



Semi-dwarf narrow rolled leaf mutant in rice: Photosynthetic efficiency and physiological response to gibberellic acid (GA₃)

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Received: July 24, 2015; Revised received: May 10, 2016; Accepted: July 21, 2016

Abstract: Leaf is the major component for fixation of carbon dioxide to produce more photoassimilates and maintains source sink relationship in plants. The present investigation was carried out to study the photosynthetic efficiency and physiological response of GA₃ of semi-dwarf narrow rolled leaf mutant. Semi-dwarf narrow rolled leaf mutant exhibited reduced plant height, reduced leaf width, reduced panicle length and number of grains per panicle when compared to wild type. Photosynthetic efficiency study revealed that the mutant showed higher photosynthetic efficiency than wild type. Complementation test with GA₃ clearly revealed that this mutant is sensitive to GA₃ and revert to normal plant height. Scanning Electron Microscope study revealed that mutant had exhibited minimum number of inter-veins between veins, minimum distance between midrib and has slightly larger cell size. Hence, this loss of function mutant is very useful to map the regions associated with panicle length, number of grains per panicle and other traits related to enhancement of yield. The result provides an important clue for further understanding the mechanism of rice leaf development and plant architecture.

Keywords: Gibberellic acid, Photosynthetic efficiency, Scanning electron microscope, Semi-dwarf

INTRODUCTION

Rice (*Oryza sativa* L.) being a model plant species for most of the monocotyledons and investigation of their leaf morphology such as size and shape are major determinants of plant architecture and strongly affect high-yield performance. However, leaf morphology, as well as other yield-related components, is a highly complex trait. In rice, many quantitative trait loci (QTLs) controlling such traits have been identified and QTLs for the size of leaves were found to be co-located with sink-related traits (Cui *et al.*, 2003). Leaf is the main factor for photosynthesis and its morphology is closely related to the photosynthetic efficiency and consequently contributing to the yield formation. Recently, there is a report that combination of heterosis utilization with plant ideotype is the only way to achieve higher yield (Yuan, 1997). The morphology of rice leaf is one of the major components of the ideotype in rice. And being appropriate curled can be useful for maintaining the rice leaf upright and helping the population to receive more light (Lv *et al.*, 1991; Su *et al.*, 2003). Because of the importance of leaf shape and size in constructing ideotype in rice, many scientists have put efforts in dissecting the genetic mechanism underlying rice leaf formation by analyzing the leaf shape mutants (Zuo and Li, 2014). To date, a few genes involved in regulating the development of leaf

shape, especially the curled leaf shape, have been cloned and functionally analyzed. *SLL1/RL9*, a member of the KANADI family, encodes a transcription factor (Yan *et al.*, 2008; Zhang *et al.*, 2009), and its mutation leads to the failure of programmed cell death of abaxial mesophyll cells and the suppression of the differentiation of the abaxial cells, and finally to generate adaxially rolled leaves.

Studies of leaf-shape development mainly focus on leaf rolling and size. Several rolled-leaf genes have been cloned and some breakthroughs in this regard have been achieved in rice recently (Zou *et al.*, 2011). However, few narrow-leaf traits have been studied because of the lack of functional deficiency mutants. At present, only seven narrow-leaf mutants are reported to be controlled by a single gene (nal1, nal2, nal3, nal4, nal5, nal6 and nal7), of which Nal1 and Nal7 have been isolated but the molecular mechanisms are still unclear (Wang *et al.*, 2009). Creation and identification of novel narrow leaf mutants are important in order to isolate regulatory genes by a map-based cloning strategy and to further understand the mechanisms of rice leaf-shape development. In the present study, the semi-dwarf narrow rolled leaf mutant derived from the mega variety White Ponni via gamma irradiation was used as material to study the photosynthetic efficiency, leaf morphology using scanning electron microscope (SEM) and was also observed for the physio-

logical response to gibberellic acid (GA₃).

MATERIALS AND METHODS

Plant materials: Biological material used in the present study was semi-dwarf narrow rolled leaf mutant identified in M₂ generation using gamma radiation. The M₂ seeds of the mutant were forwarded to M₃ generation where leaf morphology, vein patterning in leaves, cell pattern in nodal and internodal regions under scanning electron microscope, photosynthetic efficiency of the mutant using chlorophyll fluorometer and GA₃ response were studied along with the wild type White Ponni.

Study on Photochemical efficiency (Chlorophyll Fluorometer) : Photochemical efficiency of semi-dwarf narrow leaf mutant and wild type were recorded by using the handy and portable photochemical efficiency analyser or chlorophyll fluorometer (Model – OS30p). Simple fluorescence measurements can be made by the pre-darkening of the leaf sites followed by a short exposure to a saturating light intensity. Measuring the fluorescence intensities over time produces a Kautsky induction curve. The shape of this curve can be used as an indication of environmental stress damage on the photosynthetic apparatus. Measured parameters include Fo, Ft, Fv, Fm and Fv/Fm and OJIP. Photochemical efficiency of the genotype was calculated by the formula given below.

Photochemical efficiency = Fv/Fm

Where,

Fv = Variable fluorescence

Fm = Maximum fluorescence

Study of GA₃ response in mutants: Seeds of the mutant was selected in M₃ generation were surface sterilized with 2 per cent HgCl₂ (Mercuric chloride) solution for 30 minutes and then washed with sterile water. They were placed in wet filter paper and then kept in dark at 30°C for two days for germination. Elongation of shoot was quantified by microdrop method (Mitsunaga *et al.*, 1994). After germination, ten uniformly germinated seeds were placed on 1 per cent agar plate and allowed to grow at 25°C under fluorescent light till the emergence of second leaf sheath. After two days, 1 µl of GA₃ solution (10 mg/ml) was applied to coleoptile region of rice seedlings. Five days after this treatment, the length of the second-leaf sheath was measured and an average of five seedlings was taken to calculate GA₃ response (GAR) by the following formula. This GA₃ response study was conducted in three replicates under *in vitro* condition.

$$GA_3 \text{ response (GAR)} = \frac{\text{Mean length of second leaf sheath in } GA_3 \text{ treatment}}{\text{Mean length of second leaf sheath in control}} \times 100$$

Scanning electron microscope (SEM): Transverse sections of leaf, nodal region and internodal regions of the semi-dwarf narrow rolled leaf mutant and control were

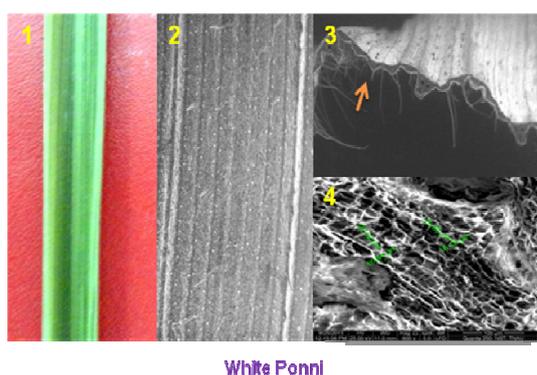
studied for their difference in internal cell arrangement patterns under Scanning Electron Microscope (SEM) (Model : FEI quanta 200 SEM) built in the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

RESULTS AND DISCUSSION

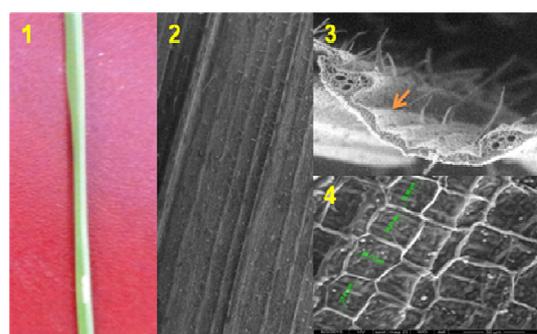
Leaf rolling is an important agronomic trait in rice breeding and moderate leaf rolling maintains the erectness of leaves and minimizes shadowing between leaves, leading to improved photosynthetic efficiency and grain yields. The present investigation identified a unique mutant with some defects in their leaf morphology and plant architecture (Fig 1). This mutant exhibited narrow rolled leaves and reduced height when compared to White Ponni wild type. Rolling leaf material of rice were also helpful in the research of genes related with leaf growth and development as same as regulation mechanisms to osmotic stress (Hsiao *et al.*, 1984). Rolling leaf is easily distinguished from other leaf type. As marker gene rolling leaf characterizer are widely applied in rice genetic and physical mapping research (Shi *et al.*, 2007; Wu *et al.*, 2010), abundance rolling leaf materials in rice which the genetic mechanisms were different have been identified (Hu *et al.*, 2010; Luo *et al.*, 2007). Determination of photochemical efficiency of narrow rolled leaf mutants during the peak vegetative phase using photosynthetic efficiency analyzer (Portable chlorophyll fluorometer – Model: OS30p). This study clearly revealed that, semi-dwarf narrow rolled leaf mutants of White Ponni exhibited higher value of photosynthetic efficiency when compared to wild type (Table 1). Most of the rolling leaf mutant increased the colony yield which depends on the decrease yield of single plant. However, the paradox was found that in some research, the net photosynthesis rate of single plant was higher in rolling rice than that of wild-type (Chen *et al.*, 2005). Wang *et al.* (2012) indicated that rolling leaf mutant decrease the efficiency of light utilization compared to the wild type. This was because of the reduction of leaf area



Fig 1. Semi-dwarf narrow rolled leaf mutant of White Ponni.



White Ponni



Semi-dwarf narrow rolled leaf mutant of White Ponni

Fig 2. Variation in leaf morphology and cell structural pattern of third internodal region in white ponni and semi-dwarf narrow rolled leaf mutant. 1. Leaf morphology variation, 2. Vein pattern variation, 3. Intervein variation and 4. Cell pattern variation.

and chlorophyll contents and the dissipation of more excitation energy as non-photochemical quenching as a result of avoiding potential damage of membrane structure. These narrow rolled leaf mutants also exhibited semi-dwarfness, less panicle length and reduced number of filled grains per panicle but their grains was slender when compared to grains of wild type. This may be due to lack of source sink relationship. Thus, the study of rolled leaf mutant will be meaningful for

Table 1. Photosynthetic efficiency of sem-dwarf narrow rolled leaf mutant of White Ponni.

Mutants	Fv/Fm
Semi-dwarf narrow rolled leaf mutant	0.7107
White Ponni (wild type)	0.6902

Table 2. GA₃ response of Semi-dwarf narrow rolled leaf mutant of White Ponni using microdrop method.

Mutant	GA ₃ non treated		GA ₃ treated		GA ₃ response	
	5 DAT	15 DAT	5 DAT	15 DAT	5 DAT	15 DAT
	Length of 2 nd leaf sheath (cm)					
Semi-dwarf narrow rolled leaf mutant	1.90	2.77	2.78	3.75	146.32	135.54
White Ponni (wild type)	1.07	2.17	1.78	3.46	166.88	159.69

breeding crops with improved photosynthetic efficiency and subsequently higher yield (Lang *et al.*, 2004).

GA₃ response of semi-dwarf narrow rolled leaf mutant of White Ponni was estimated using microdrop method of GA₃ application to the coleoptiles region of the seeds three days after germination. Narrow rolled leaf mutant recorded low response to GA₃ application (Table 2) (Tanaka *et al.*, 2000). Hence from the study it is better to understand that the mutant is more sensitive to GA₃ application. The complementation test with gibberellic acid exhibited that the identified mutant reverts to its normal plant height when GA₃ applied externally. Neeraja *et al.* (2009) reported that, occurrence of DGWG allele of *sd1* gene and GA₃ response were analyzed together, existence of two kinds of dwarfs was noticed *viz.*, dwarf accessions with DGWG allele and dwarf accessions without DGWG allele of *sd1* allele exhibiting varying responses to GA₃. *Sd1* gene with varying response to GA₃ could be used as excellent alternate sources for DGWG allele of *sd1* gene.

In addition to this, the leaf of narrow rolled leaf mutant had exhibited minimum number of inter-veins between veins, minimum distance between midrib and has slightly larger cell size and minimum cell number unit area (Fig 2). These results of our study were supported by the findings of Qi *et al.* (2008) in a classic rice dwarf mutant named narrow leaf1 (*nal1*) with affected vein patterning and polar auxin transport. Fujino *et al.* (2008) reported that, in narrow and curled leaf mutant, mutation resulted in reduced leaf width, no significant morphological changes at the cellular level in leaves were observed. The same study was conducted by Feng *et al.* (2012) who reported that, the mutant displayed narrow and upper-albino leaf blades with significantly decreased photosynthetic pigment contents throughout their development and narrow leaf trait is caused by a decreased number of small veins.

Lee *et al.* (2011) also reported that, the mutant leaves had fewer veins and smaller bulliform cells comparing to those of Koshihikari. Mutant had fewer large veins (lateral veins) and small veins (intermediate veins) than those of Koshihikari, resulting in the narrow-leaf phenotypes. Cross-section analysis of mutant leaves showed reduced bulliform cells and exists specifically in the upper side of leaves in rice, and their number and density affects determination of the leaf rolling

and stretching movement (Li *et al.*, 1979). Therefore, developmental defects of veins and bulliform cells affected the leaf rolling morphology. It has been shown that under drought conditions, bulliform cells lose turgor pressure and shrink, leading to the rolling up of leaves. Once water is sufficient, the bulliform cells expand and the leaves open again (Price *et al.*, 1997; Alvarez *et al.*, 2008). However, the molecular and genetic mechanisms of leaf rolling and the function of bulliform cells in leaf rolling remain to be elucidated. Therefore the elucidation of the genetic basis of the control of leaf shape and size could be of use in the manipulation of crop traits, leading to more stable and increased crop production.

Conclusion

One of the most important targets to improve crop yield is leaf photosynthetic capacity. The leaf morphology, particularly in crop, is one of the most important agronomic traits because it influences the yield through the manipulation of photosynthetic capacity and transpiration. A new rolling leaf rice mutant was identified which showed an apparently straighter longitudinal shape normal transverse rolling characters at all developing stages. This study also found that the net photosynthesis efficiency of single plant was higher in rolling rice than that of wild-type. This mutant also served as loss of function mutant for various yield related traits *viz.*, reduced plant height, reduced leaf width, reduced panicle length and number of grains per panicle. Therefore, further genetic analysis is necessary to map the genes associated with the above mentioned traits in future breeding programmes.

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