ELITE TRANSGENIC LINES OF BASMATI-370 REVEALED HIGH LEVEL OF LODGING RESISTANCE UNDER FIELD CONDITIONS

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Abstract

Lodging decreases crop yield, photosynthesis and grain quality. Three transgenic lines of Basmati-370 containing two Bt genes (*cry1Ac* & *cry2A*) were evaluated in the field along with non-transgenic parent as control. The experiment was repeated for consecutive two years. Transgenic rice revealed tremendous morphological variations associated with lodging such as short stature, more number of nodes, less internodal length, etc. The transgenic plants were 33% short in stature while average number of nodes was 3.4 as compared to 4.2 in control. Internodal length in transgenic plants ranged 6.41-8.61cm while in control it was found 14.0cm. These altered characteristics of plants made them resistant to lodging by conferring stiffness to the stem. The elite transgenic lines were 88% more resistant to lodging during the first year while they revealed 78.5% resistance in the second year. The transgenic lines were statistically different from the control during both of the years. Thus, it can be concluded that the elite transgenic lines of Basmati-370 are resistance to lodging as compared to its untransformed counterpart.

Keywords: Basmati; Bt; *cry1Ac*; *cry2A*; Lodging Resistance; Transgenic

Introduction

Rice (*Oryza sativa* L.) is the staple food for about 40% of the world's population. Almost 90% of the rice is grown and consumed in Asia [1]. It feeds more than two billion people in the developing countries of Asia [2]. The rice consuming community is increasing day by day and the number of rice consumers will be two fold by the year 2020 [3]. In Pakistan, during the financial year 2010-11, rice contributed 4.4% of value added in agriculture and 0.9% to GDP [4]. It is a valuable source of foreign exchange earnings for Pakistan. Pakistan is the third major exporter of rice, after USA and Thailand [5].

Lodging is the state of permanent displacement of stem from the upright position [6]. It is a common problem in most cereals and various other crops, including wheat, barley, oat, corn, sorghum, soybean, tomato, tobacco and rice [7]. It can reduce yield and quality of production [8]. Lodging has negative effects on cereal production and may reduce grain yield by 40% [9]. Lodging is usually caused by strong winds and/or rain and can be intensified by insects. The basis of lodging resistance has been studied in several crops and has focused on mapping of genes linked to stem stiffness, plant height or other genes associated with lodging resistance as in hexaploid wheat [10-11], rice [12], barley [13], maize [14-16] and soybean [17-18].

Bt rice is being developed at several institutions for the last several years [19]. Bt genes along with resistance to insect pests brought about

morphological and physio-chemical variations [20-21]. Transgenic plants are short in stature, have less number of nodes and less internodal length. These characteristics are directly associated with lodging. At National Centre of Excellence in Molecular Biology (CEMB), Lahore, Pakistan, Bt rice expressing two pesticidal genes (*cry1Ac* & *cry2A*) was evaluated in the field for two years. Transgenic rice showed characteristics that made it resistant to lodging. These traits impart strength to plant so that it can bear the stress applied due to wind and/or rain.

The local variety Basmati-370 transformed with Bt genes was tested in the field for insect resistance and environmental adaptability. The transformed plants showed greater level of resistance against target insects [19-20] along with lodging resistance. The aim of the study was to evaluate the performance of Bt rice under field conditions. Another objective was to make comparison between Bt and non Bt rice in various agronomic and morphological characteristics including lodging resistance.

Materials and Methods

Plant Material

Three elite transgenic lines (L-8-22-2, L-8-22-32 and L-8-22-35) of Basmati-370 containing two Bt genes developed at National Centre of Excellence in Molecular Biology (CEMB), Lahore, Pakistan [19-20,22] were selected for their evaluation in the field alongwith non-transgenic control parent. The experiment was repeated for consecutive two years at National Centre of Excellence in Molecular Biology

(CEMB), Lahore, Pakistan and two other locations of Punjab, Pakistan (Table-1).

Experimental Design

Three transgenic lines were evaluated for consecutive two years according to Randomized Complete Block Design (RCBD) with four replications. Bt rice was grown in central Punjab province that is rice growing area of Pakistan. Each replication had 140 plants of individual lines/variety with total 700 plants in a replication. Date of seed sowing and date of transplanting nursery are given in Table-1. Plant-to-plant distance was maintained at 45cm and row-to-row distance was 60cm to facilitate data recording and easy handling of plants. All agronomic requirements were fulfilled and studies were carried out according to the recommendations and biosafety guidelines [23].

Lodging Incidence (LI)

Percent lodging incidence was recorded at various growth stages and maturity after wind storm and/or rain. The weather conditions for both of the years during the rice growing season are provided in Table-2. Total number of plants and the plants that lodged were counted and expressed in percentage of the total number of plants. The damage due to lodging in untransformed control plants was expressed as 100% and the damage in different transgenic lines was expressed as percent lodging in comparison with the control.

Agronomical Characters associated with Lodging

Different agronomical characters including average plant height, number of nodes, internodal length, panicle length, stem length, number of leaves, yield per plant and number of spikelets per panicle were studied according to the standards described by the INGER [24]. Plant height was measured from the surface of the soil upto the top of the panicle and expressed in centimeters (cm). The number of nodes was counted per plant and the distance between two nodes was measured in cm. Panicle length was measured in cm starting from the last node upto the top of the longest sub-panicle. Stem length was calculated by subtracting panicle length from total plant height and denoted in cm. The yield of each plant was recorded separately in grams (g).

Statistical Analysis

All the data regarding lodging incidence and associated agronomical traits were subjected to Analysis of variance (ANOVA) followed by LSD-test to determine the significance of agronomical traits and lodging incidence. Mean values were used

for phenotypic correlation analysis to determine the relationship between physiological traits and lodging resistance.

Results

Lodging Incidence (LI)

The elite transgenic rice lines of Basmati-370 containing two Bt genes (*cryIAc* & *cry2A*) were found fairly resistant to lodging caused by wind storm and/or rain at different growth stages of the plant. The data recorded as a result of the two year's field experiments showed that the control plants lodged more as compared to transgenic. The elite transgenic lines were 87-89% more lodging resistant in the first year while they showed 78-79% increased resistance in the next year (Figure-1).

The correlation co-efficients between different parameters associated with lodging showed positive correlation between morphological data and Lodging Incidence, i.e. plant height ($r = 0.9948$ and $r = 0.9947$), stem length ($r = 0.9939$ and $r = 0.9937$), internodal length ($r = 0.9538$ and $r = 0.9546$) and no. of nodes ($r = 0.9909$ and $r = 0.9913$) during the year 2004 and 2005 respectively (Table-3).

Analysis of Lodging Resistance in association with Agronomical Traits

Plant Height (cm): The height of transgenic plants when measured in the field was significantly different from control. The transgenic lines were 105-110cm in height as compared to 156cm height of control (Table-4). Plant height was positively correlated with other important traits like number of nodes, internodal length and stem length which are associated with lodging resistance when correlation co-efficients were calculated (Table-5).

Number of Nodes & Internodal Length (cm): Number of nodes and internodal length are also important traits regarding lodging resistance. More no. of nodes and less internodal length were observed in transgenic elite lines conferring strength to the stem (Table-4). Moreover, no. of nodes and internodal length were found positively correlated with plant height (Table-5).

Stem Length (cm): The stem is strong if it has more number of nodes and less internodal length. The control plants had 43-49cm longer stems as compared to transgenic plants. So, it was found that transgenic plants have stronger stems than control (Table-4).

Yield per Plant (g): Yield is associated with several traits like plant height, strength of the stem, panicle length, etc. if the plant is high; it is likely to be

lodged due to the weight of the grains. The elite lines of transgenic rice have strong stem, which can bear

Table-1: Experimental Sites and Sowing Dates of Plant Material

S. No.	Year	Location	No. of Plants/Replication	Date of Nursery Sowing	Date of Transplanting
1.	Year-1	CEMB* Punjab University**	700 2000	June 10 June 10	July 14 July 17
2.	Year-2	CEMB	700	June 14	July 20

* National Centre of Excellence in Molecular Biology (CEMB), West Canal Bank Road, Lahore, Pakistan.

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Table-2: Monthly weather conditions in Lahore during rice growing season.

S. No.	Month	Av. Temperature (°C)		Av. Wind Speed (km/h)			No. of Thunderstorms	No. of Rains
		Min.	Max.	Min.	Av.	Max.		
YEAR-1								
1.	July	27	36	0	9	74	5	5
2.	August	25	34	0	11	171	10	9
3.	September	24	35	0	6	37	2	4
4.	October	16	29	0	6	37	2	4
5.	November	11	26	0	2	21	1	1
YEAR-2								
1.	July	25	34	0	8	45	7	14
2.	August	25	35	0	6	48	12	12
3.	September	22	33	0	7	196	5	6
4.	October	16	32	0	3	40	1	1
5.	November	09	26	0	3	21	0	0

Av. = Average

Min. = Minimum

Max. = Maximum

Source: Weather Data Available Online at [<http://www.wunderground.com/history/airport/OPLA/2004/9/1/MonthlyHistory.html>].

Table-3: Correlation co-efficient (r) analysis between lodging incidence and some associated agronomical traits

S. No.	Name of Agronomical Trait	Correlation co-efficient (r)	
		Year-1	Year-2
1.	Plant Height (cm)	0.9948	0.9947
2.	Stem Length (cm)	0.9939	0.9937
3.	Internodal Length (cm)	0.9538	0.9546
4.	No. of Nodes	0.9909	0.9913

Table-4: Morphological Characters associated with Lodging Resistance

S. No.	Line No./ Variety	Plant Height (cm)	No. of Nodes	Internodal Length (cm)	Panicle Length (cm)	Stem Length (cm)	No. of Leaves	Yield/ Plant (g)
1.	L-8-22-2	109 ^a	3.5 ^a	8.68 ^a	26.9 ^a	82.9 ^a	111.8 ^a	19.6 ^a
2.	L-8-22-32	105 ^a	3.4 ^a	6.56 ^a	26.4 ^a	78.6 ^a	97.2 ^b	18.3 ^a
3.	L-8-22-35	110 ^a	3.4 ^a	6.41 ^a	26.7 ^a	83.8 ^a	95.6 ^b	19.3 ^a
4.	Control	156 ^b	4.2 ^b	14.0 ^b	28.3 ^b	127.6 ^b	52.5 ^c	16.6 ^b

Note: The values with the same letters are statistically same and the values with different letters are significantly different from each other according to LSD-test at P = 0.05

Table-5: Correlation Co-efficients (*r*) associated with Lodging Resistance

Trait	Plant Height	No. of Nodes	Internodal Length	Panicle Length	Stem Length	No. of Leaves	Yield/Plant
Plant Height							
No. Nodes	0.9223						
Internodal Length	0.8943	0.9074					
Panicle Length	0.2249	0.4561	0.4932				
Stem Length	0.9976	0.9031	0.8732	0.1589			
No. of Leaves	-0.8934	-0.8104	-0.8184	-0.3418	-0.8778		
Yield/Plant	-0.6956	-0.5194	-0.6776	-0.2380	-0.6821	0.8299	
No. of Spikes/Panicle	0.7891	0.8198	0.8978	0.6595	0.7584	-0.7343	-0.5190

Figure-1: The percent lodging resistance shown in transgenic lines and non-transgenic control. The transgenic lines revealed high level of resistance to lodging as compared to control plants.

Note: the bars with the same letters are not statistically different according to the LSD-test at $P = 0.05$

heavy load of grains conferring lodging resistance to the plants (Table-4).

Discussion

Breeding for lodging resistance has focused on the selection of short plants with short, thick internodes [25-26]. For example, in a population derived from a cross of wheat $\times$ spelt, plant height explained 77% of the phenotypic variance of lodging across three environments [10].

The data from the two years of the field trials

showed higher lodging resistance in elite transgenic lines of Basmati-370. They showed higher correlation coefficients in major lodging related traits (Table-5). Plant height, stem length, no. of nodes and internodal length were statistically different during both of the years. Lodging Incidence (LI) showed significantly different correlation analysis between morphological data like plant height, stem length, internodal length and no. of nodes during the first and second year respectively. During the first year, the transgenic lines were 87-89% resistant to lodging and they were 78-79% lodging resistant in the second year. This difference is probably due to the varied weather conditions during these years. The weather was more intense in second as compared to that of first. (Table-2).

The cause of the lodging is the lack of proportionality between the weight of the upper part and the sturdiness of the basal part. In breeding for lodging resistance, the target has been limited to reduce plant height, which affects the weight of the upper part and the centre of gravity, through the use of semi-dwarf and dwarf genes [27]. Although major genes related to plant height (*Rht* genes) were responsible for increasing lodging resistance, several other traits independent of plant height were shown to be important such as root and shoot traits, and various component of plant yield. Due to unknown reason, the integration of Bt genes resulted in the reduction of plant height [19, 21] that is the desirable character as far as lodging resistance is concerned.

It is obvious from several reports that the most important trait in this study, lodging in the field, was correlated with plant height and the length of the internode. Plant height is the single trait that influences lodging more than any other trait [28]. There are numerous studies that have been conducted to show the genetic control of these characters [29-31]. Yield components such as grain number and weight were shown to be an indicator of plant susceptibility to lodging [28].

The detection and introduction of semi-dwarf lines was one of the main factors responsible for the higher yields of rice and wheat in the "Green Revolution" [10, 32]. Crook and Ennos [33] reported that, in modern varieties, stem lodging is relatively un-common as a result of selecting for shorter, more rigid stems over the last 40 years. The optimum plant height for maximum photosynthetic capacity is between 70 and 100 cm in wheat [34]. Rice lodging resistance could be improved by means of shortening the length of basal internodes, strengthening the hardness of the basal culm [35]. Hence, the transgene insertion is a random process. Usually, the transgenic plants are different from their parents in number of traits. These differences are due to disruption of host genome and causes pleiotropic effects including plant height, maturity, production, etc. [36-37]. These altered traits may be beneficial or otherwise. In this study, the beneficial pleiotropic effect due to transgene insertion is discussed and reported.

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