Development of a rice herbicide, fenquinotrione

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Fenquinotrione is a novel rice herbicide that was discovered and developed by Kumiai Chemical Industry Co., Ltd. It can control a wide range of broadleaf and sedge weeds with excellent rice selectivity at 30 g a.i./10 a and is as effective as the wild type on acetolactate synthase inhibitor-resistant weeds. Our metabolic and molecular biological studies showed that CYP81A6-mediated demethylation and subsequent glucose conjugation are responsible for the safety of fenquinotrione in rice. Fenquinotrione was registered in Japan in 2018, and various products containing fenquinotrione have been launched. With its high efficacy and excellent rice selectivity, we believe that fenquinotrione will contribute to efficient food production in the future.

Keywords: fenquinotrione, oxoquinoxaline, paddy rice, herbicide, CYP81A6, 4-hydroxyphenylpyruvate dioxygenase (HPPD).

Introduction

In the current Japanese agricultural setting, rice cultivation systems are diversifying, including direct seeding and transplant cultivation. In addition, planting of new rice varieties, that are in demand for feed and processing, has increased. From the viewpoint of weed management, the following weeds, that show resistance to acetolactate synthase (ALS)-inhibiting herbicides, have become problematic in Japanese rice paddy fields: Schoenoplectus juncoides, Monochoria vaginalis, Monochoria korsakowii, Sagittaria trifolia, and Lindernia spp. Under these circumstances, there is a need for herbicides that have long application periods, can be applied to various cropping systems and cultivars, and can control the growth of a wide range of weeds, including ALS-resistant weeds, at low concentrations.

To meet the needs of such herbicides, we focused on 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors in this study, and succeeded in discovering a novel herbicide, fenquinotrione (trademark name: EFFEEDA®, Fig. 1). In addition, we revealed its biological properties as well as rice safety factors using molecular biology methods.

1. Discovery of fenquinotrione

1.1. Discovery of an oxoquinoxaline derivative

Following a review of commercialized HPPD-inhibiting herbicides (Fig. 2) and patents-related HPPD inhibitors, we confirmed that substituents at the 2-, 3-, and 4-positions of the benzoyl group have a significant impact on herbicidal activity, crop safety, and herbicidal spectrum. Based on this knowledge, we designed and synthesized 2-pyridone derivatives. However, although these 2-pyridone derivatives showed potent HPPD inhibitory activity as well as high herbicidal efficacy, we were unable to identify a compound with practical applications. Subsequently, we focused on the heterocyclic structure with a carbonyl group at the 2-position and identified an oxoquinoxaline derivative, which is the basic structure of fenquinotrione, and used it as a lead compound to convert the acid moiety, N-position, and fused benzene ring moiety (Fig. 3).1)

1.2. Structure and biological activity of acid site structure

The selection of compounds with promising biological activity was based on their herbicidal efficacy against S. juncoides at the pre-emergence application. The cyclohexadiene ring was identified as the most active acid, whereas other acid structures, such
as pyrazole rings, were significantly less active. Therefore, the acid moiety was fixed to the cyclohexadione ring, and the other moieties were converted.1)

1.3. N-position structure and biological activity
Phenyl and heterocyclic groups at the N-position showed high herbicidal efficacy; however, their safety against rice was low. Therefore, we introduced a substituent on the N-phenyl ring to improve the crop injury in rice plants. The introduction of methoxy or methyl groups at the 3- or 4-positions of the phenyl ring decreased herbicidal efficacy but improved the selectivity between rice and paddy weeds (Table 1). In particular, the 4-methoxy group was the most suitable because of the resulting excellent balance between herbicidal efficacy and safety against rice. 4-methoxyphenyl was not sufficiently safe against rice, and we continued to investigate the substituent effect on the fused benzene ring.1)

Table 1. Effect of substituents

| R4       | ED20 (g a.i./10 a) | ED90 (g a.i./10 a) |
|----------|--------------------|--------------------|
|          | ORYSA              | ECHOR              | MOOVP              | SCPJU              |
| H        | 0.4                | 6.3                | 1.6                | 1.6                |
| 3-Me     | 6.3                | 25                 | 6.3                | 25                 |
| 4-Me     | 6.3                | 25                 | 6.3                | 6.3                |
| 2-OMe    | 6.3                | 100                | 25                 | 25                 |
| 3-OMe    | 6.3                | 100                | 6.3                | 6.3                |
| 4-OMe    | 6.3                | 100                | 6.3                | 6.3                |

Evaluation: Herbicidal activity and crop injury were visually evaluated using a rating of 0 (no effect) to 100 (complete kill). ED20: Dosage at 20% crop inhibition. ED90: Dosage at 90% weed control. Crop and weeds tested were ORYSA, Oryza sativa cv. Kinmaze; ECHOR, Echinochloa oryzicola; MOOVP, Monochoria vaginalis; and SCPJU, Schoenoplectus juncoides.

Table 2. Effect of substituents

| R3       | R4       | ED20 (g a.i./10 a) | ED90 (g a.i./10 a) |
|----------|----------|--------------------|--------------------|
|          |          | ORYSA              | ECHOR              | MOOVP              | SCPJU              |
| H        | OMe      | 6.3                | 100                | 6.3                | 6.3                |
| 5-Cl     | OMe      | 6.3                | 25                 | 6.3                | 25                 |
| 6-Cl     | OMe      | 6.3                | 100                | 6.3                | 25                 |
| 7-Cl     | OMe      | 6.3                | 25                 | 1.6                | 25                 |
| 8-Cl     | OMe      | >100               | >100               | 6.3                | 6.3                |
| 8-Br     | OMe      | >100               | >100               | 6.3                | 6.3                |
| 8-Me     | OMe      | 100                | 25                 | 6.3                | 25                 |
| 8-Cl     | H        | 1.6                | 25                 | 1.6                | 1.6                |

Evaluation: Herbicidal activity and crop injury were visually evaluated using a rating of 0 (no effect) to 100 (complete kill). ED20: Dosage at 20% crop inhibition. ED90: Dosage at 90% weed control. Crop and weeds tested were ORYSA, Oryza sativa cv. Kinmaze; ECHOR, Echinochloa oryzicola; MOOVP, Monochoria vaginalis; and SCPJU, Schoenoplectus juncoides.
1.4. Structure and biological activity of the fused ring moiety

The introduction of various substituents at positions 5, 6, and 7 of the fused benzene ring moiety did not improve its safety in rice plants. However, the introduction of a halogen or methyl group at the 8-position tended to improve the selectivity between rice and paddy weeds (Table 2). In particular, the 8-chloro form (fenquinotrione) was selected as the development compound because it showed sufficient crop safety and herbicidal efficacy.1)

2. Physicochemical properties

Common name (ISO name): Fenquinotrione
Development code: KUH-110

Chemical name (IUPAC): 2-[(8-chloro-3,4-dihydro-4-(4-methoxyphenyl)-3-oxoquinazalin-2-yl)carbonyl]cyclohex-1,3-dione
CAS registry number: 1342891-70-6
Molecular formula: C_{22}H_{17}ClN_{2}O_{5}
Molecular weight: 424.83
Appearance (physical state, form, and color): Pale yellow powder
Melting Point: 157.6°C
Solubility in water: 17.3 mg/L (20°C)
Log $P_{ow}$: 2.91 (pH 1.0), 1.59 (pH 4.0), 0.33 (pH 7.0)

3. Biological properties

3.1. Herbicidal spectrum

The herbicidal activity of fenquinotrione against each weed species was confirmed in the greenhouse trials. Fenquinotrione showed high herbicidal activity against paddy sedges and broadleaf weeds such as *S. juncoides* and *M. vaginalis* except for *Echinochloa oryzicola* and *Eleocharis kuroguwai*, from pre-emergence to early post-emergence application at 30 g a.i./10 a (Fig. 4),2,3) and was considered to have a broad spectrum of weed-killing activities.4,5)

3.2. Herbicidal activities at post-emergence application window

The herbicidal efficacy of fenquinotrione on *S. juncoides*, *M. vaginalis*, and *S. trifolia*, at different leaf stages, was confirmed in greenhouse trials. At a dose of 30 g a.i./10 a, fenquinotrione showed high herbicidal efficacy against these weed species, including at the high-leaf stages, such as the 4-leaf stage of *S. juncoides* and *M. vaginalis*, and the 1-arrowhead-leaf stage of *S. Trifolia* (Fig. 5).

Fenquinotrione was also effective against ALS inhibitor-resistant biotypes similar to the wild type (Fig. 6).

3.3. Residual activity

The residual activity of fenquinotrione on *S. juncoides* and *M. vaginalis* was confirmed in greenhouse trials. The weeds were seeded on the soil surface of the pots at 10, 20, 30, and 40 days after treatment.
after application, and residual activity evaluated. Fenquinotri- one at a dose of 30 g a.i./10 a was as effective as, or more effective than, the control (Fig. 7).  

3.4. Influence of overflow on herbicidal efficacy

Various environmental factors affect herbicidal efficacy such as overflow due to rainfall, temperature, weed seeding depth, rice transplanting depth, and water leaching. The effect of overflow on the herbicidal efficacy of fenquinotrione was confirmed in a greenhouse trial. At a dose of 30 g a.i./10 a, fenquinotrione showed less variation in efficacy and higher stability in herbicidal effects than the reference, under conditions assuming an overflow of 6 cm in three days (2 cm/day) (Fig. 8).  

3.5. Effect of environmental factors on rice phytotoxicity

The phytotoxicity of transplanted paddy rice (Oryza sativa cv. Kinmaze) was confirmed in the greenhouse trials. In the field, water leakage conditions are assumed to have stronger herbicidal phytotoxicity. However, fenquinotrione, at a dosage of 30 g a.i./10 a, showed high paddy rice safety in a trial in which 10 cm of water leakage occurred in 10 days (1 cm/day) (Fig. 9).  

In a trial under high-temperature (assuming cultivation in the western part of Japan), low-temperature (assuming cultivation in the northern part of Japan), and intermediate temperature conditions, the phytotoxicity of fenquinotrione varied depending on the temperature. However, the safety of fenquinotrione was as good as, or better than that of the control at all temperatures (Fig. 10).
4. Mechanism of rice safety

4.1. Elucidation of safety factors of fenquinotrione in paddy rice

To estimate the safety factors of fenquinotrione in paddy rice, we first assessed the difference in the susceptibility of the target enzymes of *Arabidopsis* and rice to fenquinotrione. Fenquinotrione potently inhibits 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity in *Arabidopsis* (IC$_{50}$ = 44.7 nM) and rice (IC$_{50}$ = 27.2 nM). In addition, the high similarity in the amino acid sequence of HPPD among plants (Fig. 11) as well as the high conservation of fenquinotrione-binding sites of the HPPD protein (Fig. 12) suggest that the selectivity between rice and weeds was not due to differences in affinity for the HPPD protein. Therefore, it was assumed that the high safety of fenquinotrione in rice was due to its metabolism in rice.

To determine the metabolic mechanism of fenquinotrione, we examined the metabolites of fenquinotrione in rice. The major metabolites of fenquinotrione were M-1, M-2, and their glucose conjugates (Fig. 13). M-2 is a hydrolysis product of the triketone moiety; such metabolites are commonly found in existing HPPD inhibitors. M-1 is a demethylated form of methoxybenzene on the oxoquinoxaline ring, which is unique to fenquinotrione. Cytochrome P450 is known to be involved in oxidation reactions such as demethylation. Therefore, we focused on CYP81A6,
which has been reported to be involved in herbicide metabolism in rice\textsuperscript{11} and assessed whether this enzyme metabolizes fenquino-
trione using a \textit{CYP81A6} gene expression-suppressed line. The
results showed a 20-fold increase in susceptibility to fenquinotrione in the \textit{CYP81A6}-suppressed line as compared to that in the
wild-type (Fig. 14).\textsuperscript{12} These results indicated that the demethyl-
ating metabolism by \textit{CYP81A6}, followed by glucose conjugation
in rice, was responsible for the safety of fenquinotrione.

4.2. \textit{Crop safety and CYP81A6 relationship in triketone HPPD
inhibitor highly sensitive varieties}

It has been reported that some new rice varieties, such as for-
age rice, are highly susceptible to triketone HPPD inhibitors.\textsuperscript{13,14} Therefore, we assessed the safety of fenquinotrione, which has a
triketone structure, in these rice varieties, and examined its rela-
tionship with the function of \textit{CYP81A6}. The correlation be-
tween \textit{CYP81A6} transcript levels in each cultivar and suscepti-
bility to fenquinotrione was examined using real-time RT-PCR.
\textit{CYP81A6} gene was expressed in all cultivars, including the cul-
tivar highly susceptible to triketone HPPD inhibitors as well as
the indica variety Kasalath (Fig. 15). The safety of fenquinotri-
one in these varieties correlated with the expression level of the
\textit{CYP81A6} gene (Fig. 16). Furthermore, the \textit{CYP81A6} gene se-
quences of these cultivars are identical to those of Nipponbare.\textsuperscript{15}

These results suggest that \textit{CYP81A6}, which is involved in the
safety of fenquinotrione in rice, is ubiquitous in rice, and that
fenquinotrione has sufficient crop safety potential in many rice
cultivars.
Concluding remarks

Fenquinotrione was registered in Japan in 2018, launched in 2019, and many fenquinotrione mixture products have been developed since then.\(^{16-18}\) In Japan, the need for cultivation technology that contributes towards cost reduction, labor savings, and diversification of cultivation systems, is expected to increase. Fenquinotrione meets the needs of the current agricultural scene, as it can control a wide range of weeds, including ALS inhibitor-resistant weeds, and has a wide application window. Thus, fenquinotrione is expected to contribute to stable food production in the future.

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Fig. 16. Fenquinotrione susceptibility test for each rice variety. Photos were taken one week after seeding on Hogland’s No.2 medium containing fenquinotrione. The fenquinotrione concentrations were, from left to right, 10, 1, 0.1, 0.01, and 0 µM.