Transgenic Approaches for Development of Disease Resistance in Banana

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Abstract

Banana (Musa spp.) is an important food and cash crop worldwide. Diseases and pests pose the most serious constraint to banana cultivation. Among the diseases, Fusarium wilt and banana bunchy top virus (BBTV) are the most important economically. We have explored different transgenic approaches for development of efficient resistance in banana against these two diseases. For countering Fusarium wilt, we have overexpressed Petunia floral defensins using a strong constitutive promoter in transgenic banana plants. We have also tested a host-induced gene silencing strategy targeting two vital fungal genes to obtain Fusarium resistant banana plants. For development of BBTV resistant banana plants also, we have used a host-induced gene silencing approach utilizing the full and partial coding sequence of the viral replication initiation protein. Successful bioassays performed in controlled greenhouse conditions have shown the efficacy of using these strategies to develop disease resistant banana plants.

Fusarium wilt of banana

Fusarium wilt (also called as the Panama disease) is the most important and devastating disease of banana. *Fusarium oxysporum*, which causes wilt disease in several crop plants, is known to be a soil borne ubiquitous species complex of plant pathogenic fungi that includes several *formae speciales*, each possessing a high degree of host specificity (Ploetz, 2006). *Fusarium oxysporum* f. sp. *cubense* (Foc) is the causal organism of Fusarium wilt of banana. Foc infects and subsequently occludes the xylem vessels of the banana roots leading to wilting and ultimately fruit develops parthenocarpically. Biotechnological approaches such as genetic transformation methods can be employed to incorporate specific useful characters relatively rapidly in proven elite cultivars without compromising their fundamental genetic makeup. Establishment of an efficient and reproducible protocol for plant regeneration using in vitro methods is a crucial prerequisite for the successful genetic transformation of banana for the incorporation of desirable traits. We have described herein our results on efficient transgenic approaches developed for Fusarium wilt and banana bunchy top virus disease resistance in banana plants.
death of the banana plant. Fusarium infected banana plants show progressive yellowing of leaves, cracking of the pseudostem and also discoloration of the corm tissue. As the fungus resides deep inside the plant in the xylem vessels, application of external fungicides is not effective in controlling Foc. Foc chlamydospores survive for decades in the soil, thereby making it permanently unsuitable for growth of healthy banana plants. Out of the four recognized races of Foc, race 1, which caused the epidemic in ‘Gros Michel’ plantations, also damages ‘Lady Finger’ (AAB) and ‘Silk’ (AAB) subgroups. Foc Race 2 infects cooking varieties like ‘Bluggoe’ (ABB) and race 4 can attack all known edible banana varieties including the ‘Cavendish’ (AAA) varieties (which are resistant to race 1) (Buddenhagen, 2009). Foc Race 4, presently is the biggest concern as it has now spread to the African continent also. As there are no natural sources of resistance available against Foc race 4 from any cultivated banana, the only option available is the development of Fusarium wilt resistance in elite banana varieties by genetic engineering. This is especially relevant to banana owing to its triploid nature and the parthenocarpic fruit development in most of the edible elite varieties.

In this direction, we have implemented a multidimensional approach towards engineering banana with efficient Fusarium wilt resistance. This includes the use of potent anti-microbial defensins with C-terminal prodomains like Petunia floral defensins as the initial first barrier to the Foc hyphal entry followed by host-induced gene silencing of vital fungal genes mediated by siRNAs during the intermixing of fungal and plant cytosol to ultimately the prevention of programmed cell death in banana (possibly induced by Foc infection) by overexpression of select anti-apoptotic genes.

**Use of novel *Petunia* floral defensins for development of efficient resistance against Fusarium wilt in banana**

Antimicrobial peptides form a potent group of defense related proteins which have been used in development of resistance against a range of plant pathogens (Thomma et al., 2002). Defensins constitute one of the biggest families of these antimicrobial peptides in plants. Plant defensins, which generally possess 3-4 disulfide linkages, are particularly potent against fungal targets. Defensins target specific lipids on the fungal membranes thereby permeabilizing them to inhibit fungus growth. Floral defensins, derived from floral tissues of plants, have been demonstrated with potent growth inhibitory activity towards pathogenic filamentous fungi especially belonging to the *Fusarium oxysporum* species complex (Lay et al., 2003). In our laboratory, we amplified full length coding sequences of two *Petunia* floral defensins, *PhDef1* and *PhDef2* (having C-terminal 31 and 27 amino acids long prodomains) from *Petunia hybrida* floral tissues derived cDNA (Ghag et al., 2012). These were constitutively overexpressed in transgenic banana plants using embryogenic suspension culture cells as explants for *Agrobacterium*-mediated genetic transformation as described previously (Ganapathi et al., 2001). Efficient expression of these antifungal defensins in an elite banana cv. *Rasthali* resulted in significantly enhanced resistance against infection of *Fusarium oxysporum* f. sp. *cubense* in the transgenic plants as compared to equivalent controls as indicated by in *vitro* and ex *vivo* bioassays. Transgenic banana lines which were expressing any of the two ectopic defensins were seen to be less chlorotic and showed significantly less discoloration of the corm region of the banana plant as compared to untransformed controls (Fig. 1). Further these transgenic banana plants were phenotypically normal and no growth stunting was observed at any regeneration or growth stage. In contrast, another group of transgenic banana plants which constitutively expressed C-terminal truncated defensins (without the coding region for the pro-domains) were stunted from initial stages of regeneration and the corresponding transformation efficiency was also significantly less (Ghag et al., 2013). This indicated that expression of such potent defensin proteins without their cognate inactivating C-terminal pro-domains runs the risk of severely affecting the growth and development of the transgenic plants.
Host induced gene silencing (HIGS) of vital fungal genes in transgenic banana plants for development of sustainable resistance against Fusarium wilt

Although potent defensin molecules have been used for development of resistance against a host of fungal diseases in plants, they mostly function in a nonspecific manner at the fungal entry barrier level and hence are vulnerable to the evolution of resistance in the fungal pathogens against these defense molecules. Also, due to their broad spectrum non-specific mode of action several beneficial fungi, most importantly the arbuscular mycorrhizal fungi, are unnecessarily targeted by these defensin molecules affecting the normal growth and development of the host plants. To overcome these limitations, we investigated whether intron hairpin RNA (ihpRNA) (Wesley et al., 2001) mediated in plants expression of small interfering RNAs (siRNAs) targeted against important fungal genes (like velvet and fusarium transcription factor 1) in transgenic banana plants can lead to development of effective resistance against Foc in banana. Since this phenomenon works at the level of sequence homology between the siRNAs and the targeted fungal genes, no unintended off targets are expected to be affected by this strategy. Towards this goal, two fungal specific vital genes were selected and their partial sequences were assembled as ihpRNAs in plant binary vectors (Ghag et al., 2014). These binary vectors which were designed to express the two ihpRNA cassettes in constitutive fashion in transgenic banana plants were transformed into embryogenic cell suspensions of banana cv. Rasthali by Agrobacterium-mediated genetic transformation. Transformed banana lines which were confirmed to
express the fungal genes targeted siRNAs tolerated the 6 weeks long greenhouse Foc resistance bioassays without demonstrating any external and internal symptoms of Foc (Fig. 2). Presence of specific siRNAs expressed from the two ihpRNAs in transgenic banana plants was shown through Illumina sequencing of the total small RNAs isolated from the two groups of transgenic banana plants.

Banana Bunchy Top Disease (BBTD) of banana

Banana Bunchy Top Virus (BBTV) derives its name from the typical ‘bunchy top’ appearance shown by the infected banana plant. Under severe infection, the plant top gets choked with a rosette of narrow, short, erect and brittle leaves having yellow margins, which ultimately appear to be burnt. The infected leaves characteristically show dark green dots and dashes beside the minor leaf veins, which appear like hooks as they move into the edge of the midrib. Infected banana plants seldom produce a bunch and in case of late infections, a distorted bunch may be formed. BBTV is most commonly transmitted by banana aphid, Pentalonia nigronervosa and it can readily transmit through all different forms of vegetative planting material like suckers, corms and tissue culture plantlets (Hafner et al., 1995). Since no natural sources of resistance are known, use of planting material which is free from BBTV is the best way to control BBTD. Since the aphid vector is widespread and difficult to treat via systemic insecticides, a localized chance infection runs the risk of developing into a full-blown outbreak resulting in total loss of production. The severity of the disease coupled with complete lack of resistant cultivars necessitates the development of BBTV resistant banana cultivars through genetic engineering.

Host induced gene silencing (HIGS) of replication initiation protein gene of BBTV for development of sustainable resistance against BBTD

Using an approach similar to the one used to develop Fusarium wilt resistant banana plants, we explored the use of ihpRNA transcripts corresponding to viral master replication initiation protein (Rep) (Horser et al., 2001) to generate BBTV resistant transgenic banana plants. Two ihpRNA binary vector constructs namely ihpRNA-Rep and ihpRNA-ProRep were generated using Rep full coding sequence or Rep partial coding sequence along with its 5’ upstream region respectively and castor bean catalase intron. The Rep coding and 5’ upstream region were amplified from genomic DNA isolated from leaves of
a BBTV infected banana plant. The two constructs were successfully transformed into banana embryogenic cells of banana cv Rasthali. ihpRNA-Rep and ihpRNA-ProRep derived transgenic banana plants were assayed for resistance towards BBTV infection using viruliferous aphids (Shekhawat et al., 2012). The transgenic banana plants generated using either of the constructs were completely resistant to BBTV infection as indicated by total absence of BBTD symptoms after 6 months of viruliferous aphid inoculation (Fig. 3). The resistance to BBTV infection in the two groups of transgenic plants was also proved by the fact that cDNAs coding for viral coat protein, movement protein and Rep protein could not be detected by RT-PCR in the inoculated transgenic leaves. Further, siRNAs specific to the sequence of Rep gene were detected in small RNAs isolated from transgenic leaves, establishing the basis of resistance of these plants towards BBTD.

Conclusions and future directions

The three strategies described above have successfully demonstrated the immense importance of the transgenic technology for development of improved banana varieties. Apart from disease resistance, we have also developed abiotic stress tolerance in transgenic bananas (Shekhawat et al., 2011) signifying the versatility of our approach (Fig. 4). Currently, our efforts are underway to develop transgenic bananas.
showing multiple disease resistance by stacking two or more of the transgenes in the same banana plant. These transgenic plants need to be tested in field trials after obtaining the necessary regulatory approvals.

References


