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Botanical indices of ploidy levels in some African accessions of *Oryza punctata* Kotschy ex Steud

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ABSTRACT

Twenty-nine accessions of *Oryza punctata* Kotschy ex Steud, from local and other African habitats were studied to establish the attributes that can delineate the two ploidy levels based on agro-botanical, foliar epidermal and nodal anatomical characteristics. The diploid plants of *O. punctata* are early-maturing annuals with a small biomass, while the tetraploids are large, vigorous perennials. From the anatomical and morphological traits: the diploids have narrower leaves (0.95 - 1.36 cm wide) than the tetraploids (1.9 - 2.3 cm); the culms of diploids are generally thin (0.28 - 0.35 cm in diameter) compared to the tetraploids (0.37 - 0.57 cm); the diploids have longer spikelets (6.20 - 7.04 mm) with length/breadth > 3; longer awns (52 - 86 mm) than the tetraploids (20 - 31 mm). The basal cells of microhairs in the diploids are about twice the length of apical cells, but only slightly longer than the apical cells in the tetraploids; prickle hairs are sparse in the intercostal zone of diploids, but abundant in tetraploids; short cells are paired and abundant in the intercostal zone of diploids, but sparse and solitary in the tetraploids. These indices delineated the ploidy levels and are reliable for use as identification aids.

Keywords: Agro-botanical characteristics, diploid *punctata*, tetraploid *punctata*, delineation, identification aids.

INTRODUCTION

Sano (1980) compared the adaptive strategies in diploid and tetraploid forms of *Oryza punctata* Kotschy ex Steud. He reported that diploid strains had a high reproductive effort, well developed awns, low regeneration of excised stem segments, short anthers and a high degree of seed dormancy. The diploid strains were from open grasslands while the tetraploid strains were confined to the forest. Faluyi and Nwokeocha (1993) reported a widespread distribution of diploids and an overlap in the distribution of diploids and polyploids (4n=48) but with the polyploids spreading beyond the range of diploids. Watanabe et al. (1993) attributed the inability of diploid *O. punctata* to adapt to low light to the total amount of chlorohyll produced or the partitioning of the thylakoid protein into the core complex of photosystems II.

The taxonomic importance of cuticular features of plant surfaces had been revealed as early as 1965 by Stace. Srivastava (1978) described the leaf epidermis as the second most important character after cytology for

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solving taxonomic problems. For decades, anatomical and foliar epidermal features have been shown to possess great significance in the taxonomy of many taxa. Many of these attributes have been found to be diagnostic at generic or specific levels while others are important only in combination of other characters. This fact is illustrated in the works of Stace (1984), Ogundipe and Olatunji (1991), Nwokeocha (1996), Jayeola et al. (2001), Adedeji and Faluyi (2001), Adedeji and Illoh (2004), Kadiri et al. (2005), Ayodele and Olowokudejo (2006), Adedeji and Jewoola (2008), Ogie-Oda et al. (2010), Adedeji (2011), Munir et al. (2011), Adeniji and Ariwaodo (2012), Thakur and Patil (2014). The outcome of these works is a better understanding of the taxonomy of the taxa involved. These successes have been attributed to the fact that the features involved are subject to limited environmental variation and therefore are reliable.

The taxonomy and identification of the species in the *O. officinalis* complex, sometimes also referred to as *O. latifolia* complex, are difficult due to similar morphology and overlapping distribution of some of the species. The difference in ploidy levels of some species has been reported to add to the complexity (Faluyi and Nwokeocha, 1993; Bao et al., 2005). Also, the efficient utilization of rice genetic resources and efficient management of the germplasm collections rely on the correct identification of the germplasm (Ge et al., 2001; Li et al., 2001). This paper, therefore, investigated the agro-botanical and morphological characteristics, in addition to some nodal and foliar epidermal features, of the two ploidy levels of *O. punctata*. This was with a view to distinguishing between diploid and tetraploid *O. punctata*, which belong in the *latifolia* complex, and providing identification aids.

MATERIALS AND METHODS

Plant source

A total of 29 accessions of *Oryza punctata*, collected from the Genetic Resources Unit of International Institute for Tropical Agriculture (IITA) and from our locality, as seen in Table 1, were studied. The plant materials collected (seeds and rootstocks) were grown in both screen house and field and were maintained for several years. Measurements of quantitative and qualitative morphological characters from culm, leaf, ligule, panicle, spikelet, awn, anther, pollen grain, among others, were taken for all the accessions based on the IBPGR-IRRI Rice Advisory Committee (1980) Descriptors for Rice, *Oryza sativa* Linn.

Foliar epidermal studies

Mid portions of the abaxial and adaxial surfaces of leaf blade were scraped with sharp razor blade to prepare the epidermides according to Cutler (1978). The peels were decolourized in 5% solution of sodium hypochlorite (domestic bleach) for 30-60 minutes after which they were preserved in 50% ethanol. The cleared epidermal peels were stained in Alcian blue and counter-stained in Toluidene blue to enhance contrast. To enhance the identification of cork and silica cells, epidermal peels were stained in 1% Sudan IV. Peels were mounted on microscope slides in 25% glycerol. Illustrations of epidermal features were made with the aid of Camera Lucida outfit at X25 and X40 objective powers. Measurements of cells, microhairs and stomata were made in ocular units and converted to micrometers. Stomata frequency per mm^2 was determined.

Nodal anatomical studies

Transverse sections (T.S.) of the first three nodes above the ground were made on the Reichert sliding microtome at 15 µm. The sections were preserved in FAA (Formalin-Acetic-Alcohol), stained in 1% aqueous solution of Safranin 'O', counter-stained in Alcian blue for 3-5 minutes, differentiated and dehydrated in different grades of concentration of alcohol up to 95% and mounted in DPX. Photomicrographs of the

T.S. of nodes were taken under X4 objective power.

Statistical analysis

Quantitative data were analyzed using ANOVA with a significant level of p = 0.05.

RESULTS

Habitat/Habit

The local accessions were found on marshy ground, river beds of stagnant water except where man has interfered with the natural ecosystem as shown in Table 1. Two types of habit were encountered: diploids (2n = 24) which are annuals and tetraploids (2n =48) which are perennials. The diploids are earlier maturing (98 - 105 days) than the tetraploids (124-148 days).

Plant type

Three major plant types were observed: the open, the erect and the spreading to procumbent. The open plant type, with culms angle c.50° comprises TOP 5702, TOP 14097 and TOP 14460, all diploids, whose culms are thin, heavy tillering with leaves long and narrow; the accessions from CAR and TOP 13596 from Tanzania whose culms are tall and fat, heavy tillering with leaves long and broads. The erect plant type (with culms angle c.30°) comprises TOP 6788 and TOP 8222 which are low tillering with leaves long, broad and densely pubescent. The diploid TOP 13546, representing the third plant type, is fairly heavy tillered with thin culms which are between procumbent and prostrate at late vegetative phase. The length/breadth ratio of leaves separated the diploids from the tetraploids without overlap as shown in Table 2.

Reproductive biology

The result is shown in Table 3.

Panicle

The panicle type encountered in this study is open with loose ascending or spreading to descending primary branches. The length is variable among accessions but generally shorter in the diploid forms. The panicle density is also variable.

Spikelet

Shattering habit is present in all the accessions. The pericarp colour is brown; the stigma is dark-purple. Awn is present in all the accessions. The diploid *0. punctata* has very long and broad spikelets (l:b ratio > 3) with very long awns while the tetraploids have long and very broad spikelets (l:b ratio < 3) with short awns.

Anther

The anther colour is dirty-brown except in TOP 6788 and TOP 8222 which have milk-coloured anthers. The anther sizes among the accessions vary but of medium size.

Pollen grain

The pollen grain types/forms in all the accessions are of a typical grass: monoporate, spherical with thick exine which is raised around a circular pore; surface psilate or reticulate; of medium size. The size and sculpturing pattern under light microscope did not distinguish between diploid and tetraploid *O. punctata.*

Foliar epidermal studies

The result is shown in Tables 4 and 5, and Figure 1.

Costal zone

Long cells rectangular, many times longer than broad, cell walls markedly sinuous; papillae numerous per cell, of uniform size, circular and dome-shaped; short cells abundant in continuous rows; silica bodies cross-shaped (Oryza-type; according to Metcalfe, 1960), in pairs. Prickle hairs frequent, also on the margin of the leaf blade. Macrohair sparse to frequent in all, except TOP 6788 and TOP 8222, long and slender with large, raised base and pointed apex. Microhair and Stomata not seen.

Intercostal zone

Long cells rectangular, longer than broad, generally longer on abaxial than on the adaxial epidermides; cell walls markedly sinuous; papillae numerous per cell, rounded, small-sized and uniform on adaxial, a

combination of small and largely inflated ones on abaxial epidermides; short cells sparse usually solitary in tetraploids, numerous and usually paired in diploids; stomata abundant, paracytic, 2 bands per intercostal region, 1 - 2 rows per band; subsidiary cells predominantly triangular in shape. Prickle hairs abundant in tetraploids but sparse in diploids. No macrohair was seen. Microhair numerous, bicellular, basal cells slightly longer than apical cells in tetraploids but about twice the length of apical cells in diploids

Nodal anatomy

The transverse section through the node in all accessions studied revealed the following general features as presented in Figure 2.

The epidermis composed of 1-layered cell, generally isodiametric, occasionally substended by 1-2 layered sclerenchyma cells. Outer ground tissue composed of 2 - 5 layers of parenchyma cells, occasionally lignified and sometimes intercepted by 4 - 8 layers of sclerenchyma cells extending to epidermis and traverses the entire inner ground tissue; small vascular bundles are embedded within the sclerenchyma at intervals. Inner ground tissue composed of bundles of primary vascular bundles surrounded by extraxylary fibres, smaller vascular bundles in-between primary vascular bundles. Towards central ground tissue is 1 circle of large vascular bundles surrounded by extraxylary fibres and 1 - 2 layers of sclerenchyma cells. Sclereids occasionally seen. Central ground tissue composed of schizogenous cells. Pith at node is solid.

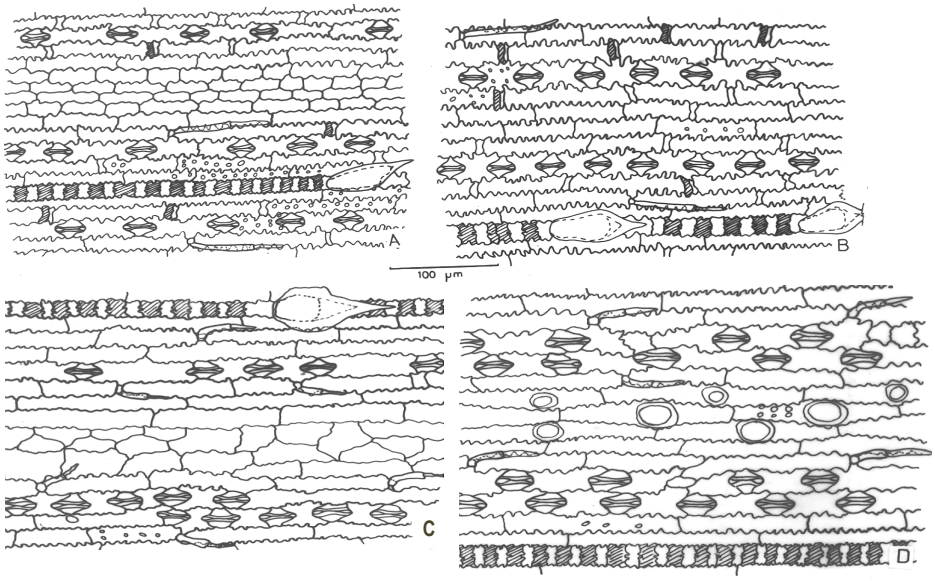

Figure 1: Representatives of Foliar Epidermal features of *O. punctata* studied. A: Adaxial of diploid; B: Abaxial of diploid; C: Adaxial of tetraploid, D: Abaxial of tetraploid.

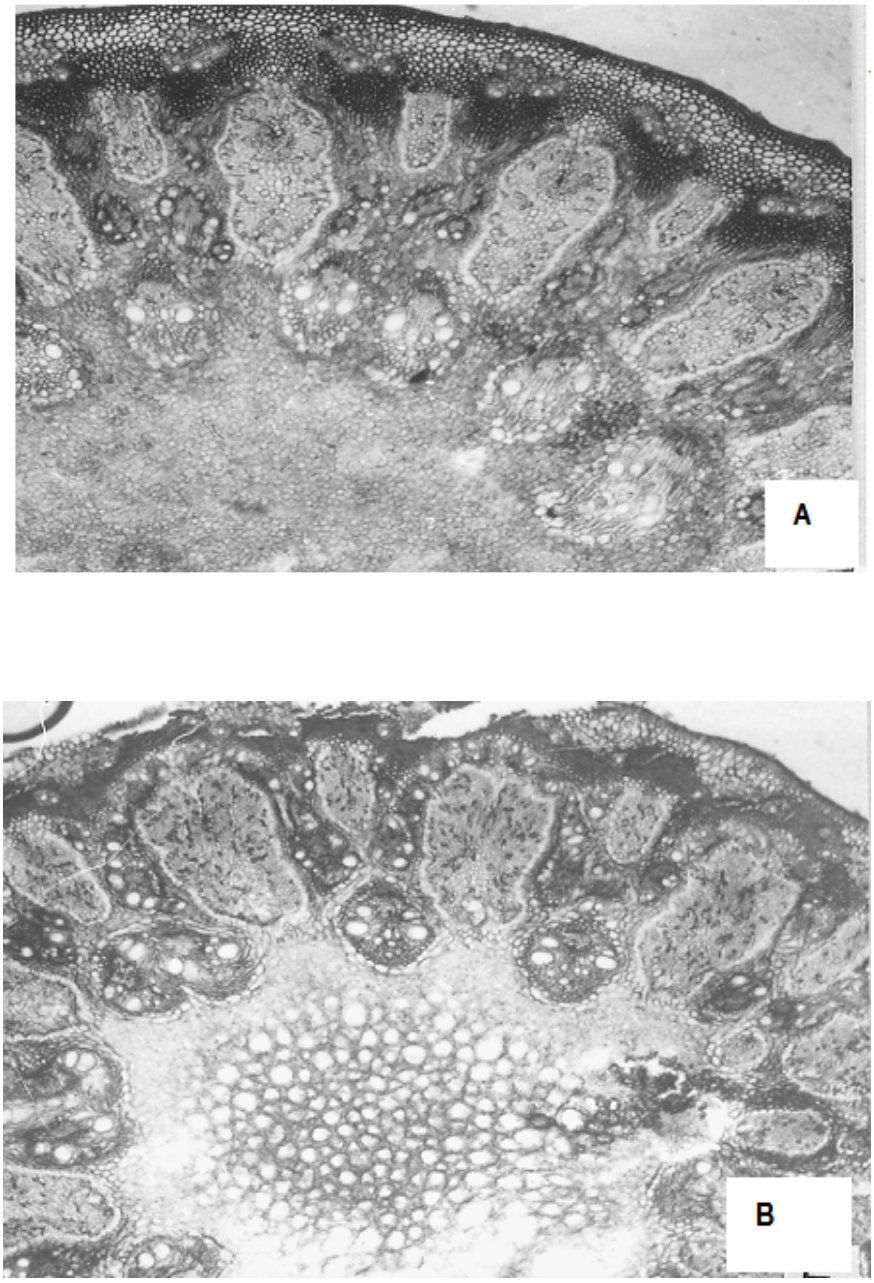

Figure 2: Representatives of Nodal Anatomical Features of *O. punctata* Species Studied. A. 2n = 48. B. 2n = 24.

Table 1: Accessions of *Oryza punctata* studied and their sources.

Accession Number	Collector and Location	Collection Date	Chromosome Number	Description
TOP[a] IPETU	NWOKEOCHA: Ipetumodu	28/9/90	48	Large Population on marshy ground around pool of water, close to human habitation; open location.
01	7°30"N 4°45"E, Nigeria	”	48	
02		”	48	
03		”	48	
04		”	48	
05		”	48	“
06		”	48	”
07		”	48	”
08		”	48	”
TOP MORO	NWOKEOCHA: Moro	28/9/90	48	Few stands on the bed of a river, sandy soil, located under shade of *Alchornea cordifolia*
09	7°33"N 4°45"E, Nigeria	”	48	
10	“ ”	”	48	
11	“ ”	”	48	
TOP RD7[F]	FALUYI: RD 7[F] O.A.U.	5/10/90	48	Along the valley of a river. Location is swampy, open in part, largely under shade.
12	Campus, Ile-Ife;	”	48	
13	7°33"N 4°31"E, Nigeria	”		
14	“	”	48	
TOP SEKONA	NWOKEOCHA: Sekona	12/10/90	48	Few stands on river bed, road side. Location is open
15	7°33"N 4°38"E, Nigeria	”		
16	“			
TOP 8222	IITA[b]: 6°59"N 9°35"E, Nigeria	11/3/90	48	Wild on fallow land
TOP 6788	“		48	“
TOP 15114	IITA: 4°22"N 18°30"E, CAR[C]	”	48	“
TOP 15115	IITA: 4°23"N 18°30"E, CAR	”	48	“
TOP 15116	IITA: 6°53"N 19°07"E, CAR	”	48	”
TOP 15117	IITA: 6°55"N 19°06"E, CAR	”	48	”
TOP 15118	IITA: 7°02"N 18°50"E, CAR	”	48	”
TOP 15119	IITA: 4°20"N 18°30"E, CAR	”	48	”
TOP 13596	IITA:--------Tanzania	”	48	--
TOP 13546	IITA: ------- Tanzania	”	24	--
TOP 5702	IITA: 7°22"N 7°50"E, Nigeria	”	24	Wild Vegetation
TOP 14097	IITA: ------- Tanzania	”	24	--
TOP 14460	IITA: ------- Nigeria	”	24	Wild on fallow land

a – Tropical *Oryza punctata*

b – International Institute for Tropical Agriculture

c – Central African Republic; OAU – Obafemi Awolowo University; -- No available information

Table 2: Morphological features of the accessions of *Oryza punctata* studied.

Accession Number	Flag leaf (cm)		Leaf below[a] (cm)		Culm Length (cm)	Culm Diameter (cm)	No.	Ligule length (cm)	Days to heading*
	l	b	l	b	l	b			
Ipetu-Moro	27.10±1.36	1.90±0.05	41.10±3.15	1.96±0.09	152.02±1.24	0.42±0.02	38	6.58±0.03	145
Rd 7^F	25.80±2.26	1.80±0.03	43.80±3.04	1.90±0.06	1.56.18±1.42	0.45±0.03	27	5.80±0.02	145
Sekona	23.70±2.22	1.80±0.04	36.22±1.57	1.96.004	151.90±1.50	0.43±0.03	34	6.62±0.04	148
8222	36.50±2.21	2.60±0.02	44.96±2.90	1.96±0.09	1.36.96±0.65	0.36±0.02	17	2.10±0.02	124
6788	31.00±2.24	2.50±0.03	45.52±1.87	1.94±0.05	131.48±0.74	0.35±0.02	13	2.20±0.02	126
15114	17.80±1.93	1.67±0.04	35.84±2.67	1.84±0.05	72.50±1.48	0.35±0.08	7	5.40±0.04	141
15115	25.70±2.08	2.50±0.10	51.22±3.50	1.94±0.08	66.87±1.60	0.43±0.03	19	3.25±0.04	132
15116	17.30±1.97	2.00±0.09	37.40±2.76	2.00±0.05	101.77±1.48	0.43±0.03	12	4.51±0.03	145
15117	20.70±1.95	2.20±0.06	39.32±2.93	2.12±0.13	129.80±1.02	0.43±0.03	14	5.22±0.03	137
15118	26.60±2.20	2.00±0.04	41.36±2.53	2.06±0.06	114.00±1.06	0.42±0.03	19	5.51±0.02	132
15119	24.70±1.86	2.20±0.11	44.22±2.60	1.92±0.07	134.70±1.31	0.50±0.02	21	5.10±0.03	145
13596	23.70±2.05	1.80±0.08	38.12±1.94	1.96±0.08	106.50±2.21	0.47±0.03	11	6.15±0.05	136
13546	32.20±2.15	1.50±0.03	44.56±2.16	1.32±0.04	104.60±1.35	0.32±0.03	15	6.20±0.04	139
5702	35.10±2.08	1.30±0.02	52.34±2.09	1.02±0.04	86.35±1.09	0.32±0.02	25	3.53±0.03	105
14097	36.50±2.17	1.30±0.02	48.22±0.90	0.99±0.03	87.10±1.12	0.30±0.02	25	4.10±0.04	98
14460	37.40±2.19	1.30±0.03	43.72±1.03	0.97±0.02	72.10±0.93	0.30±0.02	16	3.70±0.03	100

a- leaf below the flag leaf

l - length; b- breadth

Tabulated values are Mean ±Standard Error of 5 determinations; Significant level of p = 0.05.

* Number of days from germination to 50% panicle exsertion or heading

Table 3: Floral attributes of the accessions of *Oryza punctata* studied.

Accession Number	Panicle Length* (cm)	Panicle Density[a]	Number of Spikelets	Awn length* (mm)	Grain Size length * (cm)	Grain Size breadth * (cm)	Grain Mean l:b ratio	Seed Set (%)	Pollen Fertility (%)	Anther length* (mm)	Pollen Grain size** (µm)
Moro	27.9±1.01	1.53	240	21.50±0.50	6.20±0.11	2.25±0.04	2.71	80.0	75.3	2.44±0.03	43.81±0.35
Ipetu	28.0±1.04	1.52	193	21.70±0.65	6.20±0.11	2.25±0.03	2.76	79.8	73.4	2.42±0.02	43.73±0.41
Rd 7^F	27.8±0.66	1.66	260	21.80±0.77	5.75±0.11	1.98±0.04	2.90	80.8	96.1	1.84±0.03	38.54±0.25
Sekona	21.5±0.50	1.55	117	20.20±0.70	5.78±0.11	2.05±0.03	2.82	76.1	79.4	1.75±0.03	39.15±0.22
8222	26.1±0.59	1.40	167	25.90±0.75	6.49±0.09	2.31±0.04	2.81	85.6	92.1	1.24±0.02	36.85±0.54
6788	20.1±0.54	1.21	93	26.60±1.04	6.11±0.07	2.32±0.04	2.63	84.9	96.5	1.74±0.03	35.54±0.25
15114	20.5±0.60	1.05	82	21.70±0.67	5.72±0.07	1.92±0.03	2.98	87.8	95.9	2.30±0.02	48.20±0.54
15115	21.6±0.49	1.06	95	28.10±0.88	5.38±0.10	1.91±0.07	2.82	92.2	97.3	2.07±0.02	37.40±0.30
15116	22.8±0.51	1.27	78	22.90±0.72	5.78±0.09	1.98±0.04	2.92	65.4	93.1	2.23±0.02	37.40±0.40
15117	27.7±0.54	1.24	156	29.10±0.95	5.62±0.05	2.00±0.05	2.81	91.0	92.1	2.19±0.02	47.33±0.46
15118	23.9±0.49	1.13	123	26.10±0.93	5.91±0.06	2.00±0.03	2.96	87.2	91.2	2.14±0.02	35.21±0.30
15119	22.3±0.52	0.74	47	25.70±0.83	5.68±0.06	1.92±0.04	2.96	78.7	94.9	2.35±0.03	39.35±0.19
13596	25.4±0.51	1.27	144	29.80±0.93	5.62±0.05	2.00±0.02	2.81	81.9	95.3	1.95±0.02	41.03±0.31
13546	21.4±0.54	1.28	107	54.60±2.79	6.27±0.09	2.08±0.03	3.01	87.9	92.2	2.00±0.03	36.43±0.21
5702	20.5±0.52	0.93	56	78.70±2.28	6.98±0.06	2.09±0.02	3.34	85.7	97.6	1.60±0.02	32.58±0.13
14097	16.7±0.50	1.13	54	83.50±2.74	6.87±0.05	2.13±0.03	3.22	85.0	93.3	1.74±0.03	36.48±0.13
14460	15.2±0.55	1.12	59	63.40±1.85	6.79±0.06	2.16±0.03	3.14	83.1	92.5	1.84±0.03	35.42±0.12

a - computed as a ratio of total spikelets to total length of radius and primary branches (Futsuhara et al., 1979).

l – length, b- breadth

***** - Tabulated values are Mean±Standard Error of 20 measurements; Significant level of p = 0.05.

****** - Tabulated values are Mean±Standard Error of 100 measurements

Table 4: Foliar epidermal features of the accessions of *Oryza punctata* studied.

Accession Number	Stomata Frequency (mm^2)**		Stomata Size (µm)*				Long Cell Size (µm)*			
			Adaxial		Abaxial		Adaxial		Abaxial	
	Adaxial	Abaxial	l	b	l	b	l	b	l	b
Ipetu-Moro	145.18	209.63	22.25±0.59	3.75±0.32	28.00±0.38	3.50±0.36	82.50±3.94	18.00±1.28	87.50±3.97	15.25±0.58
Rd 7^F	147.59	173.49	24.00±0.67	3.75±0.37	28.50±0.40	4.00±0.40	91.00±4.04	17.75±0.87	97.78±3.76	16.40±0.57
Sekona	148.79	216.26	21.00±0.55	3.63±0.34	23.50±0.36	3.50±0.40	80.00±4.12	17.50±0.00	122.50±6.40	14.50±0.62
8222	118.07	163.85	23.25±0.28	2.88±0.19	25.88±0.45	4.75±0.25	74.00±5.06	22.00±0.83	77.50±3.94	18.25±0.38
6788	118.67	189.76	24.00±0.64	3.50±0.31	25.25±0.55	3.88±0.29	61.50±2.14	21.25±0.67	78.50±2.91	20.00±0.64
15114	152.83	185.54	22.13±0.38	3.38±0.32	25.63±0.33	4.00±0.40	91.95±2.52	18.00±0.06	90.25±3.62	16.50±0.76
15115	156.62	297.83	22.50±0.56	3.38±0.32	26.00±0.41	4.38±0.33	101.50±2.74	21.50±0.92	112.00±4.11	18.00±0.62
15116	125.30	217.88	21.75±0.50	3.38±0.37	23.63±0.39	4.00±0.45	85.50±4.58	19.50±1.04	86.00±4.46	22.00±0.72
15117	163.25	172.29	22.13±0.48	4.50±0.27	25.50±0.53	3.25±0.33	110.50±4.54	17.00±0.06	103.50±5.86	19.00±0.76
15118	153.61	181.92	23.75±0.32	2.88±0.26	26.25±0.37	4.38±0.20	95.50±3.97	23.50±0.40	85.75±5.45	17.75±0.44
15119	170.48	180.12	22.13±0.37	2.88±0.26	26.88±0.68	2.75±0.16	89.00±5.27	19.00±0.55	92.50±4.12	20.75±0.38
13596	136.56	221.08	24.63±0.53	4.50±0.28	26.00±0.40	3.38±0.37	70.00±1.97	17.75±0.88	84.73±4.60	16.25±0.42
13546	181.30	228.91	23.87±0.47	4.00±0.36	25.75±0.33	3.75±0.35	90.75±4.86	18.25±0.75	102.50±4.36	20.25±0.58
5702	178.91	182.53	24.50±0.48	4.75±0.31	27.50±0.26	4.38±0.28	77.25±5.18	18.00±0.50	84.25±3.42	21.25±0.67
14097	187.95	252.41	24.00±0.76	4.00±0.25	23.25±0.38	3.13±0.20	82.50±5.28	20.50±0.62	87.50±2.86	16.75±0.65
14460	184.93	239.15	23.13±0.69	4.25±0.33	20.88±0.75	3.88±0.34	103.50±4.41	16.25±0.41	113.50±6.20	11.75±0.53

l – length, b- breadth

***** - Tabulated values are Mean±Standard Error of 100 measurements

****** - Tabulated values are Mean±Standard Error of 100 measurements; Significant level of p = 0.05.

Table 5: Length and breadth (µm) of the microhairs in the accessions of *Oryza punctata* studied.

Accession Number	Adaxial			Abaxial			Total Length		Basal (l):Apical (l) ratio	
	Basal Cell (l)	Apical Cell (l)	Max. breadth	Basal Cell (l)	Apical Cell (l)	Max. breadth	Adaxial	Abaxial	Adaxial	Abaxial
Ipetu-Moro	33.10±1.16	25.50±0.72	5.13±0.12	30.00±0.52	26.60±0.64	5.13±0.12	58.60±1.88	56.60±1.16	1.298	1.128
Rd 7^F	30.95±0.91	25.50±0.38	5.00±0.00	31.50±0.76	26.75±0.38	5.98±0.24	56.25±1.25	58.25±1.14	1.206	1.178
Sekona	28.00±0.62	25.75±0.65	5.00±0.00	37.00±0.89	32.25±0.87	5.20±0.27	53.75±1.27	69.25±1.76	1.087	1.147
8222	32.00±0.72	24.25±0.65	5.06±0.07	33.00±0.89	25.75±0.83	5.50±0.23	56.25±1.37	58.75±1.73	1.320	1.282
6788	32.50±0.83	21.83±0.87	5.18±0.09	33.50±0.76	23.50±0.55	5.45±0.16	54.33±1.70	57.00±1.31	1.489	1.426
15114	38.00±1.22	29.75±0.69	5.00±0.00	31.00±0.55	25.50±0.62	5.00±0.00	67.75±1.92	56.50±1.17	1.277	1.216
15115	32.00±0.82	26.25±0.85	5.00±0.20	33.50±0.76	31.00±1.00	4.88±0.08	58.25±1.67	64.50±1.76	1.219	1.081
15116	24.00±0.36	21.63±0.75	5.00±0.00	25.50±0.97	24.45±0.66	5.00±0.00	45.63±1.11	49.95±1.63	1.110	1.043
15117	30.75±0.75	25.00±0.98	4.95±0.07	32.00±0.89	28.00±0.62	5.25±0.16	55.75±1.73	60.00±1.52	1.230	1.143
15118	33.50±1.06	25.68±1/28	5.00±0.00	32.50±0.64	25.50±0.50	5.00±0.00	59.18±2.34	58.00±1.14	1.305	1.275
15119	29.50±0.81	30.25±1.14	5.00±0.00	30.50±0.72	31.25±1.13	5.63±0.19	59.75±1.96	61.75±1.86	0.975	0.976
13596	38.00±0.81	24.50±1.43	5.13±0.29	32.50±0.83	33.00±0.72	5.00±0.18	62.50±2.25	65.50±1.56	1.551	0.985
13546	50.45±1.31	25.50±110	5.00±0.00	52.25±1.08	26.75±0.79	5.13±0.12	75.95±2.42	79.00±1.88	1.978	1.953
5702	43.10±1.22	24.50±0.81	4.95±0.05	52.25±0.75	31.25±1.67	5.00±0.00	67.50±2.04	83.50±2.42	1.755	1.736
14097	40.00±1.78	22.75±1.08	4.95±0.00	42.00±1.28	23.75±1.35	5.00±0.00	62.75±2.87	65.75±2.63	1.758	1.768
14460	40.00±1.05	23.50±0.85	4.88±0.12	39.00±0.66	25.00±0.61	5.00±0.00	63.50±1.90	64.00±1.28	1.702	1.560

l = length

Tabulated values are Mean ± Standard Error of 20 measurements.

DISCUSSION

The features of both diploid and tetraploid *O. punctata* revealed by this investigation agree with the report of Sano (1980) except for the anther length which was not significantly different between the two ploidy levels and the significantly taller polyploids found in this work. It was found that anther sizes are reliable only when they are measured at a uniform growth stage and that dehisced anthers are generally shorter than undehisced ones. The anthers measured in this study were those subtending primary branches whose spikelets had just bloomed but undehisced.

The diploids have longer spikelets (with length/breadth > 3), longer awns and narrower leaves than the tetraploids. Also the culms of diploids are generally thin as compared to those of tetraploids which are thicker. These morphological attributes form the field first aid to the rice collector before any laboratory examination.

The leaf epidermis in the rice under study, as in some other grasses, is demarcated into costal and intercostal zones within which the epidermal features are uniquely arranged and distributed so that certain features could be referred to as 'grass-type' or 'oryza-type'. This is supported by Metcalfe (1960) classification and agrees with the findings of Ogie-Oda et al. (2010). Of all the epidermal characters studied, the length of microhair, abundance of short cells and prickle hairs on intercostal zone showed significant differences between the diploid and tetraploid *O. punctata*. The basal cells of the microhair are about twice the length of apical cells in diploids contrary to the situation in tetraploid forms. Added to these are the abundance of short cells mostly paired in the intercostal zone of all the diploid forms but sparse and mostly solitary in tetraploids; and the prickle hairs which were abundant in the intercostal zone of the tetrapolids but very sparse or none seen in diploids. The above characters have delineated the diploid from the tetraploid *O. punctata* studied. Adeniji and Ariwaodo (2012) elucidated the taxonomic importance of foliar epidermal features of the genus *Pericopsis* (Papilionaceae) in Nigeria and presented a complimentary data which would aid identification of the species even when only leaf fragments are available. By comparism, the diagonistic features of the leaf epidermis in the present study revealed that even when only leaf fragments of *O. punctata* are available, the ploidy level could still be determined. These identification aids or indices are easily accessible, visible, constant and consistent. They are taxonomically important, as they would facilitate and fast-track the efficient utilization and management of rice genetic resources as suggested by Ge et al. (2001) and also enhance the understanding of the taxonomy of the species complex (*O. latifolia* complex).

Other characters, like long cell length and breadth; stomata type, length and breadth, shapes of the subsidiary cells; macrohairs and shape of the silica bodies, anther length, etc, showed slight variation among the accessions studied but could not reliably distinguish diploids from tetraploidy *O. punctata*. The features of the transverse section of the node also showed no significant variations among the accessions and between the two ploidy levels. Also the nodal anatomical features, especially the supporting tissues (collenchyma, sclerenchyma) did not correlate with the ploidy levels and did not support the plant types observed.

Conclusion

Several indices involving the habit, leaf breadth, culm diameter, awn length, microhair, short cells and prickle hair abundance, and spikelet length/breath ratio, have been established. These indices are reliable because they are subject to very limited environmental variation and they provide instant identification aids for the rice collector. They also serve as a base for germplasm cataloguing for future research and utilization.

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