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EVALUATION OF GRAIN QUALITY AND NUTRITIONAL QUALITY OF DOUBLE HAPLOID DHP6, AN ELITE RICE LINE IN MADAGASCAR

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*Anther/pollen culture technology is being efficiently used for accelerating rice breeding progress and improving grain quality characters. Using pollen culture technique, we obtained elite rice (*Oryza sativa *L.) line DHP6, which is well adapted to the highlands of Madagascar. Here we show that certain grain, nutritional, and culinary quality characteristics have been significantly improved in line DHP6. Morphometric and physico-chemical analysis demonstrated that physical (colour, transluscency, and chalkiness), chemical (amylose content, alkali spreading value, and gel consistency) and cooking (volume expansion, elongation ratio) features were greatly improved compared to the parental line. Culinary and nutritional quality analyses showed a significant increase in nutritional values and mineral richness. Some desired concentrations of minerals like Mg and Cu were much higher (e.g. three times richer in Cu) than those of its parental line (variety IR58614). Taken together, such ameliorated grain quality traits will promote its wide-scale planting by the farmers and should thus help to improve the nutritional quality of the population of Madagascar.*

Key words: Oryza sativa *L. double haploid line, pollen culture, grain quality, nutritional quality.*

INTRODUCTION

The advantages of haploids and doubled haploids (DHs) in accelerating conventional plant breeding programmes and crop improvement have been well described (Sangwan and Sangwan-Norreel, 1990; Maluszynski *et al.,* 2003; Dunwell, 2010). In addition, DHs are an important constituent of germplasm and are extensively used in genetic studies, such as gene mapping, marker/trait association studies, location of quantitative trait locuses (QTLs), genomics and as targets for transformations (Khush and Virmani, 1996; Dunwell, 2010). *In vitro* haploids are produced by either gynogenesis (ovary/ovule culture) or androgenesis (anther and pollen culture); however, androgenesis, due to its high effectiveness and applicability to many plant species, has outstanding potential for plant breeding and commercial exploitation of doubled haploids. One of the most interesting features of haploids is the possibility to produce DH individuals. DHs are useful to plant breeders because they enable shortened breeding periods and efficiency in selection of useful recessive agronomic traits. DH technology is not only applicable to breeding, but also to transformation programmes of desired genes. In rice, anther and pollen culture technology has been efficiently used to speed up variety development, and several rice varieties with improved characteristics of shape, good grain quality, high yielding and pest resistance have been produced (Brar and Kush, 2006; Silva, 2010). Although rice grain yield needs to be continuously increased for food security, grain and nutritional quality is becoming increasingly important from the commercial point of view, e.g. the consumer preference for rice is determined mainly by its appearance and cooking qualities. Most of the quality traits are complex and grain quality has been the biggest bottleneck for hybrid breeding in rice and increasing efforts are being centred on improving rice grain quality (Nelson *et al*., 2011). Hence, rice needs attention toward improvement in its cooking qualities as well as several biochemical and morphological characteristics (Nelson *et al*., 2011). Interestingly, several studies have deepened our understanding of the genes and molecular mechanisms determining overall quality traits in rice (Fitzgerald *et al*., 2009).

Rice is one of the most important staple foods in Madagascar. Anther/pollen culture technique is an ongoing part of the rice-breeding programme in the University of Madagascar at Antananarivo, which has the goal to improve rice varieties for Madagascar growers. The Plant Breeding and Biotechnology (UBAP) laboratory of the University of Antananarivo has introduced in the national collection of Madagascar several irrigated rice varieties obtained from *in vitro* anther/pollen culture, including an elite high yielding doubled haploid line number 6, referred here as DHP6 (Rakotoarisoa *et al*., 2008). The main objectives of the present study were to perform morphometric and the physico-chemical analyses to assess grain nutritional and culinary quality features, such as physical characteristics (hulling, length, and breadth (L/B), grain classification, chalkiness), chemical characteristics (alkali spreading value (ASV), amylose concentration (AC), gel consistency (GC), and cooking quality parameters (volume expansion, elongation ratio) of line DHP6 and to compare these characteristics with the parental line IR58614. Ameliorated lines such as DHP6 represent an important step for future breeding studies, and which potentially promote wide-scale planting by farmers in order to reach national self-sufficiency in rice.

MATERIALS AND METHODS

Plant materials and the site of study. Pollen culture technique was used in rice (*Oryza sativa* L.) to obtain the DHs lines. For our study we used anther and pollen of the parental line IR58614 (*indica* rice variety Rojomanja).

Dehusked seeds of the DH line DHP6 and variety IR58614 (parental line) were used for the morphometric and physico-chemical analysis. Unless otherwise stated, we used the International Rice Research Institute (IRRI), Los Bänos, Laguna, Philippines protocols (De la Cruz, 1996; De la Cruz and Khush, 2000). In the field, both of these lines received the same type and quantity of biological fertiliser "Taroka" and all of the recommended agronomic and plant protection practices were uniformly applied throughout the crop growth period as described previously (Rabealaina, 2010). Twenty plants were selected every year, from each of three growing seasons: 2003–2004, 2004–2005 and 2006–2007, i.e. a total of 60 plants, to record observations on grain yield and grain quality characters viz., grain length, grain breadth, length: breadth ratio, amylose content, gel consistency, alkali spreading value and cooking quality. Quality tests and physico-chemical analysis were conducted in the food laboratories of the Department of Technological Research (FOFIFA, Antananarivo, Madagascar). Nitrogen, mineral and micro-element concentrations were determined in the Pedology Laboratory of FOFIFA.

<u>Morphometric analysis of the physical traits. Grain classification.</u> Length and width of de-husked entire rice grains were measured using a dial micrometer. Rice grain appearance is mainly estimated by grain shape as defined by grain length, width and the length–width ratio (L/B ratio), and chalkiness of the endosperm (Zhang, 2007). Values obtained from 10 to 20 husked grains were classified according to Rabealaina (2010) and based on the L/B ratio: very long slender 4>L/B>3; long slender 3>L/B>2.7; semi-long slender 2.7>L/B>2.4; semi-round 2.4>L/B>2; round 2>L/B>1.5.

<u>Colour</u> was estimated using scores: covered white (score = 1) and pinked white (score = 2). Ten grains were observed. When rice grain was neither white nor pink white, a score 0 was given.

<u>Translucence</u> was determined as the percentage of translucence of dehusked grain using a modified Dobelmann (1976) method based on visual appearance of the white belly using a score between 1 to 0, according to the surface on the transversal section of the grain: no chalkiness (score = 1), maximum chalkiness (score = 0), white belly less than 20% (score = 0.60), chalkiness 25% (score = 0.25), chalkiness 50% (score = 0.10), chalkiness 75% (score = 0.050). Ten grains were observed for each of three samples.

Chemical traits. <u>Grain quality analysis and culinary characteristics.</u> Grains harvested from the experiment were milled and grinded. Powdered samples were analysed for amylose content, Alkali spreading value, and gel consistency, following the protocol of the IRRI-Plant Breeding Department (De la Cruz, 1996). Chemicals and reagents used for quality analysis were obtained from Sigma.

The <u>amylose content</u> of milled rice floor was determined by the relative absorbency of starch–iodine colour in a digested solution of 100-mesh rice flour using the method of Perez and Juliano (1978). For gelatinisation, 1 ml of 95% ethanol and 9 ml of 1.0 N NaOH were added to 100 mg of milled rice floor, and the mixture was heated in a boiling water-bath for 10 min. Samples were then diluted to 100 ml with distilled water. To 5 ml of this suspension, 1 ml of acetic acid (57.75 ml in one litre water) was added to acidify the sample, and 1.5 ml iodine solution (0.2% iodine + 2% potassium iodide) and distilled water (up to 100 mL) were added. After 20 min incubation at room temperature, absorbance of the suspension was measured at 620 nm using a spectrophotometer (SHIMADZU UV-160A). The amylose concentration was calculated based on absorbance of known standards (Perez and Juliano, 1978).

Alkali test. The alkali test (Alkali spreading value) measures rice caryopsis hardness and its gelatinisation temperature. It was determined according to the procedure reported by Biswas and Juliano (1988). Rice grains were soaked in potassium hydroxide 1.7% for 23 h at 30 °C. Then the alkali spreading value was estimated visually as low, low-intermediate, intermediate or high (Perez and Juliano, 1978) according to the following scores: 1: unaffected grain; 2:

swollen grain, low alkali value; 3: swollen grain, with narrow incomplete collar, intermediate alkali value; 4: swollen grain with width complete collar, intermediate alkali value; 5: grain split with complete collar, intermediate to high alkali value; 6: dispersed grain merging with collar, high alkali value; 7: completely dispersed and intermingled grain, high alkali value. Lower scores correspond to harder grains.

Gel consistency. The gel consistency test was based on the method of Tang *et al.* (1991) using the scores: L > 61 mm, soft consistency; 60 > L > 41 mm: medium consistency; L < 40 mm: hard consistency.

Cooking characteristics: Grain cooking elongation. Grain elongation during cooking was estimated by the length of grain after soaking in boiling water for 10 min, and then in ice water for 20 min (De la Cruz, 1996). Ten cooked rice kernels were selected (intact at both ends) and length of the kernels measured using graph paper (De la Cruz, 1996).

Nutritive values. Total starch concentration was determined by the method of De la Cruz (1996). Total sugar concentration was determined by titration in the same manner as starch concentration. After defecation of the hydrolysed solution by 1 ml of lead sub acetate and addition of 2 ml concentrated HCl, the resulting solution was placed in a boiling water bath for 30 mn, neutralised with NaOH 10 N, and distilled water was added up to 50 ml before titration (De la Cruz, 1996.)

Total lipid concentration was determined using the method of Cheng *et al.* (2011). Oil in 5 g flour was extracted in a soxhlet extractor with hexane for 6 hours. Oil was weighed after removal of the solvent in a rotative evaporator with a drying balloon flask and cooling at room temperature (De la Cruz, 1996).

Total protein concentration in rice flour samples was estimated by the Kjeldahl's method according to the procedure described by De la Cruz (1996).

The ash content in each dry sample was determined by incinerating 1 g of flour sample in an oven at 600 °C for 3 h. The ash, i.e. the remaining residue, was weighed (De la Cruz, 1996).

Mineral analysis. The concentrations of K, P, Mg, Ca, Fe, Mn, Zn, and Cu were determined by an atomic absorption spectrophotometer (Perkin-Elmer Model AA6300) after wet digestion with nitric acid (Rivero-Huguet *et al*., 2006). The digests were diluted to volume 100 ml with double distilled water (De la Cruz, 1996). Triplicate measurements were performed for each sample.

Statistical analysis. The estimates of morphological characters of whitened grains such as length, translucency and colour were replicated 20 times. Determination of starch, total sugars, total lipids, ash, protein, mineral elements and microelements was replicated 5 times. All data were treated statistically with SPSS 10 software, version 10.07 (2 June 2000).

RESULTS

Morphometric analysis: Physical characteristics of husked grain. In 2003, we focused on the anther culture technique of the parental line IR 58614 and produced several hundreds of DHs lines. IR58614 is a high yielding pure breeding line. The field performance trials of 250 anther culture-derived DHs lines conducted in 2002 to 2003 resulted in selection and advancement of over 10 superior lines (including the DHP6 line) for further yield, grain quality, stress, or disease-resistance tests. We then focused on the morphometric and chemical characterisation of the DHP6 line. The growth and development characteristics of line DHP6 grown in the field are illustrated in Figure 1. Briefly, the morphology of DHP6 plant is described in the vegetative phases (including germination, seedling, and tillering stages, Fig. 1a, b and the reproductive phases (including panicle initiation and heading stages, Fig. 1c, d. At maturity the DHP6 plant has a main stem and a number of tillers. The panicles of line DHP6 were long, droopy, and loose (Fig. 1c, d). Line DHP6 has a pinkish white caryopsis and has semi-translucent grains with a white belly (Fig. 1e, detailed below). The growth duration of the rice DHP6 plant is 142 days with yield 7.9 ton/ha, while that of the parental line IR58614 is 161 days with yield of 7.5 ton/ha (Rabealaina, 2010). The morphological and physiological evaluation of the IR58614 variety and HDP6 line were conducted during three growing seasons: 2003–2004, 2004–2005 and 2006–2007 (Tables 1 to 4). Morphometric analysis of size, colour, and translucency of line DHP6 and parental line IR 58614 are summarised in Table 1. We ob-

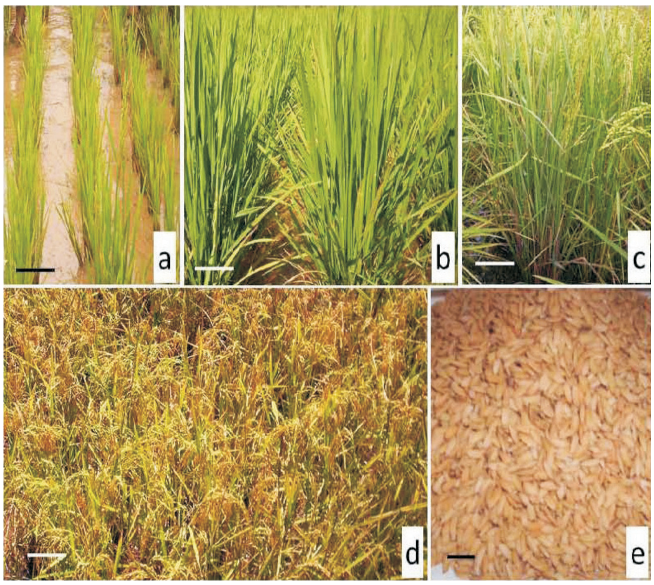

Fig. 1. The growth and development characteristics of rice line DHP6 in the field for agronomical evaluation. These plants were used for morphometric and chemical characterisation of the DHP6 line. Close views of two developmental stages of vegetative growth (a–b), early flowering and maturity stages (c–d). Harvested seeds of rice line DHP6 (e). Bars: a – d = 10 cm; e = 1 cm.

Table 1

MEAN VALUES (±SD) OF HUSKED GRAIN (PHYSICAL CHARACTERISTICS)

Line	Colour	Transluscency	Length, mm	Width, mm	Length/Width
HDP6	1.8 ± 0.42	0.40 ± 0.22	5.88 ± 0.27	2.56 ± 0.15	2.31 ± 0.20
IR58614	0.7 ± 0.48	0.14 ± 0.07	6.30 ± 0.25	2.65 ± 0.17	2.40 ± 0.21

The morphometric analysis of the IR58614 variety and HDP6 line was conducted during three growing seasons: years 2003–2004, 2004–2005, and 2006–2007. Results presented here represent mean ± standard deviation of three independent experiments.

Table 2

AVERAGE VALUES (±SD) OF CHEMICAL AND CULINARY CHARACTERISTICS

Line	Alkali spreading value	Amylose %	Gel consistency, mm	Cooking elongation, mm
HDP6	7.0 ± 0.00	28.73±0.21	96.4 ± 0.55	1.26 ± 0.11
IR58614	6.48 ± 0.15	28.42±0.26	85.2 ± 0.84	1.06 ± 0.12

Results represent mean ± standard deviation of three replicated experiments.

The physiological (chemical and culinary) analysis of the variety IR58614 and line HDP6 were conducted during three growing seasons: years 2003–2004, 2004–2005, and 2006–2007. Results presented here represent mean ± standard deviation of three independent experiments.

Table 3

AVERAGE (±SD) OF NUTRITIONAL PARAMETERS (%) CALCULATED AS CONCENTRATION FOR DRY MATTER

Line	Starch	Sugars	Lipids	Proteins	Ashes
HDP6	76.54 ± 0.29	0.18 ± 0.01	1.70 ± 0.10	10.02 ± 0.06	1.59 ± 0.15
IR58614	74.30 ± 0.10	0.27 ± 0.02	2.07 ± 0.11	10.40 ± 0.11	1.16 ± 0.14

Results represent mean ± standard deviation of three replicated experiments.

The nutritional values of the IR58614 variety and HDP6 line were determined during three growing seasons: years 2003–2004, 2004–2005, and 2006–2007. Results presented here represent mean ± standard deviation of three independent experiments.

served that line DHP6 line had a pinkish white caryopsis, while the parental line IR 58614 had a matted white colour with a red grain. Line DHP6 had semi-translucent grains with a white belly (Table 1, score 0.60). Its translucency was much higher than that of its parental line (Table 1, 25% chalkiness, scores 0.25). These results were in accordance with those of Rabealaina (2010), but translucency was lower than that of MK34 (Rasoazanakolona, 2003). The chalkiness of the rice grain was classified into white belly, white centre and white back. DHP6 has a medium size grain (Table 1, L: 5.85 mm, L/B 2.31), slightly inferior to its parent (L: 6.30 mm, L/B 2.40). Both the HDP6 and parental lines have half round grains according to L/B. Rice grain width of the DHP6 was 2.75 mm, which was relatively higher than that of the of the parental line (2.54 mm). Indeed, rice grain width and shape play a crucial role in determining grain quality and yield.

Grain quality analysis: Chemical and culinary quality characteristics. Amylose concentration defines several eating and cooking qualities of the rice grain, e.g., amylose concentration is important because firmness and stickiness are two properties of cooked rice that also influence consumer preference. Among rice genotypes of the same apparent amylose type, characteristics such as alkali spreading value (ASV) and gel consistency (GC) can be used as quality indicators. Table 2 summarises chemical and culinary characteristics such as amylose content, alkali spreading value, gel consistency and cooking elongation of the DHP6 and parental lines. Amylose concentration (AC) of DHP6 is significantly higher (25.9%) than that of the parental line, reflecting its firm cooking texture and its translucency. In addition, the DHP6 line has much higher GC, alkali spreading value, and cooking elongation (Table 2) than of the parental line, thus giving quick cooking quality. GC of the grains after cooking was estimated as 96.4 mm (soft consistency), which was superior to the parental line. Gel consistency is a measure of firmness of the rice after cooking and is performed to classify rice varieties of the same AC, particularly in the high AC class, into hard, medium, or soft texture. Cooking elongation of raw rice grain resulted in length 1.26 mm for the DHP6 line and 1.06 mm for the parental line. Interestingly, higher elongation ratio (ER) of the cooked rice is preferred than lower ER. Taken together, our analysis of the culinary quality characteristics showed superiority of the DHP6 line over the parental line.

Nutritional values and micronutrients mineral richness. As a primary dietary source of energy, especially for the low-income and rural population, rice plays an important role in providing plant proteins, carbohydrates, and other nutrients. However, from a human nutritional point of view, rice grain is relatively low in some micronutrients such as iron (Fe), zinc (Zn), and calcium (Ca), compared with other staple crops such as wheat, maize, and legumes. Table 3 summarises the nutritional values of the DHP6 and parental lines. DHP6 had higher starch and ash concentration and slightly lower sugar and protein concentrations. The lipid concentration of DHP6 was slightly lower (1.53 %) than that of the parental line (1.84 %), but higher than that of white rice (Favier *et al*., 1995). In addition, we determined the concentrations of eight minerals: five macro elements (P, K, Ca, Na and Mg) and three microelements (Cu, Fe, and Mn). Table 4 showed large variation for grain mineral concentrations between the two lines. Of all eight minerals, DHP6 grains had substantially higher concentrations of P, K, Ca, Mg, and Cu than those of the parental line. For example, DHP6 had three times higher concentration of Cu (3.40 $\mu g \cdot g^{-1}$) than its parental line (1.40 $\mu g \cdot g^{-1}$). In comparative terms, in the DHP6 line concentrations of P,

Table 4

AVERAGE VALUES (±SD) OF MINERAL ELEMENTS P, K, CA, MG, NA (‰) AND MICRONUTRIENTS CU, MN, FE (PPM)

Line	P	K	Ca	Mg	Na	Cu	Mn	Fe
HDP6	1.87 ± 0.02	2.80 ± 0.17	0.14 ± 0.02	1.16± 0.05	0.10 ± 0.02	3.8 ± 0.10	18.13 ± 0.11	38 ± 2
IR58614	1.81 ± 0..01	2.04 ± 0.02	0.08 ± 0.01	1.11± 0.03	0.10 ± 0.01	1.57 ± 0.25	18.39 ± 1.13	55.96 ±2.28

The mineral elements and micronutrients of the IR58614 variety and HDP6 line were determined during three growing seasons: years 2003–2004, 2004–2005, and 2006–2007. Results presented here represent mean ± standard deviation of three independent experiments.

K, Ca, Mg, and Cu concentrations were higher than those on Na and Mn concentrations (Table 4). In general, our data indicated that line DHP6 is rich in certain mineral elements, including P, K, Ca, Mg, and Cu.

DISCUSSION

Comparisons of our results with those obtained by Favier *et al.* (1995) showed that the two local rice lines (DHP6 and IR58614) were richer in nutritious elements. The new double haploid DHP6 line was more nutritious in elements than its parental line. Previous analysis of white imported varieties (stock tampon) also confirmed our results (Rasoazanakolona, 2003).

Eating and nutritional quality is the most important end-use trait of cooked rice. Consequently, eating quality has become increasingly important for selecting rice varieties, especially for consumers in rice-producing countries (Allahgholipour *et al*., 2006). Rice grain appearance is mainly defined by grain shape: grain length, width and the length–width ratio, and chalkiness of the endosperm (Zhang, 2007). Moreover, cooking and eating characteristics of rice are determined by a combination of objective and subjective methods, e.g. amylose concentration strongly influences the cooking and eating characteristics of rice. Rice with a high amylose concentration (25–30%) tends to cook firm and dry, whereas rice with a intermediate amylose content (20–25%) tends to be softer and stickier and rice with a low amylose content (<20%) is generally quite soft and sticky (Juliano, 1985). The high level of AC of DHP6 is interesting, as amylose concentration is regarded as the most important indicator in classifying rice varieties (Juliano, 1985). It affects texture and retrogradation potential of cooked grains. Rice varieties are classified into high (>25%), intermediate (20–25%), low (10–19%), very low (3–9%), or waxy (0–2%) amylose classes (Kumar and Khush, 1986). Eating quality of indica rice is generally characterised by its higher amylose content and long grains (Bhattacharjee, and Kulkarni, 2000). The amylose concentration of DHP6 is high (25.9%), reflecting to its firm cooking texture and its translucency. Moreover, DHP6 has a low alkali value (=7); its gelatinisation temperature is low (55° to 69.5°C) resulting in quick cooking. In addition, the mean length of the gel flow was 96.4 mm (soft consistency). Thus, high amylose concentration, low gelatinisation temperature and soft gel consistency are important characters of the DHP6 line. Regarding culinary quality, grain of the line DHP6 is fast to cook and the cooked grain is firm and becomes soft after cooling. Moreover, the major chemical characteristics of the parental line (amylose concentration, alkali test, gel consistency) were well preserved and even improved in the DHP6 line. The present study revealed that DHP6 line has potential for improved consumer preferences and it could be used for breeding programmes for the improvement of valuable grain quality traits.

CONCLUSION

This study focused on the physical, chemical and cooking characteristics of an elite double haploid DHP6 of Madagascar. Rice grain quality consists of many components: cooking texture, palatability, flavour, grain appearance, milling efficiency, and nutritional quality. Among these, the cooking, eating, and appearance qualities constitute key economic concerns that influence rice production in many rice-producing countries of the world. Clearly, the new DHP6 line offers new opportunities for improvement of culinary and nutritional quality, due to its richness in mineral and microelements. Our results also suggested that concentrations of some desired minerals, like Mg and Cu, can be improved, which would be helpful in the selection of rice genotypes with low lipid concentrations and high mineral nutrient concentrations for further breeding strategy without sacrificing their high yields. For example, DHP6 is three times richer in Cu (3.40 ppm) than its parental line (1.40 ppm). Its low lipid concentration grants a longer time for better conservation.

In general, our data suggest that the new DHP6 line has excellent grain quality, culinary quality and will have important economic benefits to Madagascar rice growers. The present study provides evidence that DH technology has become an important part of rice breeding progress and improving grain nutritional and culinary quality characteristics. Thus, DHP6 line has high breeding and commercial value.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

The author(s) have made the following declarations about their contributions: conceived and designed the experiments: RSS VR NVR AA; performed the experiments: VR BBR XR NVR; analysed the data: RSS VR NVR AA; contributed reagents/materials/analysis tools: VR BBR XR NVR; wrote the paper: RSS VR NVR

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ELITES DUBULTOTĀS HAPLOĪDU RĪSU LĪNIJAS DHP6 GRAUDU UN PĀRTIKAS KVALITĀTES NOTEIKŠANA MADAGASKARĀ

Rīsu dubultotā haploīdu līnija DHP6 ir izveidota ar putekšņu kultūras metodi no šķirnes IR58614 un ir labi pielāgota audzēšanai Madagaskaras augstienes apstākļos. Parādīts, ka līnijas pārtikas un kulinārās īpašības ir vērtīgākas, nekā izejas šķirnei.