

## Elevated temperature and DNA methylation Affects Mago nashi Expression and Sexual Development in *Ceratopteris richardii*

**Category:** Plant Sciences

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### A. Rationale:

Elevated temperature, a distinct characteristic of climate change, has previously been correlated with significant decreases in crop yield. Elevated temperatures have also been shown to reduce pollen production and fertility in various plants such as *Phaseolus vulgaris* (Gross and Kigel, 1994), *Brassica napus* (Young *et al.*, 2004), and *Lycopersicon esculentum* (Abdul-Baki and Stommel, 1995). With the world population predicted to grow to about 9 billion in 2050, it is crucial that a stable food supply is established.

Elevated temperature is known to reduce crop yield. An increase in temperature is detrimental to pollen production and development. Specifically, both microsporogenesis and microgametogenesis phases are extremely sensitive to heat (Mueller and Rieu, 2016). However, this mechanism of heat sensitivity is poorly understood.

Mago nashi is a RNA-binding protein that is involved in germ cell differentiation. It is ubiquitous, being highly conserved throughout numerous species of plants and animals (Swidzinski *et al.*). Mago nashi has been found in plants such as *Oryza sativa*, *Physalis floridana*, *Marsilea vestita*, and *Arabidopsis thaliana* (Swidzinski *et al.*, 2001; He *et al.*, 2007; Weele *et al.*, 2007). It affects the sex determination in the early development in plants, including spermatogenesis (He *et al.*, 2007), male fertility (Weele *et al.*, 2007), and seed viability (Park *et al.*, 2009).

Mago nashi has been shown to partake in the export of mRNA from the nucleus to the cytoplasm in eukaryotic organisms. It has co-evolved with another mRNA binding protein, Y-14 (Gong, *et al.*, 2014), in the export of mRNA into the cytoplasm with export factors barentz (Btz) and eukaryotic initiation factor 4a-III (eIF4AIII) (Roignant and Treisman, 2010). These export factors, along with Mago nashi and Y-14, also play a key role in the function of the Exon Junction Complex (EJC). The knockdown of key EJC components such as Mago nashi and Y14 in *Oryza sativa* have resulted in much shorter plants compared to the wild type (Gong and He, 2014). This suggests the significance of EJC components play in the early plant development.

Further examination of Mago nashi in higher plants is challenging due to the complex nature of pollen and seeds (Bushart *et al.*, 2007). Mechanisms including Mago Nashi-mRNA binding is not well understood in plants. Studying these mechanisms in ferns may provide insight into gametophyte development in higher plants (Banks, 1999).

A more viable plant model may be the aquatic fern, *Ceratopteris richardii* (Cooke *et al.*, 1995; Chasan, 1992). Gametophytes of *Ceratopteris* are either characterized as male or hermaphrodites. Since ferns have characteristics of a higher plant (sporophyte) and lower plant system (gametophyte), examination of *Ceratopteris* is highly advantageous as development can be examined separately in each developmental stage (Hickok *et al.*, 1995; Banks, 1999). The sexual expression of gametophytes in *Ceratopteris* is determined by a chemical messenger, antheridiogen, which promotes the antheridia, the male sex organ (Warne and Hicock, 1988). Hermaphrodites produce antheridia as well as archegonia, the female sex organ. Two mutants characterized in *Ceratopteris* are the *him1* and *her1* (Banks, 1994; Banks, 1999). *him1* and *her1* display abnormalities in sexual development (Spiro *et al.*, 2008; Banks 1994). *him1* favors the development of males; *her1*, the development of hermaphrodites.

The focus of this study is to investigate the role of Mago nashi in sexual differentiation of males and hermaphrodites in response to temperature changes. We will examine the effects of high temperature and inhibition of DNA methylation on the expression of Mago nashi in *Ceratopteris richardii*. Investigation will shed light on the mechanisms of sexual differentiation and development in plants in response to the effects caused by climate change.

### **Research Questions/Hypothesis/ Expected Outcome**

*Problem:* How does elevated temperature due to climate change affect sexual differentiation of gametophytes in *Ceratopteris richardii*?

*Hypothesis:*

Elevated temperature (28 °C) and inhibition of DNA methylation by 5-Azacytidine will lead to a greater disparity between male and hermaphrodite gametophytes in *Ceratopteris richardii*, and resulting increases in the number of males will affect fertility and cause a subsequent decrease in quantity of sporophytes developed. Mago nashi will be expressed more in *Ceratopteris* spores grown at higher temperatures and spores treated with 5-AC than those at standard condition.

### **B.**

#### **Procedures:**

##### **Measuring Germination:**

C-Fern® agar, Liquid C-Fern® media, and pre-sterilized dry spores of *Ceratopteris richardii* (Catalogue #156728) will be obtained from Carolina Biological. C-Fern® agar and Liquid C-Fern® media will be prepared according to manufacturer's procedures. Gloves, goggles, and lab coats will be worn when handling C-Fern® agar.

Spores will be incubated in the dark for 7 days with the 25 µM 5-Azacytidine, or 5-AC (Sigma Aldrich; Product ID: A2385-100MG). Since this chemical was highly toxic, it was prepared and handled by mentor. Personal protective devices includes goggles, gloves, lab coats and fume hood. After 7 days of incubation, spores will be plated onto C-fern media (Carolina Biological) and be allowed to develop under continuous white light. The ratio of males to hermaphrodite will be assessed at 10 days. The number of sporophytes produced will be measured after 30 days.

##### **RNA Isolation/RT-PCR:**

Spores will be grown in the presence of 5 AC and elevated temperature as previously described. To determine Mago nashi expression, RT-PCR will be conducted. Gloves, goggles, and lab coats will be worn. Mentor will assist in handling and disposing of hazardous chemicals. RNA will be isolated using the Invitrogen RNAqueous-4PCR Total RNA Isolation Kit (Catalog # AM1914). 0.5-0.75 mg tissue samples will be used. Samples will be disrupted with Lysis/Binding Solution. An equal volume of 64% Ethanol will be added. The lysate/ethanol mixture will be drawn through a Filter Cartridge. It will then be washed with 700µl Wash Solution #1 as well as with the 2×500µl Wash Solution #2/3. RNA will be eluted with 40-60µl preheated Elution Solution. It will then be eluted with a second 10-60 µl aliquot of Elution Solution.

RNA will be reverse transcribed using Invitrogen SuperScript III One-Step RT-PCR System with Platinum *Taq* High Fidelity DNA Polymerase from Thermo Fisher Scientific (Catalog # 12574-030). These procedures will be followed according to manufacturer's instructions. The thermal cycler will be programmed so that cDNA synthesis is automatically followed with PCR amplification.

#### **Agarose Gel Electrophoresis:**

Agarose Gel Electrophoresis will be used to analyze the amplification of PCR products. Mentor will oversee the procedure. Gel tray will be assembled and 1% agarose gel in 1xTBE solution (89mM Tris Base, 89mM Boric Acid, 2mM EDTA) will be prepared. Agarose gel will be run in 1xTBE buffer. DNA will then be visualized using SYBR Safe DNA stain and UV light.

#### **PCR Conditions and Agarose Gel Electrophoresis:**

| <b><u>Step</u></b>   | <b><u>Temperature (°C)</u></b> | <b><u>Time (Minutes)</u></b> | <b><u>Cycles</u></b> |
|----------------------|--------------------------------|------------------------------|----------------------|
| Initial Denaturation | 94°C                           | 3 minutes                    | 1                    |
| Denaturation         | 94°C                           | 0.5 minutes                  | 30 -35               |
| Annealing            | 45-68°C                        | 0.5 minutes                  |                      |
| Extension            | 72°C                           | 2 minutes                    |                      |
| Final Extension      | 72°C                           | 10 minutes                   | 1                    |
| Hold                 | 4°C                            | Indefinitely                 | N/A                  |

#### **Risk and Safety:**

Material Safety Data Sheets from US Research Nanomaterials, Cayman Chemicals, Sigma Aldrich, ThermoFisher Scientific, and Carolina were used for the chemicals.

**5-Azacytidine** - may cause genetic defects, cancer, and is harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed according to official regulations by the chemical safety officer of G.W. Hewlett High School.

<https://www.fishersci.com/store/msds?partNumber=AC226620500&productDescription=5-AZACYTIDINE%2C+99%25+50MG&vendorId=VN00032119&countryCode=US&language=en>

**C-Fern Agar/Liquid C-Fern** - more than minimal risk may cause eye, skin, and respiratory irritation and can cause gastrointestinal discomfort if swallowed. Gloves, goggles, lab coats will be worn when handling. <https://www.carolina.com/teacher-resources/Document/msds-basic-c-fern-medium-prepared/tr-msds-basic-c-fern-medium-prepared.tr>

**Acetone-** highly flammable liquid and vapor; more than minimal risk may cause serious eye irritation; toxic to aquatic life; potentially harmful if inhaled or absorbed through skin; potentially harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed according to official regulations by the chemical safety officer of G.W. Hewlett High School.

<https://www.fishersci.com/shop/msdsproxy?productName=AC177170010&productDescription=ACETONE>

**Invitrogen RNAqueous®-4PCR Total RNA Isolation Kit-** more than minimal risk can potentially harmful if inhaled, absorbed through skin, or when in contact with the eyes, causing irritation; potentially harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instructions and according to official regulations by the chemical safety officer of G.W. Hewlett High School.

[https://assets.fishersci.com/TFS-Assets/LSG/manuals/cms\\_059300.pdf](https://assets.fishersci.com/TFS-Assets/LSG/manuals/cms_059300.pdf)

**Invitrogen SuperScript® III Platinum® One-Step RT-PCR Kit-** more than minimal risk can potentially harmful if inhaled, absorbed through skin, or when in contact with the eyes, causing irritation; potentially harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instructions and according to official regulations by the chemical safety officer of G.W. Hewlett High School.

[https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/superscript\\_onestep\\_qrtPCR\\_man.pdf&title=Product%20Info%20Sheet:%20SuperScript%20III%20Platinum%20One-Step%20qRT-PCR%20Kit](https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/superscript_onestep_qrtPCR_man.pdf&title=Product%20Info%20Sheet:%20SuperScript%20III%20Platinum%20One-Step%20qRT-PCR%20Kit)

**SYBR Safe® DNA Stain-** nonhazardous; nonetheless, gloves, goggles, lab coats will be worn when handling.

<https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/PG1290-PJ8342-CO128458-SYBRsafe-Green-Fact-Sheets-Corp-FHR.pdf>

**1xTBE** has no health effects and is not flammable. Gloves, goggles, lab coats will be worn when handling.

<https://www.fishersci.com/shop/msdsproxy?productName=BP24304&productDescription=1X+TRIS-BORATE-EDTA>

**Ethanol** - more than minimal risk of eye, skin, gastrointestinal, and respiratory irritation, ignition, reaction with oxidizers, and formation of carbon monoxide and carbon dioxide via decomposition. Will be disposed according to official regulations by the chemical safety officer of G.W. Hewlett High School.

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E7023&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2Fe7023%3Flang%3Den>

**Agarose gel** - more than minimal risk of skin, eye, respiratory, and gastric irritation. Gloves, goggles, lab coats will be worn when handling.

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en>

<https://www.sigmaaldrich.com/catalog/product/sigma/P5472?lang=en&productNumber=P5472&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fp5472%3Flang%3Den>

**Wash Solution #1** - more than minimal risk of skin, eye, respiratory, and gastrointestinal irritation, instability caused by excess heat and moisture, and reaction with oxidizing agents. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instruction.

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=W0263&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fw0263%3Flang%3Den>

**Wash Solution #2/3** - more than minimal risk of eye and gastric irritation. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instructions.

<https://www.sigmaaldrich.com/MSDS/MSDS/PleaseWaitMSDSPage.do?language=en&country=US&brand=SIGMA&productNumber=W4639&PageToGoToURL=https://www.sigmaaldrich.com/catalog/product/sigma/w4639?lang=en&region=US>

**Elution Buffer**- more than minimal risk of eye and gastric irritation. Gloves, goggles, lab coats will be worn when handling.

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=C0241&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fc0241%3Flang%3Den>

### **Activities and Devices**

**Agarose Gel Electrophoresis**- more than minimal risk of electric shock. Mentor will oversee use and assist with using device.

**PCR Thermo-cycler**- more than minimal risk of skin irritation and slight burns minor risk of burns to hand/fingers. Mentor will oversee use and assist with using device

### **Describe the Safety precautions and procedures that will be used to reduce the risks.**

We will wear gloves, goggles, lab coats, and use the fume hood when necessary. Face masks will be used when handling chemicals that are hazardous with inhalation. Mentor handles safety precaution training, hazardous chemicals, and will assist with the use of potentially harmful equipment.

### **Describe the disposal procedures that will be used.**

Hazardous and organic chemicals will be stored in hazardous and organic chemical waste containers to be properly disposed by the Chemical safety officer. Most other chemicals are safe to dispose of down the drain diluted with water according to manufacturer's instructions.

## **Data Analysis:**

### **Measurements of Germination:**

After collecting data on the emergence of rhizoids, the area of the prothalli, the number of cells in the gametophyte stage, and the percentage of ferns developing into the sporophyte stage, an average will be taken. Then, an ANOVA test will be conducted between the experimental data sets and the control data set of each data type to determine statistical significance (p-values lower than 0.05 indicate statistical significance between two sets of data).

### **Gene Identification of Mago nashi isolated from *Ceratopteris richardii*:**

To find an orthologue of Mago nashi in *Ceratopteris*, an Expressed Sequence Tag (EST) library will be screened. Since the Mago nashi sequence from *Arabidopsis thaliana* has already been sequenced, the sequence will be entered into Basic Local Alignment Search Tool (BLAST) to obtain protein sequences of orthologues. Selected genomes of plants will be found from [www.phytozome.net](http://www.phytozome.net). MEGA6 (<http://www.megasoftware.net/>) will be used to create a phylogenetic tree to compare the evolutionary relationship of the Mago nashi sequence from *Ceratopteris* to other organisms. Clustal omega will be used to compare known protein sequences of Mago nashi from *Arabidopsis* and other plants to the partial Mago nashi sequence from *Ceratopteris*.

#### D. Bibliography

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## Addendum

No changes were made to the research plan