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Characterization of Rice (*Oryza sativa* L.) Hybrids and their Parental Lines Based on the Seed, Seedling, and Plant Morphological Traits

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ABSTRACT

Increasing interest in the descriptive characterisation of plant varieties in the context of intellectual property rights is stimulated by the recent agreements within the framework of the World Trade Organisation (WTO). The requirements of these activities vary, for example, the varietal registration process (involving testing for distinctness, uniformity and stability-DUS) requires that a description of a newly bred variety be produced and compared to all existing varieties of common knowledge. The genuineness of variety is one of the most important characteristics of quality seed. In addition, seed certification, which forms a link between variety registration and seed production, involves an assessment of both varietal identity and purity to assure the quality of seed marked to farmer or grower. Hence characterization of two Indian bred public hybrids such as KRH-2 and DRRH-2 including their parental lines *viz*., IR-58025A, IR-58025B, KMR-3R, IR-68897A, IR-68897B, and DR-714-1-2R based on the seed, seedling, and plant morphological traits play an important role from many points of view to be able to distinguish between crop varieties, Out of 38 qualitative and quantitative morphological traits (as per the National DUS Test Guidelines) observed, flag leaf attitude, flag leaf length and width, days to 50% flowering and maturity, degree of panicle exertion, presence of awns, panicle secondary branching, days to maturity, leaf senescence and seed traits such as 1000-seed weight, grain length and width and shape of grain were found to be more useful for grouping of genotypes. The characters such as leaf length (varied from 22.55 cm (KRH-2) to 35.70 cm (IR-68897A), days to 50% flowering (from 73 days (IR-68897B) to 102 days (KMR-3R), panicle secondary branching (from weak to clustered), days to maturity from 112 days (IR-68897B) to 134 days (IR-58025B)), 1000-seed weight from 18.63 (IR58024A) to 24.94 g (IR-68897B)), and decorticated grain shape (from semi-spherical to elongated) exhibited more variation among the parents and hybrids.

Keywords: hybrid rice, characterization, qualitative and quantitative traits
Abbreviations: AICRP, All India Coordinated Rice Project; **PPV&FRA**, Plant Variety Protection and Farmers Right Act; **ISTA**, International Seed Testing Association; **ZARS**, Zonal Agricultural Research Station

INTRODUCTION

Rice is a staple food crop of India which occupies the largest area of 44 million ha among all the crops grown in the country and is the largest in the world. To meet the demands of increasing population, the present production level of around 93 million tons of rice, need to be increased to 120 million tons by the year 2020. Hybrid rice technology is one among the immediately adoptable new technologies that play an important role in achievement of the targeted production increase. Hybrid rice seed production in the country, starting with less than 200 tones of total production in the year 1995 has reached almost 20,000 tons from 15,000 ha in 2007. The requirement of the hybrid seed during 2010 and 2020 is expected to be around 50,000 tons and 100,000 tonnes, respectively (Anon 2008).

Internationally, Distinctness, Uniformity and Stability (DUS) testing is coordinated by the International Union for the Protection of New Varieties of Plants (UPOV), which produces guidelines detailing lists of characters to be used for examination of different species.

The ability to distinguish and clearly identify the varieties of cultivated species is fundamental for the operational aspects in the seed trade. With the introduction of Indian legislation on 'The Protection of Plant Varieties and Farmers Rights (PPV & FR) Act, 2001', it is essential that the new varieties developed in agricultural and horticultural crops should be distinct from other varieties. The legally recognized traditional method in India for cultivar identification and genetic purity assessment is based on field plot or Grow-Out Test (GOT) including only the morphological characteristics of a variety. Such methods have been highly successful and efficient (Virakthamat *et al*. 2009).

Intensive crop improvement programme has resulted in the development of large number of hybrids and varieties in rice. However, there is lack of identification of diagnostic characters of these hybrids and their parental lines. The variety identification serves the important goals, such as maintenance of genetic purity and confirming intellectual property rights. The morphological characters have been major components of variety identification. This plant description should start from its early growth habit to maturity. With this study was taken up with the objective of characterizing and identifying distinguished features for KRH-2 and DRRH-2 hybrids including their parental lines also.

MATERIALS AND METHODS

The materials for this study comprised of two hybrids, two CMS lines, two maintainer lines and two restorer lines of rice. The pure seeds of all these parents and hybrids were collected from AICRP on Hybrid Rice, ZARS, VC Farm, Mandya and Directorate of

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Table 1 Details of hybrids and their parents used in the study.

Particulars	Hybrids	
	KRH-2	DRRH-2
CMS line	IR-58025A	IR-68897A
Maintainer line	IR-58025B	IR-68897B
Restorer line	KMR-3R	DR-714-1-2R
Source	AICRP on Hybrid rice, VC Farm, Mandya	DRR, Hyderabad

Rice Research, Hyderabad. The materials used in the present study are listed in **Table 1**.

The field experiment was carried out during Kharif 2009 at wet lands of AICRP on Hybrid Rice, ZARS, VC Farm, Mandya, Karnataka (IND) with three replications. All the agronomic and plant protection measures were adopted as per recommended package of practices for raising a healthy crop. The characteristics given as per the National Test Guidelines for DUS (PPV&FR 2007) were recorded at different stages of crop growth period.

Ten hills were selected at random and tagged in each genotype and replication. The data on DUS characters were recorded on single plant basis in each of the parents and hybrids and days to heading and maturity were recorded on plot basis.

Seed morphology

Seed samples of two hybrids and their parents were evaluated for their morphometrics characters like seed length, seed width, seed thickness, dehusked grain length and width by using grain micrometer. In each cultivar, 25 seeds of four replicates were used for recording these measurements. Besides, seed colour and 1000 seed weight were also recorded.

Seed length (mm)

Seed length was measured in mm using grain micrometer as the distance from the base of the lower most sterile lemma to the tip of the lemma or palea. In the case of awned parents and hybrids, length was measured to a point comparable to the tip of the apiculus. Based on seed length cultivars were grouped (Rosta 1975). The groupings of cultivars based on seed length were as follows:

Category	Length (mm)
Very short	< 6
Short	6.1-8.5
Medium	8.6-10.5
Long	> 12.5
Very long	> 10.0

Seed width (mm)

Seed width was measured using grain micrometer as the distance across lemma and palea (the widest point) and the mean of seed width was expressed in mm. Based on the mean seed width, the cultivars were categorized according to Ramaiah and Rao (1953).

Category	Length (mm)
Very narrow	< 2
Narrow	2.1-2.5
Medium	2.6-3.0
Broad	3.1-3.5
Very broad	> 3.5

Seed thickness (mm)

Seed thickness was calculated as height of seed when placed horizontally and expressed in mm. The thickness is also used for computing the profile value of the seed.

Seed size (mm^3)

Based on length (L), width (W) and thickness (T) the seed size was calculated using formula as below:

Seed size (mm^3) = $(L \times W \times T)^{1/3}$

Seed shape

Based on length to width ratio the cultivars were grouped into four categories according to Rosta (1975):

Category	Range
Spherical	< 2.0
Semi-spherical	2.0-2.4
Semi-long	2.4-3.0
Elongated	> 3.0

Dehusked grain length (mm)

The lemma and palea of seeds were carefully removed (dehulled) and the length was taken from the base to the tip of the kernel and expressed in mm. Based on the mean length, cultivars were classified into four categories according to Rosta (1975):

Category	Length (mm)
Short	< 7.5
Medium	7.5-9.0
Long	9.0-10.0
Very long	> 10.0

Dehusked grain width (mm)

The lemma and palea of the grain were removed carefully and the width was measured across the seed at the widest point and expressed in mm. The mean width was used to determine the decorticated seed shape.

Dehusked grain shape

The shape of the dehusked grain was determined based on the mean dehusked grain length to width ratio the cultivars were classified into four groups according to Rosta (1975):

Category	Range (mm)
Spherical	< 2.0
Semi-spherical	2.0-2.4
Semi-long	2.4-3.0
Elongated	> 3.0

Seed colour

The colour of the husk was recorded under natural day light condition using Munsell colour chart (Anon., 1954). Based on the colour groups, the cultivars were classified into five categories as pale yellow, yellow, yellowish brown, brownish yellow and very pale brown.

1000-seed weight (g)

One thousand seeds of eight replications were counted randomly and weighed up to two decimal places. The mean 1000-seed weight was expressed in grams and accordingly the cultivars were classified into four groups as follows:

Category	Weight (g)
Very light	15
Low	15-20
Medium	21-25
Heavy	> 26-30
Very heavy	> 30

Seedling morphology

Four replications of 50 seeds each of selected cultivar were tested for germination by between paper method as per ISTA (2007). The rolled towels were incubated at 25 ± 1°C. At the end of the 14^{th} day 25 normal seedlings from each replication were taken randomly and seedling morphological characters like root length and shoot length were measured in centimeters. The ratio of root and shoot was determined.

Plant morphological traits

To study the plant morphological traits, small seed sample drawn from the seed stock of parents and hybrids was sown in the field during Kharif, 2009 at the Hybrid rice research plot, VC Farm, ZARS, and Mandya. The seedlings were raised by providing recommended package of practices. Ten plants were selected at random from each variety and were observed for various stable and distinguishable characters according to DUS guidelines (2007).

Various plant morphological traits recorded at different plant growth stages were as follows:

1. Seedling stage (before transplantation)

Seedling height (from the base of root to the tip of primary leaf) was recorded at 14th and 25th day after sowing. Besides this anthocyanin pigmentation of seedlings was also recorded.

2. After transplantation

At the boot leaf stage: The characters that have been recorded at boot leaf stage included the number of tillers, anthocyanin coloration of leaves, stem character like pigmentation at nodal region, flag leaf characters like length and width, pigmentation, presence of ligule, colour of ligule and auricle colour.

After the panicle initiation stage: The observations recorded after panicle initiation stage were plant height, days to panicle emergence, days to 50% flowering, duration of flower opening, panicle type, number of tillers, number of panicles, panicle length, awn characters, secondary branches of panicle, panicle attitude, panicle exertion, flag leaf senescence and days to maturation.

RESULTS AND DISCUSSION

Seed size and shape have been used to differentiate the rice genotypes by various scientists (Oka 1958; Bhattacharya and Sowbhagya 1980). In the present study the rice parents and hybrids were grouped into five categories as short, medium, long and very long based on the seed length. However, based on the seed width the parents and hybrids were classified as narrow, medium and broad. The seed length varied from 6.69 mm in KMR-3R to 9.73 mm in IR-58025A and from 2.04 in KRH-2 to 2.62 mm in KMR-3R (**Fig. 1**).

The results indicated that, the longer grains tend to be narrow and shorter grains tend to be broader. The significant difference was observed among the parents and hybrids for shape of seed, which was governed by length to width ratio. Larger the ratio, the more slender is the seed. There is a definite association between the length and length to width ratio. The longer the grain the finer it tends to be. So, it is suggested that the genes governing length also partly govern the seed shape (Ramaiah and Rao 1953). In the present study, the length to width ratio was ranged from 2.55 (KMR-3R) to 4.52 (KRH-2) (**Table 2**). However, the length to thickness ratio varied from 3.31 (KMR-3R) to 8.10 (IR-58025A). Similar observations were made in case of dehusked seeds.

Seed colour, which was a heritable character (Gupta and Agarwal 1988). Based on the seed colour, the genotypes were grouped into five classes *viz*., pale yellow, yellow, very pale brown, yellowish brown and brownish yellow and based on dehusked seed colour it is classified as white, pale yellow and red. Thus the rice hybrids and their parents could be classified into several groups based on seed colour. However, the seed colour is also influenced by environmental conditions during ripening besides the genetic effect

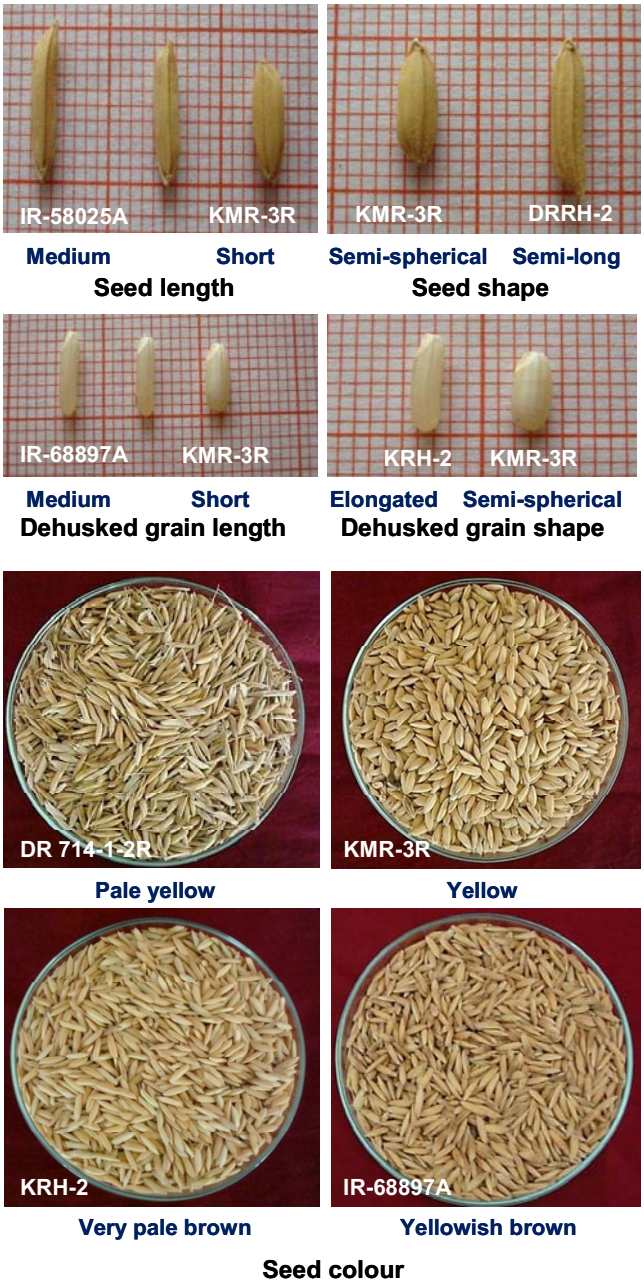

Fig. 1 Seed morphological characters of rice parents and hybrids.

Table 2 Seed morphological characters in rice hybrids and their parental lines.

Parents/hybrids	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	L/W ratio	L/T ratio	1000-seed weight (g)
IR-68897A	8.82	2.34	1.59	3.78	5.58	23.46
IR-68897B	8.84	2.16	1.63	4.09	5.45	24.94
DR 714-1-2R	7.92	2.46	1.41	3.21	5.65	19.10
DRRH-2	8.52	2.24	1.42	3.82	6.04	23.75
IR-58025A	9.73	2.36	1.20	4.12	8.10	18.63
IR-58025B	9.34	2.18	1.35	4.28	6.91	19.36
KMR-3R	6.69	2.62	2.03	2.55	3.31	23.63
KRH-2	9.24	2.04	1.49	4.52	6.24	18.75
Mean	8.64	2.301	1.51	3.79	5.91	21.41
SEm±	0.325	0.118	0.112	0.271	0.562	0.532
CD (P =0.05)	0.974	0.353	0.033	0.811	1.680	1.590

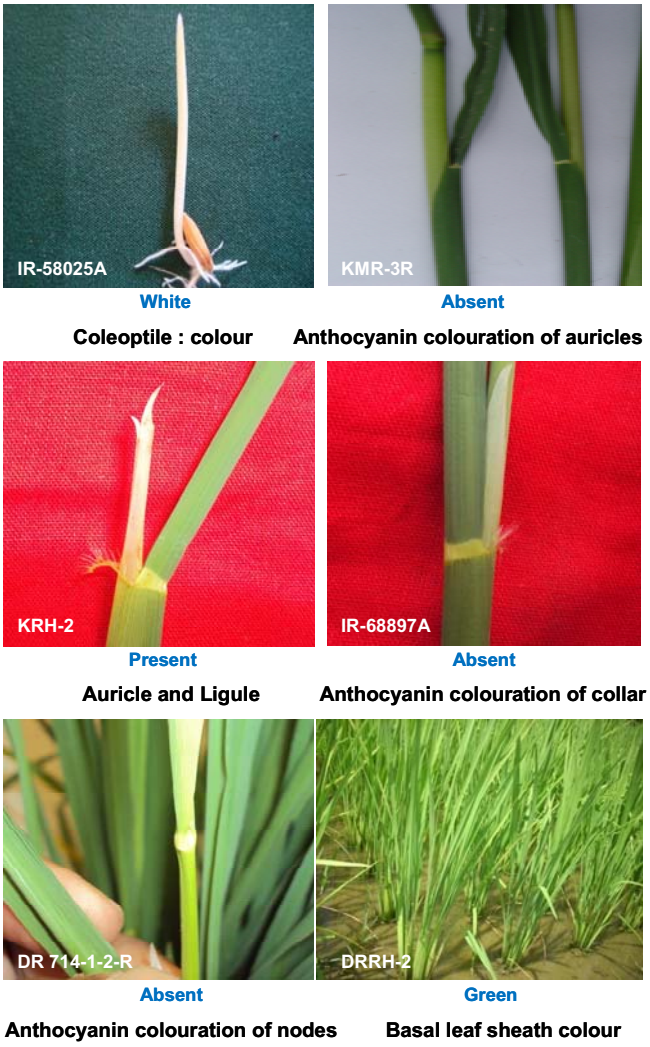

Fig. 2 Plant morphological characters of rice parents and hybrids.

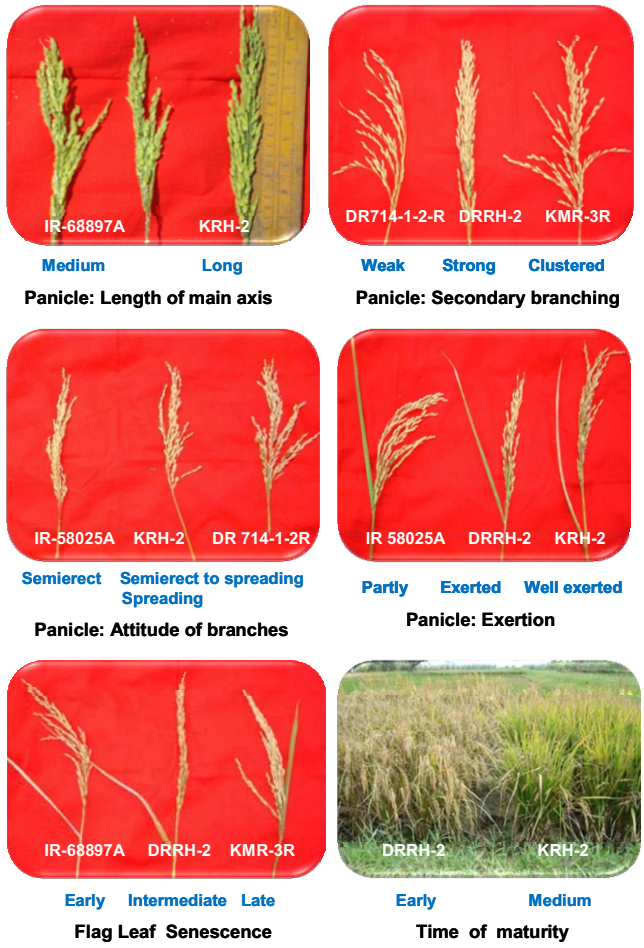

Fig. 3 Plant morphological characters of rice hybrids and parents.

Table 3 Seedling morphological characters in rice hybrids and their parental lines.

Parents/ hybrids	Shoot length (cm)	Root length (cm)	Mesocotyl length (cm)	Root to shoot ratio
IR-68897A	12.387	15.97	1.28	1.29
IR-68897B	13.283	14.10	1.60	1.00
DR 714-1-2R	13.917	15.35	1.37	1.10
DRRH-2	13.747	17.58	1.44	1.32
IR-58025A	11.377	13.99	1.13	1.19
IR-58025B	11.273	15.81	1.20	1.42
KMR-3R	14.110	17.39	1.29	1.23
KRH-2	12.390	13.99	2.00	1.16
Mean	12.810	15.52	1.41	1.21
SEm±	0.549	0.754	0.0785	0.019
CD (P=0.05)	1.642	2.253	0.234	0.058
CV (%)	7.41	8.39	9.59	2.75

(Pascual *et al.* 1993). Thus seed characters could be used for grouping of the hybrids and parental lines into distinct classes on the basis of each of these characteristics.

Joshi *et al*. (2007) noticed the variability and heritability coupled with moderate to high genetic advance for most of the morphological traits signified their utility in varietal characterization in 19 varieties of rice. Behla and Rang (2007) suggested quantitative traits like plant height, panicle numbers per plant, panicle length, days to maturity etc., effectively used for clustering of rice genotypes. Siddiqui *et al.* (2007) evaluated the rice grain quality characters pertaining to morphology within Pakistan local rice germplasm and suggested that a wide variation present in grain size, shape and weight with respect to altitude of collection site. Veasey *et al.* (2008) used several morpho-agronomic traits to characterize the genetic variability among species and populations of South American wild rice. Rimpi *et al.* (2008) characterized 12 rice varieties on the basis of hulled and unhulled grain characters like grain length, grain colour, grain width, decorticated grain length, decorticated grain width, decorticated grain colour, L/B ratio and 1000-grain weight.

In the present study significant differences were observed among the parents and hybrids with respect to shoot length, root length and mesocotyl length and based on these parameters the parents and hybrids were grouped as short, medium and long. The shoot length varied from 11.27 (IR-58025A) to 14.11 cm (IR-68897B). However, the root length was highest in DRRH-2 (17.58 cm) and lowest in IR-58025A (14.10 cm) whereas, mesocotyl length showed greater variation, which ranged from 1.60 (IR-68897BA) to 2.00 cm (KRH-2) (**Table 3**). The variation in mesocotyl length in rice genotypes was reported by Miyagawa (1984) and Rohini Devi (2000). Though, these characters are in use for a long time for varietal classification, their utility appears to be doubtful as these characters are quantitative in nature and are subject to fluctuations due to environment (Grabe 1957) (**Fig. 2**).

The plant morphological characters differed significantly among the hybrids and parental lines. The plant height varied from 31.26 (IR-58025B) to 37.42 cm (IR-58025A) at 30 days, 38.48 (DR 714-1-2R) to 53.78 cm (KMR-3R) at 60 days. However, at 90 days it varied from 52.80 (DR 714-1-2R) to 72.17 cm (KRH-2), and at maturity it ranged from 77.00 (DRRH-2) to 124.33 cm (KMR-3R), which suggest that significant variations in plant height at various intervals was observed. Similar variation was reported by Rosta (1975). Significant differences were observed for the panicle attitude of branches. The parents and hybrids

Table 4 Qualitative characters in rice hybrids and their parental lines.

Characters	IR-68897A	IR-68897B	DR 174-12R	DRRH-2	IR-58025A	IR-58025B	KMR-3R	KRH-2
Basal leaf sheath colour	Green	Green	Green	Light purple	Green	Green	Green	Green
Leaf intensity of green colour	Dark	Light	Medium	Dark	Dark	Dark	Medium	Medium
Leaf anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf anthocyanin distribution	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf sheath anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf sheath intensity of anthocyanin colouration	Very weak	Very weak	Very weak	Very weak	Very weak	Very weak	Very weak	Very weak
Leaf pubescence of blade surface	Weak	Weak	Weak	Weak	Absent	Weak	Weak	Weak
Leaf auricle	Present	Present	Present	Present	Present	Present	Present	Present
Anthocyanin colour of auricle	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Leaf collar	Present	Present	Present	Present	Present	Present	Present	Present
Leaf ligule	Present	Present	Present	Present	Present	Present	Present	Present
Shape of ligule	Acute	Split	Split	Split	Acute	Split	Split	Acute
Colour of ligule	Green	Green	Green	Green	Green	Green	Green	Green
Leaf length of blade	Medium (42 cm)	Medium (40 cm)	Medium (36 cm)	Long (55 cm)	Medium (32 cm)	Medium (35 cm)	Medium (41 cm)	Long (46 cm)
Culm altitude	Semi erect	Semi erect	Erect	Semi erect	Semi erect	Semi erect	Erect	Semi erect
Basal sheath colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Leaf senescence	Intermediate	Early	Late	Intermediate	Late	Late	Late	Late

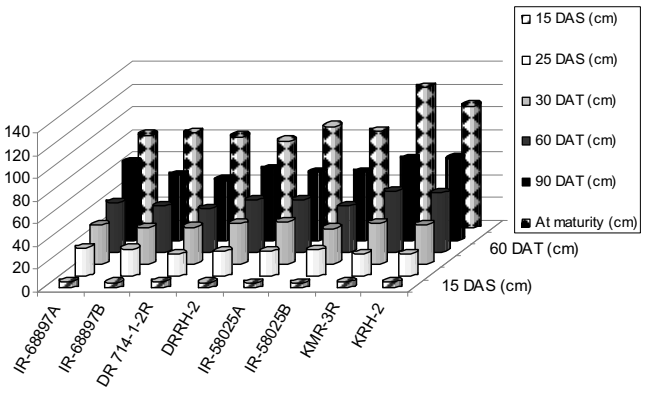

Fig. 4 Plant height at different growth stages in rice hybrids and their parental lines. DAS = days after sowing; DAT = days after planting.

were grouped as semi erect, semi erect to spreading and spreading, Based on the panicle exertion parents and hybrids were classified as partly exerted, exerted, and well exerted (**Fig. 3**).

Observations for 30 qualitative traits taken from ten randomly selected plants in all parental lines and hybrids indicated that 15 traits (basal leaf sheath colour, leaf anthocyanin colouration and distribution, leaf sheath anthocyanin colouration and intensity, leaf auricles & ligule and colour of ligule, leaf collar, lemma anthocyanin colouration of keel and anthocyanin colouration of area below apex, spikelet colour of stigma, anthocyanin colouration of nodes and internodes on stem, basal sheath colour) did not show any variation (**Table 4**).

Time of maturity and leaf senescence was observed at the ripening stage on the basis of toughness of the seed and colouration of seed. The number of days taken to maturity ranged from 112 days (IR-68897B) to 134 days (IR-58025B) and grouped into early, medium and late, whereas DRRH-2 Hybrid and its parents were early maturity (**Fig. 4**). However, leaf senescence was visually observed at caryopsis hardening stage and significant differences observed for IR-68897A, IR-68897B, DRRH-2 and DR 714-1-2R where as KRH-2 and their parents were grouped under late.

Thus from the study it is concluded that the morphological characteristics of seed, seedling and plant were found useful for varietal characterization in rice hybrids and parents. Some of the distinguishing character like attitude of flag leaf blade, flag leaf length and width, days to 50% flowering and maturity, stem length, degree of panicle exertion, presence of awns and seed traits such as 1000-seed weight, grain length and width and shape of grain were found to be more useful for identification and grouping of hybrids and parents to maintain genetic purity during seed production.

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