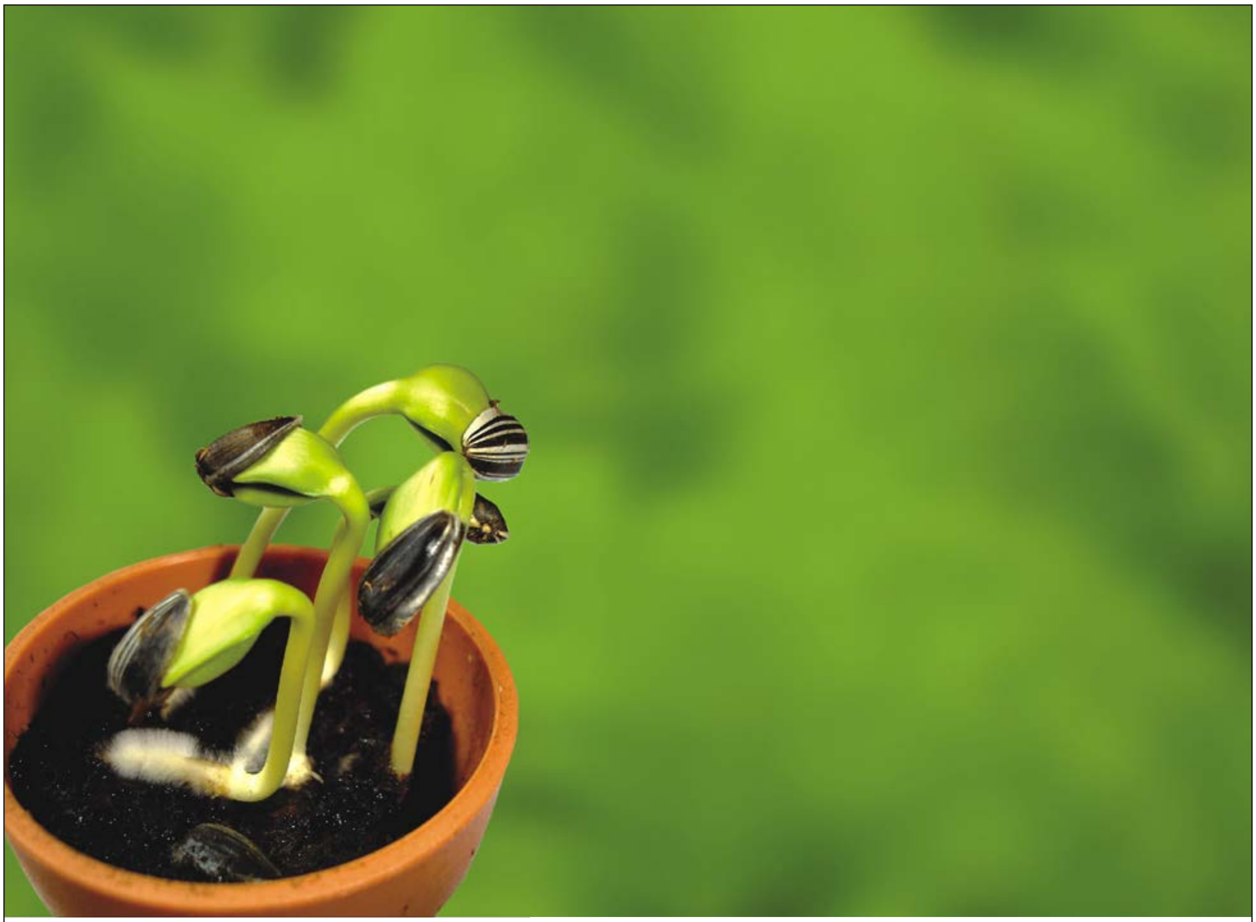

International Journal of
Plant Breeding and Genetics

ISSN 1819-3595

Academic Journals Inc.

www.academicjournals.com

International Journal of Plant Breeding and Genetics 6 (4): 252-255, 2012
ISSN 1819-3595 / DOI: 10.3923/ijpbg.2012.252.255

Occurrence of Trifid Stigma Morphotype in a Maintainer Line of Rice (*Oryza sativa* L.)

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ABSTRACT

Morphological characteristics of florets are of utmost importance in increasing the outcrossing rate in hybrid rice seed production. Among the different floral traits, stigma exsertion is considered to be of highest significance. Normally rice has stigma with two branches mentioned as bifid stigma. We reported a new natural mutant morphotype of stigma having an extra branch (hereafter mentioned as trifid stigma) which was identified in an *indica* maintainer line of rice. It occurs at a frequency of 0-35% in the population. Morphometric data showed that the extra branch is shorter than the other two branches of trifid stigma which were shorter than the mean length of bifid stigma. The possible use of the new variant in hybrid rice breeding is discussed.

Key words: Hybrid rice, stigma, maintainer, restorer, outcrossing

INTRODUCTION

Breeding for high-yielding hybrid rice is one of the pragmatic solutions in addressing a food shortage problem that is caused by a marked increase in the global population coupled with decreasing trend in available land and limited water for agriculture (Shanti *et al*., 2010). Hybrid rice exhibits a yield advantage of 15-20% (or more than one ton of paddy per hectare) over the best traditional varieties in a large-scale production worldwide (Xu, 2003). Commercial seed production of hybrid rice plays a key role in successful utilization of hybrid rice technology (Sreedhar *et al*., 2011). However, as opposed to the case of open-pollinated plants such as maize or many vegetable plants in which the hybrid system is common, it is difficult to reliably produce an acceptable quantity of hybrid rice seeds owing to its self-pollinating nature (Azzini and Rutger, 1982). Therefore, improvement of hybrid seed production efficiency is an essential factor for large scale commercialization of hybrid rice (Tiwari *et al*., 2011).

There are several phenotypic traits contributing to the seed production efficiency of hybrid rice, such as days to heading or blooming time, number of pollen (pollen load), pollen longevity and morphological traits of floret such as dimension of spikelet, stigma, style, stigma exsertion, stigmatic receptivity and spikelet opening angle and duration (Virmani, 1994). Among them, stigma exsertion is especially emphasized as a major component in increasing pollination and seed set (Kato and Namai, 1987).

The rice flower consists of the stamens and pistil. The six stamens are composed of 2-celled anthers borne on slender filaments. The pistil contains one ovule and the short style that bears the bifurcate, plumose, stigma. We report here a new phenotype of stigma identified in a maintainer line of rice.

MATERIALS AND METHODS

In our study on characterizing pistil of different genotypes, we found an unusual three branched stigma (trifid stigma; Fig. 1) in an *indica* maintainer line IR80155B of rice, during dry season 2010-11. This line was developed at the International Rice Research Institute (IRRI), Philippines, in hybrid rice breeding program. Out of curiosity, we started looking at the different characteristic features of the third stigma branch. Seed from the plant showing trifid stigma was harvested separately and a total of 267 plants were planted in wet season 2011. Three panicles from each plant were sampled to calculate the overall frequency of trifid stigma, which was defined as rate (%) of the number of spikelets with trifid stigma to the total number of stigmas on spikelets. Length and width of stigma and style were then measured under a light microscope with an ocular micrometer. For three plants bearing trifid stigma, measurement was taken on ten spikelets from each of the three panicles of plant. The stigma exsertion percentage was measured by counting the total number of stigma outside the spikelets divided by total number of spikelets in a normal bifid and trifid stigma bearing plant after anthesis.

RESULTS

It was found that the frequency of trifid stigma in the population ranged between 0-35% and out of 267 plants only 34 plants had trifid stigma morphotype. Further, in a given plant, the frequency of trifid stigma was found to be stable. Since, the flowering in rice is from panicle tip to panicle base, the panicle was divided into three parts. The number of trifid stigma in the top, middle and bottom parts of panicle was nearly the same (data not shown). The distribution of trifid stigma in the panicle was found to be random and not limited to a particular location within the panicle. Variation was observed between different pistil parts of normal bifid and trifid sigma (Table 1). It was observed that a 27, 3.0, 30.0 and 2.5% reduction in the length of non-brush-shaped part of stigma, brush-shaped part of stigma, total stigma length and stigma width, respectively, in the third branch as compared with the other two branches of the trifid stigma. Moreover, when compared with the normal bifid pistil, the reduction in first and second branch of trifid stigma (Fig. 1) was 12.6% of the total length of stigma. Further, the stigma exsertion was calculated for plants bearing

Table 1: Variation of different pistil parts of normal bifid and trifid sigma in the rice genotype IR80155B

Pistil	Pistil part[1]	Stigma non-brush-shaped part (mm)	Stigma brush-shaped part (mm)	Total stigma length (mm)	Stigma width (mm)	Style length (mm)
Bifid	First branch of bifid stigma	1.44±0.04	0.63±0.04	2.07±0.05	0.55±0.03	0.29±0.03
	Second branch of bifid stigma	1.42±0.04	0.62±0.04	2.05±0.06	0.54±0.04	
Trifid	First branch of trifid stigma	1.30±0.03	0.56±0.04	1.89±0.06	0.51±0.03	0.31±0.04
	Second branch of trifid stigma	1.29±0.01	0.57±0.04	1.86±0.04	0.49±0.02	
	Third branch of trifid stigma	0.94±0.05	0.52±0.03	1.46±0.07	0.44±0.03	

[1]Pistil part as depicted in Fig. 1. Standard error was calculated from ten spikelets each from three panicles each from three accessions (i.e., number of accessions = 90)

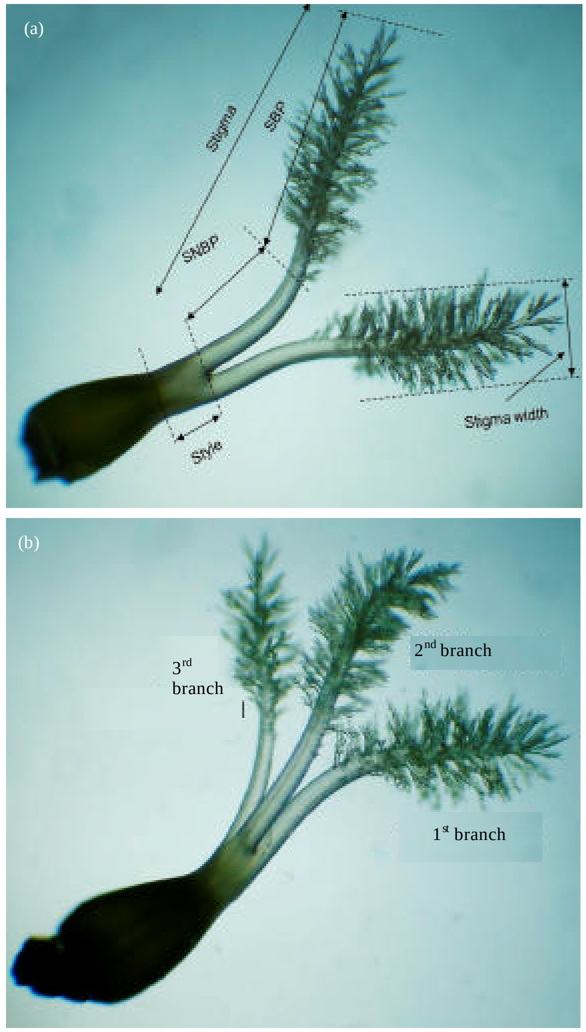

Fig. 1(a-b): Morphotypes of stigma in the rice line IR 80155B. (a) Normal bifid stigma with two branches, (b) Trifid stigma depicting the three branches, SNBP: Stigma non-brush-shaped part, SBP: Stigma brush-shaped part (Adapted from Takano-Kai *et al.*, 2011)

only bifid and plants with trifid stigma, in three panicles each from ten plants, it was found to be 52 and 55%, respectively. Furthermore, out of the total number of spikelets with exserted stigmas in a trifid stigma bearing panicle, 60% were of trifid morphotype. Further, the presence of trifid and bifid stigma has no detrimental effects in other flower related traits such as style length, total number of spikelets per panicle, anther length, spikelet length, spikelet breadth and spikelet width (data not shown).

DISCUSSION

Floral morphology in rice plant plays a critical role in determining the outcrossing in hybrid rice seed production. To the best of our knowledge the identification of a natural mutant plant in an *indica* maintainer background, bearing a trifid stigma, is the first report of its kind. Kim *et al.*

(2009), while studying the effect of over-expression of methyl jasmonate on drought stress by developing transgenic rice, observed various mutant morphotypes of rice pistil including trifid stigma. Further, due to the orthogonal position of the third branch with respect to the other two branches of trifid stigma, an extra pressure is exerted onto the glumes while closure of anthesis, which could force the other two branches to diverge outside and might lead to increase in stigma exsertion. Though, there is a significant reduction in pistil characteristics of trifid stigma, the extra stigma branch holds promise to increase the overall stigma exsertion percentage. Also, the trifid mutant would be helpful in understanding the molecular mechanism of pistil development in rice. Further studies are underway to understand the anatomy and genetics of this trait.

ACKNOWLEDGMENTS

The authors thank Mr. Dinesh C. Joshi, Executive Director and Dr. Usha B. Zehr, Director, Barwale Foundation, for providing all the facilities for the execution of this research work.

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