



# *C-Fern*<sup>™</sup> Sport Reports:

Descriptions and Culture Suggestions for Mutant *C-Fern*<sup>™</sup> Stocks

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## Descriptions and culture suggestions for mutant *C-Fern* stocks

The term *sport* is sometimes used to refer to unusual forms of plants or animals that typically arise by mutation. The following short descriptions or *sport reports* provide basic information about a variety of *C-Fern* mutant stocks that are available through Carolina Biological Supply Company. Ordering information is found in the table on pg. 7.

### **Mutant: polka dot; gene symbol, *cp***

This is a very striking visual mutant that exhibits a distinct green polka-dot appearance in cells of both gametophytes and homozygous sporophytes when viewed with a low-power microscope. In sporophytes, older leaves of individuals homozygous for this mutation have an attractive silver-green appearance. Although there is a slight reduction in growth and a decrease in spore viability\* in spores produced from homozygotes, gametophytes carrying the polka-dot mutant and homozygous polka-dot sporophytes grow nearly as well as the wild type. This recessive trait has been observed in several independent selections that have been generated using both X-rays and the chemical mutagen EMS. A study of the trait, using transmission electron microscopy, was not successful in discerning the structural basis of the phenotype. The phenotype is associated with a clumping of chloroplasts and other organelles around the nucleus. It may involve some disruption of the cytoskeleton. The pleiotropic effect of reduced spore viability is associated with somewhat fragile spore walls. Different degrees or *strengths* of the clumping phenotype are positively associated with increased spore wall weakness. Available both as a pure line and as F<sub>1</sub>-produced spores.

**\*Note:** Because of the decreased spore viability associated with spores produced by *cp/cp* homozygotes, presterilized and bulk spores are supplied in larger quantities than other stocks. Although inviable spores will be apparent in cultures, the increased number of spores provided will produce adequate numbers of gametophytes.

Vaughn, K. C., L. G. Hickok, T. R. Warne, and A. C. Farrow. 1990. Structural analysis and inheritance of a clumped-chloroplast mutant in the fern *Ceratopteris*. *Journal of Heredity* 81:146–51.

### **Mutant: pale; gene symbol, *pall***

Ghost-like *pall* gametophytes appear to have decreasing amounts of chlorophyll as they enlarge. Nonetheless, they grow to sexual maturity within 2 weeks and can self or cross-fertilize. At 1–2 weeks, *pall* gametophytes are slightly smaller than wild type, and their pale phenotype makes them clearly recognizable in a segregating population (use of transmitted light is best). This is a recessive mutation in sporophytes, and leaves of *pall/pall* homozygotes show a yellow-green phenotype when viewed with reflected light (from the side or top). These attractive sporophytes are quite viable, but slower growing than wild type. This EMS-induced mutation is available only in an F<sub>1</sub> hybrid stock. The F<sub>1</sub> spores can be used very effectively in the Genetics in Action Kit (15-6708) as a substitute for polka dot (*cp*).

### **Mutant: glyphosate tolerant; gene symbol, *glt 1***

This mutation was induced by X-irradiation of spores and selected for tolerance of gametophytes to the active ingredient in the herbicide Roundup\*. This is a very popular, effective and widely used herbicide that interferes with aromatic amino acid synthesis. The active ingredient, glyphosate [N-(phosphonomethyl) glycine], competitively inhibits an enzyme (EPSP synthase) within this metabolic pathway. A number of glyphosate-tolerant mutants have been identified in several plant and microbial strains. Some of these mutants exhibit an altered form of EPSP synthase that is not competitively inhibited by the herbicide. In the case of this *C-Fern* mutation, the mechanism of tolerance is not known.

Tolerance and sensitivity in the gametophyte generation can be observed when mutant and wild-type gametophytes, respectively, are grown on nutrient agar medium containing RoundUp®. A 1:5 dilution of a standard (18%) commercial solution, filter sterilized, can be added to autoclaved medium at a rate of 30–100 µL per 100 mL. If one-week-old gametophytes are transferred to this medium, differential tolerance of wild type and *glt1* can be observed after 1–2 weeks of culture. Available only in a double-mutant, herbicide-tolerant line with *pq45* (unlinked).

Tai Chun, P and L. G. Hickok. 1992. Inheritance of two mutations conferring glyphosate tolerance in the fern *Ceratopteris richardii*. *Canadian Journal of Botany* 70:1097–99.

#### **Mutant: paraquat tolerant; gene symbol, *pq45***

This mutation was induced by X-rays and selected for its tolerance to the herbicide paraquat. Paraquat is a broadly effective herbicide that acts rapidly in full sunlight by accepting an electron from Photosystem I, which results in production of the highly reactive superoxide anion. This free radical causes extensive damage to membranes and results in rapid bleaching and desiccation of plants exposed to light. In *C-Fern*, this is a recessive mutation that is expressed in both gametophytes and homozygous sporophytes. Tolerance is very clear-cut, and it is quite easy to identify tolerant individuals from sensitive wild-type individuals. However, in contrast to glyphosate, paraquat is toxic to humans and wildlife. Currently, its use and availability are restricted. It can be obtained for research purposes under the name methyl viologen or paraquat dichloride. The wild type and *pq45* can be clearly distinguished by assay of gametophyte growth on nutrient agar or sporophyte leaf bleaching in distilled water at a concentration of 0.5 mM paraquat. Available only in a double-mutant, herbicide-tolerant line with *glt1* (unlinked).

Carroll, E. W., O. J. Schwarz, and L. G. Hickok. 1988. Biochemical studies of paraquat-tolerant mutants of the fern *Ceratopteris richardii*. *Plant Physiology* 87:651–54.

Hickok, L. G. and O. J. Schwarz. 1986. Paraquat tolerant mutants in *Ceratopteris*: Genetic characterization and reselection for enhanced tolerance. *Plant Science* 47:753–58.

#### **Mutant: dark germinator; gene symbol, *dkg1***

This is a fascinating mutation that shows a reversal in the light requirement for germination of *C-Fern* spores. In wild type, light (red) is necessary for spore germination. However, in spores containing the *dkg1* mutation, germination occurs readily in the complete absence of light. Use of this mutant allows investigations of gametophyte growth and development to be conducted in the dark or by using specific wavelengths of light without the requirement to expose spores to red or white light to initiate germination. Another interesting aspect of this mutation is that germination in white light is substantially reduced relative to its germination in the dark.

Cooke, T., L. Hickok, W. J. Vanderwoude, J. Banks, and R. Scott. 1993. Photobiological characterization of a spore germination mutant with reversed photoregulation in the fern *Ceratopteris richardii*. *Photochemical Photobiology* 57:1032–41.

Cooke, T. J., L. G. Hickok, and M. Sugai. 1995. The fern *Ceratopteris richardii* as a lower plant model system for studying the genetic regulation of plant photomorphogenesis. *International Journal of Plant Science* 156:367–73.

#### **Mutant: non-etiolated; gene symbol, *det30***

If *C-Fern* gametophytes are given an initial light exposure (1–2 days under standard conditions) to initiate germination and then placed in the dark, their growth form will be dramatically altered. The alteration in some ways resembles the etiolation response that is well known for higher plants, in which plants subjected to dark conditions grow substantially longer than those in light. In dark-grown *C-Fern* gametophytes, some of the basal cells undergo extreme elongation, which results in strap-shaped

structures consisting of a few highly elongated cells just above the spore coat and a pad of smaller cells at the tip of the gametophyte. In gametophytes containing the *det30* mutation, which was induced by X-rays, this elongation response is reduced. In contrast to flowering plants, both *C-Fern* wild type and *det30* gametophytes grown in the dark still have the capacity to synthesize chlorophyll, although they are typically a very light green.

Cooke, T. J., R. H. Racusen, L. G. Hickok, and T. R. Warne. 1987. The photocontrol of spore germination in the fern *Ceratopteris richardii*. *Plant and Cell Physiology* 28:753–59.

Cooke, T. J., L. G. Hickok, and M. Sugai. 1995. The fern *Ceratopteris richardii* as a lower plant model system for studying the genetic regulation of plant photomorphogenesis. *International Journal of Plant Science* 156:367–73.

Murata, T., A. Kadota, and M. Wada. 1997. Effects of blue light on cell elongation and microtubule orientation in dark-grown gametophytes of *Ceratopteris richardii*. *Plant Cell Physiology* 38:201–09.

#### **Mutant: day-night responder; gene symbol, *dnr1***

Gametophytes and homozygous sporophytes containing this mutation accumulate massive amounts of starch in their plastids when grown under constant light conditions. This can be easily observed by viewing the plastids under a compound microscope; staining with I<sub>2</sub>KI can enhance observations. The massive starch grains give the plastids a very lumpy appearance. The accumulation of starch suggests that under constant light conditions the cells are unable to use photosynthate effectively and, as a result, gametophyte growth is severely impaired. This is a conditional mutation in that near-normal growth and depletion of the abnormal starch accumulation occur when gametophytes are grown under day-night conditions. Sporophytes grow well under normal greenhouse day-night conditions. This X-ray induced mutation is recessive in sporophytes.

#### **Mutant: abscisic acid tolerant; gene symbol, *abr48***

This mutation confers tolerance to the typical effects of abscisic acid (ABA) on gametophytes. Wild-type gametophytes cultured in the presence of ABA exhibit multiple effects involving decreased growth rate and altered development. The number of antheridia on hermaphroditic wild-type gametophytes is reduced and the number of rhizoids is increased. In some wild-type hermaphrodites, the presence of ABA can cause the marginal notch meristem to become more centrally located. This results in an interesting pattern of growth that resembles a tube sock, with the tip of the toe representing the position of the meristem. The number of male gametophytes is also reduced. These effects, which can be induced in the wild type at a concentration of 5.0–50.0 mM ABA, are not evident in the mutant.

Hickok, L. G. 1985. Abscisic acid-resistant mutants in the fern *Ceratopteris*: Characterization and genetic analysis. *Canadian Journal of Botany* 63:1582–85.

Warne, T. R. and L. G. Hickok. 1991. Control of sexual development of *Ceratopteris richardii*: antheridiogen and abscisic acid. *Botanical Gazette* 152:148–53.

#### **Mutant: maleless; gene symbol, *her1***

The *her1* mutation, which is one of many mutations that affect sexual differentiation in *C-Fern*, was induced by X-irradiation of spores. The effects of this mutation can be seen clearly in populations of gametophytes. Populations of wild-type *C-Fern* gametophytes show two sexual types, males and hermaphrodites. In contrast, this *maleless* mutant does not contain male gametophytes in populations. This mutation renders gametophytes insensitive to the presence of the male-inducing pheromone, antheridiogen (*A<sub>c</sub>*). On the other hand, development of hermaphrodite gametophytes occurs quite normally and appears no different from the wild type. It is also possible to see the effects of the mutation by using staggered sowings, over time, of spores on the same petri dish (as is done in the Battle of the

Sexes kit (15-6712). For direct testing, it is possible to obtain a crude source of antheridiogen by growing multispore cultures of gametophytes for 2–3 weeks on basic *C-Fem* medium and then extracting the liquid from the medium. Extraction can be readily done by taking the older cultures, scraping most of the gametophyte material away, and then freezing the remaining agar. After freezing, the agar can be thawed to effectively separate the solid portion from the liquid. The solution can then be filtered and used as a portion of the liquid to formulate new antheridiogen-supplemented *C-Fem* medium, which can be referred to as CFM +  $A_{C_e}$ . If spores of the wild type are sown on CFM + 50%  $A_{C_e}$ , most of the gametophytes in the culture will be male. In contrast, if spores carrying the maleless *her1* mutation are sown on such medium, all of the gametophytes will be hermaphrodites.

Banks, J. A., L. Hickok, and M. A. Webb. 1993. The programming of sexual phenotype in the homosporous fern, *Ceratopteris richardii*. *International Journal of Plant Science* 154:522–34.

Banks, J. A. 1997. Sex determination in the fern *Ceratopteris*. *Trends in Plant Science* 2:175–79.

Warne, T. R., L. G. Hickok, and R. J. Scott. 1988. Characterization and genetic analysis of antheridiogen insensitive mutants in *Ceratopteris richardii*. *Botanical Journal of the Linnean Society* 96:371–79.

#### **Mutant: highly male; gene symbol, *him1***

Initial characterization of this EMS-induced mutation suggests that it is highly sensitive to the pheromone,  $A_{C_e}$ . Typically, *C-Fem* gametophytes always develop as hermaphrodites when cultured as isolates. In contrast, *him1* types can spontaneously develop as males in isolate culture, and there are correspondingly higher numbers of males in multispore cultures. In addition, *him1* hermaphrodites tend to have much higher numbers of antheridia, especially as they age. The higher numbers of males and antheridia combine to produce cultures that have the capacity to produce many sperm that can be observed in large masses.

Banks, J. A., L. Hickok, and M. A. Webb. 1993. The programming of sexual phenotype in the homosporous fern, *Ceratopteris richardii*. *International Journal of Plant Science* 154:522–34.

Banks, J. A. 1997. Sex determination in the fern *Ceratopteris*. *Trends in Plant Science* 2:175–79.

Warne, T. R., L. G. Hickok, and R. J. Scott. 1988. Characterization and genetic analysis of antheridiogen insensitive mutants in *Ceratopteris richardii*. *Botanical Journal of the Linnean Society* 96:371–79.

#### **Mutant: salt tolerant; gene symbol, *stl2***

This mutation, which was induced with X-rays, shows an interesting collection of co-segregating traits. It was selected for tolerance to salt (NaCl), and gametophytes carrying the mutation show a high level of tolerance to this agent. Sporophytes also show NaCl tolerance, but at a lower level and in a semi-dominant fashion. In addition, *stl2* confers tolerance to magnesium salts, but sensitivity to moderate levels of potassium in the medium. Sensitivity of the wild type to sodium salts is associated with the level of calcium in the medium. In the presence of 150 mM NaCl, the calcium level in basic *C-Fem* medium results in very poor growth and necrosis of the wild type with little apparent effect on the mutant. At higher calcium levels commonly found in other plant nutrient formulations, the wild type and *stl2* show similar responses at 150 mM NaCl. This mutation can be used to show a simple salt-tolerance response and also can be used to demonstrate the complexities of mineral and nutrient interactions in the environment.

Vogelien, D. L., L. G. Hickok, and T. R. Warne. 1996. Differential effects of  $Na^+$ ,  $Mg^{2+}$ ,  $K^+$ ,  $Ca^{2+}$  and osmotic stress on the wild type and NaCl-tolerant mutants, *stl2*, of *Ceratopteris richardii*. *Plant, Cell and Environment* 19:17–23.

Warne, T. R., L. G. Hickok, T. B. Kinraide, and D. L. Vogelien. 1996. High salinity tolerance of the *stl2* mutation of *Ceratopteris richardii* is associated with enhanced  $K^+$  influx and loss. *Plant, Cell and Environment* 19:24–32.

**Mutant: FUDR tolerant; gene symbol, *fdr1***

This mutation, which was induced by X-rays, confers tolerance to 2'-deoxy-5-fluorouridine (FUDR). This substance is a pyrimidine nucleoside analog and is highly toxic to many organisms. As a nucleotide analog, FUDR interferes with normal metabolism and nucleic acid biosynthesis. This mutation confers a very clear tolerance to FUDR and when gametophytes carrying it are grown in medium containing 10 mM FUDR, wild-type gametophytes are killed shortly after germinating while mutant gametophytes grow quite normally.

Wu, K. and J. King. 1994. Biochemical and genetic characterization of 5-fluoro-2'-deoxyuridine-resistant mutants of *Arabidopsis thaliana*. *Planta* 194:117-22.

**Mutant: sleepy sperm; gene symbol, *zzz1***

Wild-type *C-Fern* sperm, when released from the antheridium, are encased in a thin-walled vesicle. Several seconds after release, the sperm break free of the vesicle and quickly swim away. In contrast, most *zzz1* sperm remain in the vesicles after release and some eventually break free only after several minutes. The free sperm typically move only slightly or swim very slowly to moderately, with some showing faster movement. The more normal types apparently allow *zzz1* gametophytes to self fertilize, despite the abnormalities in most *zzz1* sperm. This EMS-generated mutant is excellent for observations at both low and high (>50) magnifications.

Duckett, J. G., E. J. Klekowski, and L. G. Hickok. 1979. Ultrastructural studies of mutant spermatozooids in ferns. I. The mature nonmotile spermatozoid of mutation 230X in *Ceratopteris thalictroides* (L.) Brongn. *Gamete Research* 2:317-43.

**Mutant: slow-mo sperm; gene symbol, *slo1***

Like wild type, *slo1* sperm are released from the antheridium in thin-walled vesicles and then, shortly after release, break free and swim away. However, most *slo1* sperm swim very slowly and can be easily observed, even under high (>50x) magnification. A few moderately fast-swimming types allow for self-fertilization of *slo1* gametophytes. This mutation is EMS generated.

Duckett, J.G., E. J. Klekowski and L. G. Hickok. 1979. Ultrastructural studies of mutant spermatozooids in ferns. I. The mature nonmotile spermatozoid of mutation 230X in *Ceratopteris thalictroides* (L.) Brongn. *Gamete Research* 2:317-43.

**Mutant Hunt Mix**

This mixture of wild type and a variety of mutant stocks (many uncharacterized) is an excellent way to introduce students to the wide variety of EMS-induced visible mutant types that can be observed in *C-Fern* gametophytes. Mutants such as albino, pale, polka dot, early gametophyte lethals, and a variety of other types are present in a combined frequency of at least 10%. Some are very easily observed, while others require more careful observations. Using isolation and selfing or crossing techniques, students can discover, confirm, and characterize mutant phenotypes and determine dominance/recessiveness.

### **C-Fern™ Spores**

Available premeasured and presterilized in a kit-sized vial (enough to inoculate 35 petri dishes with 300+ spores/dish) or unsterilized in a bulk-sized vial (enough for 140 dishes). The bulk-sized vial comes with directions for surface sterilization of spores. Store spores at room temperature in the dark; do not refrigerate.

**Kit-Sized Vial** (presterilized) . . \$7.50

**Bulk-Sized Vial** (unsterilized) . . \$15.00

<b>Stock</b>	<b>Symbol</b>	<b>Presterilized Kit-Sized Vial</b>	<b>Unsterilized Bulk-Sized Vial</b>
wild type	<i>RNWT1</i>	<b>15-6728</b>	<b>15-6729</b>
<b>Single Gene Nuclear Mutants</b>			
polka dot	<i>cp</i>	<b>15-6732</b>	<b>15-6733</b>
dark germinator	<i>dkg</i>	<b>15-6738</b>	<b>15-6739</b>
abscisic acid tolerant	<i>abr48</i>	<b>15-6742</b>	<b>15-6743</b>
maleless	<i>her1</i>	<b>15-6744</b>	<b>15-6745</b>
non-etiolated	<i>det30</i>	<b>15-6746</b>	<b>15-6747</b>
salt tolerant	<i>stl2</i>	<b>15-6748</b>	<b>15-6749</b>
FUDR	<i>fdr1</i>	<b>15-6750</b>	<b>15-6751</b>
highly male	<i>him1</i>	<b>15-6764</b>	<b>15-6765</b>
sleepy sperm	<i>zzz1</i>	<b>15-6766</b>	<b>15-6767</b>
slow-mo sperm	<i>slo1</i>	<b>15-6768</b>	<b>15-6769</b>
day-night responder	<i>dnr1</i>	<b>15-6770</b>	<b>15-6771</b>
<b>Double Mutants</b>			
glyphosate tolerant/ paraquat tolerant	<i>glt1 pq45</i>	<b>15-6752</b>	<b>15-6753</b>
<b>F<sub>1</sub> Stocks (single gene mutants × wild type)</b>			
F <sub>1</sub> polka dot	<i>cp/CP</i>	<b>15-6760</b>	<b>15-6761</b>
pale	<i>pal1</i>	<b>15-6772</b>	<b>15-6773</b>
<b>Mutant Hunt Mix</b>		<b>15-6762</b>	<b>15-6763</b>

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