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Comparing the Agronomic and Grain Quality Characteristics of Transgenic Rice Lines Expressing *cry1Ab* vs. Non-Transgenic Controls

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Abstract: This study aimed to investigate and compare the agronomic and grain quality attributes of three advanced backcross-derived transgenic rice lines expressing synthetic *cry1Ab* gene vs. non-transgenic control in a Randomized Complete Block Design (RCBD) under field conditions. The data exhibited that transgenic rice lines, Neda and Nemat were higher in height, earlier in maturity and highly resistant to striped stem borer (*Chilo suppressalis*) in comparing with non-transgenic varieties. In contrast, no significant difference was observed for transgenic Khazar as compared to its control, except for 1000-grain weight. Laboratory tests for grain physicochemical properties showed no significant variations between transgenic lines and non-transgenic controls. However, some variations for traits like Amylose Content (AC) and Gel Consistency (GC) were seen for transgenic Neda and Khazar, respectively. As regards the rice striped stem borer natural infestation, field-release experiment indicated that all three transgenic rice lines conferred a very high degree of resistance to rice striped stem borer as compared to non-transgenic check varieties.

Key words: *Bt*-Rice, *cry1Ab*, insect resistance, agronomic, grain quality traits

INTRODUCTION

Rice is the most important food crop and the staple food for 40% of the world population. More than 90% of rice is produced and consumed in Asia (Khush and Brar, 2002). The global population exceeded 6 billion in 2000 and it is necessary to produce 50% more food to meet the increasing needs of growing population by 2025 (Khush, 2001).

Rice productivity is severely affected by several abiotic and biotic factors, including damage caused by pests and diseases. Stem borers are chronic pests in all rice-growing environments in Asia. They cause more yield loss than any other group of rice insect pests (Savary *et al*., 2000) and 50% of insecticides employed in rice fields are targeted at *Lepidopteran* insects (Heong *et al*., 1994; Huesing and English, 2004). Use of chemicals not only increase the rice production cost but also causes health harms to rice farmers as well as deteriorates the rice field environment (Litsinger *et al*., 2005).

Recent advances in genetic engineering of crops opened new avenues for production of transgenic plant with new genetic properties. Transformation of rice with genes from a soil bacterium *Bacillus thuringiensis* (*Bt*) is a common approach to confer resistance to insect infestations. Insecticidal crystal proteins that produced by *Bt* genes are highly toxic to *Lepidopteran*, *Dipteran* and *Coleopteran* insects (Hofte and Whiteley, 1989) and there are several successful reports on effective control of insect species by transgenic rice plants (Ghareyazie *et al*., 1997; Shu *et al*., 2000; Tu *et al*., 2000; Ye *et al*., 2003; Ramesh *et al*., 2004; Bashir *et al*., 2005).

Commercial success of a cultivar is dependent on morphological and agronomic characteristics. Thus field evaluation of transgenic plants is necessary to determine success of transformation in breeding programs. Schuh *et al*. (1993) observed tremendous heritable genomic changes which more common in transgenic rice plants transformed via *Agrobacterium* as compared with biolistic transformation. In the evaluation of agronomic and morphological characteristics of *Agrobacterium*-transformed *Bt* rice plant by Shu *et al*. (2002), they observed tremendous variations in plant height, seed fertility, grain size of T_1 lines. Jiang *et al*. (2000) reported the predominated aberrations were dwarfism and early-or

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late-maturity in transgenic plants produced by particle bombardment method whereas phenotypic variation such as plant height obtained in transgenic plants produced by *Agrobacterium*.

The transgenic rice could be used as a precious insect-resistant germplasm to be utilized in traditional breeding programs. Therefore, the breeding utilization of transgenic rice by means of crossing and backcrossing with elite cultivars is being undertaken and rapid progress has been made in the development of insect-resistant transgenic rice in Iran. Despite the knowledge available on development of *Bt* rice lines through traditional breeding strategy, the data on agronomically important traits has not yet been presented. Thus this study aimed to investigate whether the agronomic and grain quality traits of the recurrent parents were full recovered in the backcross-derived lines.

MATERIALS AND METHODS

Plant materials and experiment design: The genetic materials used in this study were three transgenic *Bt* lines namely Khazar, Neda and Nemat, displayed stable resistance to rice pests, confirmed by bioassay and PCR analysis (Kiani *et al*., 2008). These lines were improved through classical backcrossing method using transgenic Tarom molaii, containing a synthetic *cry1Ab* gene (Ghareyazie *et al*., 1997) as donor parent. Seeds were provided from Rice Research Institute, Amol, Iran. These transgenic lines along with their non-transgenic controls transplanted with a delay of one month on early June (normal transplantation is early May) to ensure that maximum tillering and booting stages coincide with the peak damage of stripped stem borer (*Chilo suppressalis*) that occurs on late August in north of Iran. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications under field conditions at Sari University of Agricultural Sciences and Natural Resources during 2007. Each plot was 6 m^2 with 25×25 cm planting pattern (a single plant hill^{-1}). NPK fertilizers applied at the rate of 250, 100 and 50 kg ha^{-1}, respectively. Field management was traditional except pesticide application.

Data collection: Agronomic traits like days to heading, plant height (cm), tiller number, panicle length (cm), panicles plant^{-1}, days to maturity, 1000-grain weight (g), filled and unfilled grains panicle^{-1} and yield (t ha^{-1}) were recorded. Field resistance to insect pests was investigated by observation of White Head (WH) and Dead Heart (DH), symptoms for sensitivity to Stripped Stem Borer (SSB), during the whole growth period of plants.

The grain quality properties such as Gelatinization Temperature (GT), Gel Consistency (GC), Amylose Content (AC), elongation ratio, milled rice (%) and head rice (%) were also measured (Graham, 2002).

Analysis of data: Data was analyzed by analysis of variance using MSTATC software and mean comparisons were performed by Duncan's Multiple Range Test at 1% statistical level.

RESULTS AND DISCUSSION

Agronomic characteristics: The analysis of variance for agronomic traits showed significant differences between genotypes. Table 1 presents the mean performance of transgenic lines in comparison with their controls.

Based on results obtained, no significant differences were found between transgenic lines and their respective controls for traits such as tiller number, panicle per plant, filled and unfilled grains per panicle (Table 1). Thus, these traits of the recurrent parents were fully recovered in the backcross-derived lines. The average number of tillers ranged between 8.3 and 19.3 plant^{-1} for Khazar- and Neda-*Bt*, respectively (Table 1). These lines exhibited the lowest and the highest panicle per plant, respectively. As regards filled grain per panicle of various lines, Neda-*Bt* along with its control produced minimum filled grain per panicle while the highest filled grains per panicle belonged to Khazar-*Bt* and its control. Similarly, these lines showed the lowest and highest unfilled grain per panicle, respectively.

Significant differences were observed for days to heading between Khazar- and Neda-*Bt* with their controls ($p<0.01$). Khazar-*Bt* was 4 days late in panicle exertion while Neda-*Bt* was 3 days early in that trait. As regards to maturity, despite of late flowering of Khazar-*Bt*, it was harvested at the same time with its control (124 days). But the early panicle initiation of Neda-*Bt* leads to its earliness as comparing to its control. However, the difference in the days to heading for Nemat-*Bt* was not found, but for days to maturing the difference was observed and Nemat-*Bt* was 6 days early in maturity as comparing with its control. The variations in days required to flowering and maturity were also reported by Bashir *et al*. (2004) and Rahman *et al*. (2007). Early or late maturity of transgenic rice plants which produced by particle bombardment method also reported by Jiang *et al*. (2000).

The plant height varied between 105.7 cm in Neda to 129.7 cm in Khazar. It seems that all of *Bt*-lines, except Khazar-*Bt*, had increased height than their controls (Table 1). Phenotypic changes in plant height of transgenic plants were also reported by Jiang *et al*. (2000) and Shu *et al*. (2002).

Table 1: Agronomic characteristic of transgenic *Bt* rice lines along with their controls

Bt lines/ Non-*Bt* control	Plant traits									
	Days to heading	Plant height (cm)	Tiller No.	Panicle length (cm)	Panicle plant^{-1}	Days to maturity	1000-grain weight (g)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Yield (t ha^{-1})
Khazar-*Bt*	101.3c	126.3a	8.3c	27.3b	8.3d	124c	27.4b	163ab	40ab	5.84a
Control	96.7d	129.7a	10.0c	25.3b	9.3d	124c	25.4c	188a	50a	4.08c
Neda-*Bt*	102.7bc	119.0b	17.0ab	30.0a	16.3ab	131b	28.7ab	119c	16cd	6.35a
Control	105.3a	105.7c	19.3a	27.0b	18.0a	137a	30.1a	117c	7d	5.96a
Nemat-*Bt*	104.0ab	116.0b	14.3b	32.0a	13.7c	133b	28.7ab	139bc	33abc	4.83b
Control	103.7ab	106.7c	15.3b	30.3a	14.3bc	139a	29.3a	127c	22bcd	5.22b

Similar letter(s) in each column refers to non significant difference at 0.01 statistical level

Table 2: Grain quality characteristic of transgenic rice lines along with their controls

Bt line/ Non-*Bt* control	Physicochemical traits					
	Amylose content (%)	Gelatinization temperature	Gel consistency (mm)	Elongation ratio	Milled rice (%)	Head rice (%)
Khazar-*Bt*	23.5	3.5	54	1.85	55.3	47.7
Control	23.6	3.2	75	1.79	58.7	48.8
Neda-*Bt*	23.1	3.2	53	1.80	70.3	66.1
Control	27.3	3.8	45	1.69	62.9	53.6
Nemat-*Bt*	26.1	7.0	40	1.86	60.8	56.8
Control	26.8	6.7	37	1.83	62.6	52.2

There is significant difference between Neda-*Bt* with its control for panicle length whereas the Neda-*Bt* had 3 cm longer panicle (30 cm) than its control (27 cm). While for other *Bt* lines, no significant differences were found for that trait. Changes in panicle length have been reported by Bashir *et al*. (2004).

Amongst transgenic lines Kazar- and Neda-*Bt* had more yield than their controls while Nemat-*Bt* had lower yield than its control (Table 1). The maximum yield belonged to Neda-*Bt* (6.35 t ha^{-1}) followed by Neda (5.96 t ha^{-1}) and Khazar-*Bt* (5.84 t ha^{-1}) while the minimum yield was obtained in Khazar (4.08 t ha^{-1}). The rest, Nemat-*Bt* along with its control, produced moderate paddy yield.

Grain physicochemical characteristics: Observations regarding physicochemical characteristics of transgenic lines in comparison with their controls are given in Table 2. The data showed that most of characteristics like Gelatinization Temperature (GT), elongation ratio, milled rice, head rice for various transgenic lines are at par with those of their counterparts. The Amylose Content (AC) of Nemat- and Khazar-*Bt* was comparable with their controls while the amylose percentage of Neda-*Bt* obtained 23.1 (intermediate) comparing with high amylose percentage in its control (27.3%). For Gel Consistency (GC), only Khazar-*Bt* showed variation for that trait, whereas the GC in this variety declined to 54 (medium GC) comparing with soft GC in its control (75).

Field evaluation for insect-resistance: Insect damage was recorded by counting Dead Hearts (DH) and White Heads (WH) in each plot and the mean performance of genotypes presented in Fig 1. Damage in all transgenic lines was less than their controls and they exhibited high degree of resistance against natural infestation of Stripped Stem Borers (SSB). No dead hearts were observed for transgenic lines in vegetative stage. The mean rates of white heads for transgenic lines were less than 1% while for their controls were more than 9%.

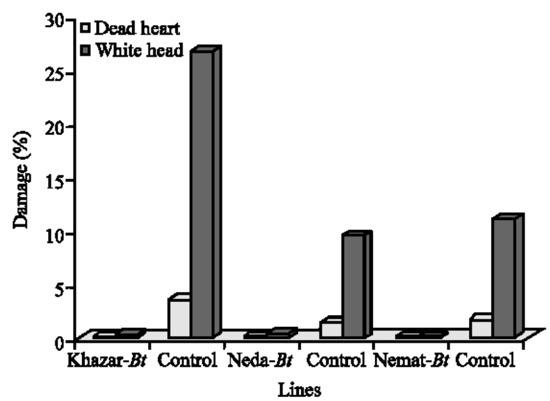

Fig. 1: Response of various transgenic rice lines to natural infestation of striped stem borer (2007)

In conclusion, Neda and Nemat transgenic lines, which were developed through classical backcrossing approach, had more height, earlier in maturity and highly resistant to SSB comparing with their non-transgenic controls. In contrast, transgenic Khazar which was improved by the same breeding method had no difference with its non-transgenic control, except for grain weight.

Laboratory tests for physicochemical characteristics showed no significant variations between transgenic lines with non-transgenic controls. However, some variations for traits like AC and GC were seen only for transgenic Neda and Khazar, respectively. The information obtained in this study has confirmed that the *Bt* gene could be transferred into adapted elite cultivars through a classical backcrossing program to enhance insect-resistant germplasm to be employed in traditional rice breeding program and development of transgenic rice with elite agronomically important traits.

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