Fusarium species can affect grain quality, producing a number of toxic compounds, including fumonisins (Munkvold, Ann. Rev. Phytopathol. 41:99-116, 2003), involved in human and animal health (CAST, Task force rep. 38. Ames, IA: CAST, 2003). Therefore, the development of maize plants carrying resistance to *Fusarium* ssp. (Lew et al., Cereal Res. Commun. 25:467-470, 1997) as well as resistance to mycotoxin production is highly desired.

In maize endosperm, a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins (Soave et al., Cell 27:403-410, 1981). It was shown that the *b-32* genes form a small gene family (Hartings et al., Genet. Res. Camb. 65:11-19, 1995).

Endosperm-derived native b-32 was shown i) to enzymatically inactivate ribosomes, through its capacity to specifically modify rRNA, inhibiting protein synthesis in vitro (Maddaloni et al., J. Genet. Breed. 45:377-380, 1991; Bass et al., Plant Cell 4:225-234, 1992), and ii) to inhibit the growth of *Rhizoctonia solani* mycelia in in vitro bioassays (Maddaloni et al., Transgenic Res. 6:393-402, 1997). Similarly, Balconi et al. (European J. Plant Pathol. 117:129-140, 2007) found that maize RIP-b-32 protein was effective in wheat transgenic lines as an anti-fungal protein by reducing *Fusarium* head blight (FHB) symptoms.

To verify if maize plants expressing b-32 in various tissues have an increased tolerance to fungal pathogens, transgenic plants were obtained through genetic transformation using a chimeric gene containing the *b-32* coding sequence downstream of a constitutive *35SCaMV* promoter. A set of four independent homozygous progenies expressing b-32 were selected for a detailed analysis of b-32 expression in leaves and for pathogenicity tests.

The integration patterns of the *b-32* transgene were determined by genomic Southern-blots using *Eco*RI-*Hin*dIII double digests as appropriate enzymes to estimate the transgene copy number. Four Basta resistant progenies (SM 3.4; SM 16.1; SM 19.4; SM 20.2), one Basta sensitive progeny (SM 20.4) and the B73 inbred line were analyzed using a *nos-bar* (resistance gene) and *b-32* probe. The *b-32* probe detected the presence of the *b-32* endogenous gene in the control B73 inbred line and in the negative control progeny SM 20.4. A band at the same position is present in transgenic progenies SM 3.4, SM 16.1, SM 19.4, and at a slightly different position in the SM 20.2 progeny, and is most likely due to a recombination event involving the endogenous gene and transgene, indicating that several insertion events have occurred. In addition to the endogenous *b-32* band (native gene), all transgenic progenies contained a few additional bands corresponding to insertions of the transgene. The unique hybridization patterns observed indicated that each progeny resulted from independent transformation events.

Comparison of b-32 expression among various individuals was performed, after immuno-blot imagine scanner acquisition, using IMAGINE MASTER 1D Elite Version 3.01 (NonLinear Dynamyc Ltd) software. A differential b-32 content in leaf protein extracts was recorded in the transgenic progenies. As expected, SM 20.4, i.e. the negative control, showed non-detectable b-32 content (n.d.) in leaf tissues. Proteomic experiments were performed on protein leaf extracts of one of the transgenic lines expressing a high b-32 level (SM 20.2) and were compared to the negative control progeny (SM 20.4). The overlapping of the two-dimensional electrophoresis maps clearly showed the presence of additional spots in SM 20.2 progeny in comparison to SM 20.4 progeny, which was Basta-sensitive and b-32 western negative. These spots were cut from gels and digested with trypsin to allow protein identification by the "peptide mass fingerprinting" (PMF) strategy (Pappin et al., Curr. Biol. 3:327-332, 1993). Both induced b-32 spots and herbicide resistance spots were successfully identified.

Transgenic progenies were tested in bioassays to evaluate the response to *Fusarium* attack in leaf tissues. Preliminary experiments supported the choice of bioassay parameters for a reliable evaluation of transgenic progenies. Results indicated that fungal colony diameters measured on the inoculated leaves of SM 20.4 (the negative control) were, at all detection times, significantly (Student's t test = $P \leq 0.05$) larger than those observed in all four progenies expressing b-32. A good correlation between the b-32 content in the leaves and the level of resistance to *Fusarium* attack was observed. In the case of progenies with high b-32 content in the leaves, in addition to reduced mycelial growth around the cut edges of the leaves, very weak growth on leaf surfaces was observed in comparison with progeny exhibiting the lowest b-32 content in leaves.

The data obtained indicate that maize b-32 is an effective antifungal protein in reducing progression of *Fusarium* infection. Additionally, the reduction in *Fusarium* induced symptoms was related to b-32 concentration in leaf tissues. The expression of antifungal proteins in plants or plant tissues in which they are not normally expressed may be very useful in reducing pathogen colonization and growth; from this perspective, a reduction of *F. verticillioides* infection in maize leaves and stalk could be very useful in limiting the spread of fungal infection to the exposed silks, and consequently, in the reduction of grain fumonisin contamination.

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Effect of water stress on performance of maize inoculated with *Glomus* sp. isolated from a tea plantation of Keonjhar, Orissa

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Arbuscular mycorrhizal (AM) fungi have been reported to help maize grow under drought and other stresses (Gupta and Routaray, Acta Agric. Scandinavica 55(2):151-157, 2005; Subramanian et al., New Phytol. 129:643-650, 1995; Subramanian and Charest, Mycorrhiza, 7:25-32, 1997). We have expanded this work to study the effects of tea plantation arbuscular mycorrhizal (AM) fungi on maize grown under well-watered and water-stressed pot culture conditions. Fungi were isolated from the drought-prone tea (*Camellia sinensis* L.) plantations of the Bhuyanpirh tea estate of M/S Orissa Tea Plantation Limited, which is situated in Tarmakanta about 48 km away from Keonjhar, Orissa, India. The plantation area has an elevation of more than 600 m and was once covered by dry and mixed deciduous sal forests. The soil is red clay-loam and poor in nutrient content. The rhizosphere soil of different tea plants was collected 10 inches below ground in polythene bags for

analysis. Bhuyanpirh tea plantations have not been previously surveyed for AM fungi.

Isolation and characterization of AM spores used published methods (Gerdemann and Nicolson, Trans. Br. Mycol. Soc. 46(2):235-244, 1963; Kormanic and McGraw, Pp. 34-45 in Methods and Principles of Mycorrhizal Research, American Phytopathological Society, 1982; Schenck and Perez, P. 245 in Manual for the Identification of VA Mycorrhizal Fungi, INVAM, 1987). 15-day-old maize seedlings were planted in earthen pots after treatment with or without AM fungi. Roots sampled after 75 days of growth had higher mycorrhizal colonization in water-stressed plants compared to well-watered plants (Fig. 1). Biomass and growth was higher in mycorrhizal than nonmycorrhizal plants irrespective of water treatments (Fig. 2). However, the plants irrigated with alter nate watering schedules showed higher biomass than those treated with daily watering.

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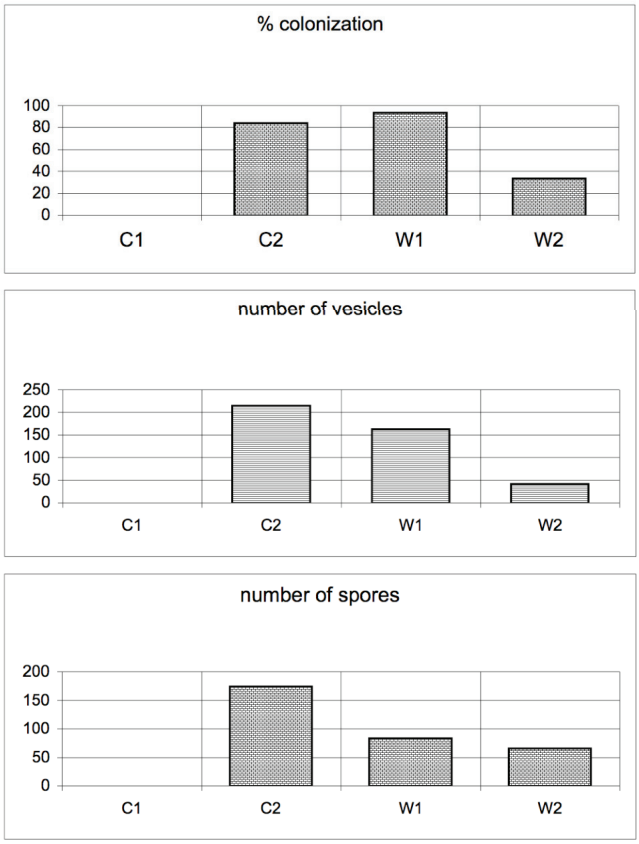

Figure 1. Status of mycorrhization in maize roots inoculated under different treatments. Abbreviations: C1 = daily watering, C2 = AM + daily watering, W1 = AM + alternate day watering , W2 = AM + watering at two day intervals.

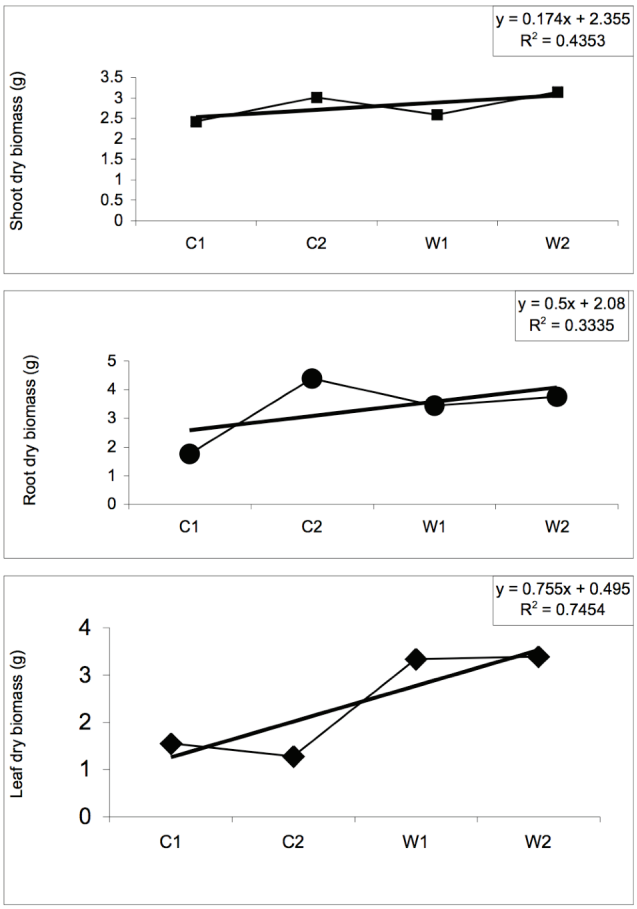

Figure 2. Effect of AM fungi on growth (measured after 75 days of experiment) of maize (host plant) grown under different watering schedules. For abbreviations see Figure 1.

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Analysis of the effect of RAD51 on the spontaneous mutation frequency in maize haploids

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Rad51p plays a central role in homologous recombination and the repair of double-strand breaks in *Saccharomyces cerevisiae*. Double mutants of the two *Zea mays* L. *rad51* homologs (*Zmrad51A1* and *Zmrad51A2*) are viable and develop well under normal conditions in diploids. However, they have meiotic abnormalities, are male sterile and have greatly reduced seed set (Li et al., Genetics 176: 1469-1482, 2007). In this article, these alleles will be referred to as *rad51A1* and *rad51A2*.

The purpose of this study was to determine if a higher spontaneous mutation frequency is present in maize plants with both the *rad51A1* and *rad51A2* mutations. For this purpose, the frequencies of mutant sectors on the 5th leaves of haploid plants that were *rad51A1* and *rad51A2* or *Rad51A1* and *rad51A2* were compared. In haploids, all mutant sectors will be detectable because they will not be covered by the non-mutant allele on the normal homolog.