Rapid Genetic Purity Test in Rice (*Oryza sativa* L.) Using Novel *Transit Albino* Mutant

R. Thangapandian[1*] and S.R. Shri Rangasami[2]

[1] *Cotton Research Station, Srivilliputtur-626 135, Virudhunagar District, TamilNadu, India*
[2] *Department of Forage Crops, TNAU, Coimbatore -641 003, TamilNadu, India*

***CORRESPONDING AUTHOR:**

R. Thangapandian
Email: ricetha@gmail.com

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ABSTRACT

The objective of this study was to develop the new variability in mega seed rice varieties by establishing the novel phenotypic marker. This new genetic variability could be immediate beneficial to the seed industry. The *green-revertible* albino mutant ‘a-16’ was developed from the mega rice variety, ASD16 by mutation breeding approach. The albino seedling was turned into normal green seedling at 3-7 leaf stage and this trait is inherited and controlled by Mendelian single recessive gene. The stable *green-revertible* albino trait was evaluated at different stages to screen the large-scale genetic admixtures at nursery level in a short period of time. A lot of measures were taken to assess the genetic purity in the field and laboratory which could reveal the results after harvest. The adoption of *green-revertible* albino mutant could reproduce the precise result at seedling level, hence, application of *green-revertible* albino mutant in seed industry requires technological and policy interventions urgently to verify the rice productivity level in major rice growing areas.

Keywords: Breeding, green-revertible albino, off-type, genetic admixtures

1. INTRODUCTION

Rice is the staple food for more than 60 per cent of the world population. About 90 per cent of all the rice grain in the world is produced and consumed in Asia. In India alone, rice is cultivated on more than 44.6 million hector (ha) with an average productivity of 3.76 tons/hector (t/ha) with the recorded attainable yields of 10 t/ha (Prasad *et al.* 2001), reveals an exploitable yield gap of nearly 6 t/ha. The yield gap could be achieved by utilizing the genetic variability, innovative approaches and engineering of more productive plant architecture in rice. Intensive research has decisively added numerous traits in rice to enhance input use efficiency cum withstand stress conditions but failed to raise potential yield (Inada & Katsura 1984). Difficulties in using genetic variability and limitations to conventional breeding-selection approaches foil attempts to breach the potential yield of 10 t/ha (Mao *et al*. 1996).

Rapid advances in plant molecular biology leading to sequencing of genome can help mine and use very large number of hitherto unknown genes through functional and applied genomics to overcome the genetic constraints to raising yield threshold. Many consider that the future of crop improvement will depend primarily on how the genomic knowledge is used to locate additional or new variability and move the genes of interest across the barrier of sexual incompatibility. Advances in molecular genetics will greatly help to transfer the new traits from distant sources. It may take as long a time as conventional breeding to breed a variety of choice by genetic engineering and overcome the hurdles in deregulating for commercial planting (Muralidharan *et al.*, 2019). Genes involved in leaf color often have a role in photosynthetic pigment production and chloroplast development. Albino, chlorosis, thermo-color, light green, brown, yellow, stay-green, stripes and zebra, green-revertible albino, dark green, and purple are among the color types resulted from leaf-color mutations (Sheng *et al.* 2014; Cao 1999). Morphological traits are often used by breeders as selection markers in traditional rice breeding

The creation of new variability in rice displayed a novel beneficial albino trait *i.e., green-revertible* albino that could be applied in hybrid rice technology to identify the off-types during the hybrid seed production in China. The male sterility in T/PGMS lines are highly influenced by higher temperatures and long day photoperiod conditions, if a dramatic change in temperature occurs during the male sterility inductive period, the male sterile lines become partially fertile and the hybrid seed would be contaminated. A new T/PGMS line with a leaf color mutation as a screening marker is one economically feasible way to increase dependability of this system in China (Shen *et al.* 1994).

Though the molecular marker technology is used to ascertain the genetic purity of rice variety, it requires high cost besides skilled technicians for assessing the genetic contamination in the random sampling of any paddy variety. In this juncture, alternate techniques might be considered as viable option to assess the genetic purity of any rice variety at farm level. Hence, *green-revertible* albino mutant can also be applied to screen genetic admixtures in a short span of time without involving skilled labour at nursery level. With this view, the study was carried out to assess the genetic purity of the released mega variety with mutation breeding approach to establish the novel *green- revertible* albino trait.

2. MATERIALS AND METHODS

2.1 Seed preparation in mutagen solution

Currently the total annual planting area of rice is more than 1600 thousand hectares in Tamil Nadu. The rice varieties viz., ADT 36, ADT 38, ADT39, ADT 43, ADT 45, BPT 5204 and ASD 16 occupy more than 75 per cent of rice cultivation area in Tamil Nadu due to its stability in yield potential and consumer preferences. Every year genetic admixture in seed production plot become menace to both public and private seed producers, ultimately the farmers are suffered. To get rid of this problem, mutation breeding was initiated at Tamil Nadu Rice Research Institute, Audthurai. Filter sterilized solution of NaN_3 (1.5 Molar Sodium Azide) was prepared in deionized water and diluted with sterile 0.1 Molar phosphate buffer (pH 3) to prepare 0.001 Molar, 0.002 Molar, 0.003 Molar, 0.004 Molar and 0.005 Molar working solution to treat the seeds of these varieties. The seeds were first placed in sterile deionized water for 30 minutes to imbibe seeds and then submerged in mutagen solution for 60 minutes. Seeds submerged in deionized water for the same period of time served as control.

2.2. M1 plants and seed harvest

However, there was good reason not to use the highest mutagen dose because that leads to unwanted mutations at some of the loci resulting in lethality with poor or no germination. This made relatively low doses attractive to reduce the amount of work in screening of M_1 plants. The treated seeds were sown in the field as per the dose level. At maturity, seeds were bulk harvested from each M_1 plant.

2.3 Production of homozygous stable *green-revertible* albino seedlings

The M_2 seeds were directly planted at South Farm, Tamil Nadu Rice Research Institute, Aduthurai during 2010. The variants with apparent leaf color changes were isolated and labeled at the 1-3 leaf stage (7 days after sowing). Seedlings that survived chlorophyll deficiency were transplanted into a paddy field. The mature mutants with similar agronomic characters to the original parent were harvested to produce M_3 plant lines. Seeds of M_3 plant lines were separated for field evaluation. The field evaluation carried out by planting the M_4 to M_8 generations plant lines. The homozygous stable *green-revertible* albino plants were obtained from the treated rice variety, ASD 16. The novel mutant 'a-16' with transit (*green-revertible*) albino marker was found to express albino leaves at the 3-7 leaf stage (7-21 days) and reverted back to normal green color periodically before transplanting. The mature mutant plant possessed mostly similar agronomic traits as the original plant of rice variety, ASD16 evaluated in progeny row and replicated row trial. The mature mutant plant expressed uniform transit albino traits and the survival rate of albino seedlings were significantly on par with original parent.

2.4 Selection of economically important agronomic traits

The selected mutant 'a-16' with *green-revertible* albino trait was reciprocally crossed with the original parent, ASD16. The F_1 plants were self-pollinated to obtain F_2 progenies. Leaf color phenotype of F_1 and F_2 plants was recorded at the 1-3 leaf stage. The seedlings were planted with the original parent, and seedling heights of the mutant 'a-16' were recorded at 6-leaf stage (18 days after sowing). The rooting pattern of the mutant along with original parent was recorded at the 3-leaf stage (7 days after sowing). The economically important agronomic traits *viz*., plant height, tiller numbers, panicle length, florets per panicle, 1000-grain weight, seed setting rate and single plant yield were also recorded in mature plants of both mutant and original plant. The important grain quality properties *viz*., amylose content, gel consistency and starch content were recorded to assess the changes in physico-chemical properties of the derived mutant from the original parent.

3. RESULTS AND DISCUSSION

3.1 Chlorophyll deficient mutations and their behavior in M_2 and subsequent generations

In the M_2 generation, about 1,54,760 seedlings were grown for isolating chlorophyll-deficient mutations. Four mutant phenotypes, *i.e.*, albino, light yellow, light green, and striped albino were identified from the normal green population. On a per plant basis, the total frequency of chlorophyll-deficient mutations reached 0.036%. Out of 58 albino mutants' only nine albino mutants were survived due to green reversibility, other mutants were neglected since they not exhibited scope for improvement of economically important traits (Inada and Katsura, 1984). The surviving mutant had albino leaves between 1- and 7-leaf stages (up to 21 days), later turned into green (revertible) color from leaf base to leaf tip. The albino trait slowly turned into green color (revertible) at 15 days old seedlings and this conversion process continued up to 21 days old seedlings and finally appeared as normal green color. The economic important agronomic traits between the mature mutant and its original parent are similar and no significant variation was recorded (Table 1). Although it had albino traits up to 6-7 leaf stage, the rooting pattern between the albino and normal green seedlings had significant variation, the albino seedlings showed longer root length than normal green seedlings. Similarly, the leaf blade width of green-revertible albino mutant was decreased than the original parent, ASD16; hence leaf area index (LAI) in mutant plant showed no conducive for the leaf folder incidence. Those were considered an important trait for practical breeding approach to develop the resistance mechanism against key pest since no adverse effect was noticed.

3.2 Stability of albino mutation under the field condition

In M_3 generation (Kharif and Rabi season, 2010), the mutant 'a-16' exhibited the green revertible albino phenotype (Fig. 1 a-d) as recorded in M_2 generation, indicating that the *green-revertible* albino mutation is fixed and heritable. Subsequent generations M_3-M_8 were evaluated (Kharif season, 2011-16) in field trials, further confirmed that the green revertible mutant 'a-16' retained the trait due to inheritance. Therefore, in terms of stability of the green-revertible albino trait, the inheritance pattern of novel trait was carried out subsequently. The *green-revertible* albino mutant (transit albino) can be used as a phenotypic marker for rapid removal of off–types or genetic admixtures in the seed production plot and the genetic purity of the variety could be fixed at seedling stage. It gives the feasibility to determine the genetic purity of the variety at nursery level before conducting grow-out test (GOT). A lot of measures were taken to assess the genetic purity in the field and this could be ensured in grow-out test (GOT) after harvest (Vigneshwari *et al.*

2014). The adoption of *green-revertible* albino mutant in seed industry requires technological and policy interventions urgently to verify the rice productivity level in major rice growing areas. Due to lack of skilled labour for rouging operation, genetic admixtures could definitely be appeared and its genetic contamination reduces the productivity level in rice. According to the research forecast; 1% impurity in seed lot may decrease the potential yield of varieties and hybrids by about 100 kg/ha; which is accounted for huge yield losses to the farmers (Mao et al. 1996).

3.3 Inheritance of *green-revertible* albino trait

The green revertible albino mutant plants were crossed with the original parent, ASD6 and reciprocal cross combination was also made between them during 2016. All F_1 plants from the reciprocal crosses had normal green leaves. A total of 512 albino plants and 563 albino plants were identified from the a-16/ASD16 population (2083 F_2 plants) and ASD16/a-16 population (2126 F_2 plants). The ratios of green plants to albino plants in the two populations were 3.017:0.983 or 2.941:1.059 respectively, fitting to the expected 3:1 ratios (Table 2). F_3 seedling observations found that the progenies derived from the F_2 albino plants were uniform for albino leaves, however, two-third of F_2 green plant derived progenies segregated in leaf color and the ratio of green plant to albino plants fitted well to the expected 3:1 ratios. This indicated that the albino trait was controlled by a single recessive gene. A novel thermo/photoperiod-sensitive genic male sterile rice mutant with green-revertible albino leaf color marker was employed in two line hybrid rice technology in China (Wu *et al.* 2001; Wu *et al.* 2003). The green-revertible albino trait was controlled by single recessive gene in this study.

At present, the study of leaf coloring mechanism in rice is mainly through the identification of leaf color mutants, senescence-associated mutants and the functional analysis of senescence. Although some genes related to aging and leaf color have been cloned successfully, it is still unclear whether and how some important metabolic pathways (such as photosynthesis, photorespiration, cytoplasmic receptor kinase pathways, anthocyanin biosynthesis pathway, etc.) are involved and regulated in rice leaf color variations (W. Li *et al.* 2022). Future studies in this area will help to further understand the molecular mechanisms of rice leaf coloring and provide in-depth theoretical basis for this morphological trait to be a more dependable marker in the breeding program of rice.

3.4 Economically important characters

There were very slight variations in seedling heights at the 3-5 leaf stage (14 days) between the albino mutant ‘a-16’ and the original parent because the nutrition for seedling development at that stage is mainly provided by the rice endosperm. The rooting pattern of green revertible albino mutant is more vigorous (Fig. 2) than normal green seedlings besides other chlorophyll deficient albino seedlings are shown poor root growth and it died before attaining at the 1-3 leaf stage. Due to poor photosynthesis caused by chlorophyll deficiency, the mutant seedling became significantly stunned at the 3-5 leaf stage (Fig. 3a & 3b). Then seedling growth was normal after turning into green (Fig. 4a & 4b) color at 5-6 leaf stage. Further observation indicated that the chlorophyll deficiency at the seedling stage did not influence subsequent development. There is more similarity in various agronomic characters between the *green-revertible* albino mutant and its parent (Fig. 5); which showed no adverse effect on economically important characters. Hence, the mutant could directly become beneficial to the farmers and seed producers respectively, provided large scale demonstration will be evaluated further to ascertain the off-type elimination during the seed production of mega rice variety. There were no differences in grain quality traits *viz.*, kernel length, kernel breadth, kernel length: breadth ratio, linear elongation ratio, and volume expansion ratio between the green-revertible mutant and original parent (Table 3). Similarly, no major changes were noticed in their chemical properties *i.e.*, alkali spreading value and amylose content (Fig. 6). This is an important observation from the practical breeding point of view. Since the mutated trait is not linked to any adverse effect on other traits, it can be used directly in rice breeding programme and seed industry.

Genes involved in leaf color often have a role in photosynthetic pigment production and chloroplast development. Albino, chlorosis, thermo-color, light green, brown, yellow, stay-green, stripes and zebra, green-revertible albino, dark green, and purple are among the color types resulted from leaf-color mutations (P. Sheng *et al.* 2014; L.Q.Q. Cao. 1999). Morphological traits are often used by breeders as selection markers in traditional rice breeding and molecular implications in useful gene identification. To help identifying hybrids, certain leaf color features, such as purple or pale green, have been introduced into sterile or restorer lines (Peng *et al.* 2012). In recent years, breeders have paid an increasing attention to the value of altered leaf color phenotypes.

4. CONCLUSION

Application of transit (*green-revertible*) albino mutant in rice mega varieties diminishes all the problems existed during the quality rice seed production, and it gives platform for seed producers to remove the genetic admixture at seedling stage (nursery level). A farmer can also be involved to remove the genetic admixture seedlings by identification of leaf color (Peng et al. 2012). The *green-revertible* albino mutant development in mega rice variety could be become beneficial to screen the large-scale genetic admixtures at nursery level and cultivation area could definitely be increased by supplying quality seeds to the farmers. The adoption of *green-revertible* albino mutant in seed industry requires technological and policy interventions urgently to verify the rice productivity level in major rice growing areas. This precise phenotyping will remain as the foundation of future crop improvement. A genomic breeding approach is used to locate the new variability and find the gene of interest (*green-revertible*) trait in the mutant background. This work provided feasibility to obtain desired yield improvement by supplying quality seeds to the farmers with assured genetic purity. Rapid advances in plant molecular biology leading to sequencing of genome can help mine and use very large number of unknown gene sources through functional and applied genomics to overcome the genetic constraints to raising yield threshold.

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Table 1: Economically important traits between the mutant and parent

Genotype	Days to 50% flowering	Plant height (cm)	No. of productive tillers per plant	Panicle length (cm)	No.of filled grains per panicle	1000 grain weight (gm)	Single plant yield (gm)
Green-revertible albino mutant	80.6	93.40	12.9	23.46	152.73	24.84	52.84
ASD 16 (parent)	81.5	95.60	13.4	23.68	154.32	25.43	57.25

(Note: data in the table is mean value; which is derived from the 50 plants randomly recorded)

Table 2: Inheritance pattern of green-revertible albino mutant ‘a-16’ in reciprocal crosses

Seedling color					
Genotype	Normal green	Green revertible albino	Expected ratio	Chi square value χ^2	Observed ratio
F_1 ASD16 / a-16	56	0			
F_2	1571	512	3:1	0.20	3.017: 0.983
F_1 a-16 /ASD16	43	0			
F_2	1563	563	3:1	2.489	2.941:1.059

Table 3: Grain quality traits between the mutant and parent

Genotype	Kernel length (mm)	Kernel breadth (mm)	Kernel length: breadth ratio	Linear elongation ratio	Volume expansion ratio	Alkali spreading value	Amylose content (%)
Green-revertible albino mutant	5.32	2.02	2.63	1.28	4.21	3.24	20.23
ASD 16 (parent)	5.38	2.02	2.66	1.24	4.10	3.33	20.11

(Note: data in the table is mean value; which is derived from the 50 grains randomly recorded)

Fig.1a: Albino seedling at 3-leaf stage

Fig.1b: Albino seedling at 3-leaf stage

Fig.1c: Green-revertion starts at 4-leaf stage

Fig.1d: Green-revertion starts at 4-leaf stage

Fig.2: Green-revertible albino mutant line - M3

Fig.3a: Green-revertible albino turns into normal seedling at 5-6 leaf stage

Fig.3b: Green-revertible albino turns into normal seedling at 5-6 leaf stage

Fig.4: Root growth differences between green-revertible albino and normal seedling

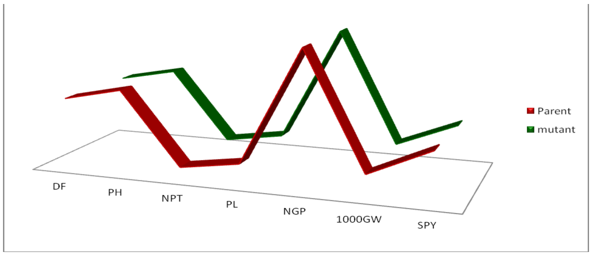

Fig.5: Comparison of agronomic traits between mutant and parent

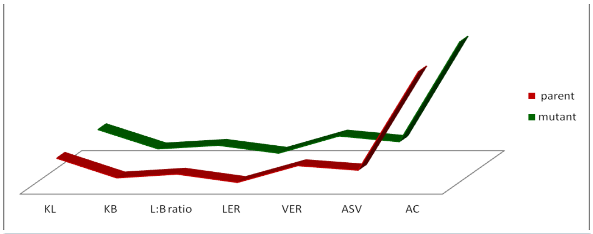

Fig.6: Comparison of grain quality traits between mutant and parent

REFERENCES

Cao, L., LiYong, C., Qian Qian, Q. Q., Zhu XuDong, Z. X., Zhen DaLi, Z. D., Min ShaoKai, M. S.,and Xiong ZhenMin, X. Z..1999. Breeding of a photo-thermo sensitive genic male sterile indica rice Zhongzi S with a purple-leaf marker and the hoterosis of its hybrid rice produced with it, Acta Agron. Sin. 25: 44-49.

Inada, K.and N. Katsura, 1984. A rice mutant entirely lacking chlorophyll b. Japan *Journal of Crop Science* 53: 445-449
DOI: 10.1626/jcs.53.445

Mao, C.X., S.S. Virmani, and I. Kumar, 1996. Technological innovations to lower the costs of hybrid rice seed production. In:Virmani,SS. *et al.*, (eds). Advances in hybrid rice technology. Proceedings of Third International Symposium on Hybrid Rice, Directorate of Rice Research, Hyderabad, India

Muralidharan, K., G.S.V. Prasad, C.S. Rao, and E.A. Siddiq, 2019.Genetic gain for yield in rice breeding and rice production in India to meet with the demand from increased human population. Current Science 116: 544-560.
DOI: 10.18520/cs/v116/i4/544-560

Prasad, G.S.V., K. Muralidharan, and C.S. Rao, 2001. Stability and yield performance of genotypes, A proposal for re-grouping world rice area into mega environments. Current Science 81: 1337-1346.

Peng, Y., Y. Zhang, J. Lv, J. Zhang, P. Li, X. Shi, Y. Wang, H. Zhang, Z. He, and S. Teng, 2012. Characterization and fine mapping of a novel rice albino mutant low temperature albino 1, J. Genetics Genomics 39: 385-396.
DOI: 10.1016/j.jgg.2012.05.001
PMID: 22884095

Shen, Y.W., M.W. Gao, and Q.H. Gai, 1994. A novel environment-induced genic male sterile (EGMS) mutant in indica rice. Euphytica 76: 8-96.
DOI: 10.1007/BF00024024

Sheng, P., J. Tan, M. Jin, F. Wu, K. Zhou, and W. Ma, 2014. Albino midrib 1 , encoding a putative potassium efflux antiporter , affects chloroplast development and drought tolerance in rice, Plant Cell Rep. 1581-1594.
DOI: 10.1007/s00299-014-1639-y
PMID: 24917171

Vigneshwari, R., A. Vijayakumar, and M. Bhaskaran, 2014. Morphological characterization of major paddy cultivars in seed chain of TamilNadu. *International Journal of Agricultural Sciences* 1: 280-285.

Weiran Li, Z. Yuchan, Md. A. R. Mazumder, R. Pan and D. Akhter. 2022. Research Progresses on rice leaf color mutants. Crop Design. Vol. 1:2. 100015 DOI: 10.1016/j.cropd.2022.100015

Wu, D.X, S. Q.Shen, H. Cui, Y.W. Xia, and Q. Y. Shu, 2003. A novel thermo/photoperiod-sensitive genic male-sterile (T/PGMS) rice mutant with green-revertible albino leaf color marker induced by gamma irradiation. Field Crops Res., 81: 141-147. DOI: 10.1016/S0378-4290(02)00218-6

Wu, D.X., Q.Y. Shu, and Y.W. Xia, 2001. A novel male sterile line suitable for ensuring and measuring the purity of hybrid rice seed. Seed Science and Technology 29: 129-133.