Utilization of Artificial Mutant Rice for Fundamental Researches of Herbicidal Mechanisms

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Preface

Artificial mutants have made great contributions to the study of biochemistry, especially to the elucidation of metabolic pathways in organisms.

On the other hand, studies on the selectivity mechanism of varieties to herbicides came to comparatively clear conclusions in the corn[1] and apple[2], and suggested an idea of the use of mutants in the study of herbicidal mechanisms to the author.

Fortunately, in our institute, the Department of Physiology and Genetics to which the author belongs has produced various mutant plants by the use of radiation and chemicals in the Genetics Section at Hiratsuka City.

Thousands of mutants have been produced from the Norin No. 8 rice variety in the 3rd Laboratory of Genetics headed by Dr. Takeshi Kawai, its chief, for the purpose of not only to obtain new good varieties but also to make researches in the mechanism of mutation itself.

Utilization of chlorophyll mutants

1) Chlorophyll mutants

Among the mutants obtained from Norin No. 8, there are CM-marked ones (chlorophyll mutants) which are defective in the biosynthetic system of chlorophyll (including defect in formation of chloroplasts), and the color of them ranges from pure white to yellow and to light green. The mutant rice used in the present study as materials are shown in Table 1.

It is needless to say that white-yellow mutant seedlings die when they have consumed reserve carbohydrate in the seeds because they are deficient in photosynthetic ability. But each of these abnormalities in chlorophyll formation is a character controled by a recessive gene, and a heterozygote for such a gene can bear seeds, a quarter of which is expected to give white or yellow seedlings in the next season again when kept in a green house during the cold season and allowed to

Table 1. Chlorophyll mutants used as materials

Color	General name	Mutant number
Pure white	Albina	CM 53
White (slightly yellowish)		CM 9, CM 33, CM 35, CM 37
Yellow	Xantha	CM 123, CM 213
Light green	Viridis	CM 46, CM 75

self-fertilize.

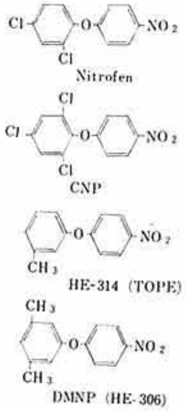
Therefore, a fairly large number of mutant individuals can be used as materials. The author carried out the following experiments in expectation of elucidating the herbicidal mechanism which has relation to the light by using of the CM series of mutant rice.

2) Photo-activation mechanism in herbicides of the diphenyl ether group

Herbicides of the diphenyl ether group are in wide use, being applied to more than 800,000 ha (in total of nitrofen and CNP) of paddy fields in 1969.

Among this group of herbicides, the ones which have substituent radical at the ortho-positions are inactive in the dark; that is, the light is necessary for their herbicidal action.

The necessity of light, however, is limited to the ones which have substituent groups at the ortho-positions of the left benzene ring (as nitrofen and CNP), and other diphenyl ether herbicides which have no substituent groups at the ortho-positions (as HE-314 and DMNP) are active in the dark[3].



In the first place, reactions of the above-mentioned white-yellow mutants which had no chlorophyll with nitrofen were examined. When just germinated seeds were placed in Petri dishes (white or yellow seedlings can be distinguished from green ones when a little germinated under the light) with the test herbicide and put under a fluorescent lamp,

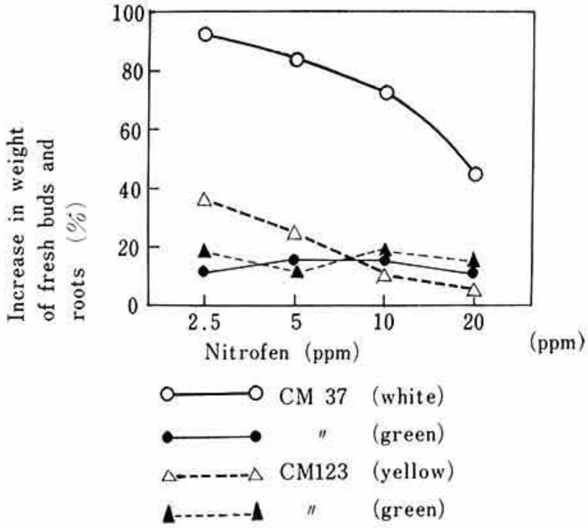


Fig. 1. Susceptibility of white and yellow mutant seedlings to nitrofen in the light condition[4]

it was observed that yellow seedlings were influenced by nitrofen, while light yellow or white ones were not affected by the chemical even in the light condition.

The growth of green segregant (segregated in the ratio of white-yellow 1: green 3) was completely inhibited by the treatment. Of course, viridis (light green) seedlings also are affected by the herbicide. An example of the results is shown in Fig. 1.

Then pigments were extracted from each kind of mutants and analyzed by thin layer chromatography. CM 213, which was a mutant susceptible to nitrofen in the light condition, contained no chlorophyll but xanthophyl at a nearly normal concentration.

White mutants did not contain any pigment, and light yellow ones had no chlorophyll but about 10% the normal concentration of xanthophyl. These results show that xanthophyl is at least one of the light-receptors in the photo-activation of nitrofen[4].

On the other hand, chlorophyll also is considered to be effective as a light-receptor since it has been proved by monochromatic-light experiments that red light is effective for activating the action of nitrofen as well.

Oxadiazon (17623-RP, G-315 [2-*tert*-butyl-4- (2, 4-dichloro-5-isopropyl-oxyphenyl) -5-oxo-1, 3, 4-oxadiazoline]) is a herbicide quite different in chemical structure from the diphenyl ether group, but it has a characteristic action similar to nitrofen, etc., and the reaction of chlorophyll mutants to this chemical is the same as to nitrofen.

Oxadiazone

3) Mechanism of the action of bipyridylium herbicides

The use of bipyridylium herbicides, especially paraquat and diquat, increases every year because of their strong and quick effect by the contact treatment of leaves and stems as well as the absence of residual effect in the soil.

The mechanism of their action has relation to the photosynthetic system as shown in Fig. 2, that is, electrons are excited in photochemical systems I and II when the photosynthetic organs absorb the light, and the excited electron act on paraquat molecules to reduce them to change into free radicals.

The paraquat free radicals are oxidized by oxygen in the air and the original form of the molecules is recovered. And it is said that at the same time an excessive power in the oxidation of free radicals produces peroxides, which will kill the leaves and stems of treated plants.

Therefore, the efficacy of paraquat is remarkably reduced in the dark or oxygen-free condition, or when it is coexistent with the Hill-reaction inhibitor (such a herbicide as monuron[7] or propanil[3]) even in the light.

When paraquat is applied to the above-mentioned chlorophyll mutants, green seedlings wilt within the day of treatment and

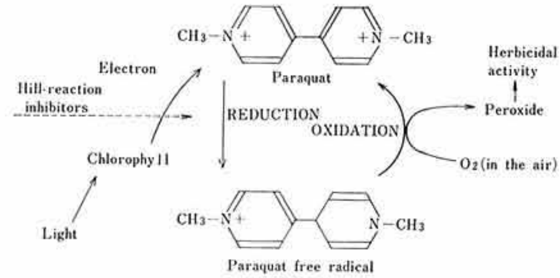


Fig. 2. Mechanism of the photo-activation of paraquat

turn brown on the next day, while yellow-white ones begin to wilt about three days after the treatment.

Paraquat does not influence the yellow seedlings of CM 213, etc. unlike nitrofen, indicating that not only light-receptors but also the photosynthetic system is necessary for its photoactivation.

Propanil-susceptive mutants

1) Selection of propanil-susceptive rice plants

Rice plants are so resistant to the action of propanil (3′, 4′-dichloropropionanilide) that propanil can safely be used even in cases where rice plants are grown by direct sowing and coexist with barnyardgrass and others under the same condition.

The chemical has a highly selective herbicidal effect on these weeds, and it is said part of this selectivity is due to the presence of an enzyme which catalyzes the hydrolysis of the herbicide in rice plants.

The resistance of rice plants to propanil has been discussed so far in comparison with barnyardgrass which is the most important weed in paddy fields and closely resembles rice plants except the susceptibility.

The propanil-hydrolyzing enzyme (a kind of acyl arylamidases) has been given attention in the sense that it is found in rice plants but not in barnyardgrass. The rice, however, is considerably different from barnyardgrass even in morphological characters, and it seems somewhat unreasonable to attribute the resistance of rice plants only to the presence of the hydrolyzing enzyme.

To confirm this point, the auther examined about 50 rice varieties in cultivation (including foreign varieties) about their susceptibility to propanil, finding that none of them were as susceptible as barnyardgrass.

The author was allowed to spray about 700 kinds of mutant rice with propanil in exchange for weeding their nursery beds by courtesy of the 3rd Laboratory of Genetics.

In this experiment the mutant rice plants were morphologically examined in detail before and one week after spraying to make a comparison between these examinations, and it was found that seedlings of mutant No. 408 were almost entirely killed by the spraying of propanil. This finding made the author very glad though the 3rd Laboratory had the misfortune of failing to grow the No. 408 seedlings in that year.

I was given reserved seeds of this mutant rice and tried to fix the character and propagate the strain at Konosu for about two years.

The following investigations were carried out after a considerable number of reliable mutants rice plants had been obtained.

2) Characteristics of propanil-susceptible mutant rice[8]

This strain was derived from Norin No. 8 and distinguished from the parental variety in the Genetics Section as a mutant because of its little smaller number of tillers.

Therefore, its growth feels a little poor when cultivated in an ordinary condition of fertilization. As compared with Norin No. 8, its earing is about five days later and the leaves are somewhat narrower tending to curl slightly.

The weak resistance of seedlings to the damping-off disease also constitutes a characteristic feature of the mutant and it is necessary to submerge nursery beds for preventing the disease.

Needless to say the most characteristics feature of the mutant is its propanil-susceptibility. When the mutant is compared with Norin No. 8, the parental variety, and barnyardgrass, it is almost the same as the latter in the susceptibility of seedlings at the stage of three–four leaves.

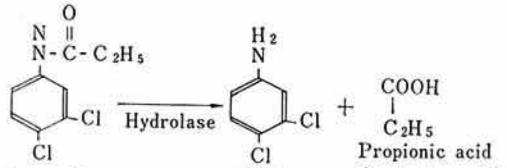
The mutant rice plants are completely killed by spraying with a propanil emulsion at an ordinary concentration, about 0.35% while Norin No. 8 is scarcely influenced by the same treatment.

Normal rice plants are able to hydrolyze propanil as shown by the following formula: If propanil is incubated with a homogenate of rice leaves or stems for some one hour, a large amount of 3, 4-dichloroaniline (DCA) is produced.

Yih and others[9] reported that the side chain of propanil was oxidized before the hydrolysis. According to this opinion, lactic acid is produced in place of propionic acid, but 3, 4-dichloroaniline is formed in the same way as mentioned above.

On the contrary, it was found that the propanil susceptible rice had almost no activity of this hydrolase. The 3, 4-dichloroaniline thus formed is further metabolized to unknown substances in the homogenate, and this activity is also observed in other plants than normal rice.

In expectation that the mutant rice would not be an exception from this, the author measured the propanil-hydrolase activity of the mutant not only by the production of DCA but also by the disappearance of 3, 4-dichloropropionanilide, but the results obtained were negative.



When photosynthetic activity is measured in normal rice plants after spraying with propanil, it is observed that the activity is completely inhibited about two hours after the spraying but begins to recover after about two days, showing almost complete recovery four days after the treatment.

It seems most reasonable to think that the sprayed propanil is hydrolyzed and detoxicated during this period. In contrast to this, in the susceptible mutant rice plants photosynthesis inhibited by spraying with propanil never recovers. This fact corresponds to the inability of the mutant to detoxicate the herbicide.

From these facts it may safely be said that the mutant rice plants are susceptible to propanil because of the absence of the hydrolase in their bodies.

3) Genetical behavior of propanil-susceptible character

To make the genetical behavior of the mutant character clear, crossing experiments were carried out following the above-mentioned investigations. By crossing the resistant rice (R: Norin No. 8) to the susceptible rice (S: mutant), a considerable number of F_1—seeds, R×S and S×R (R×S means F_1 produced by a crossing of R♀and S♂) were obtained.

If we had failed, S×R should give susceptible offspring. But all the hybrids obtained, R×S and S×R, were for more resistant to propanil than S, indicating success in crossing.

The F_1-seeds collected in autumn were sowed in a growth cabinet in winter to obtain F_2-seeds. When about 1,200 F_2-seedlings, in total, from both crosses, R×S and S×R, were tested, they segregated in the ratio of 1 to 2.92. (susceptible to tolerant). This result was close to the theoretical ratio 1:3, and suggested that the susceptibility to propanil was a character controlled by a recessive gene.

On the other hand, when 24 F_2-plants were tested individually, seven of them showed no hydrolase activity mentioned above. This ratio of segregation, 7:17, seems to be not so different from the theoretical ratio, 6:18 (1:3).

The F_2-plants were numbered individually and a leaf was taken from each of them to examine the hydrolase activity. Then the plants were sprayed with propanil to see whether or not only hydrolase-negative individuals would be killed by the propanil-treament.

The result obtained showed that propanil-hydrolase-negative plants were always propanil-susceptible. The correspondence of propanil-susceptibility to the absence of propanil-hydrolase was more directly proved by this experiment.

The F_1-individuals mentioned above were kept in a green house during winter, and the plants newly grown from them were used for backcrossing with S. The offspring thus obtained were resistant and susceptible in the ratio of nearly 1:1, giving further positive proof of the monogenic inheritance of propanil-susceptibility.

Conclusion

Besides the above-mentioned experiments, Shirakawa[10] investigated the herbicidal mechanism of solan (3′-chloro-2-methyl-p-valerotoluidide) and found that white-yellow mutant rice is resistant to the chemical. He assumed on the basis of this result that the action of solan had some relation to the photosynthetic system.

The chlorophyll mutant rice appears to be useful not only for the study of herbicides but also for the elucidation of light-dependent physiological and biochemical reactions in general.

On the other hand, the propanil-susceptible mutant rice, which was discovered very fortunately, gives us useful information about the selective action of propanil, and if we come to think of the fact that the propanil-hydrolase is an enzyme which has been present in normal rice since the time when the herbicide was not yet known, we expect that the enzyme has some physiological significance.

This point may also be clarified by a comparison between Norin No. 8 and the mutant. The above-mentioned low resistance of the mutant to the dumping off disease is interesting from a pathological viewpoint if it is a pleiotropic manifestation of the gene for hydrolase deficiency.

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