number of the ferns. In addition, the task was also intended to make students knowing how the procedure of making ferns objects. Through the scientific publication reading task, students will gain a variety of benefits, particularly related to the empowering their scientific process skill (Fain, 2009; Veit et al, 2014).

In third week, each ferns group met again with their project assistant to discuss several things. During this meeting, each group submitted scientific publications they have gotten to project assistant. In addition, each group was also explaining the information or their understanding of the scientific publications they have read. Through the task, students were not only trained to search for scientific publications, but also trained to understand and communicate the content of scientific publications that have been read. Through reading activity, they understand what previous researchers have done (Martin, 2012). In addition, the ability to read and understand the contents of scientific publications is essential for students before they do a research (Fain, 2009). Moreover, through this activity, students were empowered to access, analyze, synthesize, and communicate the information they obtain, those skills needed by a real researcher (Veit et al, 2014). After that, each ferns group discussed the fern species that will be choosen, the sampling areas, as well as the experiment design and procedures of their research. Each group was required to determine the three sampling areas, one place in highland, one in middle altitudes, and one in lowland.

In fourth week, each group met again with project assistant to ensure the sampling areas and clarified the procedures that have been discussed previously. In this week, each group also began to take the ferns from their sampling areas. After each group got ferns from their sampling areas, the ferns planted in their yard. Table 1 presents the students list names who receive the ferns project with their ferns species and their sampling areas.

Table 1. The List of The Students who got Ferns Project with Their Species and Sampling Areas

No	Name	Class	Fern Species	Sampling Areas
1	Ade Rezi Amelia	A	*Adiantum diaphanum*	Batu Lowokwaru (Malang) Tanggulangin (Sidoarjo)
2	Ismi Lailatul Rohma	B	*Dryopteris filix-mas*	Pasuruan

3	Rahma Ayu Fauziah	C	*Adiantum radiannum*	Tumpang (Malang) Kepanjen (Malang) Tajinan (Malang)
4	Gizella Ayu Wiantika	G	*Pteris vittata*	Tirtoyudo (Malang); Dampit (Malang); Batu
5	Mita Larasati	H	*Pteris vittata*	Leces (Probolinggo); Lumbang (Probolinggo) Sukapura (Probolinggo)

In the fifth until fifteenth week, each group carried out the ploidy level observation based on their procedure. The determination of fern ploidy level was based on the total number of chromosomes in root tip cells. Therefore, the step that must be done firstly was to prepare the fern root tip cells object. The root tip cells were selected as the object of observation due to the root tips are the main root growth area that actively carry cell division (Gupta, 2007). By selecting that region chances of getting the cells that are in metaphase or anaphase stages increases. By getting the cell in metaphases or anaphase stage, it will be easier for students to determine the total number of chromosomes in each of those cells. This is because when metaphase, chromosomes become highly condensed, so it easy to be observed (Cooper, 1994).

Before getting the observation objects, the first step that must be done is cutting the ferns roots. The cutting roots activity must be conducted at 9 AM. After getting ferns roots pieces, those roots were soaked in FAA solution. FAA is a fixative solution, thus, soaking the pieces of roots in FAA solution can keep root cells remain in their original state and stop the mitotic process in those cells (Bhargava & Srivastava, 2013; Stasolla & Yeung et al, 2015; Simpson, 2016).

When the students will conduct the observations, the root pieces washed with water to remove FAA and then put into the vial bottle. The next step was added 1 N HCl solution into the vial bottle. Then, after the vial bottle tightly closed, the bottle put in a water bath with a temperature of 60 ^{o}C for 15 minutes. The purpose of adding HCl is to destroy the substance that unites one cell to another (commonly pectin), but does not destroy the cell walls, while also able to stop the mitotic process (Carboni, 2010). Then, the roots pieces removed from vial bottle and those pieces washed using water.

The next step was the root pieces placed on glass object and cutting the root tips (the white part). After that, the pieces of root tips drip with acetocarmine and wait for five minutes. The aim of adding acetocarmine at this step is for dyeing the chromosomes of the root tips cells (Fukui & Nakayama, 1996). Then, the root tips pieces covered with cover glass and being run over by a pen. After that, the students were ready to observe the fern root tips chromosomes under a light microscope with a magnification of 1, 000x. Figure 1 shows some objects produced by students.

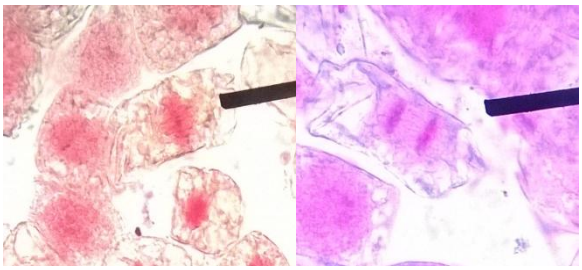

Figure 1 Some Objects Generated by Students

The determination of ploidy level is carried out by direct observation. Five cells at metaphase or anaphase stages from each of object in each ferns were observed. Every single cell was counted three times, and then the results of these calculations were averaged. The mean from each cell then were summed and then divided by five to obtain the mean of the total number of chromosomes from that fern. Furthermore, the mean of chromosomal total number was divided by the chromosomal basic number of the fern to determine the ploidy level of the fern. Five ferns were taken from each area as the replications. The determination of ploidy level by dividing the observed chromosome number with the basic chromosome number of those ferns accordance with previous research studying ploidy level in ferns, such as Zubaidah (1998); Perwati (2009); and Efendi et al (2014).

After collecting data for one semester, at the sixteenth week, the ferns group prepared a research report and presented their project. In this week, each fern group communicates the process and the results of their research to their classmates. After that, there were discussions sessions that provide an opportunity for their classmates to give advice, refutation, feedback, as well as questions related to their project. Then, the project assistant evaluating the project report have been submitted and reviewing the presentation and discussion that have been conducted. Based on the result of ferns research project activities, the general conclusion obtained is the ferns in higher altitude have higher ploidy level than ferns in lower altitude. Their results are in line with previous studies conducted by previous researchers, such as Zubaidah (1998) and Setyawati (2000).

A. The Benefits

Various benefits perceived by the students after conducting fern project research. Table 2 shows the benefits obtained by every fern project students after conducting their research. Based on Table 2, it is known that through fern research project, students can easily learn the polyploidy concept. This is because to the learning based research that applies the principles of inquiry are able to increase the concepts understanding of learners (Hassard, 2011). In addition, students were able to have the skills to conducting a research and the skills in making a ferns cells object.

Table 2 The Interview Result Reveals Some Benefits From Doing Ferns Project Research

No	Name	Benefits
1	Ade Rezi Amelia	Being able to observe the chromosomes directly, can observe the ploidy level in ferns based on altitude, acquire the skills to make object
2	Ismi Lailatul Rohma	Having knowledge from direct observation related to the effect of altitude on ploidy level in ferns and acquire the skills to make objects
3	Rahma Ayu Fauziah	Having experience and skill on conducting research and better understand the concept of polyploidy due to learn contextually
4	Gizella Ayu Wiantika	Easier to understand the polyploidy concept and acquire the skills to make object
5	Mita Larasati	Acquire the skills to make objects and can learn and observe the ploidy level in ferns directly

Besides the benefits already mentioned, there are other benefit obtained from project research activities in Genetics lecture at FMIPA UM. The benefit is students trained to be a real researcher (Fauzi et al, 2016). In the project activities, students were guided to determining a research idea, designing the research, collecting and analyzing the data, discussing research findings, as well as communicating the research. Those activities are the activities commonly performed by the real researchers.

The Obstacles

Some obstacles during the project activities were experienced by some ferns project groups. Table 3 shows the obstacles felt by every student during completing their research project. Based on Table 3 it can be seen that the main obstacle is related to the availability of microscope that support the chromosomes observation. In addition, the other obstacle was the time of cutting root that overlap with the other courses schedule and the obstacles related to the object that were not quite good. Related to the availability of microscope, it is expected this problem have been overcome in the next academic year. Related to making the object, it is normal because the skills to making good object can be obtained after practicing not in short time.

Table 3 The Interview Results Reveals Some Obstacles During Conducting Ferns Project Research

No	Name	Obstacles
1	Ade Rezi Amelia	The obstacles related to the time of cutting the roots, the difficulty in making the object that can observed easily, not all microscopes in Genetics Lab can be used to observing object under a magnification of 1000x
2	Ismi Lailatul Rohma	The difficulty in making the object that can observed easily
3	Rahma Ayu Fauziah	The obstacles related to collecting the samples from sampling areas and not all microscopes in Genetics Lab can be used to observing object under a magnification of 1000x
4	Gizella Ayu Wiantika	The difficulty in making the object that can observed easily and the obstacles related to keep ferns still alive
5	Mita Larasati	The difficulty in making the object that can observed easily, the obstacles related to the time of cutting the roots, and not all microscopes in Genetics Lab can be used to observing object under a magnification of 1000x

Conclusion

Ferns were used as a model organism in project research activity studying natural polyploidization concept in Genetics lecture at FMIPA UM. The research activity carried out during one semester. Various benefits perceived by the students after conducting fern project research, such as students can easily learn the polyploidy concept, students were able to have the skills to conducting a research and the skills in making a ferns cells object, as well as students trained to be a real researcher. However, there were some obstacles during doing the project, such as the obstacle related to the availability of microscope that support the chromosomes observation, the time of cutting root that overlap with the other courses schedule and the obstacles related to the object that were not quite good.

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