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Investigating the resistance of barnyard grass populations to pretilachlor in direct-seed rice in the Central Region of Vietnam

Nguyen Thi Thu Thuy[1], Tran Thi Anh Tuyet[2], Paul Kristiansen[3] and Nguyen Vinh Truong[1]*

[1]Department of Plant Protection, School of Agriculture and Forestry, Hue University, Hue City 530000, Vietnam.
[2]Department of Crop Production, School of Agriculture and Forestry, Hue University, Hue City 530000, Vietnam.
[3]School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia.

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Barnyard grass *(Echinochloa crus-galli* (L.) Beauv.) is a major weed widely distributed across the world and causes serious damage to rice production. Yield loss due to barnyard grass was estimated at about 7 to 27% in Vietnam. This study aimed to investigate the rice production, weed impacts, herbicide usage and herbicide resistance in the Central Region of Vietnam. A survey of farmers producing rice and yield loss due to weeds conducted in Central Region showed that men mainly educated at primary and secondary schools was the main labour source in rice production. The density of barnyard grass and weed index was 13.9 plants m^{-2} and 14.1%, respectively, in the fields that weeds had regrown after application of herbicide. Screening in the greenhouse indicated that resistance to pretilachlor was found in populations of barnyard grass in Thua Thien Hue. The field trial indicated that a concentration of pretilachlor at the recommended rate (0.3 kg a.i. ha^{-1}) was no longer effective in controlling barnyard grass. This indicates the resistance in populations of barnyard grass in Thua Thien Hue to pretilachlor in the field. The current result is the first report of resistance of barnyard grass to chloroacetamide in Vietnam.

Key words: Chloroacetamide, *Echinochloa crus-galli*, weed management, herbicide resistance.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop for about half of the world's population, providing 23 and 16% of human per capita energy and protein, respectively (Asalla and Parameswari, 2017; Singh et al., 2016). There is about 4.3 million ha planted to paddy in Vietnam, and the country is ranked the second in the world for rice exports (Jenkins, 2020). Billions of dollars are earned from rice exports, playing an important role in global food security as well and local household incomes and rural employment (BCCV, 2021; Prasad et al., 2017).

Weeds are a major problem in rice production worldwide as uncontrolled weeds can lead to rice yield losses as high as 80% (Sheeja and Syriac, 2017; Shekhawat et al., 2020; Kumar et al., 2017). In Vietnam, weeds are also

*Corresponding author. E-mail: nvtruong@hueuni.edu.vn. Tel: 84 0234 3525544.

an important constraint for rice producers, with about 400 weed species reported to occur in both lowland and upland rice fields in Vietnam (Chin and Thi, 2010). Some of the most important species are *Echinochloa* species, *Cyperus difformis*, *Fimbristylis diphylla*, *Heleocharis chaetacria*, and *Marsilea quadrifolia* (Shekhawat et al*.,* 2020; Kumar et al*.,* 2017). Barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) in particular is considered to be one of the most troublesome weed species in rice production in the world (Baltazar, 2017; Ulguim et al*.,* 2020) and especially in Vietnam (Shekhawat et al*.,* 2020). Yield loss due to this weed was estimated at about 7 to 50% (Chin, 2001; Shekhawat et al., 2020). Competition for light between rice and barnyard grass was reported to result in rice grain yield losses of 13 to 55% (Zhang et al*.,* 2017). It is well adapted to wet soils, and is common in both temperate and tropical crops, being reported in 36 crops in over 60 countries (Haghnama and Mennan, 2020; Zhang et al*.,* 2017).

The trend of using more herbicides in rice production has been observed in Vietnam in recent years, posing a range of agronomic and environmental challenges including the risk of herbicide resistance (Nguyen, 2017). The major reasons for increasing herbicide usage by farmers include the ready availability of herbicides in the market, relatively low herbicide cost, increasing labour costs and shortages particularly during the peak period, and higher benefit-cost ratios compared to hand weeding (Sheeja and Syriac, 2017).

There are about 37 herbicide compounds or proprietary mixtures formulated in 79 commercial products that are available for use in rice in Vietnam. Some of the most important compounds are chloroacetamides (pretilachlor, butachlor), pyrimidine, anilide, aryloxyphenoxypropionate, pyrimidinyl carboxy compound, triazopyrimidine sulfonamide, alkylchlorophenoxy, sulfonylurea, pyrazole, quinolinecarboxylic acid, bipyridylium, bispyribac sodium (Nguyen et al*.,* 2015). Pretilachlor, one of the most effective herbicides against grass weeds, is widely used to manage weeds in direct-seeded rice systems since being introduced into the Central Region. Pretilachlor, affects the susceptible plant by inhibition of protein, nucleic acid, lipid and gibberellic acid syntheses, at the recommended rate (0.3 kg a.i. ha^{-1}) providing effective control of grass, broadleaf, and sedge weeds for over three decades (Roberts et al*.,* 2007; Tuat and Son, 2005).

Since the first resistant weed biotype (*Commelina diffusa*) was found in the USA in 1957, 514 resistant biotypes of 262 weed species have been found in 71 countries (Heap, 2020). Prior to 1970, the few reports or observations of weeds exhibiting reduced levels of control with 2,4-D or other early herbicides received little notice or concern among farmers or scientists. However, during the 1970s, many important weed species were reported to be resistant to triazine herbicides and several other herbicides (Prather et al., 2000). Globally, the most economically important herbicide resistance weeds include rigid ryegrass (*Lolium rigidum* Gaudin), wild oat (*Avena fatua* L.), common waterhemp (*Amaranthus rudis* Sauer), tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer)], barnyard grass [*E. crus-galli* (L.) Beauv.], goose grass [*Eleusine indica* (L.) Gaertn.], kochia [*Kochia scoparia* (L.) Schrad.], horseweed [*Conyza canadensis* (L.) Cronq.], and annual blackgrass (*Alopecurus myosuroides* Huds.) (Zimdahl, 2018).

Thirty weed species have evolved resistance to herbicides in rice in all growing areas of the world (Heap, 2020; Kumar et al*.,* 2017). Resistance has occurred under all agroecosystems, except in very low input growing systems such as slash and burn upland rice. Resistance to sulfonylurea herbicides is most frequent, although this group of ALS-inhibiting herbicides is relatively new to rice (Jia et al*.,* 2020). Many weed species are known to be resistant to ALS-inhibiting herbicides (Kumar et al*.,* 2017; Gaines et al*.,* 2020). Other economically important cases of resistance are those of *Echinochloa* spp. that evolved resistance to propanil, molinate, butachlor, thiobencarb, and quinclorac and, more recently, multiple-resistance to several rice herbicides (Baltazar, 2017). In Asia, barnyard grass was confirmed for the first time to be resistant to both chloroacetamide (butachlor)- and acetanilide (propanil)-group herbicides commonly used in direct-seeded rice in China and the Philippines (Qing-ya et al*.,* 2004; Juliano et al*.,* 2010). The management strategy of herbicide resistance has been evolving in developed countries like the USA, Canada, and Australia with increasing interest in research since the early 1970s (Gaines et al*.,* 2020; Zimdahl 2018).

The study of resistance to herbicides is still limited in developing countries, including Vietnam, and there are currently few studies on herbicide resistance in rice production in particular (Le et al*.,* 2018, 2017; Le, 2017). Two populations of barnyard grass collected from rice farms in An Giang of Mekong Delta were reported to be resistant to cyhalofop-butyl (Le, 2017). Le et al*.* (2018) found that seventy-eight barnyard grass populations collected from rice fields in seven provinces in the Mekong Delta of Vietnam were resistant to bispyribac, penoxsulam and quinclorac. Weed regrowth after herbicide application has been reported by the government Plant Protection Sub-departments in many locations since the 2010s in the Central Region (personal communication).

The management of herbicide resistance is essential for the management of weeds in paddy rice fields. The shifts in weed population and development of resistant biotypes should be monitored when the single-target-site herbicides are used repeatedly in the same fields over long periods (Matloob et al*.,* 2015). This requirement applies to rice farming systems in Vietnam, where herbicide usage levels are increasing and awareness of the need to rotate herbicide mode of action is relatively low, and regulatory monitoring and enforcement is weak (Schreinemachers et al., 2015).

In order to gain a better understanding of the scope of the potential threat of herbicide resistance in the Central Region of Vietnam, a multi-disciplinary study was conducted using farmer surveys to collect data on farming practices, field-based assessments of weed impacts, and agronomic experiments to screen a wide range of barnyard grass biotypes for herbicide resistance. The specific objectives of the study were to (i) quantify weed impacts on rice production in the Central Region of Vietnam and their socio-economic drivers, and (ii) screen barnyard grass for resistance to pretilachlor under controlled environmental growing conditions.

MATERIALS AND METHODS

Survey of rice production, weed impacts and herbicide usage

A survey of rice farmers was conducted in three districts of Quang Tri, Thua Thien Hue, Quang Ngai, and Phu Yen province to investigate the rice yield loss due to weeds and herbicide usage practices (Figure 1). In each district three municipalities were surveyed and in each municipality 10 farmers were selected randomly for interviewing by the survey sheet. A survey of fields was undertaken in direct-seeded areas in nine municipalities of Quang Nam, Thua Thien Hue, and Quang Tri province near harvest time. To assess the yield loss due to barnyard grass competition under varying weed conditions, 30 fields in each municipality were chosen for the survey of weed density and weight of rice yield. A quadrat (1m^2) was used to count the weed density, measure biomass, and sample rice yield in places that were weedy and weeded. In each field, five sampling areas (1 m^2 each) were sampled for rice yield in conditions with and without weeds. All plants in the quadrat were harvested for seed weight (g m^{-2}) and yield calculated. Weed index (WI) was calculated by using the formula (Misra and Misra, 1997):

$$WI = [(\text{Yield in weeded} - \text{Yield in weedy})/\text{Yield in weeded}] \times 100.$$

Farmers were interviewed prior to the collection of samples to confirm herbicide use and crop history. Information on weed and control measures was collected at the Plant Protection Sub-department of Quang Ngai and Quang Nam provinces by a survey sheet.

A survey of herbicide usage was conducted by the Plant Protection Sub-departments of Quang Nam and Quang Ngai province. 180 extension workers and farmers from different districts were interviewed about the type of herbicide and number of applications and time by a survey sheet.

Sample collection of barnyard grass seed

Seed sources of barnyard grass to screen for resistance to pretilachlor were collected from municipalities in Quang Tri, Thua Thien Hue, Quang Nam, Quang Ngai, and Phu Yen province where pretilachlor has been applied since the 1990s together with a known susceptible population (Figure 1). Mature seeds of barnyard grass, before rice harvest was collected from 10 plants of each site at the locations of five provinces where barnyard grass has regrown after application of herbicide in direct-seed paddy fields. Seed sources of barnyard grass were stored in the cloth bags under room temperate to screen for herbicide resistance.

Screening for herbicide resistance of barnyard grass populations in greenhouse

Populations of barnyard grass collected from 63 sites in Quang Tri, Thua Thien Hue, Quang Nam, Quang Ngai, and Phu Yen were screened for resistance to chloroacetamide (pretilachlor) at the recommended dose in the greenhouse by using the pot bioassay procedure of Beckie et al. (2000). The experiment was conducted at Agronomy Laboratory, School of Agriculture and Forestry, Hue University. Five experiments were carried out in plastic pots (20 cm in height and 15 cm in diameter) for populations of barnyard grass collected from each province in the Central Region. The populations included 11 from Phu Yen, 19 from Quang Ngai, 14 from Quang Nam, 10 from Thua Thien Hue, and 10 from Quang Trị where pretilachlor has been applied frequently for a long time and eight populations from locations in Phu Yen, Quang Ngai, Quang Nam, Thua Thien Hue, and Quang Tri where pretilachlor had not been applied or only rarely.

The dormancy of barnyard grass seed was broken using the method of Sung et al. (1987). The seeds of each population were germinated by treating with sulfuric acid and soaking in water for 48 h and then placed into Petri dishes lined with moist filter paper. At one day after incubation in the Petri dishes, when 1 mm of the radicle protruded from the seed coat or shoot emerged, the seeds were transferred to the pots filled with about 0.5 kg of sterilised sand, and then herbicide was sprayed at the recommended dose (0.3 kg ai by using a micropipette) after sowing the seeds at a volume of 360 L ha^{-1}. The control treatment was applied with water only. There were 30 seeds in each pot. The number of plants that survived was observed and counted to calculate the survival rate after 15 days. A calibrated manual hand-sprayer was used for all spraying. All seedlings were treated by using two passes of the sprayer over each pot. The treatments were laid out in a completely randomised design with four replications. Pots were maintained under greenhouse conditions at a temperature of 30 ± 4°C and were irrigated daily by water sprayer until 30 days after planting. At 15 days after the herbicide treatment, populations were classified (Llewellyn and Powles, 2001) as: resistant (more than 20% of the plants survived the herbicide treatment); developing resistance (1–20% of the plants survived); or susceptible (all plants were killed) based on the number of plants that survived in each population.

Investigation of the pretilachlor resistance of barnyard grass in directed seed rice

The single-factor experiment was conducted at Huong Toan, Huong Tra, Thua Thien Hue province (16°30'35''N, 107°31'56''E, 10 m elevation). These treatments were: T1 = weed check (unweeded control); T2 = apply pretilachlor at 0.5 × the recommended rate (0.15 kg a.i. ha^{-1}); T3 = apply pretilachlor at the recommended rate (0.3 kg a.i. ha^{-1}); T4 = apply pretilachlor at 1.5 × the recommended rate (0.45 kg a.i. ha^{-1}); and T5 = apply pretilachlor at 2.0 × the recommended rate (0.6 kg a.i. ha^{-1}).

The experiment was laid out in a randomised complete block design with three replications. The layout was completed one day before sowing. Spaces of 0.5 m were maintained in between replication and unit plots, respectively as a buffer. The individual plot size was 4 m × 3 m (12 m^2). The rice variety Khang Dan was used as planting material and the amount of seed was 130 kg ha^{-1}. The experiment was conducted in a field where barnyard grass usually regrows after treatment with herbicides. Herbicide was applied by hand sprayer with a single nozzle at a volume of 320 L ha^{-1} at 1 DAS. Fertiliser was applied at 600 kg NPK ha^{-1} (16-16-8) and was applied in three equal splits; 1st at 15 days after sowing (DAS), 2nd at 30 DAS, and 3rd at 45 DAS. A 40 × 50 cm quadrat was randomly placed at five spots for recording weed data at 14, 21, and 28 days after applying herbicide and at maximum tilling and

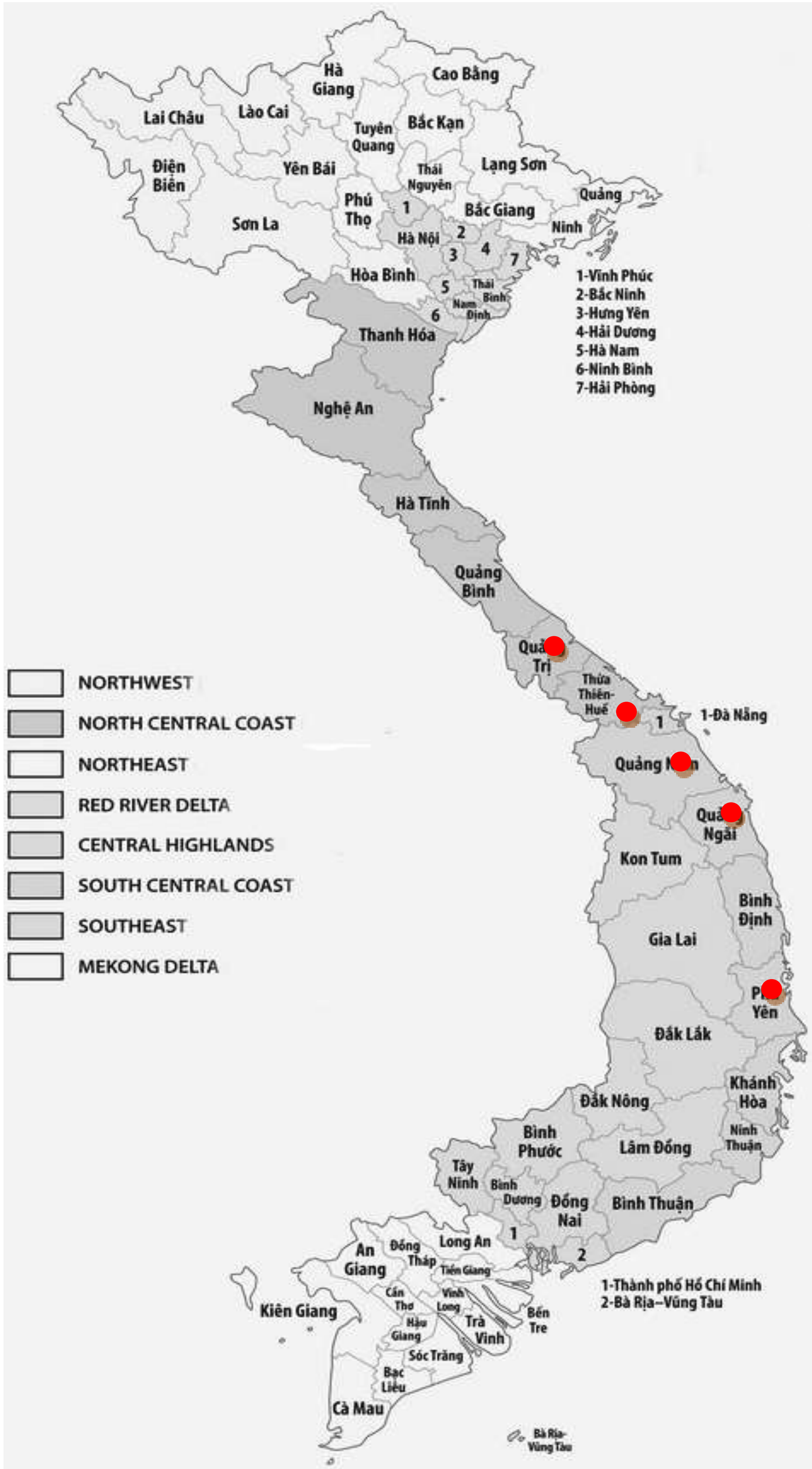

Figure 1. Map of Vietnam showing the provinces surveyed (●).
Source: Wikipedia (2020).

Table 1. Common herbicides applied by farmers in the Central Region of Vietnam.

Herbicide	Quang Nam (n = 90)	Quang Ngai (n=90)	Average (n=180)
Sofit 300EC (Pretilachlor)	22	56	24
Nominee 10SC (Bispyribac - Sodium)	17	5.4	11
Be bu 30WP (Butachlor - Bensulfuron)	15	2.2	8.6
Sunrice 15WDG (Ethoxysulfuron)	12	2.8	7.4
Dibuta 60EC (Butachlor)	5.0	8.2	6.6
Sirius 10WP (Pyrazosulfuron ethyl)	2.0.	11	6.5
Prefit 300EC (Pretilachlor)	70	2.0	4.5
Sonic 300EC (Pretilachlor)	10	3.6	2.7
Fasi 50WP (Quinclorac+ Pyrazosulfuron ethyl)	0	3.6	1.8
Echo 60EC (Butachlor)	3.0	0.1	1.6
Sontra 10WP (Pyrazosulfuron ethyl)	2.0	1.0	1.5
Anco 600SL (2,4 D Dimethyl)	0	2.5	1.3
Meco 60 EC (Butachlor)	2.0	0	1.0
Fenrim 18.5WP (Bensulfuron methyl + Propisochlor)	0.5	0.62	0.6
Tempest 36WP (Bensulfuron methyl + Quinclorac)	0.5	0.44	0.5
Michelle 62EC (Butachlor)	0.5	0	0.35
Tungrius 10WP (Pyrazosulfuron ethyl)	0.5	0.14	0.3

Source: Authors

heading stage, and 15 days after flowering. Weeds were clipped at ground level, identified, counted, and oven-dried at 70°C for 72 h for the density of weed (plant m^{-2}) and fresh and dry weight (g m^{-2}). Weed control efficiency (WCE) was calculated by the Abbott formula (Benseddik et al., 2021). Ten randomly selected hills from each plot were used to measure plant height (cm) and tillering ability (tillers m^{-2}) at the tillering, heading, flowering, and ripening stage. Crop grain was harvested and the fresh weights of grain were recorded from an area of 4 m^2 in the middle of each plot where no weeds occurred. The grains were cleaned and finally, the dry biomass grain weight was measured at a moisture content of 14%. The yield component including the number of panicles m^{-2}, the number of seeds per panicle, and the thousand seed weight of rice were determined.

Statistical analysis

For the survey data within each province, proportions, means, and standard errors of the households, weed density and weight, rice yield were computed for each municipality. For data of screening herbicide resistance, the number of plants that survived was analysed using one-way ANOVA (Gomez and Gomez, 1984) and means were compared based on Tukey's test at the 0.05 probability level with SPSS software v.16 (IBM Inc.).

RESULTS

Characteristics of rice producing households and herbicide use

Characteristics of rice households in the Central Region of Vietnam showed that male farmers and low education were the main labour source in rice production (Data not presented). Almost all the farmers used herbicides for direct-seed rice cultivation (97%). The number of herbicide applications was one to two times per crop (92%) and some farmers applied three times per crop (4.3%).

A survey collected by the Plant Protection Sub-departments of Quang Nam and Quang Ngai province showed that pretilacholor, butachlor, and ethoxysulfuron were the main pre-emergent and post-emergent herbicides introduced to farmers since the 1990s (Table 1).

Assessment of yield loss caused by barnyard grass

The yield loss caused by barnyard grass in the Central Region is presented in Table 2. The density of barnyard grass that had regrown in field subjected to herbicide application was 13.9 plants m^{-2} (Figure 2). The highest density of weeds was in Quang Tri province (22.1 plants m^{-2}) and the lowest was in Thua Thien Hue province (5.0 plants m^{-2}). The dry biomass of barnyard grass that had regrown in fields subjected to herbicide application was 147.9 g m^{-2}. The highest weight of weed was in Quang Tri province (279 g m^{-2}) and the lowest was in Thua Thien Hue province (54 plants m^{-2}). The yield sampled in weedy places was 7.0 t ha^{-1} and the yield sampled in weeded places was 8.3 t ha^{-1}. The highest yield sampled in weedy places was Quang Tri province (7.5 t ha^{-1}) and the lowest yield sampled in weedy places was Quang Nam province (5.3 t ha^{-1}). The highest yield sampled in weeded places

Table 2. Assessment of yield loss caused by barnyard grass in direct-seed rice in the Central Region of Vietnam.

Variable	**Quang Nam (n = 90)**	**Thua Thien Hue (n = 90)**	**Quang Tri (n = 90)**	**Total (n = 270)**
Density (plant m^{-2})	15±0.6	5.0±0.2	22±1.2	13.9±0.7
Weight (g m^{-2})	111±5.0	54±3.0	279±7.7	148±5.0
Yield (t ha^{-1})				
Weeded	5.5±0.2	9.5±0.2	10±0.1	8.3±0.1
Weedy	5.3±0.1	8.2±0.2	7.5±0.1	7.0±0.1
Weed index (%)	3.6	14.0	25.0	14.0

Source: Authors

Figure 2. Barnyard grass that had regrown in fields subjected to herbicide application.
Source: Authors

was Quang Tri province (10 t ha^{-1}) and the lowest yield sampled in weeded places was Quang Nam province (5.5 t ha^{-1}). The average weed index (WI) due to barnyard grass competition was 14.1%. The highest WI was in Quang Tri province (25%) and the lowest was in Quang Nam province (3.6%).

Screening for herbicide resistance of barnyard grass populations in green house

In five separate experiments, 63 barnyard grass populations in each province were screened in order to identify the biotypes that showed resistance to pretilachlor in the Central Region (Table 3). The survival percentage of populations treated with pretilachlor was significantly different in 5, 10, and 15 DAS in five provinces. The survival percentage of populations was from 0 to 50% at 5 DAS in five provinces but the ratio decreased at 10 and 15 DAS due to the phytotoxicity of herbicide. The survival percentage was the highest in the Hai Phu population of Quang Tri at 5 DAS (47.5%) and 10 DAS (20.0%) but all plants were dead at 15 DAS (0.0%). Barnyard grass populations from districts where pretilachlor has not been applied (Tra Van, Nam Dong and Huong Hiep) were totally senescent (dead) at 10 and

Table 3. Survival and resistance rating of barnyard grass populations collected from rice fields in five provinces the Central Region of Vietnam and treated with pretilachlor.

Source of barnyard grass populations	5 DAS		10 DAS		15 DAS	
	Survival (%)	Resistance rating[2]	Survival (%)	Resistance rating	Survival (%)	Resistance rating
Phu Yen						
Binh Ngoc	15.0[b]	2	1.7[a]	2	0[a]	3
Hoa Đong*	12.5[b]	2	0[a]	3	0[a]	3
Hoa Hiep Nam	7.5[ab]	2	0[a]	3	0[a]	3
Hoa An	5.0[ab]	2	0[a]	3	0[a]	3
An Dinh	2.5[ab]	2	0[a]	3	0[a]	3
Chi Thanh	2.5[ab]	2	0[a]	3	0[a]	3
Hoa Dinh Dong	2.5[ab]	2	0[a]	3	0[a]	3
Binh Kien	1.7[ab]	2	0[a]	3	0[a]	3
Hoa Binh 1*	1.7[ab]	2	0[a]	3	0[a]	3
Hoa Dịnh Tay	1.7[ab]	2	0[a]	3	0[a]	3
Hoa Kien	1.7[ab]	2	0[a]	3	0[a]	3
Hoa Hiep Bac	0.8[a]	3	0[a]	3	0[a]	3
An Nghiep	0[a]	3	0[a]	3	0[a]	3
Hoa Hiep Trung	0[a]	3	0[a]	3	0[a]	3
Hoa My Dong*	0[a]	3	0[a]	3	0[a]	3
Quang Ngai						
Nghia Phuong	30[a]	1	9.2[a]	2	0[a]	3
Nghia Ky	28.3[a]	1	4.2[ab]	2	0[a]	3
Nghia Trung	25.0[a]	1	6.7[ab]	2	0[a]	3
Hanh Dung	25.0[a]	1	2.5[a]	2	0[a]	3
Long Hiep	15.8[c]	2	0[b]	3	0[a]	3
Long Mai	12.5[bc]	2	0[b]	3	0[a]	3
Hanh Thuan	11.7[c]	2	0[b]	3	0[a]	3
Long Son	9.2[b]	2	0[b]	3	0[a]	3
Hanh Thien	6.7[c]	2	0.8[b]	2	0[a]	3
Tra Phong*	0[d]	2	0[b]	3	0[a]	3
Quang Nam						
Nam Phuoc	13.4[bc]	2	5.0[a]	2	2.5[a]	2
Binh Trung	19.2[c]	2	5.0[a]	2	1.7[a]	2
Duy Phu	27.5[d]	1	1.7[a]	2	1.7[a]	2
Đien Nam Đong	25.9[cd]	1	3.4[a]	2	0.9[a]	2
Binh An	15.0[bc]	2	2.5[a]	2	0.9[a]	2
Que Trung	11.7[bc]	2	2.5[a]	2	0.9[a]	2
Đien Minh	30[d]	1	2.5[a]	2	0.9[a]	2
Đai Đong	3.4[b]	2	0[a]	3	0[a]	3
Tra Van*	1.7[a]	2	0[a]	3	0[a]	3
Que Loc	20.9[cd]	1	5.9[a]	2	0[a]	3
Đien An	15.9[bc]	2	4.2[a]	2	0[a]	3
Binh Trieu	14.2[bc]	2	3.4[a]	2	0[a]	3
Duy Chau	10.9[bc]	2	2.5[a]	2	0[a]	3
Đai Nghia	5.0[ab]	2	1.7[a]	2	0[a]	3
Đai Quang	5.0[ab]	2	0.9[a]	2	0[a]	3
Thua Thien Hue						
Sia	30[c]	1	36.7[a]	1	35.8[a]	1

Table 3. Contd.

Huong Xuan	46.7^{d}	1	41.7^{a}	1	35.0^{a}	1
Quang Tho	33.3^{cd}	1	28.3^{a}	1	28.3^{a}	1
Huong Toan	39.2^{cd}	1	26.7^{a}	1	26.7^{a}	1
Huong Van	35.8^{cd}	1	33.3^{a}	1	20.8^{a}	1
Quang Phuoc	13.3^{b}	2	21.7^{a}	1	18.3^{a}	2
Thuy Duong	30^{c}	1	20^{a}	2	17.5^{a}	2
Huong Vinh	22.5^{c}	1	19.2^{a}	2	15.0^{a}	2
Thuy Van	8.3^{ab}	2	9.2^{a}	2	7.5^{a}	2
Thuy Thanh	19.2^{bc}	2	15.8^{a}	2	6.7^{a}	2
Nam Đong*	0^{a}	3	0^{b}	3	0^{b}	3
Quang Tri						
Hai Phu	47.5^{b}	1	20^{c}	2	0^{a}	3
Trieu Hoa	23.7^{ab}	1	13.7^{b}	2	0^{a}	3
Trieu Đong	50^{b}	1	3.7^{ab}	2	0^{a}	3
Hai Xuan	30^{ab}	1	1.2^{a}	2	0^{a}	3
Trieu Nguyen	38.7^{ab}	1	0^{a}	3	0^{a}	3
Vinh Long	37.5^{ab}	1	0^{a}	3	0^{a}	3
Vinh Hoa	32.5^{ab}	1	0^{a}	3	0^{a}	3
Hai Vinh	28.7^{ab}	1	0^{a}	3	0^{a}	3
Trieu Long	25.0^{ab}	1	0^{a}	3	0^{a}	3
Vinh Thuy	17.5^{a}	2	0^{a}	3	0^{a}	3
Mo O*	0.7^{c}	2	0^{a}	3	0^{a}	3
Huong Hiep*	0.2^{c}	1	0^{a}	3	0^{a}	3

DAS: Days after seeding. Resistance rating (Llewellyn and Powles 2001): 1 = resistant (20% survivors), 2 = developing resistance (1-20% survivors), 3 = susceptible (no survivors). Survival was analysed through one-way ANOVA, and separation of treatment means was performed using Tukey Test ($P \leq 0.05$). Within each column in each province, numbers with the same letters are not significantly different.
*Weed populations that have has not been exposed to pretilachlor.
Source: Authors

15 DAS. Phytotoxicity of pretilachlor on the barnyard grass emergence after application of herbicide at 5 days showed that the shoot was dead (Figure 3). Developing resistance to pretilachlor was found in populations of barnyard grass in Quang Nam province (Duy Phu, Nam Phuoc, Binh An, Binh Trung, Dien Minh, Dien Nam Dong and Que Trung districts) and Thua Thien Hue province (Thuy Duong, Thuy Thanh, Thuy Van, Quang Phuoc and Huong Vinh districts). Pretilachlor-resistant populations were found in barnyard grass in Thua Thien Hue province (Quang Tho, Sia, Huong Van, Huong Toan, and Huong Xuan districts).

Investigation of the pretilachlor resistance of barnyard grass in directed seed paddy

Weed control efficiency of pretilachlor at different doses in directed-seed paddy in Thua Thien Hue province presented in Table 4 showed that weed density (WD) of barnyard grass was from 0.0 plant m^{-2} (T3, T4, and T5) to 71.3 plant m^{-2} (T1) and weed control efficiency (WCE) of barnyard grass was 89.7 (T2) to 100% (T3, T4, and T5) at 14 days after spraying (DAS). From 21 to 28 DAS, WD, and WCE of barnyard grass varied among treatments applied pretilachlor. There were significant differences in barnyard grass density of treatments applied pretilachlor (T2, T3, T4, and T5) as compared to control treatment (T1) by Tukey test analysis but there were no significant differences in barnyard grass density among treatments applied pretilachlor (T2, T3, T4, and T5). Pretilachlor applied at higher rates (T4 and T5) effectively suppressed regrowth of barnyard grass from tillering to ripening stage but pretilachlor applied at the recommended rate (T3) did not effectively control weeds after 21 DAS. The data indicated that a concentration of pretilachlor at the recommended rate did not effectively control barnyard grass despite herbicide applied at the manufacturer's instructions. There was regrowth from 1.3 up to 6.7 plant m^{-2} of barnyard grass after applying pretilachlor at the recommended rate and WCE decreased from 3.7 to 17.0%.

Weed weight and yield of directed seed paddy applied with pretilachlor at different doses presented in Table 5

Figure 3. Phytotoxicity of pretilachlor on barnyard grass emergence after 5 days application of herbicide.
Source: Authors

showed that weed fresh and dry weight were significantly different among the treatments. The fresh and dry weight of barnyard grass ranged from 0.0 g m^{-2} (T4 and T5) and 0.0 g m^{-2} (T4 and T5) to 336.7 g m^{-2} (T1) and 31.8 g m^{-2} (T1) at tillering stage, respectively. Tukey test analysis showed a significant difference in fresh and dry weight of barnyard grass between the treatments applied pretilachlor (T2, T3, T4, and T5) and control (T1) but there was no significant difference in fresh and dry weight of barnyard grass among treatments applied herbicide. The fresh and dry weight of barnyard grass in pretilachlor treatments at the recommended rate ranged from 13.3 to 60.0 g m^{-2} and 0.6 to 1.6 g m^{-2}, respectively. The rice yield ranged from 6.59 (T1) to 8.25 t ha^{-1} (T5). There were significant differences in rice yield between the treatments applied pretilachlor compared with the control by Tukey test analysis but there was no significant difference in rice yield among the treatments applied herbicide. The weed index ranged from 2.1 (T3) to 26.9% (T1). Although the competition of barnyard grass in the treatment applied pretilachlor at the recommended rate has not caused a significant decrease in rice yield, this indicates that there is resistance in populations of barnyard grass in Thua Thien Hue to pretilachlor in the field.

DISCUSSION

Weed impacts on rice production and herbicide use

Barnyard grass is a widely distributed weed and seriously damages rice production in Vietnam (Shekhawat et al., 2020). Herbicides have been widely applied to control weeds in direct-seeded paddy for many years in Vietnam. This study found that the most common herbicides used in the Central Region were pretilachlor, butachlor, and ethoxysulfuron. These are the main pre-emergent and post-emergent herbicides introduced to farmers since the 1990s but the efficacy of these herbicides have decreased in recent times (Trung et al., 1995). At present, barnyard grass is the most common graminoid invasive species regrowing in direct-seeded paddy in the Central Region after applying herbicides. Rice yield loss due to barnyard grass competition ranged between 3.6% in Quang Nam and 25% in Quang Tri, with Thua Thien Hue being intermediate (14%). This indicates that barnyard grass is competitive in rice production, even after applying herbicide. Therefore, farmers, scientists and plant protectionists should identify the level of resistance to herbicides to develop a good weed management strategy such as rotating herbicides with

Table 4. Weed control efficiency of pretilachlor at different doses in directed-seed paddy in Thua Thien Hue province.

Treatment	1 day before spraying		14 DAS		21 DAS		28 DAS		Panicle		Heading		Ripening	
Barnyard grass	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**	**WD ($N.m^{-2}$)**	**WCE (%)**
T1	0.0	-	71.3^{a}	-	75.3^{a}	-	67.3^{a}	-	39.3^{a}	-	43.3^{a}	-	28.7^{a}	-
T2	0.0	-	7.3^{b}	89.7	6.7^{b}	91.1	11.3^{b}	83.2	29.3^{ab}	25.4	15.3^{ab}	64.5	4.7^{b}	83.6
T3	0.0	-	0.0^{b}	100.0	2.7^{b}	96.3	2.7^{b}	96.3	6.7^{bc}	83.0	6.7^{b}	84.5	1.3^{b}	95.5
T4	0.0	-	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0
T5	0.0	-	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0
Other weeds														
T1	0.0	-	80.7^{a}	-	238.7^{a}	-	230.0^{a}	-	114.7^{a}	-	42.7^{a}	-	42.7^{a}	-
T2	0.0	-	18.7^{b}	76.8	41.3^{b}	82.7	40.7^{ab}	82.3	48.0^{b}	58.2	6.7^{a}	84.3	4.0^{b}	90.6
T3	0.0	-	0.0^{b}	100.0	2.7^{b}	98.9	0.0^{b}	100.0	2.7^{b}	94.4	13.3^{a}	65.9	6.67^{b}	84.4
T4	0.0	-	0.0^{b}	100.0	2.7^{b}	98.9	41.3^{ab}	82.0	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0
T5	0.0	-	4.7^{b}	94.2	0.67^{b}	99.7	11.3^{b}	95.1	0.0^{b}	100.0	0.0^{b}	100.0	0.0^{b}	100.0

DAS: Day after spraying, WD: weed density, WCE: weed control efficiency, N: number of plants. Weed density: Number of plants was analysed through one-way ANOVA, and separation of treatment means was performed using Tukey Test ($P \leq 0.05$). Within each column in barnyard grass and other weed, numbers with the same letters are not significantly different.
Source: Authors

different modes of action, tank-mixing with herbicides with two effective modes of action in the coming years.

Screening barnyard grass for pretilachlor resistance

Screening for pretilachlor resistance in barnyard grass populations from the Central Region was conducted by bioassays in the greenhouse. These trials showed that the survival rate of barnyard grass after 15 DAS at the recommendation concentration was 6.7 to 35.8%. This result provides evidence that the populations of barnyard grass in Thua Thien Hue province (Quang Tho, Sia, Huong Van, Huong Toan, and Huong Xuan districts) in the Central Region are becoming resistant to pretilachlor, a commonly used herbicide in the region. The field trial of weed control efficiency also supported the results from greenhouse screening. The weed control efficiency of pretilachlor at different doses in directed-seed paddy in Thua Thien Hue showed that WCE decreased from 3.7 to 17.0%. These findings also indicate that pretilachlor applied at the recommended rate did not completely control barnyard grass under field conditions and is consistent with field observations by farmers and extension workers that the barnyard grass commonly regrows in rice paddies after pretilachlor applications.

Barnyard grass is one of the top 15 herbicide-resistant species in the world and is reported to be resistant to propanil, molinate, thiobencarb, quinclorac, penoxsulam, bispyribac, azimsulfuron, bensulfuron, cyhalofop, and clomazone, as well as imazethapyr and imazamox (Heap, 2020; Bajwa et al., 2015). Resistance to multiple herbicides in barnyard grass is a major threat in rice production and mechanisms that contribute to resistance have been unravelled (Yang et al., 2017). Barnyard grass has been found to be resistant to chloroacetamide (butachlor), acetanilide (propanil) in the Philippines (Juliano et al., 2010), and to quinclorac (synthetic auxin), penoxsulam (ALS inhibitor) and cyhalofop-butyl (ACCase inhibitor) in Brazil (Eberhardt et al., 2016), and to chloroacetamide (butachlor), and penoxsulam in China and Tuckey (Chen et al., 2016; Huang and Lin, 1993; Kacan et al., 2020). Qing-ya et al. (2004) also indicated that *E. crus-galli* [L.] Beauv. was the lowest tolerance species and *Echinochloa colona* (L.) Link was the highest

Table 5. Weed weight and yield of direct seeded paddy applied with pretilachlor at different doses.

Treatment	Weed weight (g m^{-2})								Yield	
	Tillering		Panicle		Heading		Ripening		Weight (t ha^{-1})	Weed index^{2} (%)
Barnyard grass*	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**		
T1	336.7^{a}	31.8^{a}	476.7^{a}	114.2^{a}	446.7^{a}	58.6^{a}	446.7^{a}	58.6^{a}	6.50^{a}±0.29	26.9
T2	74.7^{b}	8.3^{b}	93.0^{b}	8.9^{b}	106.7^{b}	10.0^{b}	106.7^{b}	10.0^{b}	7.75^{b}±0.25	6.5
T3	13.3^{b}	0.7^{b}	60.0^{b}	1.6^{b}	15.0^{b}	0.8^{b}	15.0^{b}	0.8^{b}	8.08^{b}±0.08	2.1
T4	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	8.17^{b}±0.17	-
T5	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	8.25^{b}±0.14	-
Other weeds	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**		
T1	80^{a}	1.3^{a}	13.3^{a}	3.9^{a}	48.0^{a}	16.3^{a}	48.0^{a}	10.6^{a}		
T2	58.7^{a}	1.6^{a}	3.3^{a}	0.24^{a}	4.0^{b}	0.7^{a}	4.0^{b}	0.7^{a}		
T3	1.3^{b}	0.7^{a}	60^{a}	3.3^{a}	13.3^{b}	4.0^{a}	13.3^{b}	4.0^{a}		
T4	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{a}		
T5	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{a}		

Fresh, dry weight and yield was analysed through one-way ANOVA, and separation of treatment means was performed using Tukey Test (P≤0.05). Within each column in barnyard grass and other weed, numbers with the same letters are not significantly different.
*Weed index = [(Yield in weeded – Yield in weedy)/Yield in weeded] × 100
Source: Authors

tolerance species to pretilachlor in China. In Korea, barnyard grass was resistant to azimsulfuron, bensulfuron-methyl, bispyribac-sodium, cyhalofop-butyl, fenoxaprop-P-ethyl, flazasulfuron, halosulfuron-methyl, imazosulfuron, metamifop, pyrazosulfuron-ethyl, and pyribimenzinobimac-methyl (Won et al., 2014; Heap, 2020).

Changes from traditional transplanting to direct-seeding culture from the 1990s onwards in Vietnam resulted in significant changes in weed control strategies, with greater reliance on herbicides. Herbicide such as pretilachlor, butachlor, and ethoxysulfuron have become the main method to control weeds in direct-seeded rice since the 1990s (Chin and Thi, 2010; Tuat and Son, 2005). Weed management approaches which rely on one or a few control methods can lead to a shift in the weed flora and the evolution of resistant populations (Le et al., 2018). The repeated use of the same herbicide or compounds with the same mode of action increases the herbicide resistance to weeds. The regrowth of weeds after herbicide application has been reported in various localities in Vietnam, including populations of barnyard grass in the Mekong Delta (Le et al., 2017; Le et al., 2018). In this study, it was confirmed that barnyard grass has evolved and shows resistance to pretilachlor in different rice growing areas in the Central Region of Vietnam. The current findings are the first reports on the resistance of barnyard grass to chloroacetamide in Vietnam. These findings provide the first known evidence of herbicide resistance in the Central Region on Vietnam. It sets a basis for further research on the weed management strategies in rice, genetic variations of barnyard grass populations in the region, and screening of a wider range of herbicides of different weed populations. Such research can be used in the development of integrated weed management systems for direct-seeded rice and related training packages in order to decrease reliance on herbicides and reduce herbicide resistance in rice production (Bajwa et al., 2015).

Conclusion

Barnyard grass was a major weed widely distributed in the country and is a serious damage species to rice production. Pretilachlor was the main pre-emergent to be used for controlling this weed since the 1990s. Greenhouse screening and field trial has found resistance of barnyard grass to pretilachlor. The current results are the first finding of the resistance of barnyard grass to chloroacetamide in Vietnam. This study provides an understanding of herbicide resistance in the country and will help researchers and managers to develop the strategies of weed management for direct-seed rice in the future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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