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Diversity of *Echinochloa* spp. in Palakkad rice tracts of Kerala

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ABSTRACT

Surveys were conducted twice in the major rice tracts of Palakkad, once during January-February, and the other during August-September in 2016. Three major *Echinochloa* types were identified infesting rice fields, causing severe crop competition. The three types were *E. colona*, with awnless spikelets and two types of *E. crus-galli*, *viz*. *E. crus-galli* (type A) having short awns and *E. crus-galli* (type B) having long awns. Other than the length and arrangement of awns, there were no significant differences in the morphological characters of the latter two types. Frequency and relative frequency was the highest for *E. crus-galli* (type B). There was no specific association between the *Echinochloa* types and soil nutrient parameters, probably as there were no drastic differences in the chemical properties among the different locations. Cluster analysis classified *Echinochloa* types in to 5 groups at 66.67% similarity level. The study concludes that in a less productive environment, the awned types of *Echinochloa* could have a better chance of survival.

INTRODUCTION

The most important biological constraint to rice production is weed infestation. Effective weed management plays an important role in rice cultivation to prevent yield loss, reduce production cost and ensure grain quality. Weed infestation causes 9-51% yield reduction in rice crop (Mani *et al*. 1968). The yield reduction due to weed infestation and expense of weed management together contribute an estimated 15% loss in rice production (Smith 1981). *Echinochloa* species are one of the most destructive weeds associated with rice crop, distributed throughout the world especially in tropical and warm-temperate regions (Michael 2003, Shultana *et al*. 2013). Severe infestation of *Echinochloa* spp. has been reported in wet seeded rice in 15 countries, and in dry seeded rice in 22 countries and also in transplanted rice (Chauhan and Johnson 2009).The genus has more than 50 species, which includes the third and fourth most important weeds in the world, *viz*. *Echinochloa crus-galli* (L.) and *Echinochloa colona* (L.) (Holm *et al*. 1977, Michael 2003). Weeds belonging to *Echinochloa* species vary in their growth habit, distribution, and morphology (Barret and Wilson 1983). Being C_4 plants, *Echinochloa* are adapted to diverse environmental conditions. Their adaptations include the ability to flower in varying photoperiods and to produce numerous seeds, which are easily dispersed. The seedlings of *Echinochloa* closely resemble rice seedlings, and sometimes they are unintentionally transplanted to rice fields or might escape from hand weeding (Rao and Moody 1988). They are very strong competitors with rice and can cause drastic reduction in rice yield (Rao and Moody 1992).

India is one of the largest producers of rice in the world, having an area of 43.49 mha under rice cultivation with an annual production of 104 mt. Kerala is an Indian state located at the south western end of Indian peninsula at latitudes between 8°18' and 12°48' N longitudes between 74°52' and 77°22'E. The warm humid tropical climate and ample rainfall ensure the suitability of rice cultivation in the state. Palakkad, '*Kuttanad*', '*Pokkali*', '*Kole*' lands and 'Kaippad' are the main rice growing tracts of Kerala. Palakkad district, known as the granary of Kerala, has the largest acreage of rice cultivation in the state and contributes more than 45% of the total rice produced. Recently, some new types and unidentified species of *Echinochloa* have been seen to occur in the rice fields of Kerala, especially in Palakkad district. These are believed to have been brought to the area through contaminated seeds and through mechanical harvesters, which were previously used in other states, or may be due to introgression of the existing *Echinochloa* types. Variation in genetic and

morphological characters of *Echinochloa* spp. may also be associated to differential response to herbicides (Altop and Mennan 2011). The new species or morphotypes are reported to be tolerant or less sensitive to conventional herbicides. It is essential to properly identify the new species and ecotypes based on important morphological traits for selecting the appropriate herbicides to be used effectively against each type. In this context, the objective of the present investigation was to study the diversity of *Echinochloa* spp. in the rice fields of Palakkad based on morphological characters.

MATERIALS AND METHODS

The study was focused on the rice tracts of Palakkad district in Kerala located between the latitudes of 10Ú21' to 11Ú14' North, and longitudes of 76Ú02' to 76Ú45' East. Stratified surveys were conducted twice, the first one in the months of January and February, and the second in August and September of 2016 in key rice growing areas of Palakkad rice tracts. Eleven locations were identified where different species or types of *Echinochloa* dominated. From each location, important morphological observations were recorded on 50 plants during the flowering stage. Observations on soil chemical properties, water management and weed management were also recorded. Seeds of different species and types were collected. Panicles of *Echinochloa* types collected in surveys were identified at the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore, India. Morphological studies of each type of *Echinochloa* were conducted as pot culture study at College of Horticulture, Kerala Agricultural University, Thrissur.

Observations such as plant height, number of tillers/ plant, number of panicles per plant, growth form, leaf arrangement and size, flag leaf size, dry matter production/plant, presence or absence of awns and panicle characters were recorded from the field. Soil chemical properties (pH, organic C, available N, P and K) and moisture status in the field were also recorded. The degree of abundance of each species/ morphotype of *Echinochloa* was noted in terms of frequency and relative frequency.

The observations recorded in lab and pot culture studies were as follows:

$$\text{Weed frequency} = \frac{\text{Number of sites where a particular weed species occurred}}{\text{Total number of sites surveyed}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Total number of frequencies of all species}} \times 100$$

Germination percentage: Germination studies on each type of *Echinochloa* were conducted in the laboratory. Seeds showing dormancy were subjected to pre-treatment with ethanol (1. M) under darkness for 3 days. For germination test, 10 seeds each were placed in petri dishes of 9 cm diameter lined with a piece of Whatman No.1 filter paper which had been moistened with distilled water. Seeds with a visible protrusion of radicle were considered to have germinated. The number of germinated seeds was counted at 15 days after sowing or until there was no further germination.

Time to emergence: In pot culture, the time required for the shoot to reach the soil surface was recorded and indicated in days.

Heading time: The duration required for the panicle to become fully visible was recorded and indicated in days.

Growth duration: The duration from germination to final drying of plants was recorded and indicated in days.

Observations on the nine quantitative morphological characters recorded at the survey locations were subjected to cluster analysis using Euclidean distance as a similarity index. The associated dendrogram was obtained using the statistical package 'Minitab Version 17'. PCA was also performed in the same statistical package with the same quantitative morphological observations to find the contribution of the observed characters in grouping of the *Echinochloa* types.

RESULTS AND DISCUSSION

The serious problem of *Echinochloa* in Palakkad rice tract is aggravated by the appearance of a number of morphotypes or biotypes. Biotypes are plants showing a random genetic variant within an ecotype (Klingman and Oliver 1994). Fifteen types of *Echinochloa* were obtained from 11 survey locations in Palakkad (**Table 1, Figure 1**). Plant morphology under uniform conditions can be influenced by environmental conditions and plant genotype. It is reported that the morphological characters of *E. crus-galli* are affected by soil type and fertility level (Martines *et al*. 1999). Most species of *Echinochloa* are highly polymorphic and variable in the characteristics usually considered, and are difficult to distinguish (Michael 1983).

Table 1. Distribution of *Echinochloa* spp./morphotypes in rice fields of surveyed areas

Location	Latitude (^{0}N)	Longitude (^{0}E)	*Echinochloa* species/ morphotypes	Code no. of *Echinochloa* types
Chithali 1	10°41'25.9"	76°34'58.6"	*Echinochloa colona*	1
Chithali 2	10°41'23.2"	76°34'59.3"	*Echinochloa crus-galli*(type A)	2
			Echinochloa crus-galli(typeB)	3
Parakkattukavu	10°39'19.0"	76°31'11.2"	*Echinochloa crus-galli*(typeB)	4
			Echinochloa colona	5
Modappallur	10°35'27.9"	76°31'02.4"	*Echinochloa colona*	6
Kozhinjampara	10°44'29.5"	76°49'48.8"	*Echinochloa crus-galli* (type A)	7
Thathamangalam	10°40'43.2"	76°42'35.9"	*Echinochloa crus-galli* (type A)	8
Koduvayur	10°40'25.7"	76°38'36.2"	*Echinochloa crus-galli* (type B)	9
Thrippalur	10°38'54.3"	76°33'56.5"	*Echinochloa colona*	10
Kunissery	10°38'19.8"	76°35'27.5"	*Echinochloa crus-galli* (type B)	11
Cheramangalam	10°37'14.1"	76°35'15.1"	*Echinochloa crus-galli* (type A)	12
			Echinochloa crus-galli (type B)	13
Ayilur	10°34'49.5"	76°34'11.5"	*Echinochloa crus-galli* (type A)	14
			Echinochloa crus-galli (type B)	15

From the survey conducted in the major rice producing tracts of Palakkad district, three major *Echinochloa* types were obtained, and they were identified at Botanical Survey of India, Southern Regional Centre located at TNAU Campus, Coimbatore, India. The three types included *E. colona* (synonym: *Panicum colonum* L.) and two types of *E. crus-galli*, one with short awns designated as *Echinochloa crus-galli* (type A) (Synonym: *Panicum crus-galli* L.) and the other with longer awns named *E. crus-galli* (type B) (*Panicum crus-galli* L., *Echinochloa glabrescens* Munro ex Eggel. or *Echinochloa oryzoides* auctnon (Ard.) Fritsch). The nomenclature *E. colona* was adopted in favour of *E. colonum* as per the conclusion reached by Michael (2009). The frequency of distribution and relative frequency of *Echinochloa* was the highest for *E. crus-galli* (type B) followed by *E. crus- galli* (type A) and *E. colona* (**Table 2**).

The two types of *Echinochloa crus-galli* were distinctly different with respect to awn length and arrangement of awns on the spikelets. In fact, the type collected and named as *Echinochloa crus-galli* (type A) had earlier been identified as *E. glabrescens* (Thomas and Abraham 1998, 2007). In *E. crus-galli* (type A), the spikelets were broadly ovate to ovate with short awns up to 1.5 mm long. The awns were abundant and either scattered throughout the panicle or confined on the spikelets at the tip of the panicle branches. In *E.crus-galli* (type B), the spikelets were ovate with long awns distributed throughout the panicle. The length of awn went up to 11 mm. *E. colona*, on the other hand, was awnless. There were no other significant differences in the morphological characters recorded of *E. colona* and *E. crus-galli* types. The major morphological characters of the three types of *Echinochloa* are presented in **Table 3**.

Table 2. Frequency and relative frequency of *Echinochloa* species/morphotypes in rice fields of surveyed areas of Palakkad

Echinochloa species/ Morphotype	Frequency (%)	Relative frequency (%)
Echinochloa colona	36.36	23.53
Echinochloa crus-galli (type A)	54.54	35.29
Echinochloa crus-galli (type B)	63.64	41.18

Table 3. Morphological characters of *Echinochloa* spp. and types

Parameter	*Echinochloa colona*	*E. crus-galli* (Type A)	*E. crus-galli* (Type B)
Duration (days)	95-120	90-120	>120
Days to germination	3-5	3-7	2-4
Days to heading	60-75	50-60	70-95
Plant habit	Erect	Erect	Erect
Height of plant (cm)	53-186	76-195	46-194
No. of tillers/plant	1-17	1-21	1-9
No. of panicles/plant	1-18	1-21	1-9
Stem colour	Greenish	Greenish	Greenish
Culm nature	Erect, ascending	Erect, ascending	Erect, ascending
Leaf colour	Light green	Light green	Light green
Leaf arrangement	Alternate-opposite	Alternate-opposite	Alternate-opposite
Length of leaf (cm)	38.8	20.4	22.5
Width of leaf (cm)	1.09	0.8	0.8-1.5
Dry smatter production, g/plant	5.32-7.68	1.5	4.86-7.84
Panicle length (cm)	10-40	4.98-7.93	25-45
Panicle arrangement	Loose/ compact	Compact	Loose to tight
No. of branches/panicle	17	18.7	18
No. of spikelets/panicle	371	487	403
Seed production/plant	1447	1682	766
Spikelet colour	Greenish/ purplish	Purplish/ green	Green to purple
Awns present/absent	Absent	Present	Present
Length of awn (mm)	-	1.50	6-13.5
Length of spikelets (mm)	3.45	4.70	14.75
Width of spikelets (mm)	1.60	1.75	1.9

Cluster analysis classified *Echinochloa* types in to 5 groups at 66.67% similarity level. Group 1 included two types, *i.e*. type 1 and 14. Type 2 occupied as a separate group (group 2). Group 3 included 7 types, *i.e*., types 3, 8, 7, 9, 12, 10, and 13. Group 4 had 3 types, *i.e*., 6, 11 and 15. Two types (type 4 and 5) occupied group 5 (**Figure 2**).

The scree plot of PCA revealed that first 3 principle components correspond to the whole percentage variance in the data as they have Eigen value greater than 1 (**Figure 3**). PC1, PC2 and PC3 together accounted for 76.9% of the total variations, of which PC1 accounted for 34.4%, PC2 for 28.3% and PC3 for 14.2% variation. The first PC was related to all the morphological observations taken and the second PC related to all morphological observations except leaf length, leaf width, and dry weight. PC3 was related to the factors number of tillers/ plant, number of panicles/ plant and dry weight. PC1 was more positively contributed to by the characters in the order of plant height, leaf width, panicle length, dry weight, flag leaf width, number of panicles plant, leaf length, flag leaf length and number of tillers/ plant. PC2 was more positively contributed to by number of tillers/ plant, flag leaf length, number of panicles per plant, flag leaf width, plant height and panicle length (**Table 3**).

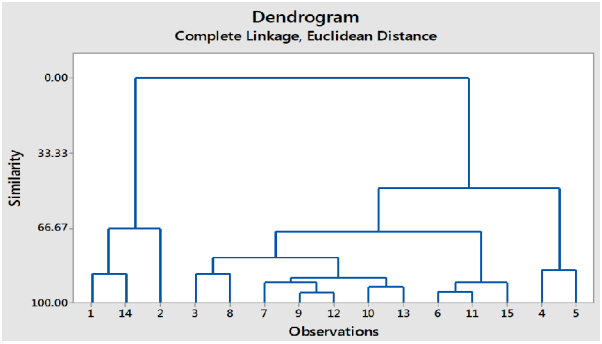

Figure 2. Dendrogram from hierarchial cluster analysis for dissimilarity among the 15 morphotypes of *Echinochloa* spp.

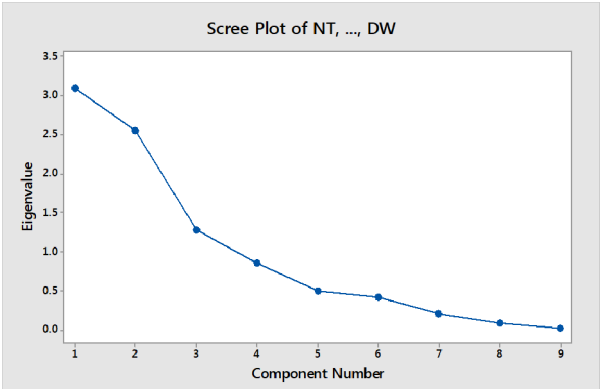

Figure3. Scree plot showing the Eigen values in response to the quantitative morphological characters of *Echinochloa* spp.

The scree plot between PC1 and PC2, which together contributed more than 60% variability, showed that the *Echinochloa* types included in different groups had different morphological characters. Many of the types coming under group 3 were distributed farther from each other, pointing to wide variation between the individuals of the group. The individuals of group 2 were distributed farther from other groups, indicating large variation from the other types (**Figure 4**).

Soil chemical properties and moisture level in the field

Semi-dry system of rice cultivation was followed throughout the survey locations in Palakkad rice tract, with saturated soil conditions. *Echinochloa* types were abundant in acidic to neutral pH prevailing in the area. Severe infestation of *Echinochloa* complex was observed in soils with high organic carbon, moderate nitrogen and phosphorus, and low to high potassium (**Table 4**). There was no specific association between *Echinochloa* types and soil nutrient parameters, probably as there were no drastic differences in chemical properties between

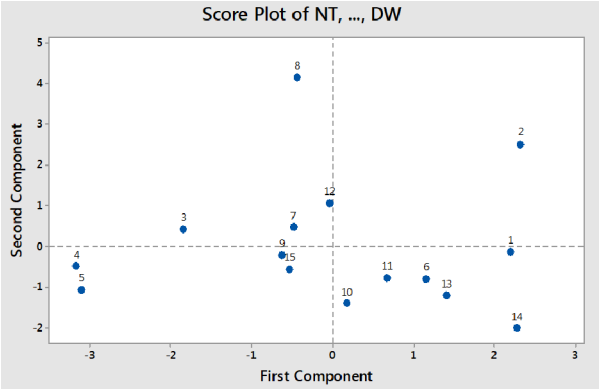

Figure4. Scree plot of first two PC indicating the variability in morphological characters of *Echinochloa* spp.

Table 4. PCA values of quantitative morphological characters of *Echinochloa* spp.

Morphological observations	PC1	PC2	PC3
No. of tillers/ plant (NT)	0.132	0.552	0.196
No. of panicles/ plant (NP)	0.274	0.256	0.543
Plant height (PH)	0.485	0.042	-0.019
Leaf length (LL)	0.260	-0.441	-0.230
Leaf width (LW)	0.403	-0.329	-0.135
Flag leaf length (FLL)	0.145	0.484	-0.404
Flag leaf width (FLW)	0.340	0.226	-0.478
Panicle length (PL)	0.400	0.032	-0.004
Dry weight (DW)	0.382	-0.198	0.451
Eigen value	3.0989	2.5474	1.2742
Proportion	0.344	0.283	0.142
Cumulative	0.344	0.627	0.769

Table 5. Soil chemical properties and moisture level in rice fields of different locations

Location	Soil pH	Organic C (%)	Available N (kg/ ha)	Available P (kg/ ha)	Available K (kg/ ha)	Soil moisture level
Chithali 1	4.41	0.76	501.76	20.00	142.20	Saturated
Chithali 2	4.46	0.80	627.20	32.17	84.00	Saturated
Parakkattukavu	6.41	0.74	376.30	17.96	142.24	Saturated
Modappallur	4.74	0.93	689.90	12.95	152.30	Saturated
Kozhinjampara	7.76	1.17	439.04	03.34	84.00	Saturated
Thathamangalam	6.28	0.62	627.20	23.40	227.33	Saturated
Koduvayur	6.49	0.78	439.04	12.53	108.60	Saturated
Thrippalur	6.02	0.81	376.02	10.03	67.20	Saturated
Kunissery	5.40	0.77	439.04	21.31	99.68	Saturated
Cheramangalam	4.85	0.87	376.00	44.29	113.12	Saturated
Ayilur	6.00	0.71	439.04	130.80	381.92	Saturated

different locations. The rice fields of Palakkad district covered in the survey had similar soil properties leading to infestation by all the three types of *Echinochloa* throughout. *E.crus-galli* (type B) recorded a slightly higher relative frequency than *E.crus-galli* (type A), pointing to a better adaptability to the existing conditions.

E. crus- galli and *E. colona* are thus the two species of *Echinochloa* identified in the rice tracts of Palakkad district. However, the presence of two types of *E. crus- galli* is a cause for concern, indicating the development of morphotypes in response to the existing environment and cultural practices used by farmers. The presence of long awns in *Echinochloa crus-galli* (type B) could be an adaptation to reduce grain number so as to increase grain size, thereby increasing probability of survival. In wheat, Rebetzke *et al.* (2016) found that awns are coupled with larger grain size and yield in less favourable environments, but reduce grain number in more favourable environments. Palakkad soils are acidic in reaction and the region is prone to drought. In such a less productive environment, the awned types of *Echinochloa* could have a better chance of survival. Such evolutionary developments could cause considerable difficulty in controlling the weed and increase the potential risk of rice cultivation in such areas.

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